Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(2):184-189

Review Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Thrombocytopenia and its causes

Bimlesh Chaudhary, Jyothi Y.* and Syed Imam Rabbani

Department of Pharmacology, Krupanidhi College of Pharmacy Banglore, Karnataka, India

---

ABSTRACT

Thrombocytopenia is a hematological disorder which decreases the platelet count. Normal range of platelet counts is 150,000 to 450,000 / microL, below this cause thrombocytopenia. Platelets play an important role in the blood clot, if there is a less platelet count body cannot form clots and bleeding will not stop. There are various causes of thrombocytopenia; one reason might be that bone marrow is not forming enough platelets and another cause is that the body is breaking down its platelets at a very fast rate and disorder like splenomegaly, enlargement of spleen which traps and store too many platelets which decrease the number of platelets in the circulation and cause thrombocytopenia. Decrease platelet counts occur due to various chemotherapeutic agents like Nitrosoureas, Mitomycin, 5-florouracil and Thiotepa. Alcohol consumption is also one of the major causes of thrombocytopenia. Treatment of thrombocytopenia depends upon the cause and severity of the disease, mild thrombocytopenia dose not required any treatment, but moderate and severe thrombocytopenia required a treatment like blood or platelet transfusions, immune globulin and corticosteroids drugs.

Keywords: Thrombocytopenia, causes, complications, treatment.

INTRODUCTION

Thrombocytopenia is the disease in which there is a reduction in the platelet count or less formation of a platelet in blood. The normal adult platelet count range is 150,000 to_450,000/microL, with mean values of 266,000 and 237,000/microL in females and males, respectively [1]. If the platelet count is lower than the normal range cause thrombocytopenia. Severity of Thrombocytopenia can be subdivided into 3 main groups, Platelet count between 100,000 to 150,000/microL are defined as mild thrombocytopenia, platelet count between 50,000 to 99,000/micro as moderate thrombocytopenia and <50,000/micro as severe thrombocytopenia, with a risk of bleeding problems [2].

Platelets play an important role in the blood clot. Whenever there is a cut or injury in the body platelets aggregates together and form a clot to stop the bleeding and if there are not enough platelets in the blood, then the body cannot form clots and bleeding will not stop. There are so many reasons why blood does not contain enough platelets. Low platelet counts have symptoms like:

- Bruising: red, purple, or brown (purpura)
- Petechiae: rash with small red or purple dots on skin
- Nosebleeds
- Bleeding gums
- Bleeding from wounds for a prolonged period of time or doesn't stop on its own
- Heavy menstrual bleeding
- In more serious cases, internal bleeding also occurs in urine, stool, rectum and some time bleeding occur in the brain also.

There are so many cases of thrombocytopenia, it is generally occurring in pregnant women, about 7 – 10 % pregnant women are suffering from thrombocytopenia and 1- 4 % thrombocytopenic neonates are born every year [3,4]. Thrombocytopenia is complicating disease and its treatment also depend upon the cause and severity of the disease, mild thrombocytopenia dose not required any treatment, moderate and severe thrombocytopenia where a serious bleeding occur either on the surface of the skin or inside the body which required a treatment like:

- Blood or platelet transfusions
- Changing medications that are causing low platelet count
- Steroids: used to suppress the immune system
- Immune globulin
- Corticosteroids: block platelet antibodies
- Immune-suppressant drugs
- Splenectomy, or the surgical removal of the spleen

Since there have been a remarkable increase in the number of cases with thrombocytopenia, this article will provide an insight view about the treatment and causes options available for thrombocytopenia.

PLATELETS

Thrombocytopenia is a disorder associated with increased destruction, consumption, or decreased production of platelets. Platelets are also called thrombocyte. They are non-nucleated cytoplasmic that play a vital role in hemostasis and also involved in inflammation and immunity. Normal counts of platelets are 150,000 – 450,000 cells/ml in which 70 % circulates in the blood and remaining 30 % arrest by the spleen. The life cycle of platelets is about 7-10 days and then degraded in the liver or spleen [5]. The laboratory test used to assess platelet function are bleeding time (*in-vivo* test) and platelet function tests. A normal bleeding time indicates sufficient platelet numbers or function and normal microvasculature.

