Analysis of Platelet Clumping in Portal Hypertension

A dissertation submitted in partial fulfillment of the requirements for

DM (Hepatology) examination of the

Tamil Nadu Dr. M.G.R. Medical University, Chennai,

to be held in August 2015.

Certificate

This is to certify that this dissertation entitled **"Analysis of platelet clumping in portal hypertension"** is a bonafide work done by Dr. Prasanna K S, Christian Medical College (CMC), Vellore in partial fulfillment of the rules and regulations for DM (Hepatology) examination of The Tamil Nadu Dr MGR Medical University, to be held in August 2015.

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Acknowledgement

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Study of plasma von Willebrand Factor level as predictor of in- hospital outcomes in patients with acute on chronic liver cell failure (ACLF).
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Ref: IRB Min No: 9146 [OBSERVE] dated 12.11.2014

Dear Dr. Prasanna K. S,

I enclose the following documents:

1.   Institutional Review Board approval   2.   Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

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Dear Dr. Prasanna K. S,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Analysis of platelet clumping in portal hypertension," on October 9, 2013.

The Committees reviewed the following documents:

1. IRB application form
2. Curriculum Vitae' Drs. Prasanna K. S, C. E. Eapen, Ashish Goel, Anup Ramachandran, Joy Mammen.
3. Proforma
4. Consent for cases
5. Consent form for controls (English & Tamil)
6. Patient Information sheet
7. No of documents 1-6

Liver is the largest organ in human body. It plays vital role in many functions like protein metabolism, detoxification, clotting factor synthesis and so on.

Liver dysfunction secondary to loss of hepatocyte function may be acute or chronic. Common causes of liver dysfunction are, viral hepatitis, alcoholic liver disease, metabolic diseases affecting liver and many a times it is cryptogenic.

The pathogenesis of chronic liver disease is not well understood in case of cryptogenic liver disease and recently number of studies has concentrated on this aspect. In our institution Non Cirrhotic Intrahepatic Portal hypertension (NCIPH) was found in significant number (48%) of patients with cryptogenic cirrhosis[1]. The major morbidity and mortality in chronic liver disease is due to portal hypertension and its consequences.

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Introduction

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Liver dysfunction secondary to loss of hepatocyte function may be acute or chronic. Common causes of liver dysfunction are, viral hepatitis, alcoholic liver disease, metabolic diseases affecting liver and many a times it is cryptogenic.

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The pathogenesis of chronic liver disease is not well understood in case of cryptogenic liver disease and recently number of studies has concentrated on this aspect. In our institution Non Cirrhotic Intrahepatic Portal hypertension (NCIPH) was found in significant number (48%) of patients with cryptogenic cirrhosis[1]. The major morbidity and mortality in chronic liver disease is due to portal hypertension and its consequences.

Thrombocytopenia is common in patients with chronic liver disease with any etiology, is seen in 30-76% of patients[2] and is multifactorial. It limits therapy in certain conditions and delays major diagnostic and therapeutic procedures as risk of bleeding in these cases is high. Whether thrombocytopenia is cause or effect of liver disease is not well understood.

Some causes proposed for thrombocytopenia in patients with chronic liver disease are splenic sequestration, bone marrow suppression and decreased production of thrombopoietin..

Studies have shown that patients with portal hypertension due to NCIPH or cryptogenic cirrhosis have decreased ADAMTS13 level. ADAMTS 13 is a protease cleaving von Willebrand factor (vWf). Due to ADAMTS 13 deficiency these patients have increased ultra large von Willebrand factor (UL vWF) in the circulation. It is hypothesized that the imbalance of ADAMTS 13 : vWf can lead to increased platelet aggregation on to ULvWF and form platelet micro- thrombus leading on to portal venular obliteration.[3,4,5]

Platelet counting is routinely done using automated counters using coulter principle and it can be done manually also. A difference in coulter and manual platelet count is noted routinely in practice.

We hypothesized that the difference in platelet count is secondary to platelet clumps which will not be counted as platelets by automated counters leading to low platelet counts compared to manual counts.

