Original Article

Morphological Spectrum of Megakaryocytic Alterations in Bone Marrow Aspiration and Biopsy of Patients with Thrombocytopenia – A Tertiary Care Hospital Based Study

Rushali Patel[1,*], Keyuri Patel[1], Zalak Parmar[1]

[1]Department of Pathology, Pramukhswami Medical College & Shree Krishna Hospital, Bhaikaka University, Karamsad, Gujarat, India

***Correspondence:** rushalipatel01@gmail.com

DOI
10.21276/apalm.3799

Article History
Received: 28-12-2025
Revised: 27-01-2026
Accepted: 08-02-2026
Published: 02-03-2026

How to cite this article
Patel R, Patel K, et al. Morphological Spectrum of Megakaryocytic Alterations in Bone Marrow Aspiration and Biopsy of Patients with Thrombocytopenia – A Tertiary Care Hospital Based Study. Ann Pathol Lab Med. 2026;13(3):A123-A134.

Abstract

Background: Megakaryocyte morphology is vital for platelet production, and abnormalities at any developmental stage can lead to thrombocytopenia ($<$150,000/μL). Bone marrow evaluation helps distinguish decreased platelet production from increased peripheral destruction. This prospective study assessed dysplastic and non-dysplastic megakaryocytic changes in thrombocytopenia, bicytopenia, and pancytopenia, including non-MDS disorders.

Aim and objectives: To study megakaryocytic alterations in a patient with thrombocytopenia, bicytopenia, and pancytopenia in BMA and BMB. The study goal was to provide a comprehensive morphological assessment and support clinical correlation in haematological disorders.

Materials and methods: The study at Pramukhswami medical college, included 55 thrombocytopenia cases. Bone marrow aspiration and biopsy were reviewed independently by two hematopathologists. Inter-observer discrepancies were resolved by consensus review to standardize findings. Megakaryocyte counts were assessed per 10 LPF, with morphology evaluated in at least 30 cells. Dysplasia was strictly defined as abnormalities in $\geq$10% of megakaryocytes.

Results: ITP(18.2%), AOCD(12.7%), and AML(10.9%) were the leading causes of thrombocytopenia. Megakaryocyte counts were increased in ITP and AOCD due to compensatory marrow response, and reduced in aplastic anaemia and AML because of marrow suppression. Dysplastic megakaryocytic features were commonly seen in ITP, AOCD, and AML, along with frequent non-dysplastic changes such as hypolobation, immaturity, emperipolesis, and bare nuclei. Bone marrow aspiration offered better cytological detail, while biopsy was superior for evaluating marrow architecture.

Conclusion: Bone marrow aspiration and biopsy are essential for assessing megakaryocyte number and morphology in thrombocytopenia, bicytopenia, and pancytopenia. Identifying dysplastic features in non-MDS conditions like ITP, IAT and AOCD highlights the need for careful clinical correlation to avoid misdiagnosis.

Keywords: bone marrow aspiration and biopsy; megakaryocytes; thrombocytopenia

Introduction

Bone marrow is the spongy tissue found in the medullary cavity of compact bones. It is the primary site of haematopoiesis in an adult. Haematopoiesis, the process by which blood cells are produced, is tightly regulated within the bone marrow microenvironment. Among the key cellular components of this system are megakaryocytes, large bone marrow cells responsible for the production of platelets. Alterations in megakaryocyte morphology, number, distribution, and maturation are critical indicators of underlying haematologic disorders and are frequently observed in patients presenting with cytopenias—conditions characterized by decreased numbers of one or more blood cell lineages.

Thrombocytopenia is a frequently encountered haematologic abnormality, defined by a platelet count below 150,000/μL.[1] These haematologic abnormalities can be manifestations of a wide spectrum of diseases ranging from benign reactive processes to serious clonal or neoplastic disorders.[17] Thrombocytopenia is commonly seen in various haematological diseases, including myelodysplastic syndrome (MDS) and non-myelodysplastic haematological conditions. It may arise in isolation or in association with other cytopenias such as anaemia or leukopenia, reflecting a wide spectrum of underlying clinical disorders. These range from benign, transient, reactive conditions to serious primary bone marrow diseases including aplastic anaemia, myelodysplastic syndromes (MDS), acute leukaemia, and marrow infiltration.[2, 4] Because platelets are produced exclusively by megakaryocytes within the bone marrow, any disturbance in megakaryocyte number, maturation, or morphology has a direct impact on platelet output and, ultimately, on peripheral platelet counts.[5]

Megakaryopoiesis is a tightly regulated process driven by haematopoietic stem cells and influenced by cytokines—most notably thrombopoietin.[6] Mature megakaryocytes undergo endomitosis, cytoplasmic remodelling, and proplatelet formation to generate thousands of platelets per cell.[7] Alterations at any of these stages may lead to dysmegakaryopoiesis and consequently, thrombocytopenia.[8] While dysplastic megakaryocytic changes are classically associated with MDS, several non-myelodysplastic conditions—including immune thrombocytopenia, infections, megaloblastic anaemia, inflammatory states, and marrow suppression disorders.[9, 10]—also exhibit significant megakaryocytic abnormalities. Recognising these changes helps differentiate between disorders caused by impaired platelet production and those driven by increased peripheral destruction.[11]

