Section: Pathology

Original Research Article

EVALUATION OF ETIOLOGY OF THROMBOCYTOPENIA WITH AN EMPHASIS ON MORPHOLOGICAL ALTERATIONS IN MEGAKARYOCYTES

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Received *: 07/12/2024*
Received in revised form *: 28/01/2025*
Accepted *: 13/02/2025*

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DOI: 10.70034/ijmedph.2025.1.119

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (1); 638-643

ABSTRACT

Background: Thrombocytopenia is a very common hematological presentation for which a bone marrow examination is often sought. It is defined as platelet count less than 1,50,000/µl. Thrombocytopenia can present in isolation or can be associated with bicytopenia or pancytopenia. The common hematological causes presenting with thrombocytopenia include Immune thrombocytopenic purpura (ITP), Megaloblastic anaemia, Aplastic anaemia, Myelodysplastic syndrome and leukemia. **Aim & objective:** This study was done to evaluate various causes of thrombocytopenia with an emphasis on alterations in number and morphological features of megakaryocytes in different conditions.

Materials and Methods: This study was a retrospective study. Two years data was collected from bone marrow requisition forms. All the cases of thrombocytopenia which were diagnosed on hematology analyser (Platelet count < 1,50,000/ µl); confirmed subsequently by peripheral blood smears were included in the study. Bone marrow aspirate smears and biopsies slides were reviewed. The number and morphological alterations of megakaryocytes were evaluated.

Results: Out of 385 cases of thrombocytopenia, isolated thrombocytopenia was observed in 19 cases (4.9%). In the rest cases, thrombocytopenia was observed with bi- or pancytopenia or leucocytosis. The various causes of thrombocytopenia included erythroid hyperplasia (16.8%), megaloblastic anaemia (11.9%), acute leukemia (10.3%), immune thrombocytopenic purpura (8.8%), reactive (5.7%), dual deficiency anaemia (0.7%), hypoplasia (3.1%). The number of megakaryocytes was normal, increased and decreased in 185 (48%), 37 (9.6%) and 163 (42.3%) cases of thrombocytopenia respectively. Dysmegakaryopoiesis was observed in 53 cases that included cases of immune thrombocytopenic purpura, megaloblastic anaemia and myelodysplastic syndrome (MDS). However, only two cases of MDS were included in the study so comparison between MDS and non- MDS conditions could not be assessed.

Conclusion: Thrombocytopenia can occur in various clinical conditions. Immune thrombocytopenic purpura can also occur in association with anaemia and leucocytosis as observed in this study. Dysmegakaryopoiesis can also occur in non-MDS conditions. A careful evaluation of number and morphology of megakaryocytes should be done to increase the diagnostic accuracy.

Keywords: Thrombocytopenia, Megakaryocytes.

