"PREDICTIVE VALUE OF PLATELET INDICES FOR DIFFERENTIATING HYPERDESTRUCTIVE THROMBOCYTOPENIA FROM HYPOPRODUCTIVE THROMBOCYTOPENIA IN PATIENTS ATTENDING TERITIARY CARE HOSPITAL"

By

Dr. D. CHANDRIKA CHINTALAPUDI

Dissertation submitted to the
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IN

PATHOLOGY

UNDER THE GUIDANCE OF

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ABSTRACT

BACKGROUND: Thrombocytopenia is one of the most critical and complicated clinical conditions to manage. Hence the need for a platelet count has increased over the years in the form of manual smear count and bone marrow examination for discriminating hyperdestructive and hypoproductive thrombocytopenia. The introduction of platelet indices like mean platelet volume (MPV), platelet distribution width (PDW), Platelet large cell ratio (P-LCR) in automated haematology analyzers has provided a window into the cause of thrombocytopenia in the recent years. These parameters are obtained rapidly in a relatively non invasive way unlike bone marrow evaluation. The recent advances in automated haematology analysers have added new dimensions to the platelet indices. The study was undertaken to analyze the utility of platelet indices in hypoproductive and hyperdestructive thrombocytopenia.

OBJECTIVE: To assess the utility of platelet indices in differentiating hyperdestructive and hypo productive thrombocytopenia.

MATERIALS AND METHODS

Patients with platelet counts less than 1.50 lakh/cumm attending to Shri B.M.Patil hospital and Research Centre were included in this study. The study period was from 1st November 2016 to 30th June 2018.

The blood samples were collected from thrombocytopenic patients in an EDTA vacutainer and immediately analyzed for CBC using SYSMEX XN 1000, 6 part haematology analyzer in CBC DIFF mode where platelet parameters like Platelet count, mean platelet Volume (MPV), platelet distribution width (PDW) and platelet large cell ratio(PLCR).

Peripheral blood smears were prepared and reviewed to estimate platelet count to rule out pseudo thrombocytopenia and fragments of cells like schistocytes. Bone marrow

aspiration was done as per the institutional protocol. Statistical analysis was performed to assess the mean standard deviation, Receiver Operating Curve (ROC) to determine the specificity, sensitivity, positive predictive value (PPV)and negative predictive values (NPV) for platelet parameters (MPV, PDW, PLCR). Analysis by Student t test to compare the mean and medians and correlation test to determine association between the continuous variables was done.

RESULT: The total number of cases included in the study are 84, where 49 cases were included in hyperdestructive and 35 were included in hypoproductive group.

MPV greater than 11 femtoliters had a 70.2% NPV in hyperdestructive TCP and MPV less than 8 femtoliters had a 100% PPV in hypoproductive TCP with 67.3% sensitivity and 65.7% specificity. PDW of > 14 fl has a NPV of 65% and < 12 fl has a PPV of 51.8% with 51.4% sensitivity and 63.3% specificity. PLCR of > 35% has a NPV of 73% in hyperdestructive TCP and PLCR of < 20% has a PPV of 83% in hypoproductive TCP with 73.5% sensitivity and 65.7% specificity.

There was statistically significant correlation and difference of platelet parameters like MPV, PDW and PLCR between the hyper destructive and hypoproductive thrombocytopenia. These platelet indices were found to be increased in hyperdestructive and decreased in hypoproductive thrombocytopenia.

CONCLUSION: The platelet indices MPV and PLCR have better ability to discriminate hyperdestructive from hypoproductive thrombocytopenia. Evaluating these parameters offers an earlier diagnosis in hyperdestructive thrombocytopenia patients avoiding, bone marrow aspiration and even prevent undesirable platelet transfusion.

KEY WORDS: Platelet indices, Thrombocytopenia, bone marrow.

LIST OF ABBREVATIONS

AA	Aplastic Anemia
ADP	Adenisine diphosphate
ADAM	A-Disintegrin-And-Metalloproteinase
ATP	Adenosine triphosphate
CCL5	Cytokine chemokine Ligand 5
CFU-MKS	Colony forming units –megakaryocytes
CLD	Chronic Liver Disease
DENVs	Dengue Virus
DHF	Dengue Hemorrhagic Fever
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
DSS	Dengue Shock Syndrome
EDTA	Ethylenediaminetetraacetic acid
GATA-1	GATA binding factor 1
GP	Glycoprotein
HSC	Hematopoietic stem cells
HIV	Human Immunodeficiency Virus
ICSH	International council for standardization of hematology
IG	Immunoglobulins
IL	Interleukins
IPF	Immature platelet fraction
ISLH	International society of laboratory hematology
ITP	Immune thrombocytopenia

JAK2	Janus kinase 2
MKs	Megakaryocytes
MPV	Mean platelet volume
MDS	Myelodysplastic syndrome
PAI-1	Plasminogen activator inhibitor 1
PAF	Platelet activating factor
PCT	Plateletcrit
PDW	Platelet distribution width
PLCR	Platelet large cell ratio
RANTES	Regulated on Activation, Normal T cell Expressed and Secreted
TCP	Thrombocytopenia
TPO	Thrombopoietin
TTP	Thrombotic thrombocytopenic Purpura
TXA2	Thromboxane A2
vWF	Von willebrand factor

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INTRODUCTION

Platelets are small sub cellular fragments which circulate in blood with characteristic discoid shapes formed from the cytoplasm of megakaryocyte.[1]

For a long time platelet count was done by phase contrast microscopy which was the gold standard method. In case of thrombocytopenia (TCP) for the assessment of definitive cause bone marrow examination was done. However, with the advancing technologies and the complex computer algorithms, the need for performing these methods are no longer done as a routine in day to day diagnosis which has been taken over partly by haematology analyzers.[2]

Evaluation of platelets using automated haematology analyzers is still inferior to manual counting methods when platelet counts are less than $50x10^9$/L, however, they offer additional parameters like mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (PLCR). These parameters are helpful in refining the cause of thrombocytopenia as they have a significant role in understanding its cause, which might be either hyper-destructive or hypo-productive thrombocytopenia.[3]

Platelet indices have improved the analyzer accuracy in determining the platelet count and the aetiology of thrombocytopenia at a rapid, relatively non invasive and reliable rate. The need for this rapid assessment is essential in managing critical conditions where repeated platelet parameters are monitored on regular basis.[4]

With the recent focus on these parameters like MPV, PDW, PLCR in analysing the cause as well as assessing the production has resulted in better patient management. To evaluate the “diagnostic predictive value of platelet indices in differentiating hypoproductive and hyperdestructive thrombocytopenia”, this prospective study has been done to further add to the data available and assessing the utility of these parameters in Indian population.

OBJECTIVE OF THE STUDY

To assess the utility of platelet indices in discriminating the hyper destructive thrombocytopenia from hypo productive thrombocytopenia.

REVIEW OF LITERATURE

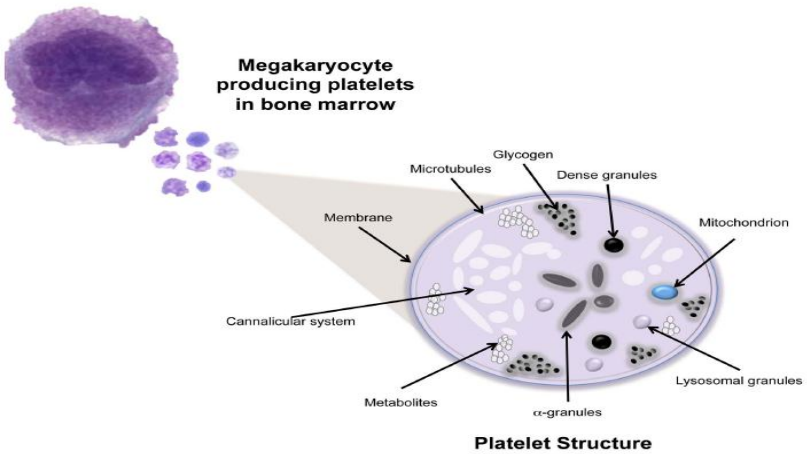
As early as 1841 Addison has described platelets as extremely minute granules in blood. Giulio Bizzozero, for the very first time has called these granules as “platelets” as early as 1882. He has also demonstrated the adhesive qualities, increased stickiness, participation in thrombosis and role in coagulation.[5]

Platelet particles were examined on the blood smears from the late nineteenth century as described by Osler and Hayem. In the late part of the 19th century Camera Lucida and Howell coined the term megakaryocyte which had led to the broader appreciation of these structures as distinct entities.

In 1906, the origin of the platelet from the cytoplasm of megakaryocyte was established by the studies of James Homer Wright.[5]

Once released from the marrow into the peripheral circulation, platelets survive for about 7 days, after which they are taken from circulation by splenic macrophages. Megakaryocytes regenerate at a rate of ~10^8 day, and each can form ~10^3 platelets thus leading to a total of 10^{11} platelets on a daily basis.[6]

Figure 1: Structure of platelet .[7]

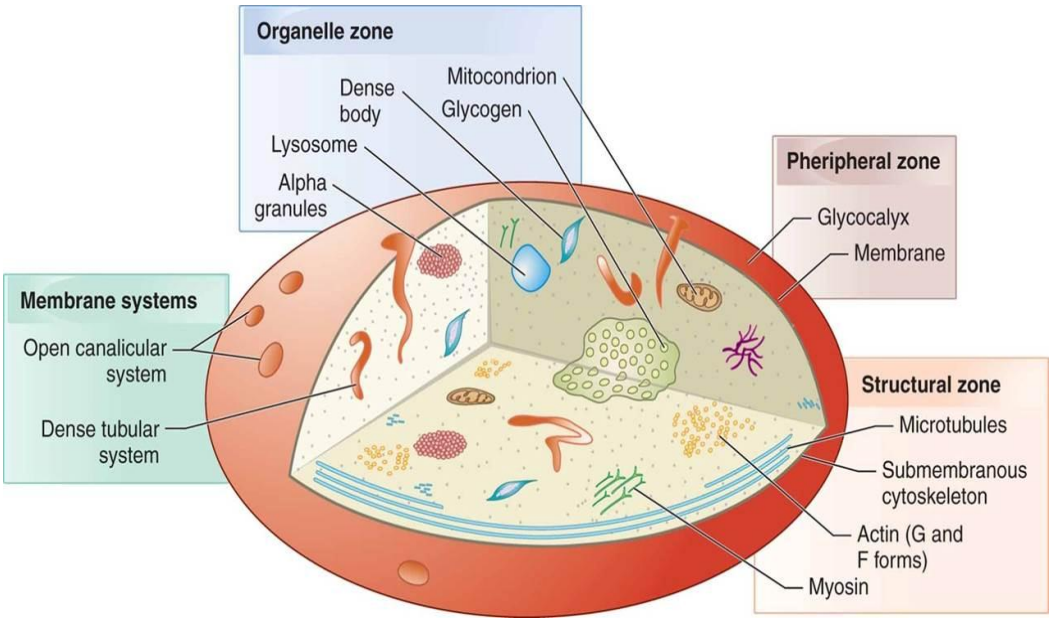


On Romanowsky – stained peripheral blood smear, platelets appear as small, lavender – blue or colourless bodies with reddish purple (Azurophilic) granules. They are generally 2-3mcM in diameter, 0.5mcM thick, and round to oval in shape. Ultra structurally, mature platelets lack nuclear material and contain only remnants of a Golgi complex, a relatively small number of ribosomes, and a small amount of RNA (Fig 1).[8]

Newly released platelets, however, contain measurable RNA as do newly released erythrocytes. These immature platelets containing RNA are called reticulated platelets. Normal healthy individuals have 5-10% reticulated platelets.[9]

The platelet ultra structure is divided into four arbitrary regions or zones : Peripheral zone, structural zone, organelle zone and membrane systems (Fig 2).[10]

Figure 2: Ultrastructure of platelet.[10]



In peripheral blood smears, platelets may adhere to the periphery showing characteristic platelet resetting around neutrophils is called platelet satellitism. This is also seen as an in vitro artefact resulting from Ig E or Ig M platelet agglutinins that are active in anticoagulated blood. Unusually large platelets – megathrombocytes or giant platelets (>5 mcM diameter) are sometimes seen, particularly in myeloproliferative disorders or during recovery from severe thrombocytopenia.[11]

Megakaryocytes (MKs)

"Megakaryocytes are the enormous (50–100 μm) and sparse cells which constitutes for ~ 0.01% of nucleated cells in the bone marrow (BM) cells. MKs originate from hematopoietic stem cells (HSCs) that are mainly occupied in the BM but are also present in the yolk sac, fetal liver, and spleen during early development".[12,13]

Megakaryopoiesis and Thrombopoiesis:

The adult human daily produces 10^{11} platelets at steady state, a level of production that can increase 10-20 fold or more in times of intensified demand. Production of platelets depends on the maturation and proliferation of haematopoietic progenitor cells to a cell committed to the megakaryocytic lineage, its maturation to a large precursor megakaryocyte and its final fragmentation of cytoplasm into platelets is a complex process of megakaryopoiesis and thrombopoiesis.[14]

Megakaryocytes are descended from pleuripotent hematopoietic progenitors through a bipotential erythroid/megakaryocytic cell. After this stage megakaryoblasts undergo maturation to megakaryocytes stimulated by erythropoietin, thrombopoietin and other cytokines and chemokines. Maturation is dependent on transcription factors GATA 1 and GATA 2 together with cofactor FOG1.[15]

The developmental stages of the megakaryocyte.[10]

Stage 1 **Megakaryoblast**

6-24mcM diameter,round nucleus basophiliccytoplasm, visible nucleoli and no visible

Stage II **Basophilic megakaryocyte**

14-30mcM diameter increased cytoplasm primarily basophilic, indented / bilobed nucleus, few azurophilic cytoplasmic granules seen

Stage III **Granular megakaryocyte**

25-50 mcM in diameter, numerous cytoplasmic granules, abundant acidophilic cytoplasm, large multilobed nucleus, no visible nucleoli.

Stage IV **Mature megakaryocyte**

40-100 mcM diameter abundant eosinophilic and granular cytoplasm, multilobulated nucleus and no visible nucleoli.

"To release and assemble platelets,MKs become polyploidy by Endomitosis which is primarily a TPO-driven process through cycles of DNA replication without cell division**.** During their life cycle, MKs first undergo a proliferative 2n stage in which their progression through the cell cycle is identical to other hematopoietic cells. Subsequently, MKs begin Endomitosis and accumulate a DNA content of 4n, 8n, 16n,

32n, 64n, and even 128n in a single polylobulated nucleus before proceeding with their final maturation and Proplatelet formation". [16,17,18]

Radley was the first to propose the idea that the long proplatelet extensions are intimately involved in the formation and release of platelets. Blair and Flaumenhaft *et al.* observed that the elongation of proplatelet plays an important role and microtubules lining the shafts of proplatelet , transportation of granules into proplatelets and assembly of platelets serves as a second function. The granules are originated from budding of small vesicles containing granule cargo from the trans-golgi network.[19, 20.]

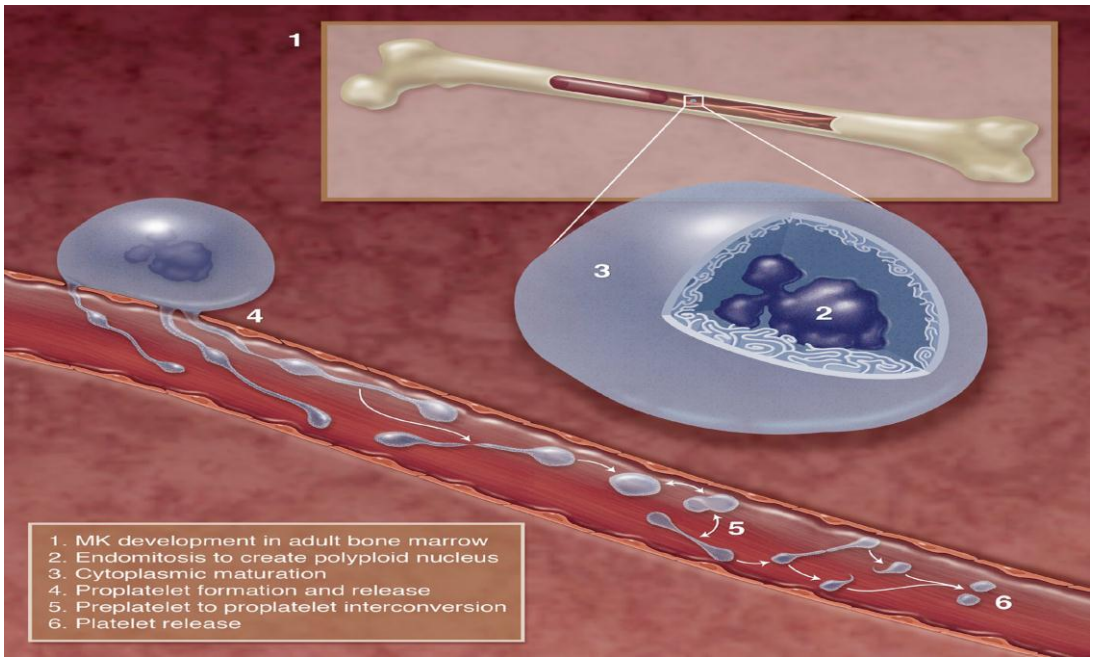
Richardson et al explained that an "megakaryocytes may extend 10–20 Proplatelets, each of which starts as a blunt protrusion that over time elongates, thins, and branches repeatedly. Organelles and granules are then sent individually from the MK cell body into the Proplatelet, where they move bidirectionally until they are captured at Proplatelet tips. In MKs, immunofluorescence and electron microscopic experiments indicate that organelles are intimately associated with microtubules, and actin drugs do not affect organelle motion.Thus, movement appears to be microtubule based".[21]

The significance of megakaryopoiesis and thrombopoiesis regulates the morbidity and mortality from bleeding due to moderate to severe TCP which is a major problem facing by a wide range of patients. The origins of thrombocytopenia are many, but include both iatrogenic and naturally occuring conditions that are frequently encountered in clinical practise.[15]

Platelet Production:

Machlus et al reported a novel potential positive feedback mechanism whereby the platelet-borne inflammatory cytokine chemokine ligand 5 (CCL5); also known as regulated on activation, normal T cell expressed and secreted [RANTES]) can stimulate megakaryocytes to produce platelets (Fig 3).[22]

Figure 3: Schematic representation of platelet production.[23]



Primarily, platelet activity is associated with the initiation of coagulation cascades. It makes the sub endothelial surface the primary target site of platelet action, where it establishes the haemostatsis at injury site. Various platelet agonists promote the action of platelet adhesion to the subendothelial surfaces. During this process, platelet changes its shape, releases its granule contents, and gradually forms aggregates by adhering with each other.[24]

The two phases of platelet formation are:

"The **first phase** of MK maturation and development requires, massive nuclear proliferation and enlargement of the MK cytoplasm occur as the MK is filled with cytoskeletal proteins, platelet specific granules, and sufficient membrane to complete the platelet assembly process. This phase occurs over a period of days and requires induction by megakaryocyte specific growth factors".