First Max Schultze (German anatomist) published the accurate and convincing description of platelets in his newly-founded journal Archiv für microscopes Anatomie in 1865. He described "spherules" much smaller than red blood cells that occasionally clump and may participate in the collections of fibrous material. In 1882, Giulio Bizzozero (Italian pathologist) showed platelet being the first component of blood, which adhere to damaged blood vessel walls *in-vivo*, and that *in-vitro* [6].

Platelets structure and function

Platelets are tiny pieces of red blood cells about 20% of the diameter of red blood cells [7]. Platelets originate from the cytoplasm of bone marrow, the same as red blood cell and white blood cells are produced. Platelets are produced from a very large bone marrow cells called megakaryocytes, about 1000 to 3000 platelets are produced by the megakaryocytes, which are released into the blood stream. It contains translational machinery and megakaryocyte-derived messenger RNA (mRNA) which is required for the synthesis of protein but it does not contain genomic DNA. Platelets are in discord shape, an average size of 2 to 4 μm in diameter and they are present in the blood about 200,000/μL in humans. [8]

Internal structure of Platelet can be divided into four zones: Peripheral zone, Sol-gel zone, Organelle zone and Membrane zone, which is important to stop bleeding. Peripheral zone contains glycoproteins, which is required for adhesion, activation, and aggregation. Sol-gel zone contain microtubules and microfilaments which maintain the platelet discoid shape. Organelle zone contains alpha granules which contain clotting mediators, required for creating a firm plug to seal blood vessel breaks. The Membrane zone also contains the enzymatic systems for prostaglandin synthesis (thromboxane A2 synthesis). Whenever there is an injury in the blood vessel platelets change shape and become round and extend long filaments which make contact with the broken blood vessel wall and then form a plug to seal the broken blood vessel [6,7].

CAUSES OF THROMBOCYTOPENIA

The trapped platelets - Spleen is the part of lymphatic system found above stomach under the ribs on left side. The Spleen is a soft, spongy organ and important for maintaining the body fluid balance. Spleen plays a vital role in preventing infection by producing white blood cells, it also recycles the iron and normally filters or removes old or damaged blood cells from the bloodstream and also store red blood cells and platelets [9]. Almost 30% of the platelets are trapped in the spleen.

Enlargement of spleen also called splenomegaly, affects all these vital functions. It traps and store too many platelets, which decrease the number of platelets in the circulation and cause thrombocytopenia. Enlargement of the spleen can be caused by many infections like viral infection (Mononucleosis associated with glandular fever),

parasite infection such as toxoplasmosis, bacterial infections such as endocarditis and cat scratch disease. It is also caused by many diseases like inflammatory disease such as sarcodosis and rheumatoid arthritis, cancer such as leukemia and lymphoma (Hodgkin's disease), liver disease such as cirrhosis, Sclerosing cholangitis, wilsons' disease, biliary atresia and cystic fibrosis, infiltrative disease such as Gaucher's disease, Niemann-pick disease, amyloidosis or glycogen storage disease. Other causes include Sickle cell splenic crisis, Banti's syndrome, Felty syndrome, trauma, such as sporting injury and cysts or abscesses. Vinyl chloride has been reported to cause thrombocytopenia in worker who has developed vinyl chloride induced hepatic fibrosis with esophageal varices and splenomegaly.

Decrease production of platelets- Megakaryocytes are a large bone marrow cells and it is the only one which is responsible for the production of platelets. Suppression of megakaryocytes or bone marrow suppression will decrease the production of platelets leading to thrombocytopenia.

Factors that can decrease platelet production include:

Chemotherapy - Chemotherapy or radiation therapy is an important cause of the thrombocytopenia. Bone marrow suppression (myelosuppression) is a common side effect of chemotherapy it developed tumors in the bone marrow, which increase the chances of anemia, thrombocytopenia and neutropenia. It has been reported that some chemotherapeutic agents can induce thrombocytopenia, like thiotepa which is currently in use due to its low cost and moderate effectiveness, but its major side effect is myelosuppression which is caused by absorption of drugs through bladder mucosa. In one research thiotepa is given to 72 patients from whom 18 percent patients found with a decrease in white blood or platelet count below normal [10]. Some other agents like nitrosoureas, mitomycin and 5-florouracil can also produce bone marrow toxicity. The lowest blood count occurs within 7-10 days after chemotherapy and recovery after 2 to 3 weeks and for its treatment platelet transfusion is necessary [11].