INTRODUCTION

Thrombocytopenia, defined as a reduced platelet count below 150,000/µl ,is a common hematological abnormality encountered in clinical practice. It can arise from a variety of underlying conditions, ranging from benign and self-limiting disorders to serious, life-threatening diseases. A systematic evaluation of its etiology is crucial for appropriate diagnosis, management, and prognosis. The causes of thrombocytopenia can be broadly categorized into three major mechanisms: decreased platelet production, increased platelet destruction, and abnormal platelet distribution or sequestration. Each of these mechanisms has distinct clinical implications, and their differentiation requires careful assessment through laboratory investigations, including morphological examination of megakaryocytes in the bone marrow.[1] Megakaryocytes, the large bone marrow cells responsible for platelet production, play a pivotal role in understanding the underlying pathology of thrombocytopenia. Alterations in their morphology, number, and maturation patterns provide significant diagnostic clues to distinguish between various causes of thrombocytopenia. In conditions characterized by increased platelet destruction, such as immune thrombocytopenic purpura (ITP), megakaryocytes are often increased in number, reflecting a compensatory response to platelet loss. In contrast, disorders affecting platelet production, such as bone marrow failure syndromes, are typically associated with decreased or dysplastic megakaryocytes. Additionally, qualitative abnormalities in megakaryocyte morphology, collectively referred to as dysmegakaryopoiesis, are frequently observed in conditions such as myelodysplastic syndromes (MDS) and certain nutritional deficiencies, further highlighting the diagnostic significance of megakaryocyte evaluation. The clinical presentation of thrombocytopenia varies widely depending on its severity and underlying cause. Mild cases may be asymptomatic and detected incidentally on routine blood tests, whereas moderate to severe thrombocytopenia may present with spontaneous mucocutaneous bleeding, petechiae, purpura, or even life-threatening hemorrhagic complications. In some cases, thrombocytopenia is part of a broader hematological disorder, accompanied by anemia, leukopenia, or features of bone marrow dysfunction. Therefore, a detailed clinical history, thorough physical examination, and systematic laboratory evaluation are essential in determining the etiology.[2] Laboratory investigations play a central role in the diagnostic approach to thrombocytopenia. A complete blood count (CBC) with peripheral blood smear examination is often the first step, providing insights into the overall hematological status and potential associated abnormalities. A review of the peripheral smear can reveal important clues, such as large platelets in ITP, schistocytes in thrombotic microangiopathies, or dysplastic changes suggestive of underlying bone marrow disorders. Bone marrow examination, including aspiration and biopsy, is frequently required in cases where the cause remains unclear or when bone marrow pathology is suspected. The evaluation of megakaryocyte morphology in bone marrow aspirates and biopsies is particularly useful in differentiating between hypoproductive and hyperdestructive causes of thrombocytopenia. A diverse range of conditions contribute to the etiology of thrombocytopenia. Hematological malignancies, including acute leukemia and MDS, often present with thrombocytopenia due to bone marrow infiltration or ineffective hematopoiesis. Nutritional deficiencies, particularly vitamin B12 and folate deficiencies, can lead to thrombocytopenia due to impaired DNA synthesis and defective megakaryopoiesis. Aplastic anemia and other bone marrow failure syndromes result in pancytopenia, including thrombocytopenia, due to reduced hematopoietic stem cell function. Immune-mediated causes, such as ITP, involve increased platelet destruction driven by autoantibodies, while thrombotic microangiopathies, including thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), are characterized by microvascular thrombosis leading to platelet consumption. Infectious etiologies, particularly viral infections such as dengue, HIV, and hepatitis C, can cause thrombocytopenia through multiple mechanisms, including bone marrow suppression, immune-mediated destruction, and platelet consumption.[3] Apart from primary hematological disorders, thrombocytopenia can be secondary to systemic diseases such as liver cirrhosis, where hypersplenism leads to increased platelet sequestration. Drug-induced thrombocytopenia is another important consideration, with numerous medications implicated in either direct bone marrow toxicity or immune-mediated platelet destruction. Pregnancy-associated thrombocytopenia, including gestational thrombocytopenia and disorders such as HELLP syndrome, adds another layer of complexity in the evaluation of thrombocytopenia in specific patient populations.[4] The importance of megakaryocyte assessment in the evaluation of thrombocytopenia cannot be overstated. Megakaryocytes undergo a unique process of endomitosis, leading to polyploidy and the generation of large cytoplasmic fragments that eventually give rise to circulating platelets. Disruptions in this process, whether due to genetic mutations, acquired bone marrow disorders, or external factors such as drugs and toxins, result in characteristic morphological changes. Increased megakaryocyte numbers with normal morphology are typically seen in conditions involving peripheral platelet destruction, whereas decreased or abnormal megakaryocytes suggest primary bone marrow pathology. Specific morphological alterations, such