"The **second phase** is relatively rapid and can be completed within hours. During this phase, MKs generate platelets by remodelling their cytoplasm first into proplatelets and then into preplatelets, which generate discoid platelets. The time required for MKs take ~5 days to complete polyploidization, mature, and release platelets in humans and 2–3 days in rodents".[25, 26, 27]

Thrombopoietin (TPO) is the most important cytokine regulating platelet production and influences all stages of megakaryocyte production and releases mature platelets**.**Once released into the bloodstream, human platelets survive 7–10 days, whereas rodent platelets survive 4–5 days.[28, 29]

Platelet function

Platelet adhesion to the extracellular matrix is the first step in primary haemostasis. Under high shear conditions, von Willebrand factor (vWF) forms a bridge between collagen and GPIb-IX-V receptor complex. Exposed collagen directly binds to platelet GP Ia/IIa and GP VI receptors. Platelets change shape during activation and release their granules of cytokines and growth factors. When the circulating platelets are low the intercellular junctions disassemble, resulting in leakage of fluid and cells

in surrounding tissue. The formation of platelet plug requires many activation events, including adhesion, contraction or shape change, secretion and aggregation.[30, 31, 32, 33]

Adhesion

The platelet receptors GPIa/IIa and GPVI, mediate adhesion and are dependent on rate of shear. Circulating vWF will not bind to GPIb/IX, but it can also bind to platelet GPIIb/IIIa receptor. High shear rates causes the conformational changes in the immobilised vWF and platelet adhesion is initiated by interacting with vWF immobilised on collagen. Interaction with collagen via GPIa/IIa and GPVI results in adhesion and platelet activation. GPVI activation induces ADP release from the DG and synthesis of TXA2 from Arachidonic acid.[34, 35]

Platelet Activation :

Platelets In resting state platelets appear smooth, discoid shaped cells having an open canalicular system (OCS) and glycocalyx exteriorly. Ca^{2+} is sequestered and released into the cytoplasm of platelets on activation by agonists such as Serotonin, ADP, Platelet activating factor(PAF) and Thromboxane A2.[36, 37]

Activation results in generation of GPIIb/IIIa receptors for binding of the fibrinogen and secretion of platelet granules further leads to formation of platelet aggregates. Once activated, the platelet response becomes self perpetuating and irreversible.[10]

Shape change and spreading

Cytoskeleton of platelets possess microtubules and microfilaments Platelets extend finger-like projections called as pseudopodia. GPIb/IX receptors are moved to the open canalicular system (OCS) and internalized whereas active GPIIb/IIIa receptors increase in density and are mobilised from α-Granules, Dense granules (DG), and

OCS membranes. This results in converting the activated platelet from an adhesion state to an aggregation state.[10]

Aggregation

During adhesion, collagen binding to GPVI triggers intracellular signaling and activates GPIIb/IIIa which then binds to fibrinogen. Resting platelets fail to express a functional GPIIb/IIIa complex and unable to bind fibrinogen. Active GP IIb/IIIa receptor has central role in mediating platelet aggregation.[38]

After activation the new platelets undergo shape change and expose their active GPIIb/IIIa sites where fibrinogen binds to activated platelets and cross linking of two adjacent platelets and GPIIb/IIIa molecules, where 40000-50000 molecules of fibrinogen bound to each activated platelet approximately.[10]

Secretion

The cytoplasm of platelets contains different types of granules, such as α-granules, dense granules and lysosomes. The most abundant granules are α-Granules with large number of proteins including GPIIb/IIIa (αIIbβ3) and P-selectin., which helps in regulating the thrombosis and fibrinolysis, such as α2-antiplasmin and plasminogen activator inhibitor-1 (PAI-1).[39,40]

The release of dense granules contributes to platelet activation and haemostasis. They contain concentrated stores of ADP, pyrophosphate, ionized calcium and ATP. Lysosomes are secreted by platelets in response to stimulation of strong agonist and has numerous acid hydrolases. Fusion and release of these granules with the OCS leads to platelet activation.[41, 42]

Pathophysiology of Thrombocytopenia

Thrombocytopenia is most common cause of abnormal bleeding, results mainly from four processes – accelerated peripheral destruction of platelets due to infection, hypoproduction of platelets due to bone marrow disease, abnormal distribution or pooling of platelets in the circulation and pseudothrombocytopenia (Fig 4).[43]

Ethylene diamino tetra acetic acid (EDTA) anticoagulant blood sample is most commonly associated with clumping. It is time dependent and varies with the type of instrumentation used for automatic counting. There is evidence that the auto antibodies bind to GP IIb/IIIa, between the presence of anticardiolipin antibody and platelet agglutinins in individual patient plasmas show over 80% concordance. [44]

Accelerated peripheral platelet destruction:

Peripheral destruction of platelets is the most common cause of thrombocytopenia, which leads to the stimulation and activation of thrombopoiesis and subsequently increases the rate of maturation of the megakaryocytes.

Deficient platelet production:

Hypoproduction of the platelets results from various processes, mostly commonly due to such as marrow injury by aplastic anaemia, myelosuppressive drugs or radiation. Disordered proliferation of precursor cells may also be one of the consequence of hypoproduction.

Abnormal distribution:

Abnormal pooling or distribution of platelet mass may produce thrombocytopenia. It is associated with splenomegaly and various disorders where platelet production is normal or increased, but platelets are mostly sequestered but splenic pool. It may also

be caused by dilution of platelets when patients are massively transfused during blood loss.[43]

Levine, et al had proposed that the mechanism of thrombocytopenia could be simplified into two groups after excluding a splenic sequestration: hyperdestructive and hypoproductive.[2]

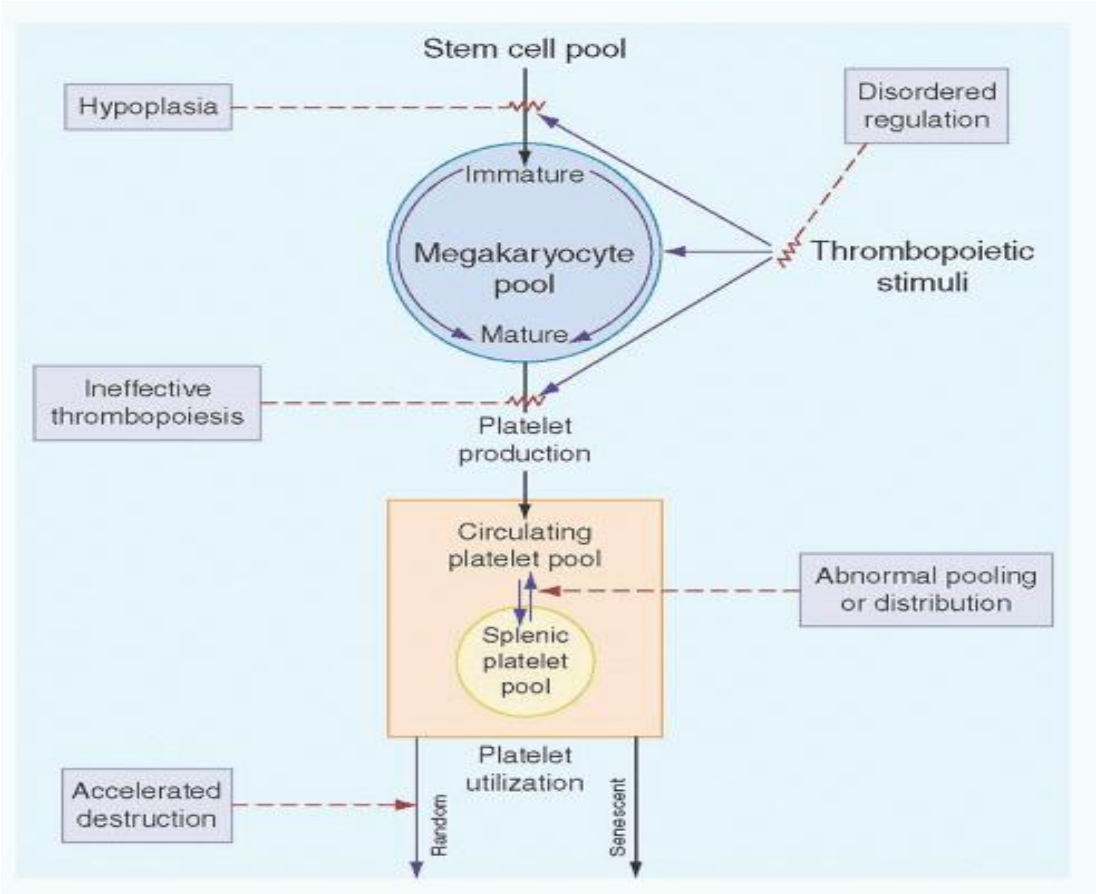


Figure 4: Pathophysiology of thrombocytopenia.[43]

Hyperdestructive thrombocytopenia is a result of extramedullary platelet destruction with normal or increased bone marrow production, e.g., ITP, secondary ITP and DIC.[45]

The glycocalicin is a major extracellular portion of the GPIb which extends from the platelet surface and can be cleaved by proteolytic enzymes ADAM10, ADAM 17 and thrombin. The plasma levels of glycocalicin can be used to differentiate thrombocytopenia resulting from decreased platelet production (low plasma glycocalicin levels) from that resulting from increased platelet destruction (high plasma glycocalicin levels).[46, 47]

The role of thrombocytopenia in several disease conditions which are categorized under hyperdestructive thrombocytopenia are follows as:

Thrombocytopenia in dengue:

Dengue viruses (DENVs) are the most important human Arboviruses worldwide and are transmitted by mosquitoes of the genus Aedes in the form of four distinct serotypes DENV-1, 2, 3, and 4. The estimated rate of dengue cases currently every year worldwide are around 50–100 million, where most commonly reported cases are dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue virus causes serious infection in humans, resulting in increased vascular permeability, hemodynamic disturbances, hypovolemia, hypotension are associated with serious manifestations and high mortality in tropical and subtropical areas of the world.[48]

Thrombocytopenia is one of the criteria used by WHO guidelines as an important potential indicator of clinical severity. In the recent WHO guidelines, the definitions generally describe a rapid decline in platelet count or a platelet count less than 150,000 per microliter of blood.[49]

There is a significant decrease in platelet count in DHF/DF on the 4th day of the illness.On the 3rd day of DHF in adults without shock the platelet counts are

decreased mild to moderately until the 7th day of illness and on 9^{th} day it reaches normal levels. In adults, a platelet count of 5×10^9 /L and packed cell volume >50 are significantly associated with bleeding manifestations.[50, 51]

When platelets confront a damaged blood vessel wall, they get activated Phosphatidylserine (PS) is exposed leads to Platelet-derived microparticles (PMPs) formation which also have prothrombinase activity. Then it undergo a change in shape, which is initiated by a Ca^{2+} influx of intracellular stores are major steps and leads to shape change, aggregation, secretion, and expression of procoagulant activity. During this reaction, the receptors on the platelet membrane for adhesive and clotting proteins increases, and activated platelets attract other platelets, which clump together and forms a plug that seals the vascular leak.[52, 53]

"Thrombocytopenia may also be due to (1) platelet consumption during ongoing coagulopathy process, (2) activation of the complement system, or (3) increased peripheral sequestration. It has also been explained that patients infected with dengue virus develop anti-platelet antibodies of the IgM isotype. Notably, antiplatelet IgM titres in patient sera are higher in DHF/DSS compared to DF. Anti-platelet antibodies cause platelet lysis, as measured using lactate dehydrogenase activity assays. In accordance with elevated IgM titres, DHF/DSS sera caused increased platelet lysis compared to DF patient sera. In addition, cytotoxicity was much higher in the presence of complement".[54, 55, 56]

Thrombocytopenia in malaria:

"Malaria is a protozoal disease of global importance caused by infection with parasites and transmitted by infected female Anopheles mosquito to man. The five

species of Plasmodium are P.vivax, P.falciparum, P.Malariae, P.Ovale, and P.Knowlesi, which causes malaria in humans".[57]

"In 2016, an estimated 216 million cases of malaria occurred worldwide. Malaria accounts for an estimated 863,000 deaths, of which 90% were in African Region followed by East Mediterranean (2%) and South-East Asia Region (7%). The incidence rate of malaria is estimated to have decreased by 18% globally, from 76 to 63 cases per 1000 population at risk, between 2010 and 2016. About 27% population in India lives in malaria high transmission area and 58% in low transmission area".[58]

The malaria paroxysm comprises of three successive stages. The cold stage is characterised by cold intolerance and shivering. Next comes the hot stage in which there is high fever , dry skin and flushes. Finally the sweating stage where fever drops rapidly and patient sweats. Malaria parasite affects multiple organs of the body such as liver, spleen, brain, gastro intestinal tract, gall bladder, pancreas, blood vessels and placenta. These stages or classic patterns of the fever may not be seen in the early onset but absence of these stages cannot rule out diagnosis of malaria. The most characteristic symptom of malaria is fever. Other clinical signs and symptoms could be of wide spectrum ranging from headache, malaise, vomiting, diarrhoea to life threatening symptoms like coma. "Other haematological abnormalities observed in patients with malaria, with anaemia, and thrombocytopenia being the most common. Fajardo and Tallent demonstrated P. vivax within platelets and suggested a direct lytic effect of the parasite on the platelets. Both non-immunological and immune mechanism involving specific platelet associated IgG antibodies that bind directly to malarial antigen in the platelets have been recently reported to play a important role in the lysis of platelets".[59, 60, 61]

“Decreased thrombopoiesis has been ruled out, because platelet forming megakaryocytes in the marrow are usually normal or increased. A good tolerance of low platelet count is well-known in malaria, which is explained by platelet activation and an enhanced agreeability. The hyperactive platelets may enhance haemostatic responses which explains, why bleeding episodes are very rare despite significant thrombocytopenia in acute malarial infections”. Thrombocytopenia in malaria may not be a cause of mortality by itself, but it can be a marker of increased severity and need of aggressive management.[57]

Thrombocytopenia in alcoholic liver disease:

Patients with chronic liver disease (CLD) most commonly encountered haematological abnormality is thrombocytopenia. It occurs in 64%–84% of patients presenting with cirrhosis or fibrosis. Cirrhosis is characterized by a loss of liver cell function, reduced diameter of the hepatic vascular bed, and increased splanchnic inflow, resulting in increased pressure in the portal vein, splenomegaly and subsequent thrombocytopenia via platelet sequestration. Depressed thrombopoietin (TPO) levels and direct suppression of bone marrow leads to decreased platelet production.[62]

Hepatic production of thrombopoietin plays a major role in thrombopoiesis and megakaryopoiesis which regulates both platelet maturation and production. TPO is secreted into the circulation at a constant rate which is primarily made in the liver by both parenchymal and sinusoidal endothelial cells.[63]

"TPO binds to the surface of platelets and megakaryocytes through the c-mpl receptor, inhibits the exposure to hormone. Stimulation of the TPO receptor results in activation of signalling pathways via Janus kinase type 2 (JAK2) and tyrosine kinase 2 (TYK2). Mitogen-activated protein (MAP) kinase activation subsequently leads to changes in gene expression, and promotes progression of stem cells along the megakaryocytic pathway, megakaryocyte maturation, and subsequent release of normally functioning platelets into the peripheral circulation. The circulating level of TPO is inversely correlated to the platelet mass, low platelet counts lead to higher TPO levels due to decreased degradation. The increased exposure of undifferentiated bone marrow cells to TPO leads to their differentiation into megakaryocytes and maturation. This increased platelet cell mass, in turn, binds increasing amounts of TPO, reducing its circulation level, and ultimately leading to decreased platelet production".[62]

Increased peripheral platelet destruction occurs in cirrhosis through increased shear stress leading to an increased rate of platelet aggregation, immunologic destruction, increased fibrinolysis, bacterial translocation, and infection. Patients with cirrhosis have inadequately low plasma TPO levels, higher platelet turnover and reduced platelet production, whereas HCV- induced cirrhosis appears to be associated with higher levels of serum antiplatelet antibodies, which could potentially lead to greater platelet destruction.[62]

Thrombocytopenia in sepsis:

Thrombocytopenia is common in critically ill patients, with an estimated incidence of 20%–40% at some point during the intensive care unit (ICU) stay which is an independent risk factor for mortality and a sensitive marker for disease severity.[64]

The development of thrombocytopenia in patients with sepsis is secondary to various mechanisms. Platelets are activated in sepsis and bound to the endothelium, resulting in sequestration and destruction. Immune-mediated mechanisms like nonspecific platelet-associated antibodies and cytokine-driven hemophagocytosis of platelets can also contribute to sepsis-induced TCP. The main concern for critically ill patients with thrombocytopenia is bleeding. Several mechanisms, acting alone or in combination, can be responsible for a low platelet count in sepsis and shock. Decreased platelet production in the bone marrow can result from pre-existing conditions or from the inhibitory effect of pathogen toxins, drugs or inflammatory mediators on haematopoiesis.[65]

Thrombocytopenia in immune thrombocytopenic Purpura (ITP):

ITP is defined as a platelet count <100 $X10^9$/L and caused by immune destruction of platelets. "The incidence of primary ITP in adults is 3.3/100,000 adults per year with a prevalence of 9.5 per 100,000 adults. There is a predilection for female patients in younger adults, but the prevalence of ITP in males and females is fairly even in the elderly (>65 years)".[66]

ITP is divided into two type: Acute and chronic ITP.