Bone marrow aspiration and bone marrow biopsy remain essential and complementary investigations in the evaluation of cytopenias.[12] BMA allows detailed cytological assessment of individual megakaryocytes, whereas BMB enables evaluation of architectural features such as clustering, fibrosis, and overall cellularity.[13] Together, they provide critical insights into megakaryocyte numbers, maturation patterns, and lineage dysplasia—parameters integral to diagnosing thrombocytopenia and guiding clinical management.[14]

Understanding the morphological spectrum of megakaryocytic alterations across various haematological conditions is particularly important in tertiary-care settings, where cytopenias commonly present with broad and overlapping clinical features. Yet, despite the centrality of megakaryocyte morphology to thrombocytopenia, literature focusing on the combined interpretation of BMA and BMB across diverse non-MDS conditions remains limited.[10]

This study aims to bridge this gap by systematically analysing megakaryocyte number, distribution, and morphological variations—both dysplastic and non-dysplastic—in patients presenting with thrombocytopenia, bicytopenia, or pancytopenia. Through integrated interpretation of aspirate and biopsy findings, the study goal to provide a more detailed morphological profile of thrombocytopenic states and support better clinicopathologic correlation in haematological disorders presenting with low platelet counts.[14]

Aim and objectives

To study megakaryocytic alterations in a patient with thrombocytopenia, bicytopenia, and pancytopenia in BMA and BMB. The study goal was to provide a comprehensive morphological assessment and support clinical correlation in haematological disorders.

Materials and methods

A prospective observational study was conducted from May 2023 to June 2025 in the Haematology Section, Department of Pathology, Pramukhswami medical college and Shree Krishna Hospital, Bhaikaka University, Karamsad. A total of 55 patients with thrombocytopenia (with or without additional cytopenias) were enrolled after institutional ethics approval (May 2023).

Both bone marrow aspiration (BMA) and bone marrow biopsy (BMB) were obtained from the posterior superior iliac spine using standard techniques.[12, 15] BMA smears were stained with Leishman and Giemsa stain, while BMB sections were processed, decalcified, paraffin-embedded, and stained with haematoxylin and eosin.[13] Two independent hematopathologists performed evaluations without prior knowledge of each other findings (double-blinded review). While clinical context is inherent to bone marrow slides, reviewers initially assessed morphology independently before resolving any discrepancies through consensus.

Bone marrow aspiration (BMA): From the aspirated material, 6 to 8 thin or crushed smears were prepared and allowed to air-dry, and then fixed the smears in methanol for 20 minutes. Then the smears by Leishman stain and Giemsa stain. The smears were then studied under the microscope.

Bone marrow aspirate smear staining procedure as per our institute protocol:

Cover the bone marrow aspirate smears with Leishman stain for 2 minutes. Add Leishman buffer and blow with air intermittently. Let it stand for 10 minutes. After 10 minutes, wash with tap water. Now, on this slide, do Giemsa stain as follows. Dilute Giemsa stain in Giemsa buffer in the proportion, two drops of stain in 1 ml buffer. Flood the slide with Giemsa buffer and keep for 10 minutes. Tilt the slide and wash gently by pouring tap water on the edge of the slide and not directly on the smear. The back of the slide is also washed and place the slide upright on draining rack to drying.

Bone marrow biopsy:

The biopsies were received in fixed with 10 % acid-zinc-formalin (AZF) overnight. The next morning (after 20- 24 hours), the solution is decanted (with a stainer) and each biopsy specimen was washed in distilled water for 30 minutes. Then kept for decalcification for 6 hours. After adequate decalcification of the biopsy, it was processed and paraffin-embedded blocks of the same were prepared. Sections of 4-5-micron thickness were cut and stained with Haematoxylin and Eosin. The samples were subsequently processed, along with special stains when required.

Haematoxylin and Eosin staining procedure as per our institute protocol:

Place the slide to be stained in a hot air oven at 58 to 60 °C for 20-30 minutes to melt the paraffin and allow contact of the tissue with the slide. Place the slide in a jar containing Xylene 1 for 5 minutes. Transfer the slide to the jar containing 100% isopropyl alcohol for 5 minutes. Transfer the slide in the jar containing tap water for 1-2 minutes or till the slide appears clear. Transfer to Harris' Haematoxylin solution for 2 minutes. Rinse the slide in tap water for 2-5 minutes. Dip the slide in 0.1% acid alcohol and then 70% and 100% isopropyl alcohol. Counterstain the slide with alcoholic eosin solution for 1 minute. Dip the slide in ascending grades (70%, 100% and 100%) of alcohol for 1-5 minutes in each. Rinse in two xylene jars for 2-5 minutes in each. Mount with a cover slip, applying DPX.

In this study, the criteria for scoring megakaryocyte number and morphological alterations were predefined before the commencement of the research. The number and morphological alterations in megakaryocytes related to thrombocytopenia shall be assessed. The bone marrow smears shall be examined as per the standard guideline, and the findings shall be documented. For each case, at least 30 megakaryocytes were assessed for:

Assessment of number of megakaryocytes - shall be expressed as the number per 10 low power fields (LPFs) and further subdivided into: Absent (0/10 LPFs), Decreased (1/5-10LPFs), Normal (1/1-3 LPFs) , Increased ($>$2/LPF).[2]

Assessment of megakaryocyte morphology shall be done by evaluating at least 30 megakaryocytes for the various megakaryocytic alterations and subdividing them into: Dysplastic features (Multiple separated nuclei, micromegakaryocytes, and hypogranular forms) and Non-dysplastic features (emperipolesis, immature form, bare nuclei, cytoplasmic vacuolization, and budding).