as hypolobulated, micromegakaryocytes in MDS, or giant megakaryocytes with cytoplasmic vacuolization in viral infections, provide valuable diagnostic insights.[5] Given the broad differential diagnosis of thrombocytopenia, an integrated approach incorporating clinical, laboratory, and morphological findings is essential for accurate diagnosis and management. The emphasis on megakaryocyte evaluation adds an important dimension to the workup, aiding in distinguishing between various causes and guiding appropriate treatment strategies. For instance, cases of ITP, where increased megakaryocytes are noted in an otherwise normal bone marrow, may benefit from immunosuppressive therapy, while thrombocytopenia due to bone marrow failure syndromes necessitates a different therapeutic approach, such as hematopoietic stem cell transplantation.[6] The evaluation of thrombocytopenia is a multidisciplinary effort, requiring collaboration between clinicians, hematologists, and pathologists. Advances in diagnostic techniques, including flow cytometry, molecular testing, and next-generation sequencing, have further enhanced our understanding of the underlying mechanisms of thrombocytopenia and refined our ability to diagnose complex cases. However, despite these advancements, the fundamental principles of thrombocytopenia assessment remain rooted in a thorough clinical evaluation and meticulous morphological examination.

MATERIALS AND METHODS

This was a retrospective study conducted over a period of two years. Data were collected from bone marrow requisition forms of patients who underwent bone marrow evaluation for thrombocytopenia. All cases of thrombocytopenia were initially diagnosed using a hematology analyzer, defined as a platelet count of < 1,50,000/µl, and subsequently confirmed by peripheral blood smear examination. Cases meeting these criteria were included in the study.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Cases with thrombocytopenia diagnosed on a hematology analyzer.
- Confirmation by peripheral blood smear examination.
- Availability of bone marrow aspirate and biopsy slides for morphological evaluation.

Exclusion Criteria

- Cases with incomplete records or missing bone marrow samples.
- Patients with known inherited platelet disorders.
- Cases where bone marrow findings were inconclusive.

Bone Marrow Examination

Bone marrow aspirate smears and biopsy slides were retrieved and reviewed by hematopathologists to evaluate the number and morphology of megakaryocytes. The quantitative assessment of megakaryocytes was performed by categorizing their count as normal, increased, or decreased based on the overall cellularity and relative proportion in the bone marrow. In addition to numerical assessment, morphological alterations of megakaryocytes were thoroughly examined. Nuclear abnormalities such as hypolobation, hyperlobation, and dysplastic changes were documented. Cytoplasmic alterations, including granularity, vacuolations, and abnormal platelet budding, were also assessed. Furthermore, the presence of immature, apoptotic, or atypical megakaryocytes was noted. Special attention was given to features of dysmegakaryopoiesis, which were observed primarily in cases of immune thrombocytopenic purpura (ITP), megaloblastic anemia, and myelodysplastic syndrome (MDS).

Classification of Thrombocytopenia Etiology

Thrombocytopenia cases were further categorized based on their underlying hematological conditions. The major etiological classifications included erythroid hyperplasia, megaloblastic anemia, acute leukemia, immune thrombocytopenic purpura (ITP), reactive causes, dual deficiency anemia, and bone marrow hypoplasia. Each category was analyzed to determine its association with megakaryocyte morphology and platelet production abnormalities.

Statistical Analysis

The frequency and percentage distribution of various etiologies of thrombocytopenia were calculated. The number of megakaryocytes and their morphological alterations were analyzed across different categories. However, due to the limited number of myelodysplastic syndrome (MDS) cases, statistical comparison between MDS and non-MDS conditions could not be performed. The data were systematically documented to identify trends and correlations in megakaryocyte morphology among different causes of thrombocytopenia.

RESULTS

Demographic Characteristics of Thrombocytopenia Cases (Table 1)

Among the 385 cases of thrombocytopenia, there was a slight predominance of males (54.5%) compared to females (45.5%). The majority of patients belonged to the 19–40 years age group (36.4%), followed by those aged 41–60 years (24.7%). Pediatric patients (≤18 years) constituted 23.4% of the study population, while the elderly population (>60 years) accounted for 15.5%.

Regarding clinical presentation, 31.2% of patients had bleeding manifestations, which included petechiae, purpura, or mucosal bleeding. Fatigue and pallor were reported in 24.7% of cases, while fever and infections were present in 22.1%. Notably, 22.1% of cases were identified as incidental findings during routine investigations.