We aim to study the difference in manual and coulter platelet count in patients with cryptogenic chronic liver disease with portal hypertension and to compare with those with hepatitis B or C related chronic liver disease with portal hypertension, healthy controls and also those with extra hepatic portal vein thrombosis, aplastic anemia, idiopathic thrombocytopenic purpura and constitutional macrothrombocytopenia.

Aim

Analysis of thrombocytopenia and endothelial dysfunction in patients with portal hypertension

Objectives:

Objective 1:

To analyze thrombocytopenia in patients with portal hypertension and in control groups of patients (with thrombocytopenia due to other causes) by

a. Study of the difference between manual and coulter platelet counts in study subjects
b. Study of platelet clumping and immature platelet fraction in study subjects

Objective 2:

To analyze the impact of thrombocytopenia and of plasma von Willebrand factor (vWf) levels on in-hospital survival in patients with acute-on-chronic liver failure (ACLF).

Platelets

Specialized blood cells discovered in 1882 by Giulio Bizzozero[13] which play central role in hemostasis. It plays important role in other functions like inflammation and tumor metastasis, some studies have shown its role in wound healing and host defense also. Platelets are the smallest of human blood cells with size 3.6-0.7µm. Megakaryocytes in bone marrow release it as anucleated cells into the peripheral circulation with a life of 7-10 days. They are dynamic but usually inactive and gets activated when blood vessel is damaged[14]

Normally only 2/3rd of total platelets are in circulation and the remaining stored in spleen. Normal platelet count is 150-400 x 10^3/ µL. A healthy adult can produce 1011 platelets/ day and old platelets are destroyed by Kupffer cells in spleen and liver[15].

Platelets are anucleated but contain mitochondria, have plasma membrane made of lipid bilayer which express various surface receptors and have lipid rafts which help in signaling and intracellular trafficking. Platelets contain two major storage granules, alpha and dense granules. These granules store biologically active molecules which initiate coagulation and recruit other cells during inflammation.

Alpha granules are more prevalent and it contains GPIIbIIIa, fibrinogen and vWf which initiate coagulation cascade and many membrane proteins essential for platelet function like P-selectin (CD62P) and CD36. P selectins are known to recruit neutrophils. Dense granules store molecules which are secreted during platelet activation like catecholamine, serotonin, calcium, ADP and ATP. ADP helps in triggering platelet shape change, granule release and aggregation.

Pathophysiology of portal hypertension

To better understand portal hypertension it is important to know portal circulation better first. It is a unique system which connects two different capillary beds; capillary beds of the wall of the small intestine and spleen to that in sinusoidal area of the liver.

The portal venous system directs blood from parts of the GI to the liver. Splenic and superior mesenteric veins join behind the neck of pancreas to form portal vein. Inferior mesenteric vein joins superior mesenteric vein just before it joins splenic vein. Portal vein also receives blood from superior pancreaticoduodenal vein, left gastric (coronary) vein and the cystic veins directly. Above veins play important role in portal hypertension and esophageal varices development. These veins together drain majority of the gastrointestinal tract into portal vein which divides into right and left branches entering corresponding lobes of the liver. Portal vein makes up 80% of the total blood flow to liver and 20% of the oxygen supply playing crucial role in liver function. Portal vein subsequently divides into smaller branches and ends up in hepatic sinusoids. In hepatic sinusoids hepatic arterial blood mixes with portal venous blood and it passes through sinusoids and then enters central vein and the blood from central vein drains into hepatic vein and hepatic veins to inferior vena cava.[16]

Hepatic sinusoids are important and it makes up a large surface area in the liver which is covered by a layer of endothelial cell. Endothelial cell encloses space of Dissse. The hepatic sinusoidal endothelial cells are unique, they are fenestrated, the size of fenestrae is variable. The subendothelial basement membrane permits free passage of substances through it as it does not have intercellular junction.

The endothelial fenestration size varies in response to various stimuli, like changes in sinusoidal pressure, circulating endotoxin, ethanol, serotonin and nicotine.