All alterations shall be considered present only when 10% or more of the megakaryocytes observed show the change. All the findings noted in BMA were corroborated by findings in BMB. The number and morphology of the megakaryocytes in non-MDS-related thrombocytopenia were assessed. Their significance was studied by comparing them with the morphological changes in MDS.

Megakaryocytic Morphology: (Alterations considered significant if present in $\geq$10% megakaryocytes) Dysplastic features: Multiple separated nuclei, Micromegakaryocytes, Hypogranular forms. Non-dysplastic (reactive) features: Immature forms, Emperipolesis, Bare megakaryocytic nuclei, Cytoplasmic vacuolization, Budding megakaryocytes.

Statistical Analysis: Data were analysed using descriptive statistics (frequency, percentage, mean). No inferential statistics were applied to claim statistically significant differences between the etiological groups due to the descriptive nature of the study.

Ethical statement: The study protocol was reviewed and approved by the Institutional Ethics Committee (IEC) of Pramukhswami Medical College, May 2023.

Results

Demographic Profile: Among the 55 patients, there was a female predominance (58.18%). The median age was 52 years.

Etiological Spectrum: The most common underlying conditions were: ITP – 18.18%, Anaemia of Chronic Disease (AOCD) – 12.72%, AML – 10.9%, Aplastic anaemia & IAT – each 9.09%, Nutritional deficiency – 5.45%, Multiple myeloma – 5.45%, Miscellaneous causes included CML, CLL, ALL, MDS, metastatic adenocarcinoma, and others.

Hypolobated megakaryocytes were universal, suggesting a strong reactive or stress response across aetiologies. Bare nuclei and budding were easier to appreciate on aspiration smears. Comparison Between BMA and BMB. BMA superior for: cytological detail, identifying micromegakaryocytes, hypogranular and budding. BMB superior for: megakaryocyte clustering, detection of fibrosis and architectural patterns, identifying focal proliferation (e.g., ITP, myeloproliferative

Table 1: Etiological profile associated with thrombocytopenia in the present study (n=55).

Conditions	Number of Patients	Percentage (%)
AML	6	10.90
IAT	5	9.09
Aplastic anaemia	5	9.09
ITP	10	18.18
AOCD	7	12.72
Iron deficiency anaemia	2	3.64
Nutritional deficiency	3	5.45
ALL	2	3.63
CML	2	3.63
Multiple myeloma	3	5.45
MDS	2	3.64
Chronic eosinophilic leukaemia	1	1.82
NLPHL	1	1.82
TTP	1	1.82
Autoimmune haemolytic anaemia	1	1.82
Myeloid hyperplasia	1	1.82
CLL	1	1.82
Metastatic Adenocarcinoma	1	1.82
Total	55	100

Table 2: Comparison of the distribution of megakaryocytes in BMA and BMB (n=55).

Megakaryocyte distribution	Bone marrow aspiration	Bone marrow biopsy
Normal	24 (43.6%)	21 (38.18%)
Increased	15 (27.27%)	13 (23.63%)
Decreased	16 (29%)	21(38.18%)

neoplasms).

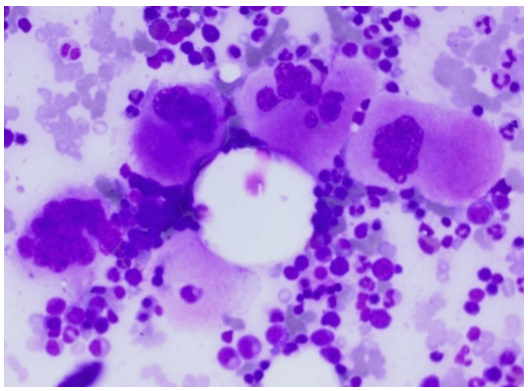

Figure 1: BMA showing clustering of megakaryocytes with dysplastic change - multiple separated nuclei (Leishman stain, 40x).

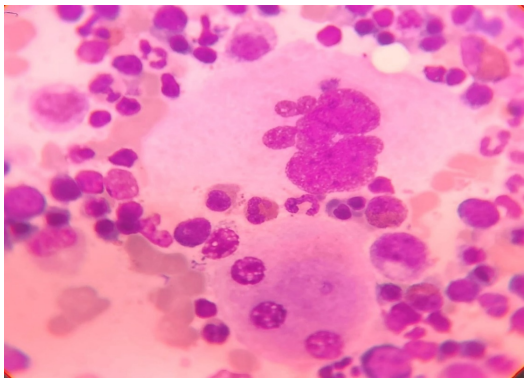

Figure 2: BMA showing multiple separate nuclei with a hypogranular megakaryocyte (Leishman stain, 100x).

Table 3: Integrated of megakaryocytes distribution in BMA and BMB (n=55).

