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**Dr. Jayanta Kumar Das**
Department of Pathology, Silchar Medical College and Hospital, Silchar, Assam, India

**Dr. Tarulata Saikia**
Department of Microbiology, Gauhati Medical College and Hospital, Guwahati, Assam, India

**Dr. Sandeep Kakhlari**
Department of Pathology, Silchar Medical College and Hospital, Silchar, Assam, India

**Dr. D Datta**
Department of Pathology, Silchar Medical College and Hospital, Silchar, Assam, India

**Correspondence**
**Dr. Jayanta Kumar Das**
Department of Pathology, Silchar Medical College and Hospital, Silchar, Assam, India

# Study of etiopathogenesis of thrombocytopenia in a tertiary health centre: A prospective study

**Dr. Jayanta Kumar Das, Dr. Tarulata Saikia, Dr. Sandeep Kakhlari and Dr. D Datta**

**Abstract**
Thrombocytopenia is defined as a platelet count less than 150 x $10^9$/liter (Lichtman AM, Beutler E *et al*. 2006). A low platelet count could result from peripheral destruction by an immune or non-immune mechanism; decreased production resulting from an inherited or acquired marrow disease; or splenic pooling. Pseudothrombocytopenia can occur in asymptomatic patients, and results from *in vitro* clumping of platelets following blood collection (Lichtman AM, Beutler E *et al*. 2006). The present study was undertaken in the department of pathology, Silchar Medical College to study the etiopathogenesis, and any bone marrow changes associated with thrombocytopenia. Total 80 patients with decreased platelet count were selected from among the patients attending the outpatient department and the patient admitted, along with concomitant bone marrow aspiration was conducted to Silchar Medical College Silchar during the period of one year, which included subjects of both sexes of different age groups. In this study, thrombocytopenia was found to be associated with megaloblastic anemia (38 cases, 47.5%), hematological malignancies (26 cases, 32.5%), infections (10 cases,12.5%), ITP (Immune- thrombocytopenic purpura) (4 cases,5%) followed by aplastic anemia (2 cases,2.5%). In the present study, a total of 80 bone marrow aspirates was performed. Megaloblastic anemia (38 cases,47.5%) with bone marrow morphology showed mostly hyperlobated megakaryocytes (34 cases out of 38 cases) followed by 2 cases with dysmegakaryopoietic feature and the remaining 2 cases showed normal megakaryocytic morphology. Second most common cause, total of 21 cases of acute leukemia (15 cases of Acute Myeloid Leukaemia & 6 cases of Acute Lymphoblastic Leukaemia) were found. Megakaryocyte morphological alteration most commonly found in the acute leukemia cases were mainly immature forms and dysplastic forms. Dysplastic forms of megakaryocytes were found in both in Myelodysplastic Synddrome and non-MDS conditions like megaloblastic anemia, Acute Myeloid Leukaemia, Chronic Myeloid Leukaemia in blast crisis. But finding of micromegakaryocytes in bone marrow were observed only in MDS. Morphological alterations of megakaryocytes in case of infection associated thrombocytopenia were mainly immature forms and presence of cytoplasmic vacuolation. Cases of ITP had an increase in megakaryocytic number and morphologically showed predominantly immature and hypogranular forms. It has been seen that from above study, megaloblastic anemia is the most common cause of thrombocytopenia followed by haematological malignancies of which acute myeloid leukemia is the leading cause. Proper clinical examination along with haematological investigations including bone marrow morphological assessment leads to a clue in establishing the final diagnosis.

**Keywords:** Thrombocytopenia, Bone marrow aspirate, Megakaryocyte, Megaloblastic anemia

## 1. Introduction
Normal hemostasis is a consequence of tightly regulated processes that maintain blood in a fluid state in normal vessels, y*et al*so permit the rapid formation of a hemostatic clot at the site of a vascular injury. The pathologic counterpart of hemostasis is thrombosis; it involves blood clot formation within intact vessels. Both hemostasis and thrombosis involve three components: the vascular wall (particularly the endothelium), platelets, and the coagulation cascade (Kumar V *et al*. 2005) [6]

It hardly needs reiteration that platelets are critical for hemostasis, since they form temporary plugs that stop bleeding and promote key reactions in the coagulation cascade. Spontaneous bleeding associated with thrombocytopenia most often involves small vessels. Common sites for such hemorrhages are the skin and the mucous membranes of the gastrointestinal and genitourinary tracts. Most feared, however, is intracranial bleeding, which is a threat to any patient with a markedly depressed platelet count (Kumar V *et al*. 2005) [6].