Portal hypertension is a complication of liver cirrhosis which determines the natural history. In the last few years significant progress has been made in understanding Pathophysiology of portal hypertension. However some issues are still not clear and needs further studies. Portal hypertension basically has two components, first is a fixed one i.e increased Intrahepatic vascular resistance, second one is dynamic i.e increased splanchnic blood flow[18].

The fixed component is mainly due to liver sinusoidal endothelial cells (LSEC's) dysfunction. Endothelial cells form the first line of defense in liver from injury. Sinusoidal endothelial dysfunction is mainly due to hypoactive endothelial cells. 98% of the total endothelial sinusoidal cell is comprised of LSEC's. LSEC dysfunction leads to increased intrahepatic resistance in the microcirculation leading to PHTN. [21]

LSECs have multiple functions. It regulates blood clearance from liver vasculature by altering vascular tone, it has role in immunity, hepatocyte regeneration, angiogenesis and sinusoidal remodeling. Thus, LSEC dysfunction results in the liver cirrhosis due to enhanced fibrosis and portal hypertension due to impaired vascular tone and in turn resistance.

LSEC's ply important role in maintaining Intrahepatic vascular tone. Vasoactive products secreted from the LSEC's act on HSCs and causes contraction or relaxation. These vasoactive products also regulate hepatic sinusoidal microcirculation.

Substances like ET-1, AT- II, nor epinephrine, PG- F2, TX-A2 can trigger contraction of HSCs. In contrast, substances such as Ach, VIP, NO, CO, PG- E2, and adrenomedullin relax HSCs. Among these agents, ET-1 and NO have been studied well and has been recognized as important regulators in sinusoidal microcirculation. vasodilatation in the microcirculation is by stimulating eNOS activity. Nitric oxide plays an important role in the vascular tone in the hepatic microcirculation.[17]

Studies have shown that in patients with cirrhosis, the liver fails to accommodate the increased portal inflow like in that caused by postprandial hyperemia, it leads to an abrupt increase in portal pressure. It is important because it determines the progression of varices in portal hypertension.

Another important thing in cirrhosis is decreased NO production by the LSEC'S as mentioned above leading to impaired vasodilator response. Increasing the NO availability in liver microcirculation is a target in the treatment of portal hypertension.

Defenestration of LSEC'S and endothelial dysfunction as described above are the initial events in the development of liver injury and as a result of defective bidirectional exchange along the endothelium, probably may play role in subsequent progression of the liver cirrhosis also[20].

Causes of LSEC dysfunction:

Oxidative stress:

Oxidative stress can cause endothelial dysfunction. Cirrhotic patients have elevated malondialdehyde levels in the circulation. It is an indicator of oxidative stress. Attenuation in postprandial portal pressure and HVPG with vitamin C was noted in some studies[22] pointing towards the role of oxidative stress in pathogenesis of portal hypertension. eNOS activity is impaired by oxidative stress by various mechanism.[23,24]

   a. eNOS association with caveolin-1, which is is an inhibitor of eNOS

   b. inhibition of ET-1 induced eNOS phosphorylation[25]

   c. Dissociation of eNOS from ET-B receptor.[26]

Together all these leads to decreased NO production leading to endothelial dysfunction.

Inflammation

Inflammation has a role in SEC dysfunction in liver cirrhosis patients. Caveolin-1 an eNOS inhibitor is increased in response to endotoxins leading to decreased NO production. LSECs also express TLR4 which is an endotoxin receptor which regulates angiogenic responses. It has been postulated to enhance fibrogenesis leading to portal hypertension.[28,29]