Acute ITP: TCP with duration of less than 6 months and spontaneous remission. The most commonly affected age group are children between 2 to 6 years of age and young adults. Chronic ITP is most commonly seen in adults with median age of 40 to 45 years with long lasting duration i.e; more than 6 months and requiring treatment to improve the thrombocytopenia.[43]

Primary ITP results from pathologic antiplatelet antibodies, impaired megakaryocytopoiesis and T-cell mediated destruction of platelets with each pathologic mechanism playing varying roles in each patient. Secondary ITP is associated with other underlying disorders such as: autoimmune disease (Systemic Lupus Erythematosis (SLE) or Rheumatoid Arthritis(RA)), HIV, helicobacter pylori, or underlying immune dysregulation syndromes such as Common Variable Immunodeficiency (CVID). The majority of adults with ITP (~80%) have primary ITP.[67]

Clinically diagnosed ITP patients have platelet specific IgG antibodies. These are generally directed at the most abundant platelet surface glycoproteins, GPIIb/IIIa and GP1b/IX/V. The type of epitope targeted by these auto reactive antibodies may influence course of the disease and some research has suggested that these different types of antibodies may differentially alter clearance, inhibit megakaryopoiesis or induce platelet apoptosis. Additionally, the presence of antiplatelet antibodies has been associated with increased risk of thrombosis. Some patients who do not have antiplatelet antibodies will have abnormal T-cells that result in platelet destruction, while in other patients it is T cell dysregulation that results in autoantibody production. Cytotoxic CD8+ T cells have been found in some patients with ITP, which are able to directly lyse platelets and accumulate in bone marrow, potentially impairing platelet production.[67]

In response to the autoantibody mediated platelet destruction the compensatory increase in platelet production takes place. In ITP patients autoantibodies have been shown to inhibit production of megakaryocytes in vitro, and megakaryocytes

apoptosis has also been observed. Megakaryocyte colony formation is increased in acute ITP and decreased in chronic ITP.[43]

Thrombocytopenia in Human immunodeficiency virus infection:

Cytopenias are one of the most common complications of HIV infection and may be broadly classified as being due either to a bone marrow production defect or to increased peripheral loss or destruction of blood cells.[68]

ITP occurs in up to 30% of HIV patients and is the most common cause of thrombocytopenia in HIV. Although the clinical presentation is similar to non-HIV-associated ITP, the mechanism is unique and thought to be due to an HIV-induced auto-antibody generated against an amino-acid sequence within the platelet surface GP IIIa. Many patients present with ITP as the first manifestation of HIV, although it can occur in both early and advanced disease.[69]

Hypoproductive thrombocytopenia is caused by decreased bone marrow production because of primary or secondary bone marrow diseases such as acute leukemia (AL), aplastic anaemia(AA), megaloblastic anemia, myelodysplastic syndrome (MDS) and post chemotherapy.[70]

Thrombocytopenia in leukemias:

Leukaemias are a group of haematological malignancies characterized by an increase in immature or abnormal white blood cells in bone marrow (BM) and peripheral blood (PB). This group of malignancies is divided into acute and chronic as well as lymphocytic and myelocytic types according to clinical manifestations and the lineage involved, respectively. Patients with leukaemia show symptoms such as anemia,

neutropenia, and thrombocytopenia due to ineffective haematopoiesis and related complications, which include fatigue, weakness, infection, and bleeding.[71]

Thrombocytopenia is observed in a large number of patients with leukaemia and may be due to several causes such as ineffective haematopoiesis, suppression of megakaryocyte progenitor cells in BM by the malignant cells clone, gene mutations, destruction of platelets in PB, and adverse effects of treatment.[71]

THROMBOCYTOPENIA IN APLASTIC ANEMIA:

"Aplastic anaemia is a rare, life-threatening bone marrow failure disorder characterized by pancytopenia and a hypocellular bone marrow. Most cases are due to autoimmune attack of marrow stem and progenitor cells leading to pancytopenia".

There is a biphasic age distribution with peaks from 10 to 25 years and >60 years. There is no significant difference in incidence between males and females. Congenital aplastic anaemia is very rare, the commonest type being Fanconi anaemia, which is inherited as an autosomal recessive disorder in most cases.

"Thrombocytopenia is a major cause of morbidity and mortality in patients with aplastic anemia. At presentation, virtually all patients with aplastic anemia are thrombocytopenic: platelet counts of < 50,000 or 20,000/lL are diagnostic criteria for moderate and severe aplastic anemia, respectively. Decreased hematopoietic stem and progenitor cell numbers and function, resulting in impaired megakaryocytopoiesis and insufficient mature platelet production, are the causes of thrombocytopenia".[72]

TPO is produced from the liver at a constant rate and regulates megakaryopoiesis and thrombopoiesis. In the circulation, it is rapidly adsorbed and internalized by platelets so that the platelet count forms a feedback loop controlling TPO concentrations.

Hepatic production of TPO and simple autoregulation, is influenced by cytokines, the most important being interleukin-6, which is produced in a variety of inflammatory conditions.[73]

Thrombopoietin binds to its receptor MPL, which is a type I cytokine receptor and lacks intrinsic kinase activation. Ligation of TPO to MPL tyrosine phosphorylation and activation of its associated proteins JAK2 and TYK2, which in turn activates STAT3 and STAT5, and its stimulation of MPL also leads to activation of LYN, LNK SOCS which acts as negative regulators. Furthermore, the TPO–MPL complex is rapidly internalized and targeted to both lysosomal and proteosomal degradation.[74]

MPL is expressed on hematopoietic stem cells, megakaryocyte colony-forming units (CFU-MKs), myeloid and erythroid precursors, early and late megakaryocytes and mature platelets. TPO is important in HSC maintenance, and individuals with MPL mutation develops Aplastic anemia.[73]

Thrombocytopenia in megaloblastic anemia:

Megaloblastic anemia is a disorder limited to red cells and the prominent feature is erythroid hyperplasia. Megaloblastic anemia is categorised under 2 types of anemias: Pernicious anemia and Folate anemia. The common feature of all megaloblastic anemias is a defect in DNA synthesis that affects rapidly dividing cells in the bone marrow and other tissues.[75]

Macrocytosis associated with a megaloblastic marrow is usually accompanied by anemia due to ineffective erythropoiesis which is accompanied by intramedullary haemolysis causing an elevated lactate dehydrogenase and indirect bilirubin in the serum. However, the reticulocyte count is low due to the abnormal maturation

process. The bone marrow is hypercellular, showing evidence of abnormal proliferation and maturation of multiple myeloid cell lines.[76]

The clinical features included anemia, leucopenia and thrombocytopenia. Thrombocytopenia sometimes may lead to bruising. The anemia and low leucocyte count may predispose to infections, particularly of the respiratory or urinary tracts. As megaloblastic anemia progresses, neutropenia and thrombocytopenia develop.[75]

Pseudothrombocytopenia:

EDTA-dependent pseudothrombocytopenia (EDTA-PTCP) is a common laboratory phenomenon with estimated prevalence of 0.1%–2% in hospitalized patients. It is due to in vitro agglutination of platelets in the blood collection tube caused by IgM/IgG autoantibodies directed against epitopes on platelet surface glycoprotein GPIIb/IIIa. EDTA induces a conformational change in GPIIb/IIIa, exposing these epitopes and resulting in platelet agglutination. The use of an alternate anticoagulant, such as citrate or heparin, may be helpful. However, up to 17% of patients with EDTA-PTCP also show this phenomenon with citrate.[77]

Bizzaro conducted a large study of EDTA-PTCP cases and found that 83% had antiplatelet antibodies. The phenomenon was not age-related or gender-related, nor was it associated with any particular pathology or use of specific drugs. It showed that EDTA-dependent PTCP is a phenomenon related to the presence of natural autoantibodies with antiplatelet activity and is not associated with any pathological significance.[78]

In 2001,the International Council for Standardization in Haematology (ICSH) and the International Society of Laboratory Haematology (ISLH) have recommended a

method to measure platelet/RBC ratio based on fluorescent flowcytometry which is expensive and cannot be done on routine basis.

A traditional method for counting platelets in a peripheral smear which has been in use for a long time is by taking the average of platelets in ten to twenty oil immersion fields and multiplying it by 15000 or 20000 to validate and estimate platelet count in peripheral smears depending on the institutional protocols. Nosanchuk *et al.* and Webb et al showed that a multiplication factor of 15000 showed better results than a multiplication factor of 20000 in the same method.

Even some other methods for estimation of platelet count on peripheral smear have been described by Brahimi *et al.* in their study by counting the number of platelets per 1000 RBCs and multiplying it by total RBC count. Torres et al described a method in 2004 in which they multiplied the average number of platelets seen per field with the haemoglobin level of the patient and multiplied it by 1000.[79]

Platelet indices:

Mean platelet volume (MPV) :

Mean platelet volume is determined in the progenitor cell, the bone marrow megakaryocyte. When platelet production is decreased, young platelets become bigger and more active, and thus leads to increase in platelet diameter. Increased MPV is a potential marker or indicator of production rate and platelet activation.[81]

Mean platelet volume, normally measured using automated blood analysers, reflects the average size of platelets in circulation. It is meant to show the relationship between platelet synthesis in bone marrow and cell destruction. A normal MPV has a

range of 7.5–11.5 fL. MPV correlates with platelet function and may be more sensitive than platelet count as a biomarker in a variety of disorders. It is also regarded as a useful surrogate marker of platelet activation or reactivity.[82]

The test is particularly useful in patients with thrombocytopenia and thrombocytosis. High MPV in TCP is seen in ITP, DIC, sepsis, and preeclampsia. Low MPV in thrombocytopenia is seen with low platelet production, associated with bone marrow diseases like aplastic anemia, megaloblastic anemia and leukaemia.[82]

Gulati Ishitha *et al.* concluded that their study showed that an MPV value of ≥8.5 fl can be used for diagnosing thrombocytopenia cases due to hyperdestructive etiology. The determination of MPV was found to be extremely useful in ascertaining the etiology of thrombocytopenia in this study.[83]

Platelet distribution width (PDW) :

"PDW is an indicator of volume variability in platelets size and is increased in the presence of platelet anisocytosis. The PDW reference intervals ranging from 8.3 to 56.6%. PDW directly measures variability in platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology".[81]

Vagdatli E *et al*. study showed increase of PDW to platelet anisocytosis, which results from pseudopodia formation. In recent publications, Ihara et al as well as Khakendar et al made the same observation in patients with ischemic heart disease. Amin *et al.* found that PDW increased in vaso-occlusive crisis in sickle cell disease. They concluded that megakaryocyte hyperplasia was responsible for PDW increase.[84]

Platelet large cell ratio (P-LCR):

"Platelet larger cell ratio (P-LCR) is an indicator of circulating larger platelets (> 12 fL) used to monitor platelet activity, and is presented as percentage. The normal percentage range is 15–35%".[81]

Plateletcrit (PCT):

"PCT is the volume occupied by platelets in the blood as a percentage and calculated according to the formula PCT = platelet count × MPV / 10,000. Under physiological conditions, the amount of platelets in the blood is maintained in an equilibrium state by regeneration and elimination. The normal range for PCT is 0.22–0.24%".[81]

Immature platelet fraction (IPF):

"Immature platelet fraction (IPF) indicates the percentage of immature platelets, as a percentage of the total platelet population measured in haematology analyser by flow cytometry, in which dye penetrates the cell membrane, staining the RNA in the cytoplasm of immature (or reticulated) platelets on the Sysmex XN-1000 analyser. The IPF percentage increases as production of platelets increases, and low values indicate suppressed thrombopoiesis".[81]

"The IPF represents the percentage of circulating platelets which still retain RNA. It is a modern parameter measuring young, reticulated, platelets in peripheral blood. IPF is a rapid and inexpensive automated biomarker for etiology of thrombocytopenia. It is usually determined by flow cytometry or haematology analysers".[82]

Platelet indices as diagnostic and prognostic markers:

"Simultaneous measurement of all of the platelet indices will provide us a valid information for measuring disease severity and an insight into the potential etiology

that resulted in changes of platelet indices. Increase in MPV and PDW suggests that bone marrow produces platelets and rapidly releases them into circulation. A simultaneous reduction of platelet count and PCT indicates that platelets have been excessively consumed".[81]

Automated haematology analyzer:

Automation in haematology have become as essential part of primary testing in haematology laboratory which is a rapid and efficient tool for handling of a large number of samples. It enables to perform multiple tests on a single platform. The Sysmex XN 1000 fully automated haematology analyzer works on the principle of hydro dynamic focusing and flow cytometry method.In hydro dynamic focusing, the RBC detector counts the RBC and PLT via the Hydro Dynamic Focusing (DC Detection) which prevents the generation of false platelet pulses.

Flow cytometry is used to analyze the qualitative and quantitative analysis of several characteristics of cells as they flow in single line through a beam of light. The Hydro Dynamic Focusing method and flow cytometric method improves blood count accuracy and reliability.[80]

Study done by Negash M *et al.* observed that 83 thrombocytopenic patients with either hyper destructive or hypo productive thrombocytopenia processes concluded that platelet indices in particular MPV and P-LCR can differentiate hyper destructive thrombocytopenia from hypoproductive thrombocytopenia and they may help in avoiding or delaying ITP patients from undergoing unnecessary, invasive bone marrow aspiration or prevent undesirable platelet transfusion.[85]

In a retrospective study done by Khanna R *et al.* the conclusion drawn was, that although MPV may provide a small initial insight into the aetiology of thrombocytopenia, it is limited by insufficient sensitivity and specificity. A bone marrow examination continues to be the gold standard to differentiate the hypo productive and hyper destructive thrombocytopenias.[2]

In the study done by Farweez *et al.* showed that MPV and P-LCR provide information about the underlying conditions of thrombocytopenia. These indices should be considered in the diagnosis of thrombocytopenia. The P-LCR can be safely relied upon for a positive diagnosis of ITP.[86]

Kaito K *et al.* suggested that the platelet indices provide clinical information about the underlying conditions of thrombocytopenia. In particular PDW and P-LCR shows marked differences between the hypo productive and hyper destructive thrombocytopenia.[87]

Study done by Khairkar PS *et al.* concluded that PDW provides plenty of clinical information about the causes and pathphysiology of thrombocytopenia and could be helpful to distinguish hyper destructive thrombocytopenia from hypo productive thrombocytopenia. They also mentioned that more attention should be paid to PDW along with other platelet indices to differentiate between hyper destructive thrombocytopenia from hypo productive and abnormal pooling thrombocytopenia.[88]

In the study done by Khaleel KJ *et al.* concluded that platelet indices provide plenty of clinical information about the causes and pathogenesis of thrombocytopenia and could be helpful tests to distinguish hyper destructive thrombocytopenia from hypo productive thrombocytopenia easily.[89]

Reddy R S *et al.* suggested that platelet distribution width is an important index in platelet parameters and along with other platelet indices it gives valuable information about the mechanism of platelet destruction. Increase in PDW indicates the increased platelet heterogenecity along with destruction and splenic pooling. PDW varies inversely with platelet destruction.[90]

Study done by Parveen S and Vimal M suggested that mean platelet volume may provide useful information in discriminating the hypoproductive and hyperdestructive thrombocytopenia and interpretation of platelet indices can help in the initial management and can avoid invasive investigations in thrombocytopenic patients.[91]

MATERIALS AND METHODS

This prospective study was conducted in patients who presented to the out patient departments of medicine, surgery, obstetrics & gynaecology, paediatrics at Shri B M Patil Medical College, who are evaluated in Department of Pathology, for thrombocytopenia over a period of November 2016 to June 2018.

SAMPLE SIZE:

With correlation coefficient between platelet count and platelet indices (MPV) of -0.38 [1] at 80% power and 95% level of significance, sample size is 84.

INCLUSION CRITERIA

Patients with platelet count less than 150 x 10^9 / L on SYSMEX XN 1000 haematology 6 part analyzer and confirmed by manual peripheral smear review.

EXCLUSION CRITERIA

Patients who, after detailed clinical assessment and laboratory tests were diagnosed to have an immunological basis for thrombocytopenia and recent manifestation of blood transfusions.

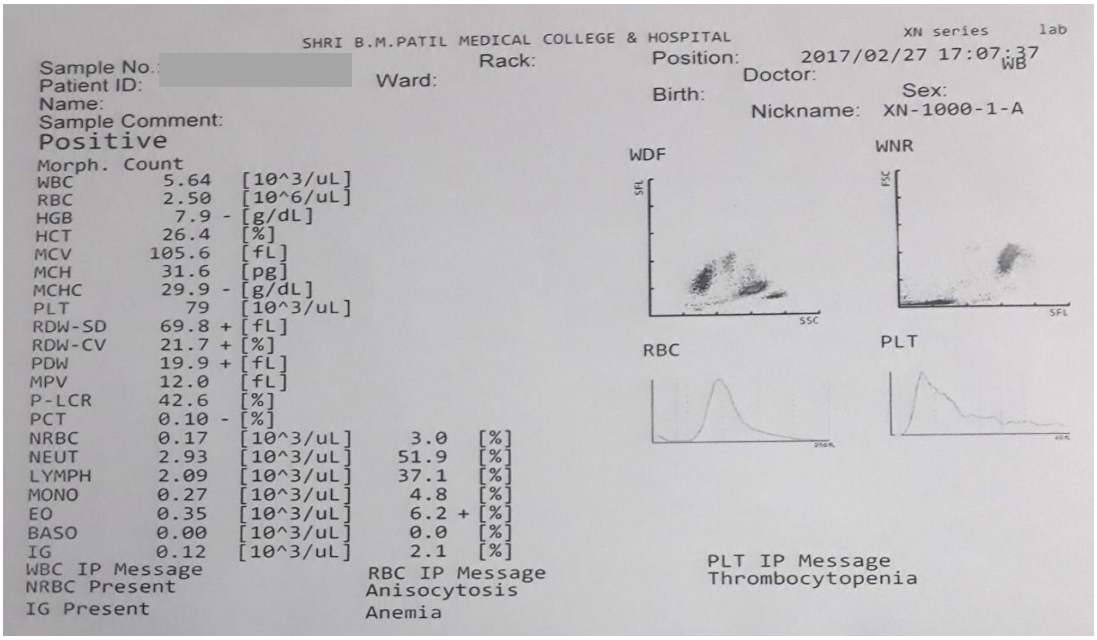
METHODOLOGY:

In cases referred for thrombocytopenia evaluation a short history and informed written consent was taken. Peripheral venous blood was obtained from antecubital venepuncture following standard protocols into tripotassium EDTA vacutainer for complete blood count and analysed within 4hrs to prevent clumping.