Bone marrow aspiration				
Conditions	No of cases	Normal	Decreased	Increased
AML	6	2 (33.3%)	4 (66.6%)	-
IAT	5	4 (80%)	-	1 (20%)
Aplastic anaemia	5	-	5 (100%)	-
ITP	10	2 (20%)	-	8 (80%)
AOCD	7	4 (57.14%)	-	3 (42.8%)
Iron deficiency anaemia	2	2 (100%)	-	-
Nutritional deficiency	3	2 (66.6%)	-	1 (33.3%)
ALL	2	1 (50%)	1 (50%)	-
CML	2	-	1 (50%)	1 (50%)
Multiple myeloma	3	2 (66.6%)	1 (33.3%)	-
MDS	2	1 (50%)	1 (50%)	-
Chronic eosinophilic leukaemia	1	1 (100%)	-	-
NLPHL	1	1 (100%)	-	-
TTP	1	-	-	1 (100%)
Autoimmune haemolytic anaemia	1	-	-	1 (100%)
Myeloid hyperplasia	1	-	1 (100%)	-
CLL	1	1 (100%)	-	-
Metastatic adenocarcinoma	1	-	1 (100%)	-
MPN	1	1 (100%)	-	-
Total	55	24	15	16
Bone marrow biopsy				
Conditions		Normal	Decreased	Increased
AML		2 (33.33%)	2 (33.33%)	2 (33.33%)
IAT		4 (80%)	-	1 (20%)
Aplastic anaemia		-	5 (100%)	-
ITP		1 (10%)	-	9 (90%)
AOCD		4 (57.14%)	-	3 (42.8%)
Iron deficiency anaemia		1 (50%)	1 (50%)	-
Nutritional deficiency		1 (33.3%)	1 (33.3%)	1 (33.3%)
ALL		-	1 (50%)	1 (50%)
CML		-	1 (50%)	1 (50%)
Multiple myeloma		1 (33.3%)	1 (33.3%)	1 (33.3%)
MDS		2 (100%)	-	-
Chronic eosinophilic leukaemia		1 (100%)	-	-
NLPHL		-	-	1 (100%)
TTP		1 (100%)	-	-
Autoimmune haemolytic anaemia		-	-	1 (100%)
Myeloid hyperplasia		1 (100%)	-	-
CLL		1 (100%)	-	-
Metastatic adenocarcinoma		-	1 (100%)	-
MPN		1 (100%)	-	-
Total		21	13	21

Table 4: Comparison of megakaryocytic alteration in BMA and BMB (n=55).

Megakaryocytes	Bone marrow aspiration	Bone marrow biopsy
Dysplastic features of megakaryocytes		
Multiple-separated nuclei	21 (38.18%)	17 (30.90%)
Micro megakaryocytes	23 (41.81%)	29 (52.72%)
Hypogranular forms	03 (5.45%)	-
Non-dysplastic features of megakaryocytes		
Immature form	22 (40%)	25 (45.45%)
Emperipolesis	13 (23.63%)	13 (23.63%)
Hypolobated or monolobated	55 (100%)	55 (100%)
Cytoplasmic Vacuolization	-	-
Bare megakaryocytic nuclei	13 (23.63%)	02 (3.63%)
Budding megakaryocytes	29 (52.72%)	(25.45%)

Table 5: Comparison of Dysplastic features: BMA and BMB (n=55).

Bone marrow aspiration				
Conditions	No of case	Multiple-separated nuclei (dysplastic form)	Micro megakaryocyte	Hypo granular form
AML	6	3 (50%)	1 (16.66%)	-
IAT	5	1 (20%)	3 (60%)	1 (20%)
Aplastic anaemia	5	1 (20%)	-	-
ITP	10	2 (20%)	8 (80%)	1 (10%)
AOCD	7	3 (42.85%)	3 (42.85%)	1 (14.28%)
Iron deficiency anaemia	2	-	1 (50%)	-
Nutritional deficiency	3	2 (66.66%)	1 (33.3%)	-
ALL	2	1 (50%)	1 (50%)	-
CML	2	2 (100%)	-	-
Multiple myeloma	3	2 (66.6%)	1 (33.3%)	-
MDS	2	-	-	-
Chronic eosinophilic leukaemia	1	1 (100%)	1 (100%)	-
NLPHL	1	1 (100%)	1 (100%)	-
TTP	1	-	1(100%)	-
Autoimmune haemolytic anaemia	1	-	1 (100%)	-
Myeloid hyperplasia	1	1 (100%)	-	-
CLL	1	-	-	-
Metastatic adenocarcinoma	1	-	-	-
MPN	1	1 (100%)	1 (100%)	-
Total	55	21	23	03
Bone marrow biopsy				
Conditions		Multiple-separated nuclei (dysplastic form)	Micro megakaryocyte	Hypo granular form
AML		1 (16.66%)	2 (33.33%)	-
IAT		1 (20%)	4 (80%)	-
Aplastic anaemia		-	1 (20%)	-
ITP		6 (60%)	7 (70%)	-
AOCD		3 (42.85%)	4 (57.14%)	-
Iron deficiency anaemia		-	1 (50%)	-
Nutritional deficiency		1 (33.33%)	1 (33.33%)	-
ALL		1 (50%)	1 (50%)	-
CML		1 (50%)	1 (50%)	-
Multiple myeloma			2 (66.66%)	-
MDS		1 (50%)	-	-
Chronic eosinophilic leukaemia		-	-	-
NLPHL		1 (100%)	1 (100%)	-
TTP		-	1 (100%)	-
Autoimmune haemolytic anaemia		-	1 (100%)	-
Myeloid hyperplasia		-	1 (100%)	-
CLL		1 (100%)	-	-
Metastatic adenocarcinoma		-	-	-
MPN		-	1 (100%)	-
Total		17	29	00