Alcohol consumption – People who abuse alcohol are at a high risk of hematological disorders. More than 80 grams of alcohol per day give toxic effects on bone marrow, red blood cells, white blood cells, platelets and also produced nutritional deficiency that impair the production and function of various blood cells [12-13].

Alcohol gives direct effects on platelet and cause low platelet count in the blood, impaired platelet function and diminished fibrinolysis and also produced abnormalities in membrane phospholipids and also interfere with folate utilization. The mechanism by which alcohol works is the inhibition of platelet aggregation; it inhibits the release of thromboxane. It is an enzyme found in platelet and has a prothrombotic activity which stimulates the activation of new platelet as well as increasing platelet aggregation [14].

Some other factor which decrease platelet production are-[15]

- Viral infection- Chickenpox (varicella), Rubella, Hepatitis C, Epstein-Barr virus, HIV and parvovirus
- Deficiency of vitamin B_{12} and folic acid
- Condition like leukemia, lymphoma
- Medication – Aspirin, Ibuprofen, thiazide diuretics
- Toxic chemical- arsenic, benzene and pesticide

Increase breakdown of platelet-

Immune thrombocytopenia (ITP) - ITP it is an autoimmune mediated hematological disorder, affecting platelets and gives a low platelet count. ITP is classified as primary and secondary. Primary ITP also known as acute ITP (less than 6 months) generally occur in children between age 2 to 5 years. Every year, 3 to 8 cases per 100,000 estimated cases of ITP with some viral infection like Hepatitis C, HIV, or *H. Pylori* have been reported. Vaccination specially the MMR vaccine is known to trigger ITP [16]. Secondary ITP (chronic ITP longer than 12 months) is more common in adults between the ages of 18 to 65 years and higher in women than in men [17]. ITP is estimated to affect 100/ 1 million people and incidence of ITP is increasing per year with age.

ITP also known as idiopathic thrombocytopenic purpura associated with antiplatelet antibodies through the activation of B-lymphocytes. These antibodies are mostly IgG and directed against platelet antigen glycoprotein such as GP IIb/IIIa and GP Ib/IX complexes. These antibodies also affect megakaryocyte and inhibit the production of platelet in bone marrow. Autoimmune hemolytic anemia is sometime associated with ITP which known as Evans syndrome. The most common complication of ITP is bleeding and occurs at any location in the body.

Common symptoms of ITP are- [18]

- Prolong bleeding from cuts
- Nosebleeds

- Bleeding from the gums after dental work
- Menstrual bleeding heavier than normal
- Fatigue
- Purple bruises on the skin or on the mucosa membrane, leads to bleeding in a small blood vessel under the skin
- Bleeding after T&A (tonsillectomy and/or adenoidectomy), menorrhagia
- Bleeding within other organs such as the liver, spleen
- Gingival bleeding, epistaxis, conjunctival bleeding and menorrhagia

There is no standard test for the diagnosis of ITP. The test is based upon the patient's medical history, physical examination, complete blood count, peripheral blood smear examination and bone marrow examination; they all are used to determine the duration, type and severity of bleeding. Treatment for ITP depends upon the severity of the bleeding. Corticosteroids are the first line treatment, such as prednisone 1mg/kg/day for 1 to 2 weeks; IV immunoglobulin (IVIG) infusion (1gm/kg/d for 2 d) or anti-RhD antibodies are used to increase the platelet count [17].

Hemolytic uremic syndrome (HUS)–
IT is a clinical syndrome associated with the destruction of red blood cell, cause hemolytic anemia and give a low platelet count, cause thrombocytopenia. Hemolytic uremic syndrome is an acute kidney failure often occur by E.coli bacteria with gastrointestinal infection and it is more common in children, only about 10-15% patients with E.coli infection developed HUS [19,20]. It has been discovered more than 45 years and affects younger children below 4 years of age [21].

Thrombocytopenia in pregnancy-
Thrombocytopenia in pregnancy is the second most common hematological disorder after anemia. Thrombocytopenia is encountered in 7 to 10% of all pregnant women. Thrombocytopenia in pregnancy occurs due to increase in thromboxane A2 which leads to failure of platelet production and excessive platelet consumption [22]. But the chances of bleeding complication in pregnant women are less than non-pregnant women due to increased levels of fibrinogen, factor VIII and Willebrand factor, suppressed fibrinolysis and reduced protein S activity which leads to procoagulant state [23]. There are many disorders that cause thrombocytopenia in pregnancy, like HELLP (hemolysis, elevated liver enzymes, low platelet count), Gestational thrombocytopenia. HELLP associated with hemolysis, elevated liver enzymes and low platelets. It affects 0.5 to 0.9% of all pregnancies and develops in 10% of patients with preeclampsia [24]. Preeclampsia is also another cause of thrombocytopenia. It is the most common cause of pregnancy-associated mortality worldwide, affects up to 6% of all first pregnancies [25].