The complete blood count was performed using SYSMEX XN 1000 (Fig 5), 6 part haematology analyzer in CBC DIFF mode where platelet parameters like platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio(PLCR) were available (Fig 6) . Peripheral blood smears were prepared for all the cases satisfying inclusion criteria and these smears were stained by Leishman stain.

Figure 5: SYSMEX XN 1000 fully Automated haematology analyzer.

Figure 6: Preliminary report showing histograms, scatter grams and parameters generated from Sysmex XN 1000 haematology analyzer.



Using Labomed Lx 400 bright field microscope under oil immersion (1000x) with a field diameter of 0.2mm(eye piece 10X/20WF) manual platelet count was performed. 20 fields were counted the average of which was multiplied with 15000 to obtain the platelet count per microliter of blood.

Platelet indices : Platelet count , MPV, PDW and PLCR were documented for each case as well as the platelet count was verified using Manual smear review . The following findings observed in the manual smear review are as follows.(Fig 7,8,9)

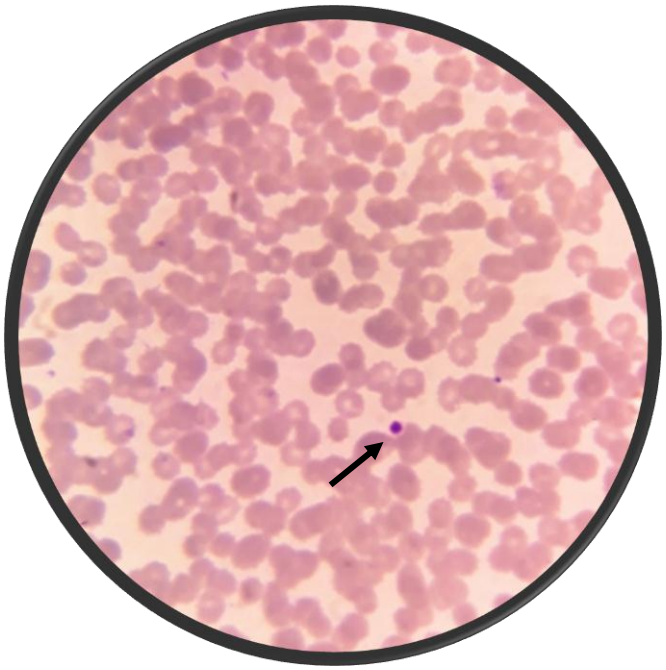


Figure 7: Microscopic image of Peripheral blood smear under oil Immersion (1000x) showing low platelets(↑)

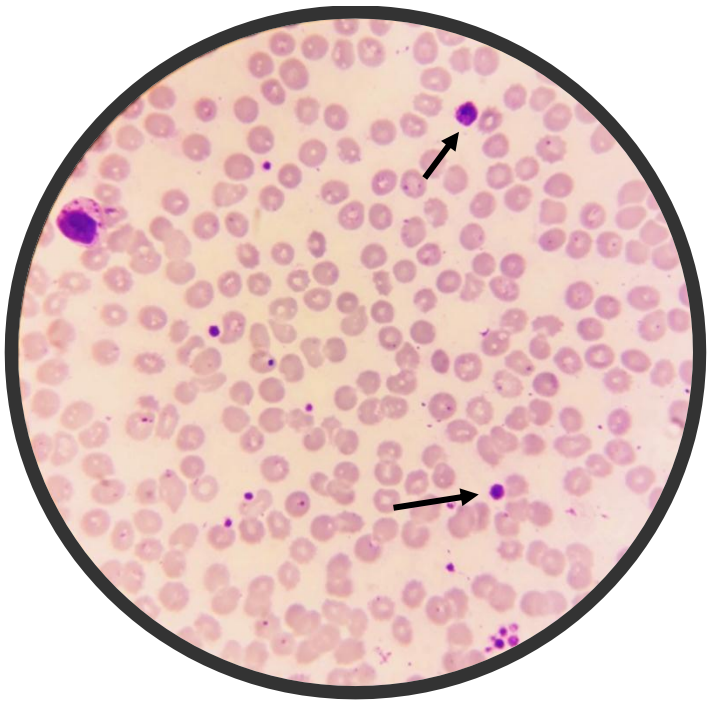
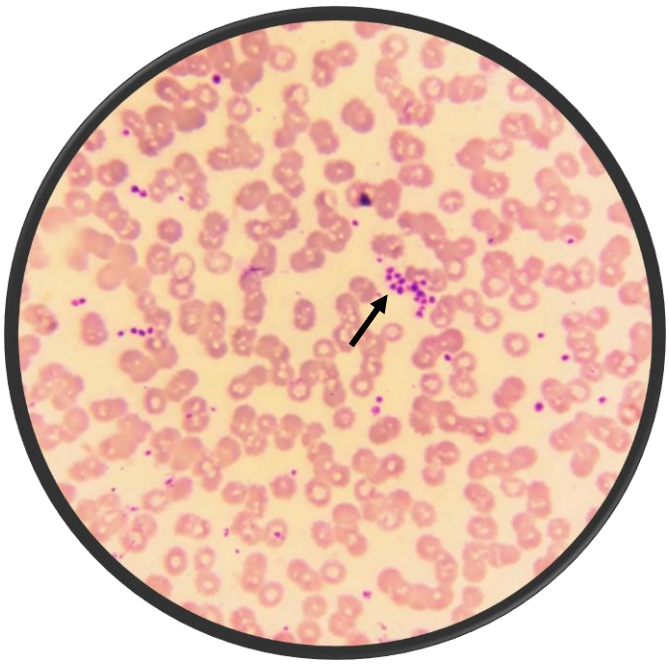
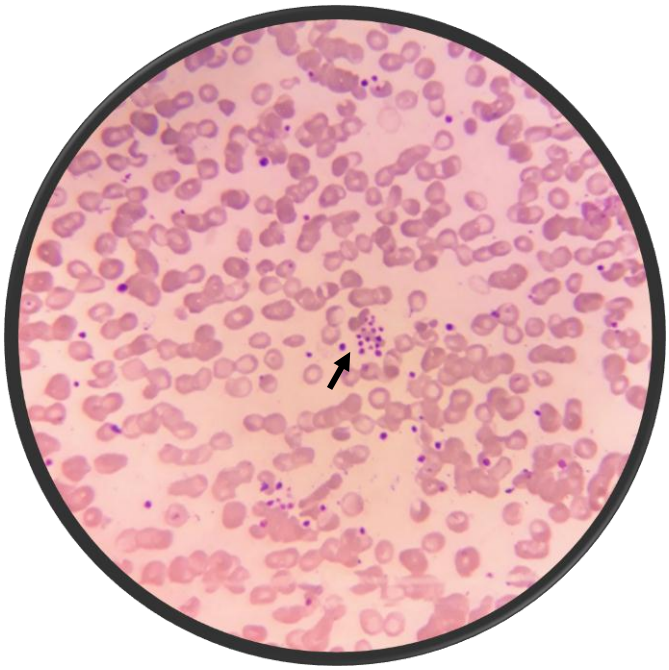


Figure 8: Microscopic images of peripheral blood smear under oil immersion (1000x) showing large platelets (↑).



A

Figure 9 (A&B): Microscopic images of peripheral blood smear under oil Immersion (1000x) showing platelet clumps (↑)



B

Table 1 : Platelet parameters with reference values

Platelet indices	Reference value	Increased value	Decreased value
MPV	7.4-11.4fl	>11.4 fl	<7.4fl
PDW	10-14 fl	>14 fl	<10fl
PLCR	11-33%	>33%	<11%
Platelet count	150-400x10^9/L	>400x10^9/L	<150x10^9/l

Based on the analysis from the data collected patients were divided into hyperdestructive and hypoproductive thrombocytopenia. The reference intervals followed for this stratification is as mentioned in the table 1. Cases falling into the category of hypoproductive group were further evaluated by bone marrow study following institutional protocols.

BONE MARROW EXAMINATION

Bone marrow Aspiration and trephine biopsy can be performed to evaluate etiology of thrombocytopenia. In a thrombocytopenic patient, when no other reason for low platelet counts can be determined, the bone marrow examination is useful for determining the presence of megakaryocytes. Absence indicates dysfunctional marrow while increased numbers suggest peripheral destruction with attempted bone marrow decompensation. Bone marrow examination can also detect myeloproliferative disorders such as acute leukaemia or metastatic malignancy.

Statistical analysis:

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean, standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries. Chi-square (χ^2)/Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means of analysis variables between two independent groups was tested by unpaired t test.

Bivariate correlation analysis using Pearson's correlation coefficient (r) was used to test the strength and direction of relationships between the interval levels of variables. Receiver operating curve (ROC) analysis for Sensitivity- specificity was done to check relative efficiency. If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0. and Microsoft office.

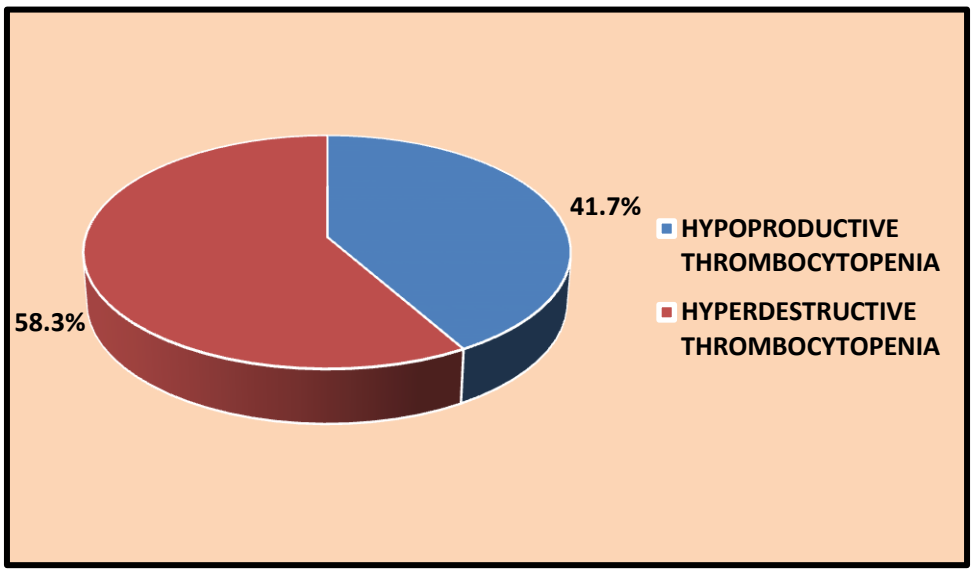
RESULTS

A total of 84thrombocytopenic patients were enrolled in the present study. Among these patients, 49 were under hyperdestructive thrombocytopenia (without bone marrow disease) and 35 were under hypoproductive thrombocytopenia (with bone marrow disease). They were categorized in two different subgroups based on etiology. The distribution of total number of 84 cases are summarized in table 2 and figure 10.

Table 2: Distribution of hypoproductive and hyperdestructive thrombocytopenia cases

VARIABLE CASES	N	%
HYPOPRODUCTIVE THROMBOCYTOPENIA	35	41.7
HYPERDESTRUCTIVE THROMBOCYTOPENIA	49	58.3

Figure 10: Distribution of hypoproductive and hyperdestructive thrombocytopenia cases



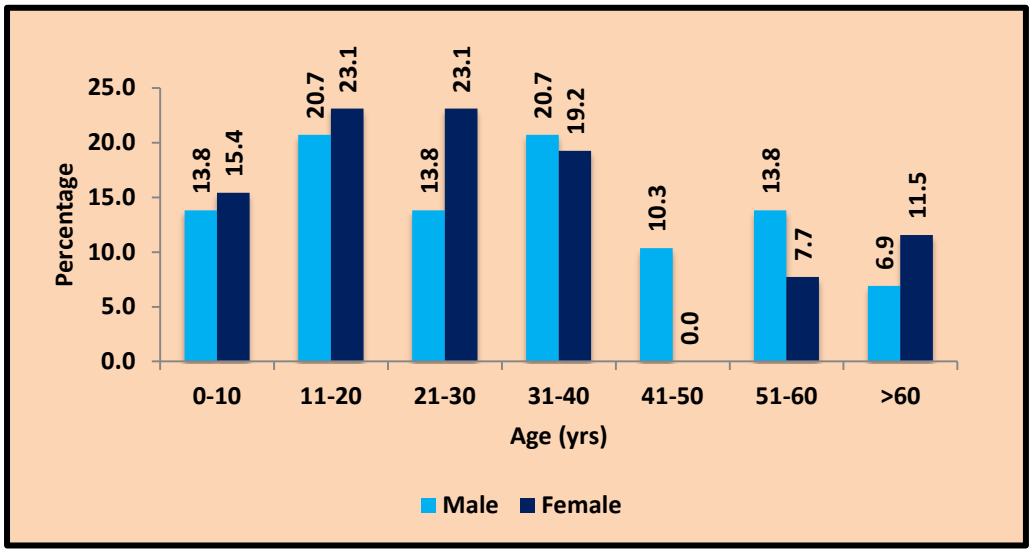
Distribution of thrombocytopenia cases according to age and sex.

Table 3 / figure 11 shows the distribution of thrombocytopenia cases according to the age and sex. The maximum number of cases in males were seen in the age group of 11-20 years and 31-40 and least number of cases were seen in those above 60 years of age. Similarly the maximum number of cases in females were seen in the age group of 11-30 years. Not a single case was detected in the age group of 41-50 years.

Table 3: Distribution of thrombocytopenia cases according to age and sex.

AGE (YRS)	Male		Female		p value
	N	%	N	%	
0-10	8	13.8	4	15.4	0.577
11-20	12	20.7	6	23.1	
21-30	8	13.8	6	23.1	
31-40	12	20.7	5	19.2	
41-50	6	10.3	0	0.0	
51-60	8	13.8	2	7.7	
>60	4	6.9	3	11.5	
Total	58	100.0	26	100.0	

Figure 11: Distribution of thrombocytopenia cases according to age and sex.



Distribution between hypoproductive and hyperdestructive thrombocytopenia cases.

Table 4 / figure 12 demonstrates the distribution of hypoproductive and hyper destructive thrombocytopenia cases according to sex. Males were predominantly affected in hypoproductive and hyperdestructive thrombocytopenia with 41.4% and 58.6%. The p value is not statistically significant for the distribution of sex in the following cases.

Table 4: Distribution of sex among hypoproductive and hyperdestructive thrombocytopenia cases

SEX	HYPOPRODUCTIVE THROMBOCYTOPENIA	HYPERDESTRUCTIVE THROMBOCYTOPENIA	Total	p value
	N (%)	N (%)		
Male	24(41.4)	34(58.6)	58(100)	0.936
Female	11(42.3)	15(57.7)	26(100)	
Total	35(41.7)	49(58.3)	84(100)	

Figure 12: Distribution of sex among hypoproductive and hyperdestructive thrombocytopenia cases.

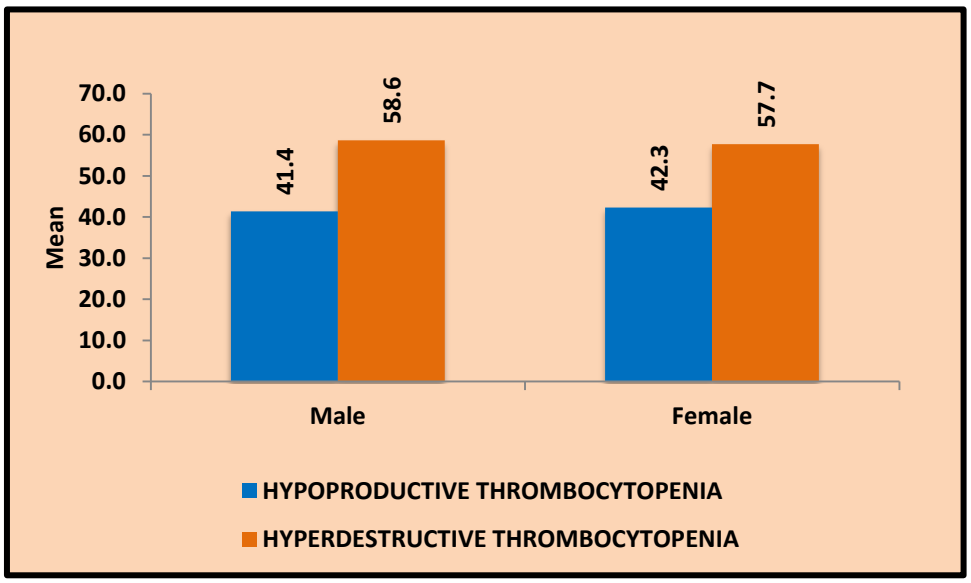
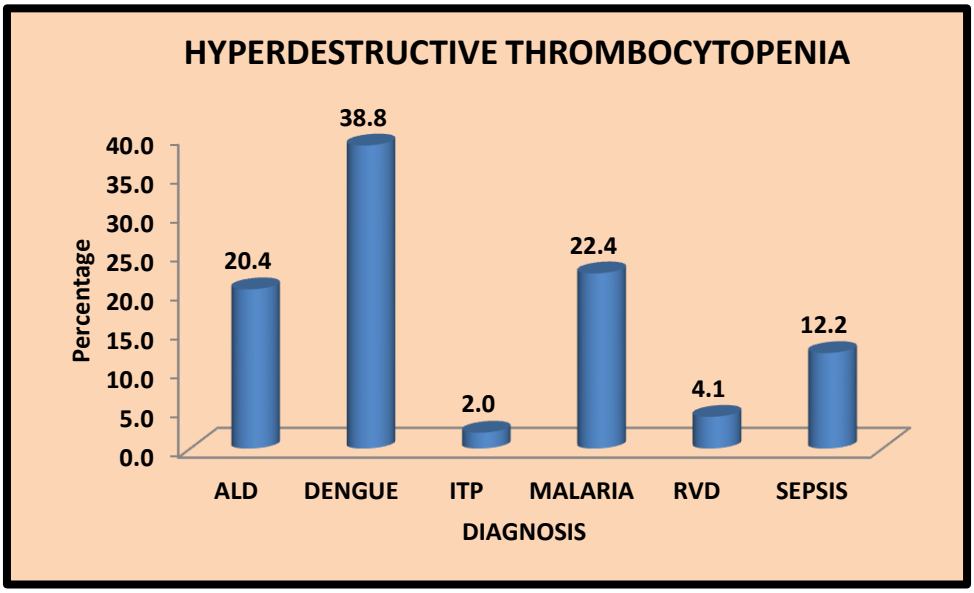


Table 5: Number of cases with hyperdestructive thrombocytopenia according to the diagnosis.