The many causes of thrombocytopenia can be classified into four major categories (Kumar V *et al*. 2005) [6]

1. Decreased platelet production.
2. Decreased platelet survival.
3. Sequestration in the spleen.
4. Dilution.

This study was undertaken to know the etiopathogenesis of thrombocytopenia along with any changes in bone marrow and megakaryocytic alterations associated with it.

## 2. Materials and Methods

This study was carried out in the Department of Pathology, Silchar Medical College & Hospital, Silchar, Assam. Eighty ( 80) patients with decreased platelet count ( <150 x $10^3$µL ) were selected from among the patients attending the outpatient departments and the patient admitted in Silchar Medical College, Silchar during the period of one year from June 2012 to July 2013 which included subjects from both sexes of different age groups. The complete blood count were done by a five part autoanalyser and followed up by manual methods like PBF (Peripheral Blood Film), Neubauer counting chamber using platelet diluting fluid. To determine the cause of thrombocytopenia, Bone marrow aspiration and biopsy was done. The marrow aspiration were done from posterior superior iliac spine taking care of all the aseptic measures with a Salah marrow puncture needle as described by and Bone marrow biopsy was done in few selected cases of thrombocytopenia where indicated. Smears were drawn and air dried from the material aspirated and stained by May Grunwald Giemsa (MGG). The biopsy tissues obtained in this way were of a length of 1.5-2cms. After fixation the tissues were decalcified in 5% nitric acid for 10 to 12 hours. After decalcification, the tissues were washed in running water. After washing step, the processing was routine as for other tissues. Section were stained by routine haematoxylene and eosine by the usual way. The bone marrow imprints were stained by May-Grunwald-Giemsa stain, Sudan Black. Sudan black staining of bone marrow smear was done to determine whether the cells belong to myeloid or lymphoid series. Myeloid series shows positivity for sudan black stain. Sudan black B (SBB) is a lipophilic dye that binds irreversibly to an undefined granule component in granulocytes, eosinophils, and some monocytes.

## 3. Results and Discussions

In this study, thrombocytopenia was found to be associated with megaloblastic anemia (38 cases, 47.5%), haematological malignancies (26 cases, 32. 5%), infections (10 cases, 12. 5%), ITP (4 cases, 5%) followed by aplastic anemia (2 cases,2.5%) (Table 1). The highest number of cases who presented with thrombocytopenia were in the age group of 21 – 30 years (25 cases, 31.25%), followed by 11 - - 20 years (16 cases, 20%) and 31 - 40 years (14 cases,17.5%).The lowest number of cases were found in the age groups 61 – 70 years (1 case,1.25%) and > 70 years (1 case,1.25%) (Table 2). In the present study, thrombocytopenia was found to be more common in females, with an incidence of 56.25% (45 cases) than in males 43.75% (35 cases) with male: female ratio of 1: 1.29. This increase in incidence in females were mainly due to megaloblastic anemia associated thrombocytopenia. Other workers also found female preponderance (Uma Khanduri *et al*., 2007) [16]. According to their study this increase in female incidence was mainly due to increase demand during growth spurt, puberty and child-bearing age group in a population already deficient in cobalamin precipitated the anemia (Uma Khanduri *et al*., 2007) [16].

This increase in the incidence in our study of thrombocytopenia in the age group of 21-30 years was also mainly due to megaloblastic anemia (19 cases, 76 %) followed by 11-20 years (10 cases, 62.5%), 5 cases (35.71 %) in the age group of 31 – 40 years & 4 cases (11.76%) in the age group of 41-50 years and It shows that diet may be a major contributory factor to low levels of cobalamin. In our study, more than 50% of their subjects were non-vegetarians, their diet contained animal protein in the form of milk, occasional eggs and very occasional meat. The diet of most subjects comprised wheat, rice, pulses, vegetables and ~250 ml of milk per day. Studies done by various workers also showed megaloblastic anaemia as the commonest causes of pancytopaenia as 40.90%, 50% respectively (Tariq Aziz *et al*.,2010; K K Kulkarni *et al*., 2006) [13, 5].