Alcohol

Alcohol being an important cause of liver diseases has been implicated in LSEC dysfunction by producing pro fibrotic factors. Acetaldehyde which is a metabolic product of ethanol forms adduts with proteins and cause deleterious effects on LSEC's. These adducts increases fibronectin expression in the LSECs and promote fibrosis by stimulating HSCs. Other mechanism like miRNA and shear stress a change at the microvascular level stimulate gene expression in LSECs and promotes fibrogenesis.[29]
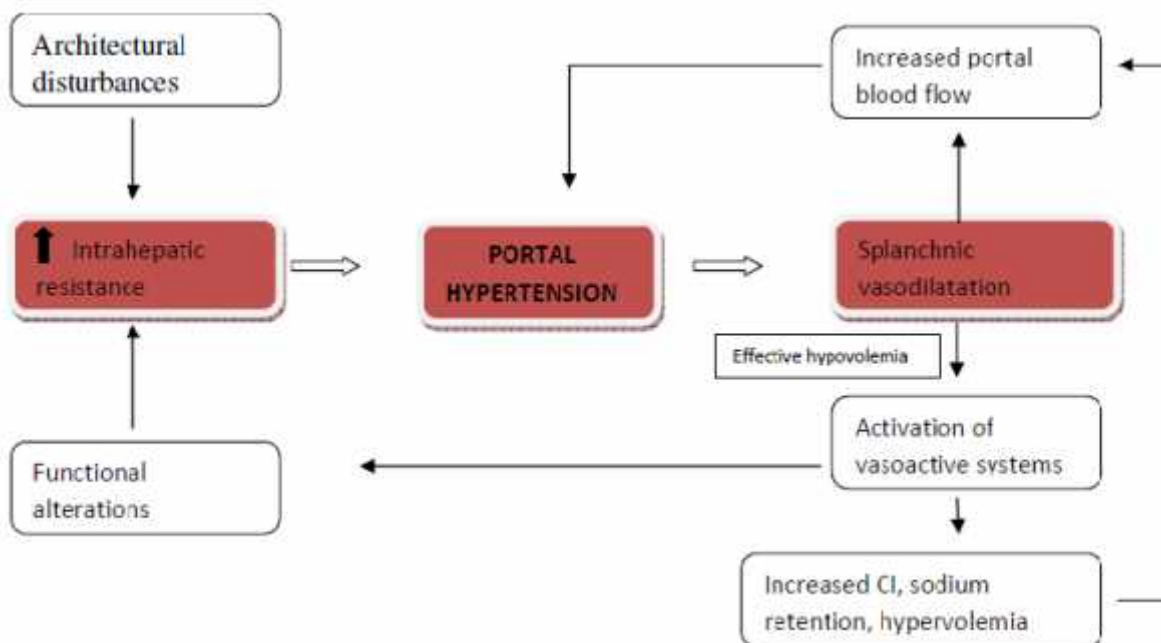
Etiology of portal hypertension

The etiology of portal hypertension varies in different parts of the world. In the western world leading causes are hepatitis C and alcohol. However in India the etiological profile is different.

Studies from our studies have shown slightly different etiological profile. A study including 583 patients including even the elderly population the most common etiology of portal hypertension was cryptogenic cirrhosis 128 (35%) followed by, alcohol 155(29%), viral, hepatitis B and C 133(23%), vascular 56(10%) and others 28(5%)[30]. In children the etiological profile was different. Extrahepatic portal vein obstruction (66%) was the most common cause of portal followed by Intrahepatic causes like wilson's disease (55%), autoimmune hepatitis (10%), NCIPH (19%) and idiopathic (24%)[31]. However European countries have mainly Intrahepatic cause of portal hypertension (~60%).[32]

Thrombocytopenia in chronic liver disease

In patients with CLD thrombocytopenia (platelet counts <150,000/lL) is common. Its incidence varies from 30-76% in cirrhotic patients[2]. The significance of mild thrombocytopenia is minimal and it does not interfere with routine treatment or management decisions. So also, moderate thrombocytopenia which is noted in approximately 13% of CLD patients. Severe thrombocytopenia (<50,000/lL) in advanced CLD may interfere in treatment decisions and be associated with significant morbidity. Mild to moderate thrombocytopenia usually is asymptomatic and it does not have any consequences, but invasive procedures like liver biopsy

and LTX carries significant risk of bleeding in severe thrombocytopenic. Intra cerebral hemorrhage or GI bleed (non variceal) is rare but can be fatal.[33]

Platelet play a very important role in liver regeneration as a source of serotonin and many studies have shown this fact recently in animal models and in post partial hepatectomy settings. However weather thrombocytopenia widely observed in chronic liver disease is cause of or effect of liver disease is not well defined.