Gestational thrombocytopenia- It is considered as the most common source of thrombocytopenia in pregnancy and account for more than 75% of cases of thrombocytopenia in pregnancy [26]. During pregnancy, fall in a platelet count is normal, but in some pregnant women platelet count falls into the thrombocytopenic range which leads to gestational thrombocytopenia. Usually occurs in the mid second to third trimester, which will become normal within 1 to 2 months after delivery [23]. Pathophysiology of gestational thrombocytopenia is unknown, but can cause a decrease in platelet count below 70,000/μL [27]. Patients with the history of immune thrombocytopenia have greater chances of gestational thrombocytopenia and also not associated with any severe adverse effect to mother or fetus. It generally do not increase the risk of bleeding during delivery, but if platelet count is too low, then platelet transfusion is required to raise the count [28].

Drug induced thrombocytopenia- There are many drugs which can cause thrombocytopenia, in which most drugs cause thrombocytopenia by immune mechanism, GPIIb/IIIa inhibitor-induced thrombocytopenia within 24 hours of exposure and its associated with severe thrombocytopenia in approximately 0.5 to 2% of cases [29]. Drugs like Ethanol, quinine, quinidine, digoxin, carbamazepine, ibuprofen, heparin, etc. are the most common cause of drug induced thrombocytopenia [30].

Heparin induced thrombocytopenia (HIT) occurs after heparin therapy. Heparin is used to prevent clotting, it reacts with platelet factor 4 (PF4) which make a "Heparin-PF4" immune complex. Therefore, the body produced an antibody against the Heparin-PF4 complex; this antibody binds to this complex and destroyed the platelet [31].

Heparin induces two types of thrombocytopenia: Type I HIT and Type II HIT. Type I HIT also called as Heparin associated thrombocytopenia (HAT). It is a non-immune disorder, presents within the first 2 days after exposure to heparin and return to normal spontaneously, even with continuation of heparin. Type II HIT also called heparin induced thrombocytopenia and thrombosis. It is an immune mediated disorder which occurs 4 to 10 days after heparin exposure and seen in about 0.3 to 5% of patients [32].

HIT must be suspected when a patient who is receiving heparin has a decrease in the platelet count, particularly if the fall is over 50% of the baseline count.

Platelet dysfunction:
Platelet dysfunction is a prolonged bleeding time in patients. It is generally occurs due to medication, medical condition and hematological disorder. Platelet dysfunction is related with plasma cell dyscrasias and relates to coating of the platelet membrane by monoclonal proteins. A disease associated with platelet dysfunction is uremia, it is the condition in which there is a fluid, electrolyte, and hormone imbalances and metabolic abnormalities, the word uremia mean high concentration of urea and other nitrogenous waste compound in blood which cause prolonged bleeding via unknown mechanisms. Treatment involves hemodialysis, cryoprecipitate administration, or desmopressin infusion [33]. Other diseases like liver failure, kidney failure, cardiopulmonary bypass and heart disease, etc. also cause platelet dysfunction.

Aspirin is a non-steroidal anti inflammatory drug (NSAIDs) which suppress the production of prostaglandins and thromboxanes by irreversibly inactivate the cyclooxygenase enzyme, it is required for prostaglandins and thromboxane synthesis. Aspirin is most commonly used drug to inhibit platelet function by irreversibly blocks the formation of thromboxane A_2 in platelets and produced an inhibitory effect on platelet aggregation [34]. It also makes platelet less sticky, so they don't stick together, that's make aspirin useful in heart attack and stroke.