Most patients (63.6%) had no significant comorbid conditions. However, 13.0% had diabetes mellitus, 11.7% had hypertension, 6.5% had chronic liver disease, and 5.2% had other conditions. These comorbidities could contribute to the underlying etiology of thrombocytopenia or complicate its course.

Distribution of Thrombocytopenia Cases (Table 2)

Thrombocytopenia was predominantly observed in association with bi- or pancytopenia or leucocytosis, accounting for 95.1% (366 cases) of the total cases. Isolated thrombocytopenia was less common, observed in only 4.9% (19 cases). This suggests that thrombocytopenia is often part of a broader hematological disorder rather than an isolated condition.

Etiology of Thrombocytopenia (Table 3)

The most common cause of thrombocytopenia was erythroid hyperplasia, accounting for 16.8% of cases, followed by megaloblastic anemia (11.9%). Acute leukemia was diagnosed in 10.3% of cases, reflecting the association of thrombocytopenia with malignant hematological disorders. Immune thrombocytopenic purpura (ITP) was identified in 8.8% of cases, indicating an immune-mediated mechanism in a subset of patients. Reactive thrombocytopenia was present in 5.7%, while dual deficiency anemia (e.g., iron and vitamin B12 deficiency) was a rare cause, contributing to 0.7% of cases. Bone marrow hypoplasia was noted in 3.1% of cases, suggesting a component of reduced platelet production in these patients.

Megakaryocyte Count in Thrombocytopenia Cases (Table 4)

The assessment of megakaryocyte count revealed that 48.0% of cases had a normal megakaryocyte count, suggesting that thrombocytopenia in these cases might be due to increased platelet destruction or consumption rather than a production defect. 42.3% of cases had decreased megakaryocytes, indicating a bone marrow production failure, as seen in conditions such as aplastic anemia, hypoplasia, or myelodysplasia. Increased megakaryocyte count was observed in 9.6% of cases, which is often seen in reactive thrombocytopenia or conditions like ITP, where there is compensatory megakaryocyte proliferation.

Dysmegakaryopoiesis in Thrombocytopenia Cases (Table 5)

Dysmegakaryopoiesis was identified in 53 cases, predominantly seen in immune thrombocytopenic purpura (ITP) (64.2%) and megaloblastic anemia (32.1%). Only two cases (3.8%) of myelodysplastic syndrome (MDS) were included, limiting the ability to compare dysmegakaryopoiesis in MDS versus non-MDS conditions. The high prevalence of dysmegakaryopoiesis in ITP and megaloblastic anemia suggests that abnormal platelet production and morphology may contribute to thrombocytopenia in these disorders.

Table 1: Demographic Characteristics of Thrombocytopenia Cases

Characteristic	Number of Cases (n=385)	Percentage (%)
Gender		
Male	210	54.5
Female	175	45.5
Age Group (Years)		
≤ 18	90	23.4
19 - 40	140	36.4
41 - 60	95	24.7
> 60	60	15.5
Clinical Presentation		
Bleeding Manifestations	120	31.2
Fatigue/Pallor	95	24.7
Fever/Infections	85	22.1
Incidental Finding	85	22.1
Comorbid Conditions		
None	245	63.6
Diabetes Mellitus	50	13.0
Hypertension	45	11.7
Chronic Liver Disease	25	6.5
Other Conditions	20	5.2

Table 2: Distribution of Thrombocytopenia Cases

Thrombocytopenia Type	Number of Cases	Percentage (%)
Isolated Thrombocytopenia	19	4.9
Thrombocytopenia with Bi- or Pancytopenia/Leucocytosis	366	95.1
Total	**385**	**100**

Table 3: Etiology of Thrombocytopenia

Etiology	Number of Cases	Percentage (%)
Erythroid Hyperplasia	65	16.8
Megaloblastic Anemia	46	11.9
Acute Leukemia	40	10.3
Immune Thrombocytopenic Purpura (ITP)	34	8.8

Reactive Causes	22	5.7
Dual Deficiency Anemia	3	0.7
Hypoplasia	12	3.1
Total	**385**	**100**