Discussion

This prospective study is aimed to thoroughly examine the range of megakaryocytic alterations observed in bone marrow aspiration (BMA) and bone marrow biopsy (BMB) samples from patients with thrombocytopenia, bicytopenia, or pancytopenia. The study analysed 55 cases to improve the understanding of both dysplastic and non-dysplastic megakaryocytic changes. The research highlights the findings underscore the critical role of combined BMA and BMB provide a comprehensive morphological assessment and support clinical correlation in haematological disorders.

Megakaryocyte number correlated with underlying disease: Increased signified compensatory production (e.g., ITP), Decreased indicated marrow failure (aplastic anaemia) or infiltration (AML, MDS)[5, 6]

The variation in disease prevalence across different studies further underscores the influence of geographical factors and population demographics. The following table provides a comparative overview of the most common underlying conditions causing thrombocytopenia in our study and other studies.

Table 6: Comparison of non-dysplastic features: BMA and BMB (n=55).

Bone marrow aspiration							
Diagnosis	No of case	Immature form	Emperipolesis	Hypo lobated or monolo-bated	Cytoplasmic vacuoliza-tion	Budding megakary-ocyte	Bare megakary-ocytic nuclei
AML	6	2 (33.33%)	2 (33.33%)	6 (100%)	-	-	2 (33.33%)
IAT	5	3 (60%)	2 (40%)	5 (100%)	-	2 (40%)	3 (60%)
Aplastic anaemia	5	-	-	2 (40%)	-	-	-
ITP	10	7 (70%)	3 (30%)	10 (100%)	-	2 (20%)	6 (60%)
AOCD	7	3 (42.85%)	1 (14.28%)	7 (100%)	-	2 (28.57%)	6 (85.71%)
Iron deficiency anaemia	2	-	-	2 (100%)	-	-	1 (50%)
Nutritional deficiency	3	-	1(33.33%)	3 (100%)	-	1 (33.33%)	2 (66.66%)
ALL	2	-	1 (50%)	2 (100%)	-	1 (50%)	1 (50%)
CML	2	-	-	2 (100%)	-	1 (50%)	1 (50%)
Multiple myeloma	3	2 (66.66%)	1 (33.33%)	3 (100%)	-	2 (66.6%)	2 (66.6%)
MDS	2	-	-	1 (50%)	-	1 (50%)	-
Chronic eosinophilic leukaemia	1	1 (100%)	1 (100%)	1 (100%)	-	1 (100%)	1 (100%)
NLPHL	1	1 (100%)	-	1 (100%)	-	-	1 (100%)
TTP	1	1 (100%)	-	1 (100%)	-	-	1 (100%)
Autoimmune haemolytic anaemia	1	1 (100%)	-	1(100%)	-	-	1 (100%)
Myeloid hyperplasia	1	-	-	1 (100%)	-	-	1 (100%)
CLL	1	-	-	1 (100%)	-	-	-
Metastatic adenocarcinoma	1	-	-	1(100%)	-	-	-
MPN	1	1 (100%)	1 (100%)	1(100%)	-	-	1(100%)
Total	55	22	13	55	00	13	29
Bone marrow biopsy							
Diagnosis		Immature form	Emperipolesis	Hypo lobated or monolo-bated	Cytoplasmic vacuoliza-tion	Budding megakary-ocyte	Bare megakary-ocytic nuclei
AML		1 (16.66)	1 (16.66%)	6 (100%)	-	-	-
IAT		2 (40%)	1 (20%)	5 (100%)	-	-	1 (20%)
Aplastic anaemia		1 (20%)	0	5 (100%)	-	-	-
ITP		8 (80%)	3 (30%)	10 (100%)	-	-	4 (40%)
AOCD		1 (14.28%)	2 (28.57%)	7 (100%)	-	1 (14.28%)	2 (28.57)
Iron deficiency anaemia		-	1 (50%)	2 (100%)	-	-	=
Nutritional deficiency		-	-	3 (100%)	-	-	2 (66.66%)
ALL		2 (100%)	-	2 (100%)	-	-	2(100%)
CML		-	1 (50%)	2 (100%)	-	-	-
Multiple myeloma		3 (100%)	-	3(100%)	-	-	-
MDS		1 (50%)	-	2(100%)	-	-	-
Chronic eosinophilic leukaemia		1(100%)	-	1 (100%)	-	-	-
NLPHL		1 (100%)	-	1 (100%)	-	1 (100%)	1 (100%)
TTP		1 (100%)	1 (100%)	1 (100%)	-	-	1 (100%)
Autoimmune haemolytic anaemia		1 (100%)	1 (100%)	1 (100%)	-	-	1 (100%)
Myeloid hyperplasia		1 (100%)	-	1 (100%)	-	-	-
CLL		-	1 (100%)	1 (100%)	-	-	-
Metastatic adenocarcinoma		-	-	1 (100%)	-	-	-
MPN		1 (100%)	1 (100%)	1 (100%)	-	-	-
Total		25	13	55	00	02	14

Megakaryocyte count emerged as a key diagnostic marker with disease-specific patterns. Increased megakaryocytes were mainly seen in ITP, AOCD, and a few cases of CML, TTP, nutritional deficiency, and autoimmune haemolytic anaemia, reflecting compensatory megakaryocytic hyperplasia due to peripheral platelet destruction or ineffective thrombopoiesis. In contrast, a marked decrease was consistently observed in aplastic anaemia, correlating with marrow failure, and was also common in AML, multiple myeloma, and metastatic adenocarcinoma due to marrow suppression or replacement by malignant cells.