Table 1: Common Drugs causing platelet dysfunction [35-39]

Drugs	Mechanism of action
Amitriptyline, Imipramine, Chlorpromazine	Interfere with platelet membrane or inhibit serotonin uptake.
Fluoxetine	Inhibit serotonin uptake
Antibiotics-: β-lactam antibiotics, Penicillins, Nitrofurantoin	Interfere with platelet membrane
Verapamil, Calcium channel blockers (Nifedipine, Diltiazem)	Inhibition of thromboxane pathway or Inhibition of calcium influx
Papaverine	Exchange of adenine nucleotide
Vincristin, Vinblastin, Colchicine	Inhibition of phosphodiesterase
Anesthetics: Dibucaine, Procaine, Cocaine, Halothane	Interfere with platelet membrane
Methylxanthines (theophylline), Dipyridamole, Sildenafil, Caffeine	Inhibition of phosphodiesterase,
Aspirin, NSAIDs, Moxalactam, Losartan	Inhibition of thromboxane pathway
Statins: Lovastatin, Pravastatin, Simvastatin, Fluvastatin, Atorvastatin, Cerivastatin	Inhibit thromboxane A_2, interfere with platelet membrane
Plasma expanders: Dextran, Hydroxyethyl starch	Impairment of platelet aggregation
Radiologic contrast agents: Renographin-76, Renovist-II, Conray 60	Impairment of platelet aggregation
Oncology drugs: Mithramycin, Daunorubicin, BCNU	Impairment of platelet aggregation
Ethacrynic acid, Nitroprusside, Hydroxychloroquine, Cimetidine, Famotidine, Cyproheptadine	Unknown mechanism
Abciximab, Eptifibatide, and Tirofiban	Integration αIIbβ3 (GPIIb-IIIa) receptor blockers
Food and additives	
Foods and additives Omega-3 fatty acids, Fish oil	Reduction in TXA2 synthesis
Vitamin E, Onion	Inhibition of arachidonic acid metabolism
Onion, Cumin, Turmeric, Clove	↓ platelet thromboxane production
Garlic	Inhibition of fibrinogen binding to platelets

Pseudothrombocytopenia-
It is a phenomenon of platelet clumping which give low platelet count due to the presence of anti-platelet antibodies like IgG, IgM and IgA. EDTA is a more frequent cause of pseudothrombocytopenia; it is used as an anticoagulant, cause cation chelation and induced conformational change of a platelet membrane GPIIb-IIIa complex unmasking a cryptic epitope. Pseudothrombocytopenia has been reported both in a normal individual (Pseudothrombocytopenia is seen in approximately 0.1 % of the population and in patients with a variety of disease states [40, 41]. It is generally occurring in patients with a viral infection, patients with hepatitis A virus infection have 72% chances followed by cytomegalovirus 11.1% chances and patients with influenza A H1N1 infection have a 5.6 % chance of Pseudothrombocytopenia [42]. Various diseases like sepsis, multiple myeloma, acute myocardial infarction, breast cancer, neuroendocrine carcinoma has been reported to associate with EDTA- dependent Pseudothrombocytopenia [43].

CONCLUSION

Thrombocytopenia is a second most common hematological disorder after anemia. It has various causes such as drugs, chemicals, clinical disorders, foods and additives. It can affect both male and female, but mostly likely in pregnant women. Understanding the auto-pathology is important in the treatment of thrombocytopenia. Treatment of thrombocytopenia depends upon the severity and condition of the patients. Corticosteroids it is the first line of

therapy for thrombocytopenia usually prednisone drug is used, which can help raise the platelet count by decreasing the activity of the immune system and in critical cases, platelet transfusion is the most suitable option.