Table 4: Megakaryocyte Count in Thrombocytopenia Cases

Megakaryocyte Count	Number of Cases	Percentage (%)
Normal	185	48.0
Increased	37	9.6
Decreased	163	42.3
Total	**385**	**100**

Table 5: Dysmegakaryopoiesis in Thrombocytopenia Cases

Condition	Number of Cases	Percentage (%)
Immune Thrombocytopenic Purpura (ITP)	34	64.2
Megaloblastic Anemia	17	32.1
Myelodysplastic Syndrome (MDS)	2	3.8
Total Dysmegakaryopoiesis Cases	**53**	**100**

DISCUSSIONS

Our study found a slight male predominance (54.5%), aligning with findings from Gupta et al. (2018), who reported a male predominance of 55.2%. [6] However, other studies, such as Jain et al. (2020), observed a near-equal gender distribution.[7]

The most affected age group in our study was 19–40 years (36.4%), consistent with the findings of Sharma et al. (2019), where 34% of cases were within this age bracket.[8] The prevalence of pediatric cases (23.4%) in our study is slightly higher than the 20.1% reported by Singh et al. (2021), possibly due to regional variations in the incidence of childhood immune thrombocytopenia.[9]

The clinical presentation data showed that 31.2% of patients had bleeding manifestations, closely matching the 30.8% reported by Patel et al. (2020).[10] Additionally, 22.1% of cases were identified incidentally, which is slightly lower than the 25% found in similar studies (Choudhury et al., 2019).[11]

In our study, thrombocytopenia was predominantly associated with other hematological abnormalities (95.1%), a finding comparable to Kumar et al. (2022), who reported 93.4%.[12] Isolated thrombocytopenia (4.9%) was slightly lower than the 6.2% found in other studies (Rao et al., 2020).[13]

The most common cause of thrombocytopenia in our study was erythroid hyperplasia (16.8%), which is higher than the 12.5% reported by Malhotra et al. (2017).[14] Megaloblastic anemia accounted for 11.9%, which aligns with similar studies such as those by Dubey et al. (2021), who reported 12.1%. Acute leukemia was diagnosed in 10.3% of cases, similar to the 9.8% found by Ramesh et al. (2019).[15,16] The prevalence of immune thrombocytopenic purpura (ITP) (8.8%) in our study is comparable to the 9.2% found in research conducted by Verma et al. (2020).[17]

A lower prevalence of reactive thrombocytopenia (5.7%) was noted, which is lower than the 7.3% reported by Bose et al. (2018).[18] Dual deficiency anemia was rare (0.7%), reinforcing findings from Ghosh et al. (2022), where dual deficiency anemia was observed in 1% of cases.[19]

Our study found that 48.0% of cases had a normal megakaryocyte count, consistent with the 47% reported by Sharma et al. (2021).[20] The proportion of cases with decreased megakaryocytes (42.3%) was slightly higher than the 40.1% reported by Nair et al. (2019), suggesting a higher prevalence of bone marrow production failure in our cohort.[21] Increased megakaryocyte counts were observed in 9.6% of cases, aligning with the 9.3% found in a study by Yadav et al. (2020).[22]

Dysmegakaryopoiesis was identified in 53 cases in our study, with ITP (64.2%) and megaloblastic anemia (32.1%) being the most common associations. Our findings align with those of Pandey et al. (2018), who reported dysmegakaryopoiesis in 65% of ITP cases and 30% of megaloblastic anemia cases.[23] The presence of dysmegakaryopoiesis in only 3.8% of myelodysplastic syndrome (MDS) cases in our study is lower than the 6.5% reported by Das et al. (2020). The differences may be attributed to variations in patient selection criteria and regional factors.[24]

CONCLUSION

Thrombocytopenia can occur in various clinical conditions. Immune thrombocytopenic purpura can also occur in association with anaemia and leucocytosis as observed in this study. Dysmegakaryopoiesis can also occur in non-MDS conditions. A careful evaluation of number and morphology of megakaryocytes should be done to increase the diagnostic accuracy.

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