DIAGNOSIS	N	%
ALD	10	20.4
DENGUE	19	38.8
ITP	1	2.0
MALARIA	11	22.5
RVD	2	4.1
SEPSIS	6	12.2
Total	49	100.0

Table 5/figure 13 demonstrates the different etiologies categorized under the hyperdestructive thrombocytopenia. The total number of cases included under this group were 49 where the maximum number of cases were diagnosed as Dengue (19) and malaria (11) i.e; 38.8% and 22.5% each.

Figure 13: Distribution of diagnosis among hyperdestructive thrombocytopenia cases



Distribution of cases of hypoproductive thrombocytopenia according to diagnosis.

Table 6 / figure 14 demonstrates the different etiologies which are included under the hypoproductive thrombocytopenia group. The total number of cases included under this subgroup were 35, where the maximum number of cases in hypoproductive group were erythroid hyperplasia (15) and acute myeloid leukemia (11) i.e; 42.8% , 31.4% each.

Table 6: Distribution of various diseases among hypoproductive thrombocytopenia cases

DIAGNOSIS	N	%
Acute Lymphoblastic Leukaemia (ALL)	8	22.9
Acute Myeloid Leukaemia (AML)	11	31.4
Aplastic Anemia	1	2.9
Erythroid Hyperplasia	15	42.8
Total	35	100.0

Figure 14: Distribution of various diseases among hypoproductive thrombocytopenia cases

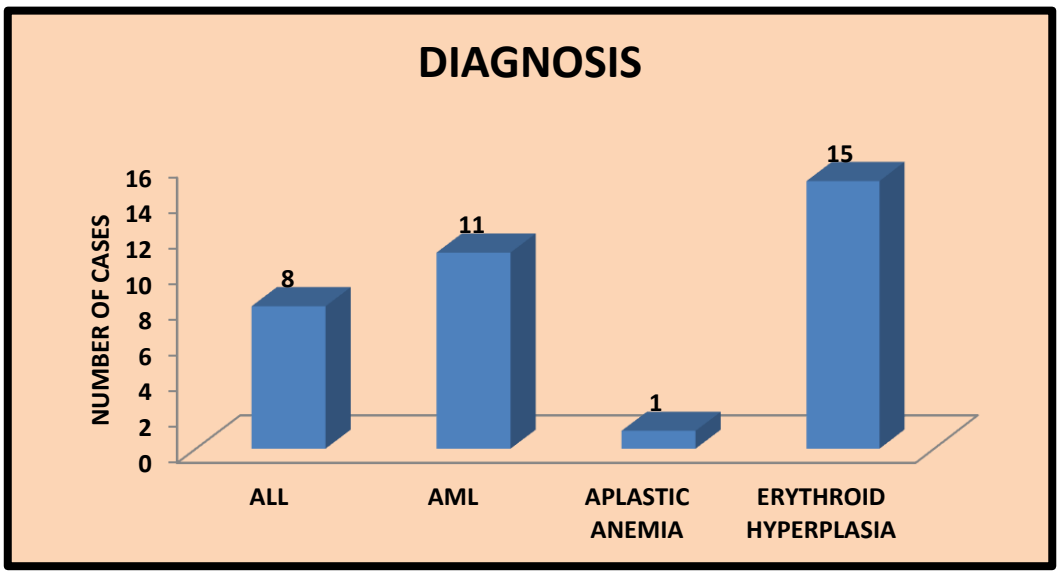


Table 7: Comparison of Mean parameters between hypoproductive and hyperdestructive thrombocytopenia cases

Parameters	HYPOPRODUCTIVE THROMBOCYTOPENIA	HYPERDESTRUCTIVE THROMBOCYTOPENIA	p value
	Mean±SD	Mean±SD	
Age(years)	37.63±23.17	26.98±18.48	0.022*
Platelet count (lakhs/cumm)	0.53±0.29	0.69±0.32	0.016*
MPV(fl)	10.40±2.05	11.52±1.20	0.002*
PDW(fl)	14.15±4.26	14.41±4.00	0.772
PLCR(%)	30.17±10.02	37.44±8.31	<0.001*

Note: * significant at 5% level of significance ($p<0.05$)

Table 7 / figure 15 demonstrates the comparison of mean and standard deviation (SD) of various parameters between the hypoproductive thrombocytopenia and hyperdestructive thrombocytopenia. The mean SD value for age was 37.63±23.17 and 26.98±18.48 for hypoproductive thrombocytopenia and hyperdestructive thrombocytopenia respectively, with a p value of 0.022 which was statistically significant. Likewise the mean SD value for the platelet count in hypoproductive thrombocytopenia shows 0.53±0.29 and 0.69±0.32 in hyperdestructive thrombocytopenia, with a p value of 0.016 which was statistically significant. The mean SD value for MPV in hypoproductive thrombocytopenia was 10.40±2.05 and 11.52±1.20 for hyperdestructive thrombocytopenia, with a p value of 0.002 which was statistically significant. The mean SD value for PDW in hypoproductive thrombocytopenia was 14.15±4.26 and 14.41±4.00 in hyperdestructive thrombocytopenia, with a p value 0.772 which was not significant. The mean SD value for PLCR in hypoproductive thrombocytopenia was 30.17±10.02 and 37.44±8.31 for hyperdestructive thrombocytopenia, with a p value of 0.001 which was statistically significant.

Figure 15: Comparison of mean parameters between hypoproductive and hyperdestructive thrombocytopenia cases

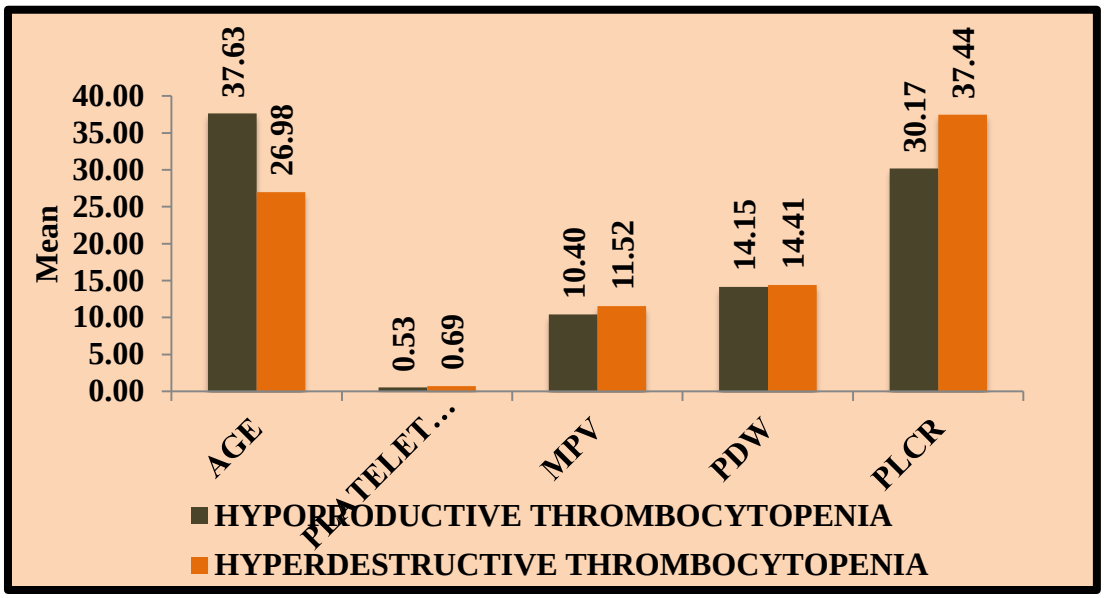


Table 8: Mean and SD values of platelet indices among hyperdestructive thrombocytopenia cases based on the diagnosis.

DIAGNOSIS	Number of cases	MPV	PDW	PLCR
		Mean±SD	Mean±SD	Mean±SD
ALD	10	11.5±1.3	13.7±2.9	36.2±8.8
DENGUE	19	11.4±1.2	14.1±4.2	35.4±9.3
ITP	1	8.4±0	1.1±0	42.1±0
MALARIA	11	11.9±1.0	16.2±3.1	41.0±7.1
RVD	2	11.6±0.1	14.2±0.5	36.5±0.1
SEPSIS	6	12.0±1.1	15.5±3.2	39.2±7.3
Total	49	11.5±1.2	14.4±4.0	37.4±8.3

Table 8 demonstrates the mean and standard deviation (SD) of the platelet indices MPV, PDW & PLCR in all the clinical conditions which were included under the hyperdestructive thrombocytopenia. In alcoholic liver disease the mean SD value for MPV was 11.5±1.3, PDW was 13.7±2.9 and PLCR was 36.2±8.8. In dengue fever the mean SD value for MPV was 11.4±1.2, PDW was 14.1±4.2 and PLCR was 35.4±9.3. In immune thrombocytopenia the mean SD value for MPV was 8.4±0, PDW was 1.1±0 and PLCR was 42.1±0. In malaria fever the mean SD value for MPV was 11.9±1.0, PDW was 16.2±3.1 and PLCR was 41.0±7.1. In retroviral diseases the mean SD value for MPV was 11.6±0.1, PDW was 14.2±0.5 and PLCR was 36.5±0.1. In sepsis the mean SD value for MPV was 12.0±1.1, PDW was 15.5±3.2 and PLCR was 39.2±7.3. The total number of cases 49 were included under hyperdestructive thrombocytopenia showing the mean SD value for MPV was 11.5±1.2, PDW was 14.4±4.0 and PLCR was 37.4±8.3.

Table 9 : Mean and SD values of platelet indices among Hypoproductive thrombocytopenia cases based on diagnosis

DIAGNOSIS	Numberof cases	MPV	PDW	PLCR
		Mean±SD	Mean±SD	Mean±SD
ALL	8	10.63±1.49	12.0±4.98	28.68±9.9
AML	11	10.61±1.79	14.38±3.98	31.56±10.83
Aplastic Anemia	1	10.7±0	13±0	28.2±0
Megaloblastic anemia	15	10.37±2.01	15.2±4.04	30.07±10.4
Total	35	10.4±2.1	14.1±4.3	30.1±10.0

Table 9 demonstrates the mean and standard deviation of the platelet indices MPV, PDW & PLCR in all the clinical conditions which are included in the hypoproductive thrombocytopenia. In acute lymphoid leukaemia the calculated mean SD value for MPV was 10.63±1.49, PDW was 12.0±4.98 and PLCR was 28.68±9.9. In acute myeloid leukaemia, the calculated mean SD value for MPV was 10.61±1.79, PDW was 14.38±3.98 and PLCR was 31.56±10.83. In Aplastic anemia , the calculated mean SD for MPV was 10.7±0, PDW was 13±0 and PLCR was 28.2±0. In Megaloblastic anemia, the calculated mean SD value for MPV was 10.37±2.01, PDW was 15.2±4.04 and PLCR was 30.07±10.4. The total number of cases 35 are included under hypoproductive thrombocytopenia showing the mean SD value for MPV was 10.4±2.1, PDW was 14.1±4.3and PLCR was 30.1±10.0.

Table 10: Pearson correlation of platelet count and platelet indices in Hypoproductive thrombocytopenia.

Parameters	HYPOPRODUCTIVE THROMBOCYTOPENIA	
	Pearson Correlation	**p value**
MPV	-0.108	0.536
PDW	0.274	0.112
PLCR	0.004	0.981

Note: * significant at 5% level of significance ($p<0.05$)

Table 10 demonstrates the Pearson correlation studies between the platelet count and platelet indices in hypoproductive thrombocytopenia. The platelet indices are showing weak negative correlation for MPV -0.108 with p value of 0.536 which is not statistically significant (Fig 16). PDW and PLCR are showing significant positive correlation 0.274 and 0.004 with a p value of 0.112 and 0.981 which is not statistically significant (Fig 17 & 18). The reason for the lower values of correlation may be due to non linear relation between MPV, PDW, PLCR with platelet count and smaller sample size.

Figure 16: Pearson correlation of platelet count and MPV in hypoproductive thrombocytopenia

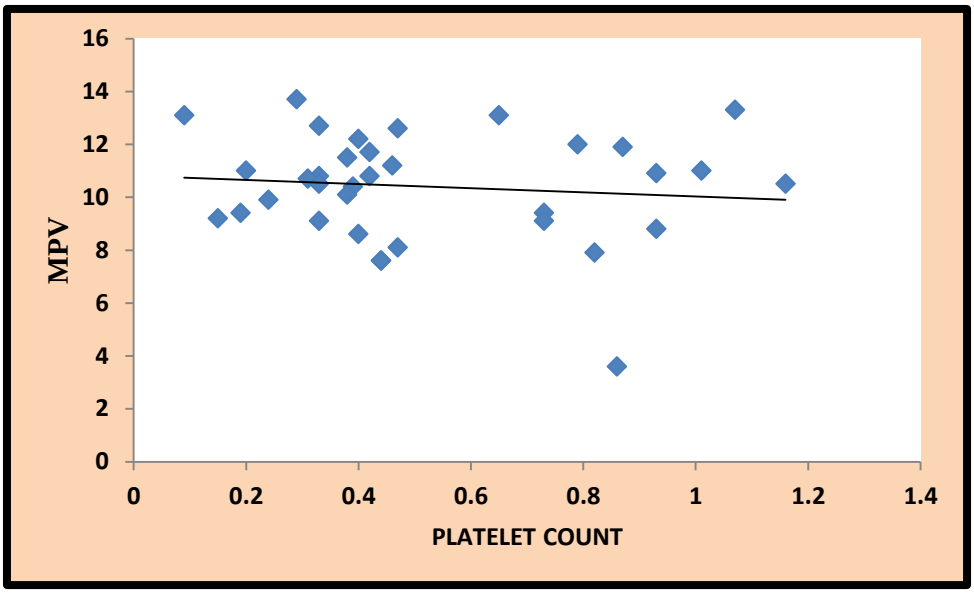


Figure 17: Pearson correlation of platelet count and PDW in hypoproductive thrombocytopenia

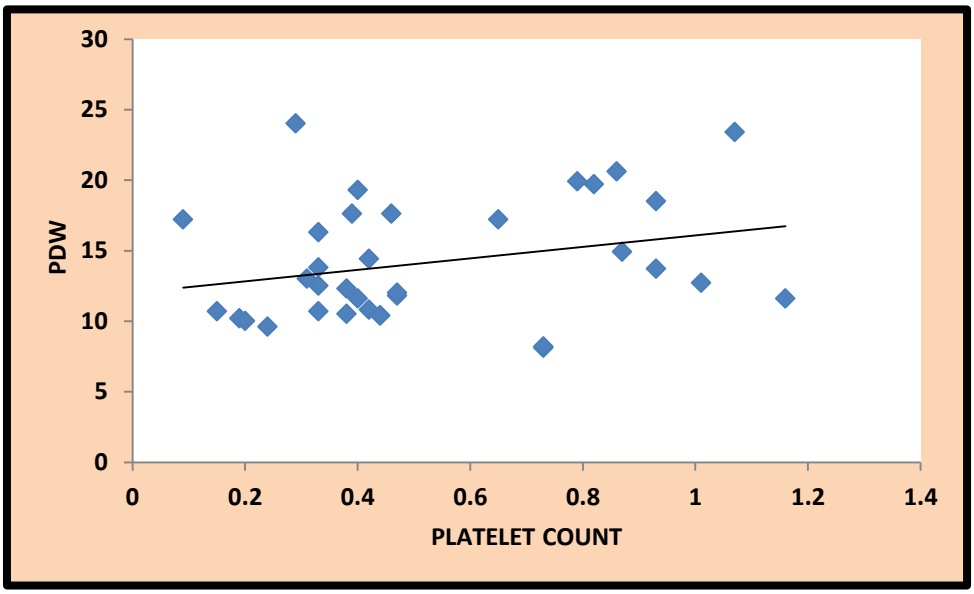


Figure 18: Pearson correlation of platelet count and PLCR in hypoproductive thrombocytopenia

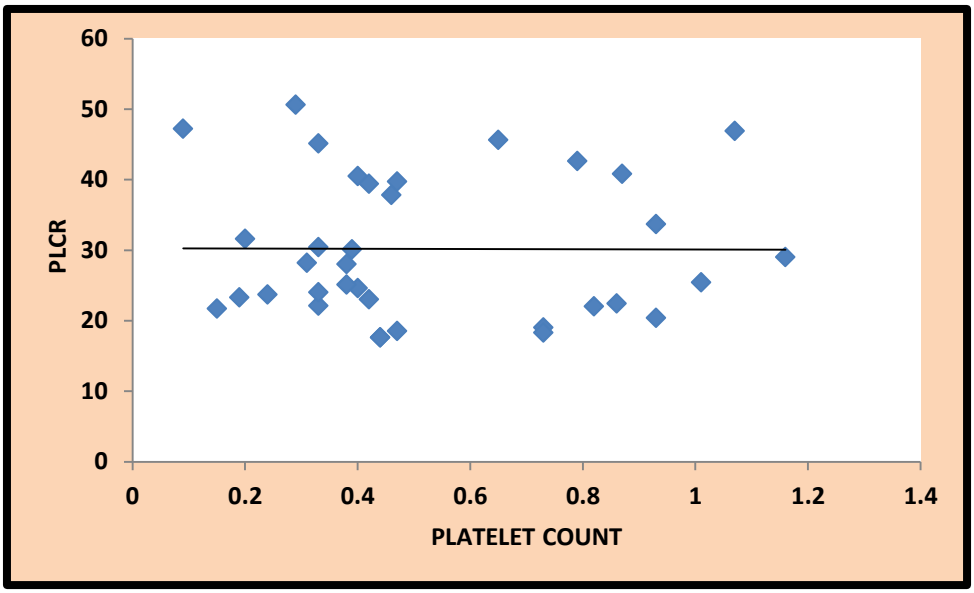


Table 11: Pearson correlation among platelet count and platelet indices in Hyperdestructive thrombocytopenia.