In the present study megaloblastic anemia mainly due to cobalamin deficiency was the most common cause of thrombocytopenia and all of these 38 cases (47.5%) of megaloblastic anemia were associated with pancytopenia. This very high incidence of megaloblastic anemia were due to low socio economic status of the people of this area and dietary deficiency of these two vitamins, cobalamin and folate plays a major role in the etiopathogenesis of megaloblastic anemia associated thrombocytopenia. In our study we found haematological malignancies (26 cases, 32.5%) as the second most common cause of thrombocytopenia. Among the haematological malignancies, AML (15 cases, 57. 69%) was the most common cause of thrombocytopenia followed by ALL (6 cases, 23. 07%), CML-blast crisis (4 cases, 15. 38%) and MDS (1 case,3.84%) (Table 3). In the present study we found, an increase in the incidence of cases of AML with increasing age. Out of total 15 cases of AML, 12 cases were found in the age group ranging from 31 – 60 years and the remaining 3 cases occured below 30 years. In the present study out of 21 cases of acute leukemia, the platelet count were ranged from < 20,000 (severe thrombocytopenia,14 cases) to 20,000—50,000 (moderate thrombocytopenia,7 cases). Platelet deficiency was more severe and frequent in acute leukemia predominantly in acute lymphoblastic leukemia followed by acute myeloid leukemia. In comparision platelet deficiency was less marked in chronic leukemia. In CML, platelet count was found to be mildly decreased in late stages. Other workers also found frequent and severe platelet depression in acute leukemia cases in comparision to chronic leukemia patients (Singh VP, 1977).

In the present study, we found out of total 80 cases, 10 cases (12.5%) with thrombocytopenia were associated with infectious origin (Figure 1). Out of these 10 cases, 4 cases (40%) were associated with P. falciparum malaria and all of them were associated with moderate degree of thrombocytopenia. The rest of the infectious cases were found to be associated with HIV (3 cases, 30%), enteric fever (2 cases, 20%) followed by tuberculosis (1 case, 10%). In this present study, 4 cases (5%) of thrombocytopenia were associated with chronic ITP. All them were associated with moderate degree of thrombocytopenia (platelet count-20 to 50 X $10^3$/µL).Most of the ITP cases in this study had a female preponderance (3 female versus 1 male) and all of

them occurred between 31-50 years of age. In the present study we found 2 cases of acquired aplastic anemia, one case in the age group of 31-40 years and the other case was above 70 years. After proper evaluation, both of the cases had a history of long term intake of analgesic (diclofenac & aspirin) due to chronic pain. Both of them had a hypocellular marrow with severe thrombocytopenia (platelet count < 20 x $10^3$/μL).

**Megakaryocytic alteration in bone marrow in various cases of thrombocytopenia**

In the present study, a total of 80 bone marrow aspirates were performed. The most common cause of thrombocytopenia in our study for which bone marrow aspiration were performed, was megaloblastic anemia (38 cases,47.5%). It was seen that in majority of cases of megaloblastic anemia, bone marrow morphology showed mostly hyperlobated megakaryocytes (34 cases out of 38 cases) followed by 2 cases with dysmegakaryopoietic feature and the remaining 2 cases showed normal megakaryocytic morphology (Table 4). Similarly, Hoffbrand AV *et al*. (2005) [4], stated that large hyperpolyploid megakaryocytes is a characteristic bone marrow finding in patient with megaloblastic anemia. Muhury *et al*. (2009) [10], in their study of megakaryocytic alteration in thrombocytopenia found that, dysplastic forms in megaloblastic anemia were seen in 9 cases out of a total of 12 cases of megaloblastic anemia. Wickramasinghe *et al*.(1995) [17], observed megakaryocytes with separation of nuclear lobes and nuclear fragments and attributed this to diminished DNA synthesis leading to nuclear maturation defect. Muhury *et al*. (2009) [10], in their study found 27 cases (18.8%) of AML and this was the most common cause of thrombocytopenia for which bone marrow examination was sought. In 17 of the cases (63%), the number of megakaryocytes was decreased with six cases (22.2%) not showing any megakaryocytes. Tricot G *et al*. (1985) [15] reported the same in marrows of AML tightly packed with leukemic blast cells with maturation arrest. The immature forms, dysplastic forms, bare megakaryocyte nuclei and emperipolesis were observed in cases of leukemias.