Morphological Alterations: The study categorised megakaryocyte morphology into dysplastic and non-dysplastic features, using a $\geq$10% dysplasia cutoff—aligned with WHO and hematopathology standards—to distinguish reactive from clonal

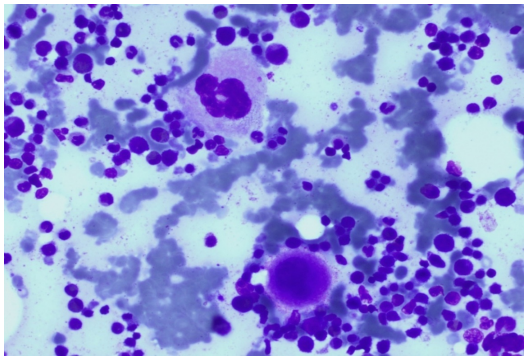

Figure 3: BMA showing dysplastic changes - micromagakaryocytes compared with normal megakaryocyte (Leishman stain, 40x).

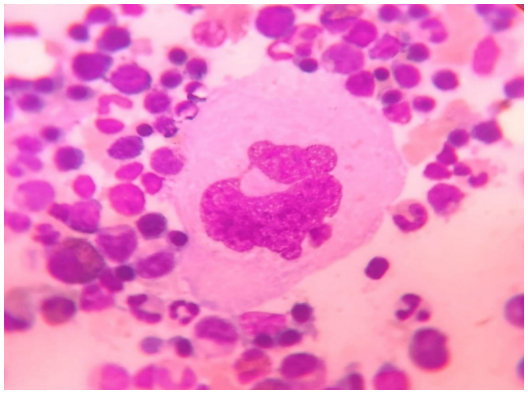

Figure 4: BMA showing dysplastic change - hypogranular megakaryocyte (Leishman stain, 100x).

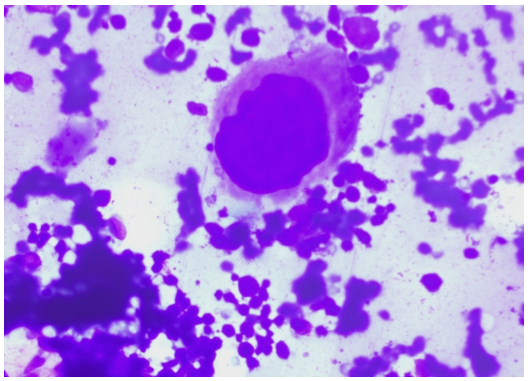

Figure 5: BMA showing non-dysplastic change - immature megakaryocyte (Leishman stain, 100x).

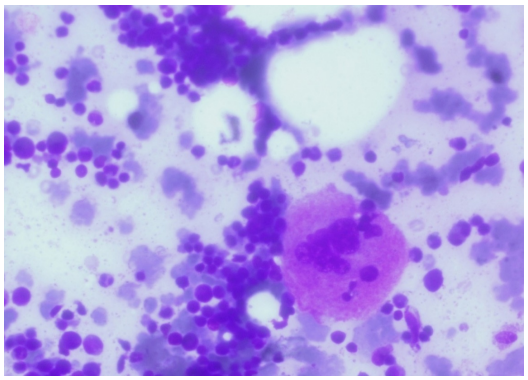

Figure 6: BMA showing emperipolesis of lymphocyte in megakaryocyte (Leishman stain, 40x).

disorders like MDS.

Dysplastic features are indicative of abnormal maturation and are particularly important in the diagnosis of MDS and certain acute leukaemias, although they can also be seen in reactive conditions. The finding of dysplastic features, such

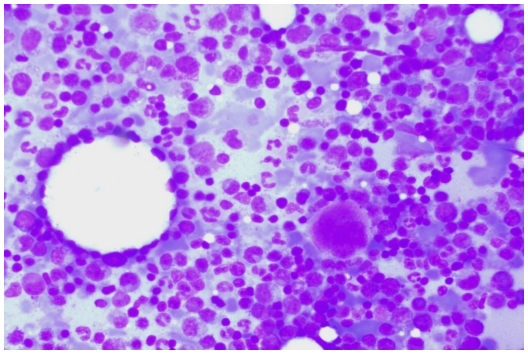

Figure 7: BMA showing non-dysplastic change - bare megakaryocytic nuclei (Leishman stain, 40x).