Parameters	HYPERDESTRUCTIVE THROMBOCYTOPENIA	
	Pearson Correlation	**p value**
MPV	0.067	0.649
PDW	-0.001	0.999
PLCR	-0.113	0.441

Note: * significant at 5% level of significance ($p<0.05$)

Table 11 demonstrates the Pearson correlation between the platelet count and platelet indices in hyper destructive thrombocytopenia cases. There is a positive correlation for MPV 0.067 with a p value of 0.64 which is statistically not significant (Fig 19). There is a negative correlation between the platelet count and platelet indices PDW -0.001 and PLCR -0.113 with a p value of 0.99 and 0.44 which is statistically not significant (Fig 20 & 21). The reason may be due to non linear relationship between the platelet count and platelet indices and a smaller sample size.

Figure 19: Pearson correlation of platelet count with MPV among hyperdestructive thrombocytopenia cases

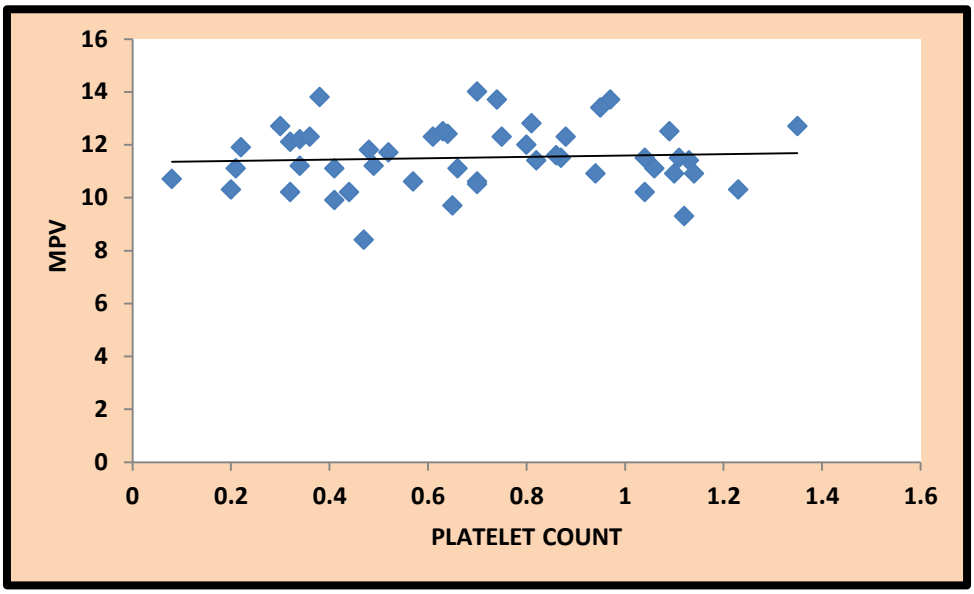


Figure 20: Pearson correlation of platelet count with PDW among hyperdestructive thrombocytopenia cases

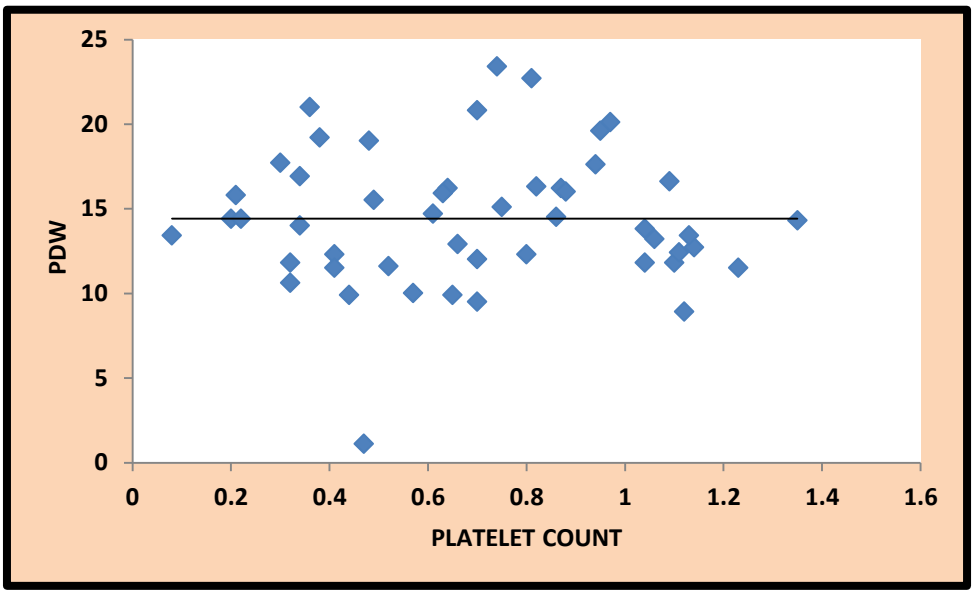


Figure 21: Pearson correlation of platelet count with PLCR among hyperdestructive thrombocytopenia cases

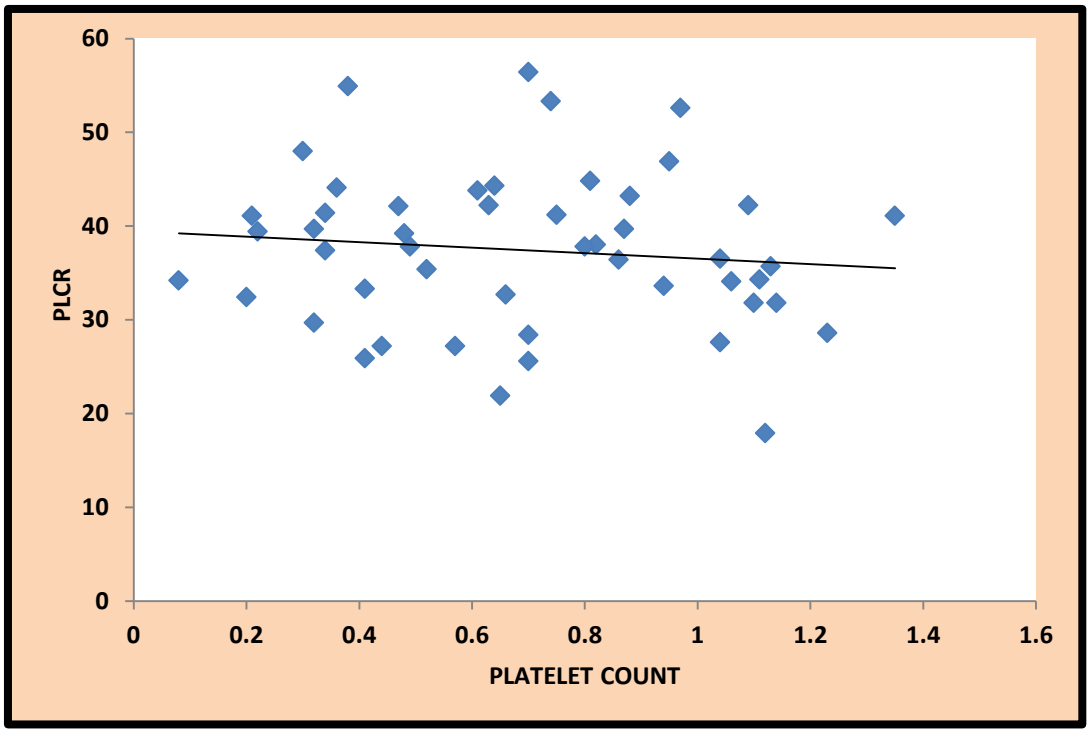


Table 12: ROC curve analysis showing MPV, PDW and PLCR in hypoproductive thrombocytopenia.

Test Variables	Area Under the Curve (AUC)	Std. Error	p value
MPV	0.673	0.062	0.007
PDW	0.548	0.066	0.457
PLCR	0.711	0.062	0.001

Table 12 / figure 22 shows that the area under the curve (AUC) gives the probability that a patient with bone marrow disease has lower values of the measurement (MPV, PDW, PLCR). The AUC shows lines shifting towards the left upper corner particularly for the MPV and PLCR giving an area of 0.673 (67.3%) and 0.711 (71.1%) respectively and PDW giving an area of 0.548 (54.8%).

Figure 22: ROC curve showing sensitivity and specificity of MPV, PDW and PLCR.

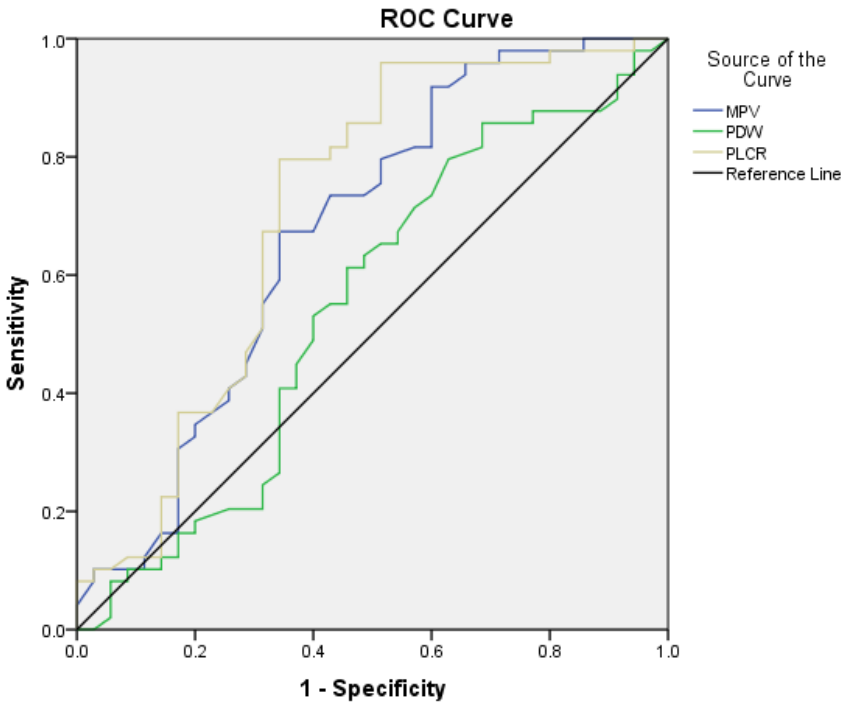


Table 13: Sensitivity and Specificity of platelet indices at different cut off points in hypoproductive thrombocytopenia.

Cut-off value	Sensitivity(%)	Specificity(%)
MPV		
<6.60	0.0%	100.0%
<8.95	20.0%	98.0%
<10.15	40.0%	91.8%
<10.75	51.4%	73.5%
<11.05	66.7%	59.2%
PDW		
<7.10	0.0%	100.0%
<10.45	20.0%	87.8%
<12.80	51.4%	63.3%
<13.75	57.1%	55.1%
<14.35	60.0%	49.0%
PLCR		
<16.60	0.0%	100.0%
<25.50	48.6%	95.9%
<33.65	65.7%	67.3%
<33.90	68.6%	67.3%
<40.65	80.0%	36.7%

As shown in the table 13 MPV of <11.05 fl can identify thrombocytopenic patients as hypoproductive with 66.7% sensitivity and 59.2 % specificity. Likewise, PDW of <12.80 fl have 51.4% of sensitivity and 63.3% of specificity. PLCR with <33.90% have 68.6% of sensitivity and 67.3% of specificity. The platelet indices MPV and PLCR in particular have better sensitivity and specificity to diagnose hypoproductive thrombocytopenia.

Table 14: Sensitivity and Specificity of platelet indices at different cut off points in hyperdestructive thrombocytopenia.

CUT-OFF VALUE	SENSITIVITY (%)	SPECIFICITY (%)
MPV		
>9.25	98.0	28.6
>10.25	85.7	40.0
>10.65	75.5	48.6
>11.05	67.3	65.7
>11.30	55.1	68.6
PDW		
>10.55	87.8	22.9
>11.90	73.5	40.0
>12.80	63.3	51.4
>13.55	55.1	54.3
>14.15	51.0	60.0
PLCR		
>22.25	95.9	25.7
>28.10	85.7	51.4
>32.55	73.5	65.7
>34.25	63.3	68.6
>36.45	55.1	68.6

As shown in the table 14 MPV of > 11.05 fl can identify thrombocytopenic patients as hyperdestructive thrombocytopenia with 67.3 % sensitivity and 65.7% specificity. PDW of >12.80 fl show 63.3% sensitivity and 51.4% specificity and PLCR of > 32.55 % show 73.5% sensitivity and 65.7% specificity. The platelet indices MPV and PLCR in particular have better sensitivity and specificity to diagnose hyperdestructive thrombocytopenia.

Comparison of negative and positive predictive value for hyperdestructive and hypoproductive groups at different MPV threshold levels

The negative and positive predictive values for the hypoproductive and hyperdestructive thrombocytopenia at different MPV, PDW, PLCR threshold levels are tabulated. As shown in the table 15 a MPV of > 11 fl has a negative predictive value of 70.21%. It also declined as the MPV threshold decreased. A MPV of <8 fl has a positive predictive value of 100%. It was observed that the positive predictive value was variable when compared with different MPV thresholds for hyperdestructive thrombocytopenia.

Table 15: Comparison of Negative and Positive predictive values for hyperdestructive and hypoproductive groups at different MPV threshold levels.

MPV threshold	No. of cases	No. of pts. without marrow disease (hyper destructive)	% of pts in this group	NPV (%)	MPV threshold	No. of cases	No. of pts. with marrow disease (hypo productive)	% of pts in this group	PPV (%)
>14	0	0	0	-	<14	84	35	35	41.67
>13	9	5	9	55.56	<13	75	31	31	41.33
>12	26	18	26	69.23	<12	58	27	27	46.55
>11	47	33	47	70.21	<11	37	21	21	56.76
>10	67	45	67	67.16	<10	17	13	13	76.47
>9	76	48	76	63.16	<9	8	7	7	87.50
>8	80	49	80	61.25	<8	4	4	4	100.00
>7	84	49	84	58.33	<7	0	0	0	-

Table 16 : Comparison of Negative and Positive predictive values of hyperdestructive and hypoproductive thrombocytopenia cases at different PDW threshold levels

PDW threshold	No. of cases	No. of pts. without marrow disease (hyper destructive)	% of pts in this group	NPV (%)	PDW threshold	No. of cases	No. of pts. with marrow disease (hypo productive)	% of pts in this group	PPV (%)
>22	0	0	0	-	<22	84	35	35	41.67
>20	8	5	8	62.50	<20	76	32	32	42.11
>18	15	8	15	53.33	<18	69	28	28	40.58
>16	28	16	28	57.14	<16	56	23	23	41.07
>14	40	26	40	65.00	<14	44	21	21	47.73
>12	57	36	57	63.16	<12	27	14	14	51.85
>10	76	44	76	57.89	<10	8	3	3	37.50
>8	84	49	84	58.33	<8	0	0	0	-

Table 16 shows PDW of > 14 fl has a negative predictive value of 65% A PDW of < 12 fl has a positive predictive value of 51.85%.

Table 17: Comparison of Negative and Positive predictive value for Hyperdestructive and hypoproductive groups at different PLCR threshold levels.

PLCR threshold	No. of cases	No. of pts. without marrow disease (hyper destructive)	% of pts in this group	NPV (%)	PLCR threshold	No. of cases	No. of pts. with marrow disease (hypo productive)	% of pts in this group	PPV (%)
>55	0	0	0	-	<55	84	35	35	41.67
>50	5	4	5	80.00	<50	79	34	34	43.04
>45	11	6	11	54.55	<45	73	30	30	41.10
>40	26	18	26	69.23	<40	58	27	27	46.55
>35	41	30	41	73.17	<35	43	24	24	55.81
>30	54	39	54	72.22	<30	30	20	20	66.67
>25	67	47	67	70.15	<25	17	15	15	88.24
>20	78	48	78	61.54	<20	6	5	5	83.33
>15	84	49	84	58.33	<15	0	0	0	-

As shown in the table 17 PLCR of > 35% has a negative predictive value of 73.17% in hyperdestructive thrombocytopenia. A PLCR of < 20%has a positive predictive value of 83.33% in hypoproductive thrombocytopenia.

DISCUSSION

Platelets are gaining importance in the present day with the list of diseases getting updated from time to time where platelet count is needed for better management as well as monitoring. So there is an active need for having a precise and accurate platelet count. The method for obtaining the count should be relatively non invasive, cost effective and rapid. Automated analyzers have covered a lot of ground over the last few decades and are on par with the manual smear count today. Platelet count when interpreted with parameters like MPV PDW and PLCR forms a valuable diagnostic modality in assessing thrombocytopenia.

To evaluate the utility of these platelet indices a total of 84 cases were analyzed and stratified as hyperdestructive and hypoproductive group in this study.