In the present study, a total of 21 cases of acute leukemia (15 cases of AML & 6 cases of ALL) were found to be associated with thrombocytopenia. Out of 15 cases of AML, all of them showed decreased number of megakaryocytes, where as all the 6 cases of ALL showed decrease in megakaryocytic number. Megakaryocyte morphological alteration most commonly found in the acute leukemia cases were mainly immature forms and dysplastic forms.

In the present study 4 cases of CML were associated with thrombocytopenia, we found number of megakaryocytes decreased in 1 case of CML in blast crisis. The remaining 3 cases of CML which were in chronic phase showed normal number of megakaryocytes. There were no significant morphological alteration in all the cases of CML in chronic phase except the one in blast crisis where dysplastic megakaryocytic morphology was noted in bone marrow. This finding of dysplastic form in CML in blast crisis in our study correlates with that of Tejinder Singh Bhasin *et al* (2013) [14].

Muhury *et al*. (2009) [10], found, dysplastic forms, bare megakaryocytic nuclei and micromegakaryocytes in cases of MDS. However, finding of micromegakaryocytes was most significant when compared to non-MDS causes of thrombocytopenia. Dyspoietic megakaryocytes are well described in MDS, however, limited literature exists regarding their significance in non-MDS cases. In their study, there was no significant difference in the dyspoietic features in non-MDS cases as compared to MDS cases except for the micromegakaryocytes with specificity of 83% in MDS. The micromegakaryocytes represent abnormal megakaryocytes that have lost their ability to undergo endomitosis, a qualitative defect of megakaryocyte maturation. Increased number of abnormal megakaryocytes, particularly the micromegakaryocytes suggests an expansion of megakaryocytic precursors, an arrest in terminal megakaryocyte differentiation and impaired nuclear development.

Similarly, in our study dysplastic forms of megakaryocytes were found in both in MDS and non-MDS conditions like megaloblastic anemia, AML, CML in blast crisis. But finding of micromegakaryocytes in bone marrow were observed only in MDS. Hence, dyspoietic features by themselves do not specify MDS, other hematological conditions causing thrombocytopenia have to be considered in the differential diagnosis. It has been seen that various dyspoietic features which were observed both in MDS and non-MDS cases, of which presence of micromegakaryocytes in MDS cases were found to be more significant in differentiating between cases of MDS from non-MDS.

In case of infection associated thrombocytopenia in our study, out of total 10 cases, 7 cases had normal megakaryocytic number where as 3 cases had decreased megakaryocytic number. All these 3 cases were associated with HIV infection.

Muhury *et al*. (2009) [10], in their study found all five cases of infection associated thrombocytopenia had an increased megakaryocytic count. Similarly Alter JH *et al*. (1969) [1] found an increase in number of megakaryocytes in infection associated thrombocytopenia.

Morphological alterations of megakaryocytes in our study for cases of infection associated thrombocytopenia were mainly immature forms and presence of cytoplasmic vacuolation. Similarly Muhury *et al*. (2009) [10], Meindersma TC *et al*. (1962) [9], found, immature megakaryocytes in cases of thrombocytopenia associated with infection who opined that this was due to the increased megakaryocyte turnover. Cytoplasmic vacuolization was also seen in cases associated with infection in this study, where Chanarin I *et al*. (1973) [2] found similar morphological alteration of megakaryocytes in infection.