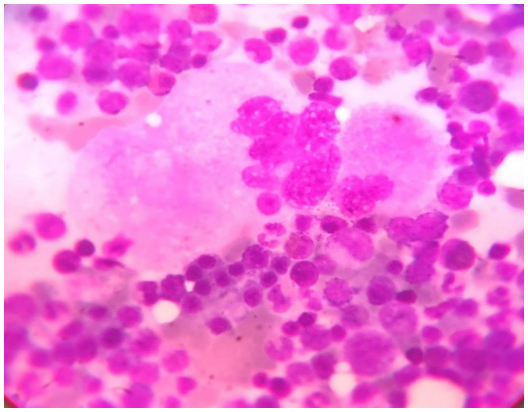

Figure 8: BMA showing non-dysplastic change - budding megakaryocyte (Leishman stain, 100x).

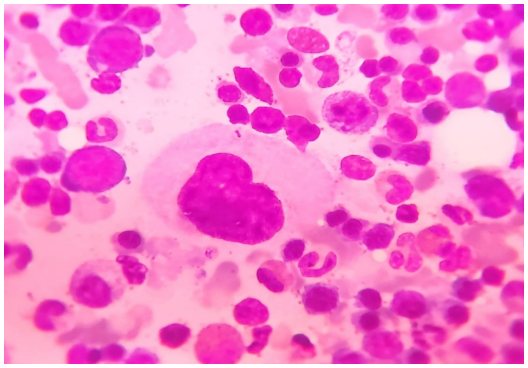

Figure 9: BMA showing hypolobated megakaryocyte (Leishman stain, 100x).

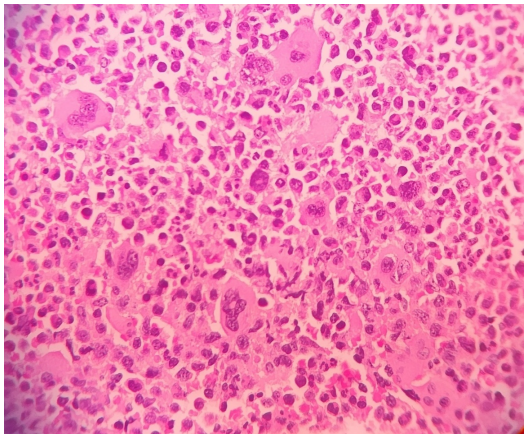

Figure 10: Bone marrow biopsy showing clustering of megakaryocytes (H & E stain, 40x).

as micromegakaryocytes (80% in ITP) and multiple separated nuclei, in non-MDS conditions is a critical observation. In ITP and AOCD, these changes likely represent 'stress megakaryopoiesis'—a reactive morphological deviation due to high

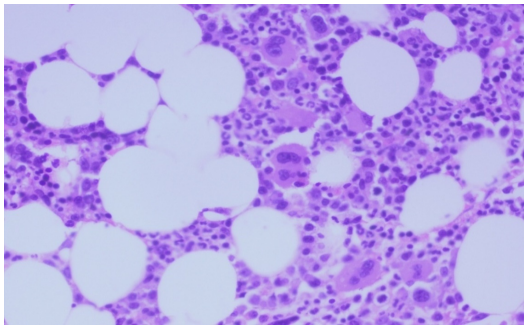

Figure 11: Bone marrow biopsy section showing emperipolesis of a lymphocyte in megakaryocyte (H& E stain, 40x).

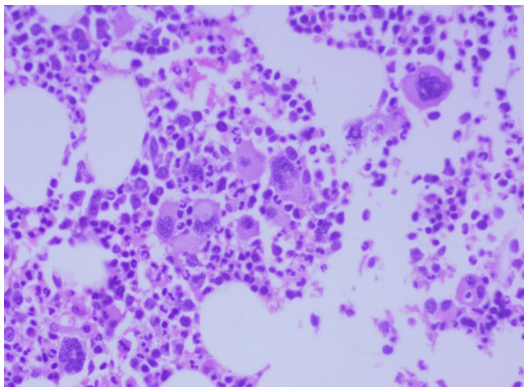

Figure 12: Bone marrow biopsy section showing hypolobated and micromegakaryocytes (H& E stain, 40x).

Table 7: Comparison of megakaryocyte distribution among various conditions.

Conditions	Our Study (BMA and BMB)	Bhasin *et al.* (2013)[16] (BMA)	Veerpaneni *et al.* (2020)[18] (BMA)	Muhury *et al.* (2009)[17](BMA)	Pokharel *et al.* (2016)[20] (BMA)	Sharma *et al.* (2019)[19] (BMA)
ITP	Increased (BMA-80%, BMB-90%)	Increased (Not specified)	Increased (100%)	Increased (94.7%)	Increased (88.3%)	Increased (100%)
Aplastic anaemia	Decreased (100%)	Decreased (Not specified)	Decreased (100%)	Decreased	Decreased	Not specified
AML	Decreased (66.6% BMA)	Decreased (Not specified)	Decreased (88.2%)	Decreased	Decreased (69.2%)	Not specified
Megaloblastic anaemia or Dimorphic anaemia	Increased (in some)	Increased (Dimorphic Anaemia)	Increased (21.7% in MA)	Not specified	Not specified	Increased
MDS	Varied (Some decreased)	Varied (Some decreased)	Decreased (100%)	Not specified	Not specified	Decreased

peripheral demand—rather than true clonal dysplasia. This necessitates a strict adherence to the $\geq$10% threshold and clinical correlation to prevent the overdiagnosis of MDS.