In the present study the mean age in hypoproductive cases was 37.63±23.17 years and in hyperdestructive cases were26.98±18.48 years. similar mean age of the study group were also noted by Islam S*et al* in hypoproductive and hyperdestructive cases [36.66(±17.04) and 29.70(±13.99) respectively].[92]

In hypoproductive group, males were 24 and females were 11, in hyperdestructive group, males were 34 and female were 15. In hypoproductive and hyperdestructive group mean platelet count was statistically significant showing0.53 (±0.29) $x10^9$/L and 0.69 (±0.32)$x10^9$/L, whereas the study done by Islam S*et al*. the most commonly affected group were females in both hypoproductive and hyperdestructive thrombocytopenia groups.

Islam S *et al.* mentioned that in Memon study, 65 ITP patients were selected and the mean age of these patients was 32.5 years, 45 were female and 20 were male. Female to male ratio was 2.25: 1. In another study Neylon, reported that the age range of the patients presenting with confirmed ITP was 16–91 years, with a median age at diagnosis of 56 years of age. There was a female to male ratio of 1.2:1(134 females to 111 males).[92]

In the present study the Mean SD of MPV in hyperdestructive and hypoproductive groups are 11.52±1.20 and 10.40±2.05 respectively. They are showing high Mean SD values in hyperdestructive group than in hypoproductive group. The sensitivity and specificity of MPV were calculated under various cut off ranges to make a diagnosis of hyperdestructive thrombocytopenia from hypoproductive thrombocytopenia. The MPV of > 11(fl) shows 67.3% sensitivity and 65.7% specificity respectively in hyperdestructive thrombocytopenia and MPV of <11 (fl) has 65.7% sensitivity and 59.2% specificity respectively in hypoproductive thrombocytopenia. In the present study an MPV of > 11 (fl) has a negative predictive value (NPV) of 70.2% in hyperdestructive thrombocytopenia and MPV of < 8 (fl) has a positive predictive value (PPV) of 100% in hypoproductive thrombocytopenia at different MPV threshold levels.

Table 18: Comparision and distribution of thrombocytopenia cases with similar studies.

Platelet indices	**Present study (84)**		**Negash et al. (83)**		**Kaito etal. (79)**		**Islam et al. (60)**	
Hyperdestructive thrombocytopenia								
Cut off ranges	sensitivity	specificity	sensitivity	specificity	sensitivity	specificity	sensitivity	Specificity
MPV >11 (fl)	67.3%	65.7%	67 %	95%	87.2 %	80%	73.3 %	80 %
PDW >14 (fl)	51 %	60 %	61 %	62%	76.9 %	90%	86.6 %	93.3%
PLCR > 33 (%)	73.5 %	65.7 %	67 %	88 %	91.4 %	73%	73.3 %	90 %
Hypoproductive thrombocytopenia								
Cut off ranges	sensitivity	specificity	sensitivity	specificity	sensitivity	specificity	sensitivity	Specificity
MPV< 10 (fl)	40 %	91%	74 %	70 %	-	-	-	-
PDW< 15 (fl)	60 %	49 %	76 %	55 %	-	-	-	-
PLCR <31 (%)	68.6 %	67.3 %	76 %	67 %	-	-	-	-

Table 18 shows study done by Negash M *et al*. reported that MPV are significantly higher in hyper destructive cases with 67% sensitivity and 95% specificity than hypoproductive cases with 74% sensitivity and 70% specificity and also with 88% positive predictive value and 81% negative predictive value respectively. Kaito K *et al.* and Islam S *et al*. reported that MPV >11 (fl) has sensitivity (87%, 73.33%) and specificity (80%,80%) respectively to make a diagnosis of hyperdestructive thrombocytopenia . Farweez B *et al*. concluded that MPV >9.7 fl has (57.5%) sensitivity and (82.5%) specificity with (76.7%) positive predictive value and (66.0%) negative predictive value. Niethammer *et al*. concluded that MPV was able to predict the presence of bone marrow metastasis in solid tumour patients with 85 % PPV and

90 % NPV. Numbenjapon *et al.* and Khaleel K *et al*. found that MPV was significantly higher in hyperdestructive group compared to hypoproductive thrombocytopenia. Borkataky *et al*. found no significant difference in the MPV between the hyperdestructive and hypoproductive thrombocytopenia.[85, 92]

In the present study the Mean SD values for the PDW values in hyperdestructive and hypoproductive group are 14.41±4.00 and 14.15±4.26. The PDW is not statistically significant in both subgroups. PDW >10.55 (fl) has 87.8% sensitivity and 22.9% specificity respectively in hyperdestructive thrombocytopenia and PDW <14. 35 (fl) sensitivity 60% and specificity 49% respectively in hypoproductive thrombocytopenia. PDW of >14 (fl) has a NPV of 65% in hyperdestructive and PDW of <12 (fl) has a PPV of 51.85% in hypoproductive TCP at different PDW threshold levels.

Negash M *et al*. reported that PDW (fl) cut off range >14.25 has 61% of sensitivity and 62% of specificity respectively, in hyperdestructive thrombocytopenia and PDW < 15 (fl) show76% sensitivity and 55% specificity respectively in hypoproductive thrombocytopenia. Islam S *et al.* and kaito K *et al.* reported that PDW of >14 (fl) (86.6%, 76.9%) sensitivity and (93.3%, 90%) specificity respectively in hyperdestructive thrombocytopenia.[85, 87]

In the study done by Farweez B *et al.* and khaleel *et al.* reported that the mean SD values for PDW are higher in hyperdestructive thrombocytopenia than in hypoproductive thrombocytopenia showing (17.11±1.87, 15.61±0.7.3) and (16.90±1.88, 13.83±1.75) respectively. Parveen S and Vimal M observed the Mean SD values of PDW in hyperdestructive and hyproductuve are 19.3±4.2 and 19.7±5.4.

however, they did not find any significance difference of PDW in both subgroups.[86, 89, 91]

In the present study the sensitivity and specificity for PDW (fl) was similar to the study done by Negash et al. Also observed that mean SD values for PDW are similar with the other studies.

In the present study, Mean SD values of PLCR 37.44 ± 8.31 are higher in the hyperdestructive thrombocytopenia than in the hypoproductive thrombocytopenia with PLCR 30.17 ± 10.02. The sensitivity and specificity of PLCR (%) cut of values were >22.25 sensitivity 95.9% respectively and specificity 25.7% respectively in hyperdestructive thrombocytopenia and PLCR (%) cut off value < 40.65 sensitivity 80% and specificity 36.7% respectively in hypoproductive thrombocytopenia. PLCR of > 35 (%) has 73.17% negative predictive value of in hyperdestructive thrombocytopenia and PLCR of < 25(%) has a PPV of 88.24% in hypoproductive TCP at different PLCR threshold levels.

Negash M *et al.* reported that PLCR >33 (%) has 67% sensitivity and 88% specificity in hyperdestructive thrombocytopenia and PLCR <31 (%) has 76% sensitivity and 67% specificity in hypoproductive thrombocytopenia. Kaito K *et al.* reported that the Mean SD value of PLCR 42.2±1.5 and 25.7±1.1shows higher values in hyperdestructive and hypoproductive thrombocytopenia cases. PLCR of > 30 (%) has 91.4% sensitivity and 73.0% specificity respectively in hyperdestructive thrombocytopenia. Islam S *et al.* reported that the Mean SD values are higher in hyperdestructive thrombocytopenia than hypoproductive thrombocytopenia. PLCR >30 (%) has 73.33% sensitivity and 90% specificity respectively in hyperdestructive thrombocytopenia.[85, 87, 92]

In the present study the PLCR values are showing similar sensitivity with other studies whereas the specificity values are variable. Platelet indices MPV, PDW and PLCR are showing high mean values in hyperdestructive group than in hypoproductive group. Among these platelet indices, MPV and PLCR are statistically significant and PDW is not statistically significant in hyperdestructive and hypoproductive thrombocytopenia in the present study when compared to other studies.

Study done by Numbenjapon *et al.* found that MPV was significantly higher in hyperdestruction group compared to hypoproductive thrombocytopenia. In hyperdestructive thrombocytopenia, bone marrow compensates actively for the platelet loss and start releasing young larger platelets ("left shift") which tend to decrease in size during its 7-10 days life span. In our study also we found a significant low (10.40 ± 2.05) mean MPV in the hypoproduction group than in the hyperdestruction group (11.52 ± 1.20).[91]

In the study done by Niethammer *et al.* reported that maximum of the histogram, that is the highest peak of the platelet volume distribution curve, has better efficiency than MPV in identifying thrombocytopenia caused by hyperdestruction and that resulted from hypoproduction secondary to receiving chemotherapy. MPV was able to predict the presence of bone marrow metastasis in solid tumor patients with 85 % PPV and 90 % NPV.[85]

In the present study the platelet count was statistically significant between the hypoproductive and hyperdestructive thrombocytopenia. Although Negash M *et al.* reported that platelet count was not statistically significantly between hypoproductive

and hyper destructive thrombocytopenia and all the platelet indices are significantly higher in hyper destructive cases than with hypoproductive cases.

In the present study there was no significant difference between the mean MPV of acute lymphoblastic leukemia (10.63±1.49) and acute myeloid leukemias (10.61±1.79). Although Khanna R *et al.* reported that the cases with leukemias which are associated with marrow suppression had variable mean MPVs where it shows mean MPV less than 9fl in acute lymphoblastic leukemias in contrast to acute myeloid leukemias had a higher mean MPV.[2]

Study done by Ntaios *et al.*, Kaito K *et al.*, Khaleel K *et al.* and Shah *et al.* found significantly higher values in MPV and PDW in hyperdestructive group than in hypoproductive group. The high PDW in hyperdestruction could be explained because of the newly formed platelets that are larger than circulating platelets, which tend to decrease in size with age in the circulation similar to reticulocytes with increased mean volume. As a result, in patients with thrombocytopenia secondary to hyperdestruction the PDW is increased, reflecting active compensatory mechanism in bone maromw with release of young platelets.[88]

Tomito *et al.*, Nakadate H *et al.*, and Baynes RD *et al.* found a low MPV or no significant difference in the MPV and PDW between the hyperdestructive and hypoproductive thrombocytopenia. Bashir AB *et al.* found significant differences in the MPV, PDW and PLT in patients with dengue infection and they suggested that these parameters can be used as probable indicators for dengue in endemic area. He also found a MPV <9 fl and high PDW >13 fl had a considerable sensitivity for dengue fever.[91]

In the present study MPV, PDW and PLCR was significantly higher and these indices were effective in distinguishing these two types of thrombocytopenia. Among all the platelet indices, MPV and PLCR are more reliable and have better discriminating potential to differentiate between the hypoproductive and hyperdestructive thrombocytopenia which are statistically significant.

"The first possible explanation for such differences between the present study and the above studies could be the kind of automated hematology analyzers that is used for enumerating the platelet count and platelet parameters. A study conducted by Kaito K *et al*. in Japan using Sysmex-XE2100 analyzer (Kobe, Japan) reported a mean MPV of 10.2 fl in hypoproductive and 12.2 fl in hyperdestructive patients, whose values are closer to the present study".

"A similar finding was also reported from a study conducted by Ntaios *et al*. as they emphasized that the difference of values in their study compared to other studies could be due to a difference in type of hematology analyzers. Large or giant platelets could be excluded from the platelet count with instruments which use impedance method like coulter STKS and coulter Gen-S used by the above studies, but the Sysmex XT2000i and XE2100 series also employ optical fluorescence detection method".

"The second possible reason could be the actual difference in the population and platelet indices from age and sex wise distribution, number of study participants and country to country. A study by Hong *et al.* in healthy Chinese adults using Sysmex XT 2100 indeed confirmed variations of platelet indices between regions. The reported value of MPV for example ranged from 10.30 ± 0.80 to 12.36 ± 1.34".[85]

In the present study we are used Sysmex XN1000 hematology fully automated analyzer which works on the principal of hydrodynamic focusing and flow cytometry.

Some studies suggest that in severe thrombocytopenia it is not always possible to record platelet indices in the presence of red cell fragmentation, a platelet histogram cannot be adequately drawn hence, the indices cannot be recorded. Babu E and Basu D also mentioned the difficulties in getting these parameters and discarded those cases lacking these parameters. [88]

We also avoided this problem by discarding cases without indices and histogram and selected only those cases which had platelet indices and provided a histogram.

Kaito K *et al.* and Islam S *et al.* reported the sensitivity and specificity of platelet indices to make a diagnosis of hyperdestructive thrombocytopenia was calculated under various cut off ranges. PDW and PLCR showed favorable sensitivity and specificity, they found MPV (fL) cut of value >11, >12 and >13, sensitivity 87%, 59% and 11% respectively, specificity were 80%, 95% and 100% respectively. PDW (fl) cut of value >13, >14 and >15 sensitivity was 92%, 76% and 71% respectively, specificity was 75%, 90% and 95% respectively. P-LCR cut off value were >25, >30 and >40 sensitivity was 100%, 91% and 62% respectively, specificity were 45%, 73% and 100% respectively. [92]

The ROC curve is useful in comparing the superiority of different laboratory tests, and a laboratory test with larger AUC is thought to be more reliable, with less misdiagnosis than a test with smaller AUC. In the present study the Receiver operating curve (ROC) analysis showed the Area under curve (AUC) showed lines shifting towards the left upper corner particularly for MPV and PLCR giving an area

of 67.3% and 71.1% respectively where PDW is decline in the AUC graph with area of 54.8% respectively.

Study done by Kaito K *et al.* reported that platelet indices showed favorable ROC curves with large AUC and among the three parameters, PDW and P-LCR were more reliable markers for distinguishing hyper-destructive thrombocytopenia from hypo-productive thrombocytopenia. Negash M *et al.* concluded that MPV and PLCR are more reliable markers, giving an area of 87.6% and 81.6% respectively to distinguish hyperdestructive from hypoproductive Thrombocytopenia.[87]

Farweez B *et al.* study reported that correlation studies with hyperdestructive thrombocytopenia has significant positive correlations between MPV and PDW (r = 0.312, p = 0.048), PDW and P-LCR (r = 0.405, P = 0.010), and MPV and P-LCR were found (r = 0.561, P < 0.010). In addition, significant positive correlations were found between MPV and PDW (r = 0.611, P < 0. 001), PDW and P-LCR (r = 0.373, P = 0.018), and MPV and P-LCR (r = 0.315, P < 0.047) in hypoproductive thrombocytopenia patients.[86]

Kaito K *et al.* reported that correlation studies between the platelet count and platelet indices showed an inverse correlation between platelet count, PDW and PLCR. However, no significant correlation between platelet indices and platelet count was found in immune thrombocytopenia. Khaleed K *et al.* reported that there were no correlations between platelet count and both MPV and PDW in hyperdestructive and hypoproductive thrombocytopenia but there was a direct correlation between platelet count and plateletcrit.[87, 89]

Negash M *et al.* observed that there was statistically significant negative correlation between platelet count and the platelet indices in hyperdestructive patients. However, the platelet count and platelet indices did not show significant correlation in Hypoproductive patients.[85]

In the present study there was a statistically significant negative correlation between the platelet count, PDW and PLCR in hyperdestructive thrombocytopenia. MPV is showing significant positive correlation. However, there is no significant correlation between platelet count and other platelet indices in hyperdestructive thrombocytopenia. The correlation coefficient between the platelet count and MPV, PDW, and P-LCR was 0.067, -0.001 and -0.113 respectively.

There was a negative correlation between the MPV in hypoproductive thrombocytopenia. There is a positive correlation between the platelet count and PDW and PLCR. However, there is no significant correlation between platelet count and platelet indices in hypoproductive thrombocytopenia. The correlation coefficient between the platelet count and AGE, MPV, PDW, and P-LCR was - 0.10, 0.27 and 0.004 respectively.

Khanna R *et al.* reported negative predictive value (NPV) for hyperdestructive at different MPV threshold levels. MPV of more than 12 fl has a negative predictive of 100%. It also gradually declined as the MPV threshold decreased. Also reported positive predictive value (PPV) of MPV for bone marrow diseases at different threshold levels which showed variable PPV when compared with different thresholds and showed no particular pattern.[2]

In the study done by Negash M *et al.* reported that an MPV of <10.75 fl shows 74 % of sensitivity, 70 % of specificity with 79 % of PPV and 64 % of NPV can identify thrombocytopenic patients as hypoproductive. Similarly, MPV of >11.05 fl can identify hyperdestructive thrombocytopenic patients with 67 % of sensitivity, 95 % of specificity, 88 % of PPV and 81 % of NPV.[85]

In the present study an MPV of > 11 (fl) has a negative predictive value (NPV) of 70.2% in hyperdestructive thrombocytopenia and MPV of < 8 (fl) has a positive predictive value (PPV) of 100% in hypoproductive thrombocytopenia at different MPV threshold levels.

PLCR of > 35% has a NPV of 73% in hyperdestructive TCP and PLCR of < 20% has a PPV of 83% in hypoproductive TCP at different PLCR threshold levels, Whereas PDW of >14 (fl) has a NPV of 65% in hyperdestructive TCP and PDW of <12 (fl) has a PPV of 51.85% in hypoproductive TCP at different PDW threshold levels. Therefore the platelet indices MPV and PLCR could be helpful to have a better prediction capacity for differentiating hyperdestructive and hypoproductive thrombocytopenia during the early diagnosis of the thrombocytopenia patients.

CONCLUSION

Automated hematology analyzers have incremental improvement in accuracy and reliability, which were comparable with manual smear count, over the years. Along with the platelet count the additional parameters like MPV PDW and PLCR were able to stratify the thrombocytopenia based on etiology into hypoproductive and hyperdestructive thrombocytopenia with a significant sensitivity and specificity. The bone marrow analysis done for the confirmation of hypoproductive cases as stratified by the analyzer yielded Pearson correlation showing significantly negative correlation with respect to the hyperdestructive cases. The analyzer classified cases were having better correlation with the clinically proven etiology. Based on these observations, analyzers stratification of these cases provides a reliable modality for the management of thrombocytopenia where in invasive procedures like bone marrow study is not feasible.