Muhury *et al*. (2009) [10], in their study found that,out of 18 of the 19 cases of ITP, there was an increase in the number of megakaryocytes which was also observed by George JN *et al*. (1994) [3] and Levine FC (1999) [8]. They attributed this to stimulation of the marrow megakaryocytes to synthesize platelets at an increased rate due to immune-mediated platelet destruction in the spleen and other reticuloendothelial tissues. A shift to young, immature, less polypoid megakaryocytes and fewer mature platelet-producing megakaryocytes was the outstanding morphological feature noted in almost all the cases of ITP in the study done by Tejinder Singh Bhasin *et al*. (2013) [14].

Similarly in our study, all the 4 cases of ITP had an increase in megakaryocytic number and morphologically showed predominantly immature and hypogranular forms.

In our study we came across 2 cases of aplastic anemia associated with severe thrombocytopenia. Bone marrow

aspiration showed dry-tap. So, bone marrow trephine biopsy was advised, which showed markedly hypocellular marrow with majority of the marrow spaces replaced by fat, lymphocytes, plasma cells and macrophages. Out of the two cases, one case showed decrease in megakaryocyte number while in the other case no megakaryocytes seen. Similarly Shadduck *et al*. (2001) [11], observed decreased or absent megakaryocytes in aplastic anemia. There was no alteration of megakaryocytes morphology in this present study. They attributed this to bone marrow suppression and significant inhibition of nucleic acid synthesis in the megakaryocytes.

Muhury *et al*. (2009) [10], in their study found that the dyspoietic megakaryocytes which were present in one case of aplastic anemia, showed that the patient was a known case of ITP who had subsequently developed aplastic anemia and these abnormal megakaryocytes had persisted in the marrow. Tricot G *et al* (1985) [15] where megakaryocytes were of normal morphology. Similarly, in the present study there was no alteration in the morphology of megakaryocytes in the case of aplastic anemia.

**Table 1:** Etiologies of Thrombocytopenia

| Etiology | Frequency | Percentage(%) |
|---|---|---|
| Megaloblastic anemia | 38 | 47.5 |
| Hematological malignancies | 26 | 32.5 |
| Infections | 10 | 12.5 |
| ITP | 04 | 5 |
| Aplastic anemia | 02 | 2.5 |
| Total | 80 | 100 |

**Table 2:** Frequencies of Causes of Thrombocytopenia At Different Age Groups

| Age | Megalo.A | AML | ALL | CML | MDS | Malaria | TB | Bact. | HIV | ITP | AA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1—10 | -- | -- | 04 | -- | -- | -- | -- | 01 | -- | -- | -- |
| 11--20 | 10 | 01 | 02 | -- | -- | 02 | -- | 01 | -- | -- | -- |
| 21--30 | 19 | 02 | -- | -- | -- | -- | 01 | -- | 01 | -- | -- |
| 31--40 | 05 | 05 | -- | -- | -- | 02 | -- | -- | 02 | 01 | 01 |
| 41--50 | 04 | 04 | -- | 02 | -- | -- | -- | -- | -- | 03 | -- |
| 51--60 | -- | 03 | -- | 01 | 01 | -- | -- | -- | -- | -- | -- |
| 61--70 | -- | -- | -- | 01 | -- | -- | -- | -- | -- | -- | -- |
| >70 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 01 |
| TOTAL | 38 | 15 | 06 | 04 | 01 | 04 | 01 | 02 | 03 | 04 | 02 |

**Table 3:** Hematological Malignancies Causing Thrombocytopenia.

| Sl No. | Hematological malignancy | Frequency | Percentage (%) |
|---|---|---|---|
| 1 | AML | 15 | 57.69 |
| 2 | ALL | 06 | 23.08 |
| 3 | CML | 04 | 15.38 |
| 4 | MDS | 01 | 3.85 |
| | Total | 26 | 100 |

**Table 4:** Morphological Changes in Megakaryocytes in Various Conditions

| Conditions | Normal | Immature Forms | Dysplastic Forms | Number of nuclear lobes | | Hypogranular Forms | Cytoplasmic Vacuolation | Micro Megakaryocyte |
|---|---|---|---|---|---|---|---|---|
| | | | | ↑ | ↓ | | | |
| Megaloblastic anemia | + | 0 | + | + | 0 | 0 | 0 | 0 |
| AML | + | + | + | 0 | 0 | + | 0 | 0 |
| ALL | + | + | 0 | 0 | 0 | 0 | 0 | 0 |
| CML | + | 0 | + | 0 | 0 | 0 | 0 | 0 |
| MDS | 0 | 0 | + | 0 | 0 | 0 | 0 | + |
| Infection | + | + | 0 | 0 | 0 | 0 | + | 0 |
| ITP | 0 | + | 0 | 0 | 0 | + | 0 | 0 |
| Aplastic anemia | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