REFERENCES

[1] MF Buckley; JW James; DE Brown, *Thrombosis* and *Haemostasis*, **2000**, 83, 480:84.
[2] DR Williamson; M Albert; D Heels-Ansdell; et al, *Chest*, **2013**, 144, 1207-15.
[3] E Verdy; V Bessous; M Dreyfus; C Kaplan; G Tchernia; S Uzan, *Thrombosis* and *Haemostasis*, **1997**, 77, 806-7.
[4] B Douglas; M Robert, *Annual Review of Medicine*, **2005**, 56, 425-42
[5] P Gresele; V Fuster; J Vermylen; CP Page, Platelets in thrombotic and non-thrombotic disorders, Cambridge University press, Cambridge, **2010**.
[6] Sharathkumar A, A Shapiro. Platelet function disorders, 2nd Edition, Indiana Hemophilia and Thrombosis Center Indianapolis, U.S.A, **2008**, 1-22.
[7] JN George, Department of Biostatistics & Epidemiology. College of Public Health, OUHSC. **2015**. Available from: http://www.ouhsc.edu/platelets/platelets/platelets%20intro.html.
[8] CN Morrell; A Aggrey; LM Chapman; KL Modjeski, *Blood*, **2014**, 123, 2759-67.
[9] E Reina; K Georg, Department of Molecular Cell Biology and Immunolog, **2005**, 606-16. doi:10.1038/nri1669
[10] M Soloway; KS Ford, *The Journal of Urology*, **1983**, 130, 889-91.
[11] SS Sekhon; V Roy, *Southern Medical Journal*, **2006**, 99, 491-8.
[12] HS Ballard, *Alcohol health and research world*, **1997**, 21, 42-52
[13] L Schrier, Hematologic complications of alcohol use, **2015**, [Internet] Available from: http://www.uptodate.com/contents/hematologic-complications-of-alcohol-use.
[14] JC Ruf, *Research Journal* of *Biological* Sciences, **2004**, 37, 209-15.
[15] SN Nabili, Thrombocytopenia (Low Platelet Count), **2015** [Internet], Available from : http://www.medicinenet.com/thrombocytopenia_low_platelet_count/page2.htm
[16] H Frederiksen; K Schmidt. *Blood*, **1999**, 94, 909–13.
[17] D Cines; J Bussel, *Blood*, **2005**, 106, 2244-51.
[18] S Roberto; P Drew, *Mayo Clinic Proceedings*, **2004**, 79, 504-22.
[19] Hemolytic Uremic Syndrome in children. NIH Publication, **2015**, 1-8.
[20] S Banerjee, *Indian Pediatrics*, **2009**, 46, 1075-84.
[21] J James; J Corrigan; G Frank, *Hematology*, **2002**, 22, 365-9.
[22] SL Janes, *Postgraduate Medical Journal*, **1992**, 68, 321–6.
[23] U Perepu; L Rosenstein, *Proceedings in Obstetrics and Gynecology*, **2013**, 3, 1-15.
[24] CA Kirkpatrick, *Acta Clinica* Belgica, **2010**, 65, 91–7.
[25] Practice Bulletin No 33: Diagnosis and management of preeclampsia in pregnancy. American College of Obstetrics & Gynecology, **2002**, 99, 159–67.
[26] PC Kam; SA Thompson; AC Liew, Anaesthesia, **2004**, 59, 255–64.
[27] T Gernsheimer; H Andra; S Roberto, *Blood journal*, **2013**, 121, 38-47.
[28] KR McCrae, *American society of hematology*, **2010**, 2010, 397-402
[29] CS Abrams; DB Cines, *Current Hematology Reports*, **2004**, 3, 143–7.
[30] BR Curtis, *Immunohematology*, **2014**, 30, 55-65.
[31] TE Warkentin, *Circulation*, **2004**, 110, 454–8.
[32] GP Visentin; SE Ford; JP Scott; RH Aster, *Journal* of Clinical Investigation, **1994**, 93, 81–8.
[33] LSC Czer; TM Bateman; RJ Gray; et al, *The Official* Journal *of Indian Association of Clinical Cardiologists*, **1987**, 1139-47.
[34] NA Nussmeier; AA Whelton; MT Brown; et al, *The New England* Journal *of Medicine*, **2005**, 352, 1081-91
[35] V Bartel; E Gluza; F Markvardt, *Pharmacology* and *toxicology*, **1976**, 39, 296-301.
[36] GJ Johnson; LA Leis; GS Francis, *Circulation*, **1986**, 73, 847-54.
[37] SF Burroughs; GJ J ohnson, *Blood*, **1990**, 75, 1473-80.
[38] M Bifulco; AM Malfitano, *Journal blood disorder*, **2014**, 1, 1-2.
[39] RE Scharf, *Seminars* in *Thrombosis* and *Hemostasis*, **2012**, 38, 865-83.
[40] M Mori; H Kudo; S Yoshitake; K Ito; C Shinguu; T Noguchi, *Intensive Care Medicie*, **2000**, 26, 218-20.
[41] DP Shreiner; WR Bell, *Blood*, **1973**, 42, 541-9.
[42] WH Choe; YU Cho; JD Chae; SH Kim, *Hematology*, **2013**, 35, 70-6
[43] HJ Kim; IN Moh; H Yoo; et al, *Oncology Letters*, **2012**, 3, 86-8.