Further studies with large number of cases in each sub groups are needed to explore the role of the platelet indices and the role of other new parameters like immature platelet fraction (IPF) in thrombocytopenia used and also to find the diagnostic role of platelet indices in various other diseases.

SUMMARY

Thrombocytopenia is the most common cause of abnormal bleeding which results from the hyperdestruction of the platelets in the peripheral circulation (without bone marrow disease) or hypoproduction of the platelets in the bone marrow (with bone marrow disease), pseudo thrombocytopenia or abnormal pooling.

With the recent advances in the automated haematology analyzers, the evaluation of platelet count and platelet indices have become rapid, cost effective for the better and beneficial management and monitor the critically ill thrombocytopenic patients.

The platelet indices MPV, PDW, PLCR have been playing a major role in differentiating hyperdestructive thrombocytopenia from hypoproductive thrombocytopenia. Various disease conditions are included under the hyperdestructive category such as dengue, malaria, alcoholic liver disease, sepsis, HIV infection and ITP. The conditions which are included in the hypoproductive thrombocytopenia are erythroid hyperplasia, leukaemias and aplastic anaemia.

The platelet indices are significantly increased in hyperdestructive thrombocytopenia than in hypoproductive thrombocytopenia with significant negative and positive predictive values, showing that the platelet indices are helpful to categorise thrombocytopenia as hyperdestructive or hypoproductive during the evaluation. These observations were in line with other studies findings with respect to stratification of thrombocytopenia.

These indices when interpreted with clinical correlation helps in guiding the management of the case in the form earlier intervention and to avoid unnecessary invasive and painful procedures like bone marrow aspiration in hyperdestructive thrombocytopenia and to request for bone marrow aspiration in hypoproductive thrombocytopenia.

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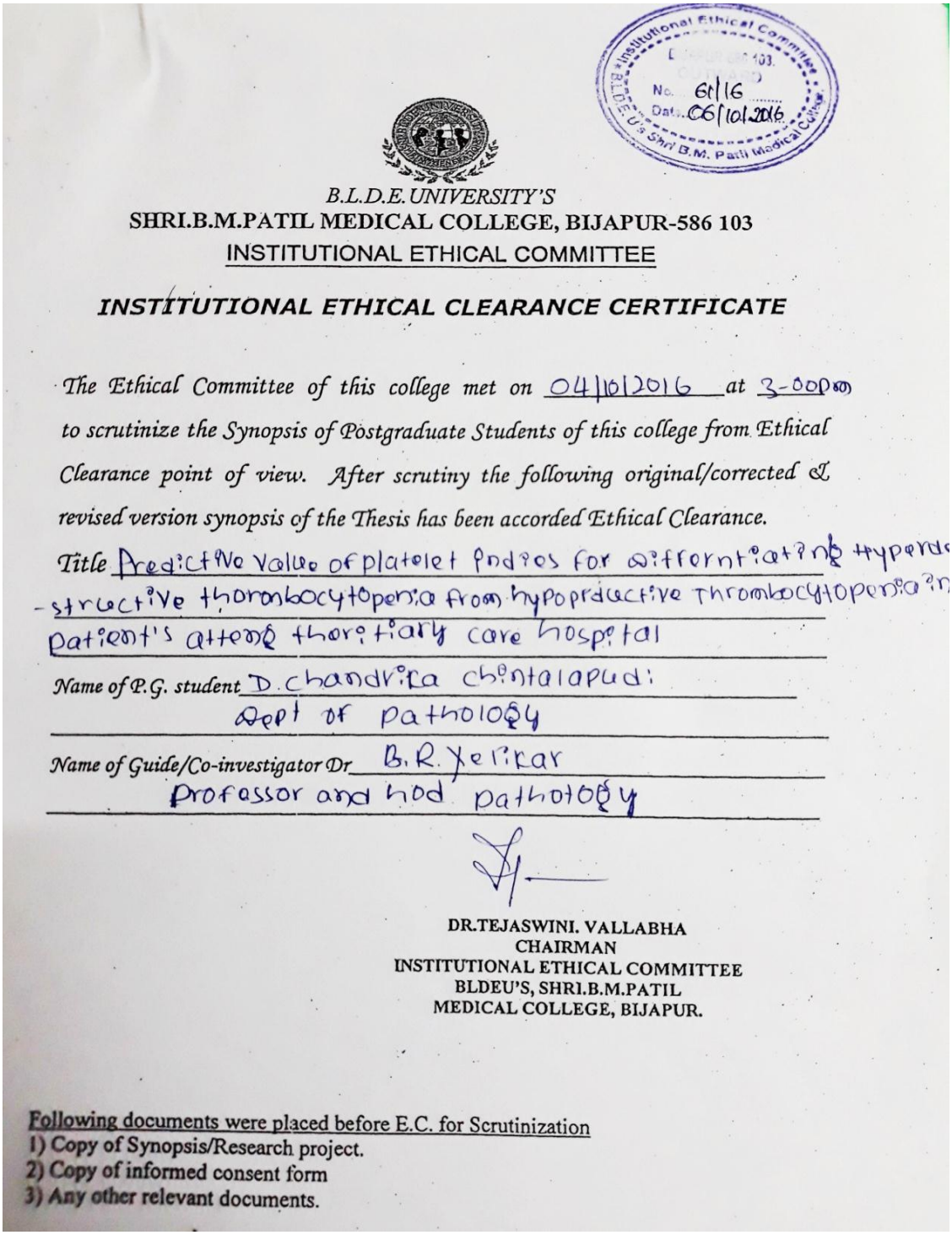
ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE



Institutional Ethical Committee
No. 61/16
Date 06/10/2016
BLDE U's Shri B.M. Patil Medical College

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04/10/2016 *at* 3-00pm *to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.*

Title Predictive value of platelet indices for differentiating hyperde-structive thrombocytopenia from hypoproductive thrombocytopenia in patient's attend tertiary care hospital

Name of P.G. student D. chandrika chintalapudi
Dept of pathology

Name of Guide/Co-investigator Dr B.R. Yerikar
Professor and HOD pathology

DR.TEJASWINI. VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE II

STUDY SUBJECT CONSENT STATEMENT

I hereby confirm that Dr. D Chandrika Chintalapudi has explained to me the purpose of the research, the study procedure, that I will undergo and the possible discomforts as well as the benefits that I may experience. I have been explained all the above in detail in my own dialect and I understand the same. Therefore I agree to render the consent to participate as a subject in this research project.

_____ _____

(Participant) Date

ANNEXURE III

PROFORMA FOR STUDY

Demographic Details:

1. Name :

2. Age : 3. Sex: 4. OPD / IPD no.:

5. Present history:

6. Past history :

7. History of chronic illness:

8. **General Physical Examination:**

Pallor :

Icterus :

Built :

Nourishment :

Petechaie :

9. **Vitals:**

Pulse :

Blood Pressure :

Respiratory rate :

Temperature :

Weight :

Height:

10.**Investigations:**

INVESTIGATIONS	RESULTS
CBC	
PLATELET COUNT	
MPV	
PDW	
P-LCR	
Peripheral smear	
Bone Marrow study (if necessary)	
HBsAg (if necessary)	
HIV (if necessary)	

ANNEXURE IV

KEY TO MASTER CHART

MPV	Mean Platelet Volume
PDW	Platelet Distribution Width
PLCR	Platelet Large Cell Ratio
E H	Erythroid Hyperplasia
ALD	Alcoholic Liver Disease
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
A A	Aplastic Anemia
RVD	Retroviral Disease
ITP	Immune thrombocytopenia

MASTER CHART

Group	S.N0	NAME	IP NUMBER	LAB NUMBER	AGE	AGE (YRS)	SEX	DIAGNOSIS	PLATELET COUNT	MPV	PDW	PLCR
HYPO	1	Nirmala S G	37046	183748	38	4	F	Erythroid Hyperplasia	0.4	10.2	19.3	40.5
HYPO	2	JanakiBai	3389	17270	75	7	F	Erythroid Hyperplasia	0.87	9.9	14.9	40.8
HYPO	3	Sanjeev Harish.CH	22573	112898	40	4	M	Erythroid Hyperplasia	0.46	9.2	17.6	37.8
HYPO	4	Ashok Kumar	3758	21880	59	6	M	Erythroid Hyperplasia	0.93	10.9	13.7	33.7
HYPO	5	Kashibai Laxman	6090	31089	23	3	F	Erythroid Hyperplasia	0.79	10	19.9	42.6
HYPO	6	Usha Ramesh	41945	208886	40	4	F	Erythroid Hyperplasia	0.47	8.1	11.8	18.5
HYPO	7	Shilpa Ramachandra	42006	208884	35	4	F	Erythroid Hyperplasia	0.33	9.1	13.8	22.1
HYPO	8	Dayanand Shankar	41771	210013	55	6	M	Erythroid Hyperplasia	0.4	8.6	11.6	24.6
HYPO	09	Yallappa Ramalingappa	43672	218014	66	7	M	Erythroid Hyperplasia	0.44	7.6	10.4	17.6
HYPO	10	Kiran Tukaram.M	44397	222771	2	1	M	Erythroid Hyperplasia	0.42	9.8	10.8	23
HYPO	11	Dundayya Gopal Katti	25358	129152	54	6	M	Erythroid Hyperplasia	0.42	11	14.4	39.4
HYPO	12	Santosh Bhimaraya	41691	206493	26	3	M	Erythroid Hyperplasia	0.33	9.7	16.3	24
HYPO	13	Rudrayya Basayya.M	27963	141502	85	7	M	Erythroid Hyperplasia	0.82	7.9	19.7	22
HYPO	14	Kishore Muragendra	44016	219504	26	3	M	Erythroid Hyperplasia	0.44	7.6	10.4	17.6
HYPO	15	Yallappa Sidappa	8211	43135	52	6	M	Erythroid Hyperplasia	0.86	7.6	20.6	22.4
HYPO	16	Bhagyashree	178764	91479	14	2	F	AML	0.39	10.4	17.6	30.1

HYPO	17	Madina	42727	2141698	69	7	M	AML	1.07	10.3	23.4	46.9
HYPO	18	Rukmuddin	214313	112431	67	6	M	AML	0.26	9.3	12.2	23.2
HYPO	19	Gangabai Shivappa	21221	106001	60	6	F	AML	0.09	11.1	17.2	47.2
HYPO	20	Jamir Lalamashak	1869	9435	2	1	M	AML	0.73	9.1	8.2	18.3
HYPO	21	Amrutha	13674	70481	2	1	F	AML	0.19	9.4	10.2	23.3
HYPO	22	Kalpesh Sharanappa	6604	33748	15	2	M	AML	0.73	9.4	8.1	19
HYPO	23	Laxmana Gouda Dandappa	19393	97747	60	6	M	AML	1.01	11	12.7	25.4
HYPO	24	Mehboob Dongrisab	43072	213547	35	4	M	AML	0.33	10.5	10.7	30.4
HYPO	25	Kandu Balu Manavar	42831	21830	45	5	M	AML	0.2	11	10	31.6
HYPO	26	SushilaBai	9463	49100	61	7	F	AML	0.38	11.5	12.3	28
HYPO	27	Shivshankar	11764	63.43	28	3	M	Aplastic Anemia	0.26	11.1	11.6	31.8
HYPO	28	Channabasu Mallikarjun	42648	203196	12	2	M	ALL	0.33	10.8	12.5	45.1
HYPO	29	Shanta Badrayya	15273	77860	25	3	F	ALL	0.15	9.2	10.7	21.7
HYPO	30	Gurushanth	103448	48709	36	4	M	ALL	0.29	11.7	24	50.6
HYPO	31	Sagar Amogasidda	44740	223978	4	1	M	ALL	0.31	10.7	13	28.2
HYPO	32	Sridhar Namadev	37955	189360	11	2	M	ALL	0.38	10.1	10.5	25.1
HYPO	33	Raju	22006	109871	25	3	M	ALL	0.24	9.9	9.6	23.7
HYPO	34	Arun Katapp Bidari	20065	106877	14	2	M	ALL	0.2	9.3	12.5	22.3
HYPO	35	Santosh	16094	85740	15	2	M	ALL	0.53	10.7	10.7	29.1
HYPER	1	Prajwal	15037	77105	14	2	M	DENGUE	1.09	12.5	16.6	42.2
HYPER	2	Shankarling	15510	81135	26	3	M	DENGUE	0.66	11.1	12.9	32.7
HYPER	3	Vittal	16620	84626	35	4	M	DENGUE	0.2	10.3	14.4	32.4
HYPER	4	Bhoominandan	17112	89087	26	3	M	DENGUE	0.74	13.7	23.4	53.3
HYPER	5	Sunanya Devi	19793	100023	18	2	F	DENGUE	0.7	14	20.8	56.4
HYPER	6	Ramesh Subash	39372	186321	21	3	M	DENGUE	0.7	10.6	12	28.4
HYPER	7	Pragathi	210263	102875	12	2	F	DENGUE	1.12	9.3	8.9	17.9

HYPER	8	Vikas	18785	101777	15	2	M	DENGUE	0.52	11.7	11.6	35.4
HYPER	9	Prathiksha	212456	103831	22	3	F	DENGUE	1.14	10.9	12.7	31.8
HYPER	10	Pooja Mahendra Jain	19979	100946	20	2	F	DENGUE	0.81	12.8	22.7	44.8
HYPER	11	Krupa Singh	40150	200633	32	4	M	DENGUE	0.41	11.1	12.3	33.3
HYPER	12	Raghu Hampayya	31244	157838	26	3	M	DENGUE	0.22	11.9	14.4	39.4
HYPER	13	Tippanna Meti	21842	109167	32	4	M	DENGUE	1.13	11.4	13.4	35.7
HYPER	14	Alhok Dileep Pawar	39411	186231	0.5	1	M	DENGUE	1.04	10.2	11.8	27.6
HYPER	15	Raju Shankar Loni	21729	109266	40	4	M	DENGUE	0.32	10.2	11.8	29.7
HYPER	16	Sachin Sidappa B	20869	107354	18	2	M	DENGUE	0.34	11.2	16.9	37.4
HYPER	17	Sridevi Matandappa	42663	2669	30	3	F	DENGUE	0.32	12.1	10.6	39.7
HYPER	18	Aslam	25500	127898	15	2	M	DENGUE	0.7	10.5	9.5	25.6
HYPER	19	Kuteja	26096	131022	2	1	F	DENGUE	1.23	10.3	11.5	28.6
HYPER	20	Rahul Pasamal	47355	24229	20	2	M	MALARIA	0.48	11.8	19	39.2
HYPER	21	Lalasab Mohammad	43194	216566	16	2	M	MALARIA	0.87	11.5	16.2	39.7
HYPER	22	Bagawwa	37755	7045	60	6	F	MALARIA	0.3	12.7	17.7	48
HYPER	23	Ramzanbi Mohammad Sab	43287	214937	30	3	F	MALARIA	0.34	12.2	14	41.4
HYPER	24	Bheerappa	41001	202970	13	2	M	MALARIA	0.38	13.8	19.2	54.9
HYPER	25	Shivshankar	352795	179426	55	6	M	MALARIA	0.36	12.3	21	44.1
HYPER	26	Sakshata shantagouda	43965	218534	30	3	F	MALARIA	0.44	10.2	9.9	27.2
HYPER	27	Padmavathi Sidappa B	43710	217455	15	2	F	MALARIA	0.21	11.1	15.8	41.1
HYPER	28	Suresh Tamanna Chand	43734	217450	60	6	M	MALARIA	0.88	12.3	16	43.2
HYPER	29	Shantabai Taranaik	4738	24499	35	4	F	MALARIA	0.82	11.4	16.3	38
HYPER	30	Faruq Budan Bepari	4268	24098	10	1	M	MALARIA	1.06	11.1	13.2	34.1
HYPER	31	Parasuram Subbarao	5666	28995	45	5	M	ALD	0.41	9.9	11.5	25.9
HYPER	32	Prakash Kallappa	7110	36358	55	6	M	ALD	0.08	10.7	13.4	34.2
HYPER	33	Siddu Chanappa	4774	25206	35	4	M	ALD	1.1	10.9	11.8	31.8

HYPER	34	Yallappa Vittal	4420	24581	35	4	M	ALD	1.11	11.5	12.4	34.3
HYPER	35	Kashinath Chandrasekhar	10664	56740	46	5	M	ALD	1.35	12.7	14.3	41.1
HYPER	36	Arjunsingh	9906	52758	85	7	M	ALD	0.49	11.2	15.5	37.8
HYPER	37	Mallikarjun P.k	4161	24747	28	3	M	ALD	0.64	12.4	16.2	44.3
HYPER	38	Mahadev Shrilshail	39279	185601	35	4	M	ALD	0.97	13.7	20.1	52.6
HYPER	39	Melappa Ramanna Pettar	21115	108592	46	5	M	ALD	0.8	12	12.3	37.8
HYPER	40	Basappa Laxman	21759	108565	45	5	M	ALD	0.65	9.7	9.9	21.9
HYPER	41	B/o Renuka	4177	21268	0.1	1	M	SEPSIS	0.94	10.9	17.6	33.6
HYPER	42	B/o Kashinath	4214	24166	0.1	1	M	SEPSIS	0.57	10.6	10	27.2
HYPER	43	B/o Tejaswini Shridar	3886	19561	0.1	1	M	SEPSIS	0.75	12.3	15.1	41.2
HYPER	44	B/o Jyothi Lakanna	42663	211594	0.1	1	F	SEPSIS	0.95	13.4	19.6	46.9
HYPER	45	Vanitha Basavaraj	38817	185989	20	2	F	SEPSIS	0.63	12.5	15.9	42.2
HYPER	46	Charan Kumar	11030	56720	11	2	M	SEPSIS	0.61	12.3	14.7	43.8
HYPER	47	Chandrakala Yalawar	19578	101768	38	4	F	RVD	1.04	11.5	13.8	36.5
HYPER	48	Kashinath	19305	97429	46	5	M	RVD	0.86	11.6	14.5	36.4
HYPER	49	Bhagyashree G	19012	100345	3	1	F	ITP	0.47	8.4	8.1	42.1