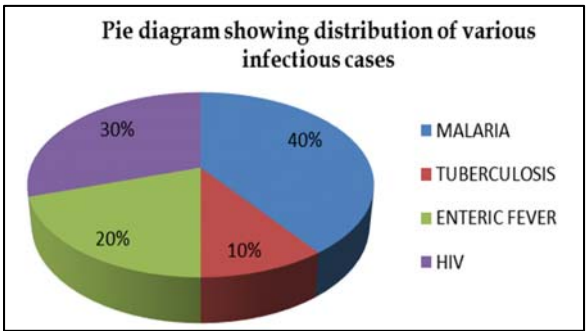


**Fig 1:** Infectious Causes of Thrombocytopenia.

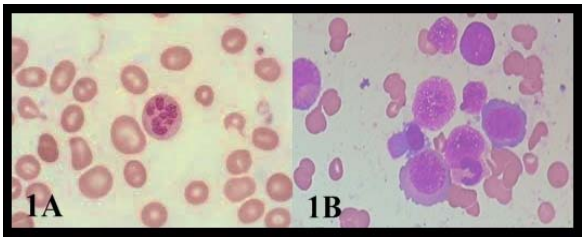


**Fig 2:** Megaloblastic anemia. (A) Peripheral blood film showing macroovalocytes and hypersegmented neutrophils and reduced platelet number. (B) Bone marrow aspirate showing megaloblasts.

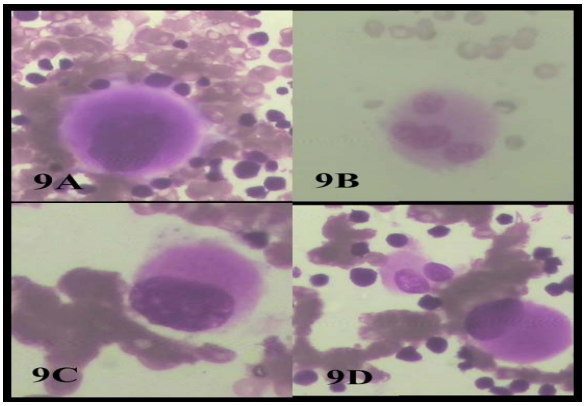


**Fig 3:** Myelodysplastic syndrome (MDS). Bone marrow aspirate showing dyserythropoiesis (A) & (B) nuclear budding and intranuclear bridging respectively. Fig. (C) & (D) showing dysthrombopoiesis, binucleated

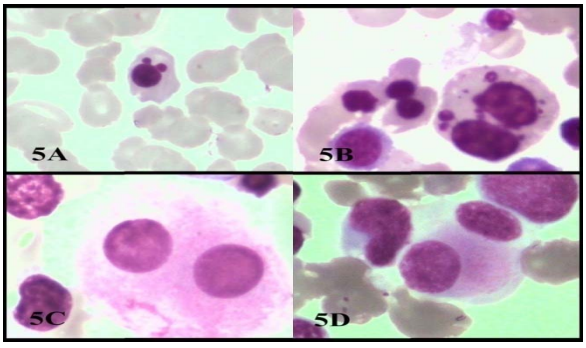


**Fig 4:** Morphological alterations of megakaryocytes. (A)Immature megakaryocyte. (B) Multinucleated megakaryocytes. (C) Micromegakaryocyte. (D) Binucleated & hypogranular megakaryocyte

## 4. Conclusion

Megaloblastic anemia was the most common cause of thrombocytopenia. Haematological malignancies was the second most common cause of thrombocytopenia of which acute myeloid leukemia was the leading cause. Proper clinical examination along with haematological investigations including bone marrow morphological assessment leads to a clue in establishing the final diagnosis. Diagnosis of the underlying cause of thrombocytopenia helps the clinicians in the further management of the patients.

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