Non-dysplastic features typically represent reactive or adaptive changes in megakaryopoiesis. Morphological findings are: Hypolobation supports its interpretation as a reactive, non-specific abnormality. Micromegakaryocytes were prominent in both reactive and clonal disorders, yet more frequent in AML and MDS, making correlation essential.[9] Emperipolesis showed variable distribution across disorders and across published studies (14–68%)[10]

BMA provided superior cytological resolution, whereas BMB excelled in architectural assessment. Their complementary nature reinforces the need for combined evaluation, particularly where aspiration yields inadequate material or when fibrosis or neoplastic infiltration is suspected.[12, 13]

Table 8: Comparison of dysplastic and non-dysplastic features in megakaryocytes (percentage frequency).

Morphological Alteration (Our Study BMA and BMB)	Our Study (BMA and BMB)	Bhasin *et al.* (2013)[16] (Overall)	Veerpaneni *et al.* (2020)[18] (BMA)	Muhury *et al.* (2009)[17] (BMA)	Pokharel *et al.* (2016)[20] (BMA)	Sharma *et al.* (2019)[19] (BMA)
Dysplastic features						
Multiple-separated Nuclei	BMA-38.18% BMB-30.90%	Not explicitly quantified	45.5% (commonest)	15.2%	Not explicitly quantified	69.5%, most common in Megaloblastic Anaemia
Micro megakaryocytes	BMA-41.81% BMB- 52.72%	Present (in MDS and a few non-MDS)	39.2%	61%	25% (in Aplastic anaemia)	Encountered in many cases (not quantified)
Hypogranular forms	BMA-5.45% BMB-0.00%	Present (in MDS and a few non-MDS)	Not explicitly quantified	Not explicitly quantified	Not explicitly quantified	6% each, seen in ITP and Megaloblastic Anaemia
Dysplastic forms (General features)	(Includes above)	Present in MDS and few non-MDS conditions (Dimorphic Anaemia, ITP, CML blast crisis)	Present in Megaloblastic anaemia, Acute or Chronic leukaemia, ITP	Present in 89.5% of cases (general dysplastic changes)	Present in MTP (33.3%), Acute leukaemia (33.3%)	Present in MDS and non-MDS conditions
Non-dysplastic features						
Immature forms	BMA-40%, BMB- 45.45%	Present (Not specified)	58.2% (commonest)	Present (Not specified)	Present (75% in AA)	93% (in ITP)
Emperipolesis	BMA-23.63%, BMB- 23.63%	Present (Not specified)	14.4%	68.4% (in ITP)	Not observed	40% (in ITP)
Hypolobated/ Monolobated megakaryocytes	BMA-100% BMB-100%	Not specified	Not explicitly quantified	Present (Not specified)	63.2%	Not explicitly quantified
Cytoplasmic vacuolization	BMA and BMB-0.00%	Present (Not specified)	Not explicitly quantified	Not explicitly quantified	Not explicitly quantified	60% (in ITP)
Budding megakaryocytes	BMA-23.63% BMB-3.63%	Present (Not specified)	Not explicitly quantified	Not explicitly quantified	Not explicitly quantified	Not explicitly quantified
Bare megakaryocytic nuclei	BMA-52.72% BMB-5.45%	Present (Not specified)	40.5%	Present (Not specified)	57.9%	65.2% (in MA)

Strengths of this prospective study design allowed for systematic data collection and consistent application of predefined criteria for megakaryocyte assessment. The double-blinded review by two independent hematopathologists enhanced the objectivity and reliability of the morphological evaluations, minimising observer bias. The inclusion of a diverse cohort of patients with various underlying conditions contributing to thrombocytopenia strengthened the generalizability of our findings regarding megakaryocytic alterations across a wide range of haematological disorders. Crucially, the direct comparison of BMA and BMB findings for each case provides valuable insights into the complementary roles of these diagnostic modalities, showing a more complete picture than studies relying on a single method.

Limitations of this study was conducted at a single centre—meaning findings may reflect local demographics and referral patterns and thus may not be generalizable to other regions or healthcare systems—and despite double-blinded review, morphological interpretations remain subjective. The study classified dysplastic features as reactive based solely on morphology and clinical context, without using advanced techniques like flow cytometry, immunohistochemistry or molecular testing to investigate clonality or specific mutations in suspected MDS case.

Conclusion

A comprehensive evaluation combining bone marrow aspiration (for cytological detail) and biopsy (for architectural assessment) is essential to accurately assess megakaryocytic changes in thrombocytopenia, bicytopenia or pancytopenia. The study highlights the diagnostic importance of assessing megakaryocyte number, morphology and noting that dysplastic

features seen in various haematological conditions (like- ITP, IAT, AML, AOCD). Future multicentre research with larger cohorts and advanced ancillary methods (like- flow cytometry, molecular studies) will refine our understanding of megakaryocytic alterations and improve diagnostic accuracy.

Acknowledgements: Not applicable

Funding: This research received no external funding. All resource and facilities for research were provided by the Department of Pathology, Pramukhswami Medical Collage and Shree Krishna Hospital, Karamsad, Gujarat.

Competing Interests: No competing interests related to this work.

Inform consent: Not applicable, because this study does not involve direct patient participants and clinical images.

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