Causes of thrombocytopenia in patients with liver disease

Thrombocytopenia in liver disease is common and causes are many[33]. Proposed causes are

a. Increased splenic sequestration of platelets (hypersplenism)

b. Suppression of bone marrow platelet production

c. Decreased activity of thrombopoietin, hematopoietic factor for platelets

Historically, thrombocytopenia in liver disease has been attributed to hypersplenism. But is not a well established cause till date. Many CLD patients with normal spleen size have low platelet counts and even the therapies aimed to reverse the same have not yielded consistent result questioning the role of enlarged spleen alone as the cause of thrombocytopenia and even other cytopenia's noted in liver disease patients. This finding prompted to think of alternative causes for cytopenias. Proposed causes are exaggerated intrasplenic cell destruction, autoantibody production by spleen and hemodilution secondary to plasma.

Increased splenic sequestration of platelets (hypersplenism)

In 1942 Wiseman and Doan" described, for the first time, a syndrome characterized by neutropenia, an enlarged spleen, and a bone marrow that was normal in its capacity to produce this type of cell. They originally termed this syndrome primary splenic neutropenia. it is important to know normal splenic anatomy and function to better understand hypersplenism.

Spleen has a capsule which sends trabaculae into the parenchyma. The capsule and trabaculae contain smooth muscles and they contract and relax in response to sympathetic inputs. Once blood enters spleen it flows sluggishly through splenic pulp and then enters venous sinuses. It is here in these sinuses blood cells come in contact with reticulo-endothelial system and the old and worn out cells gets destroyed. Spleen has three important functions normally. To destroy red blood cells, to store blood and to produce lymphocytes. In hypersplenism, apparently there exists a pathologic increase of this normal function.[37]

Kracke has stated that four premises must be established before the diagnosis of hypersplenism can be made. (1) Palpable spleen (2) decrease in the circulating cellular elements of the blood including neutropenia, thrombocytopenia, anemia, or various combinations of these; (3) a hyperplastic/normal bone marrow; and (4) demonstration of splenic over-activity by the epinephrine test. However in some rare cases spleen may not be enlarged and this does not rule out hypersplenism.

In hypersplenism cytopenias noted is due to increased destruction of the cells or is just trapped in spleen leading to peripheral cytopenias is not established. As mentioned above some studies have shown increased intrasplenic antibodies which may be the cause of cytopenias in hypersplenism cases, however antibodies have been shown to be against platelets only, then explaining cause of pancytopenia in hypersplenism will be difficult with this theory.

There is no established direct relationship between size of spleen and severity of hypersplenism. The specific mechanisms by which the spleen traps cells that are so slightly altered as to escape destruction elsewhere in the reticuloendothelial system are not fully understood; in large part, however, the filtration appears to result from physical alterations in the cell, particularly changes in size and shape (which includes agglutination), in viscosity (or rigidity) and probably in the ill-defined property of surface stickiness. studies by Cohen, Gardner and Barnett'6 in patients with congestive splenomegaly and thrombocytopenia revealed very little diminution in platelet survival. Thus, despite the remarkable capacity of the spleen to sequester and destroy slightly altered or abnormal cells, and even despite the fact that this filtering capability is increased by 2- or 4-fold in patients with splenomegaly, including those with congestive splenomegaly, this need not bear upon the hypersplenic state. Human studies with Cr51 shows that in normal human beings the red blood cells reaches an equilibrium in 2 min after intravenous injection. Taking into consideration the arm-to-spleen circulation time and systernic mixing it appears that the transit time averages no more than about 30 seconds, with an upper limit of about 1 minute. In most patients with splenomegaly, regardless of cause, the Cr51 mixing pattern is quite different and frequently shows two distinct components.

While a rapid, nearly normal-mixing component accounts for from 50 to 80% of the splenic radioactivity, a second, slower component emerges having a half-time of as long as 20 or 30 minutes and resulting in a delay in the turnover time which may exceed one hour in patients with very large spleens. Contemporaneously, of course, the quantity of labeled cells in the circulation continues to decline. Portal hypertension is a common cause of splenomegaly and hypersplenism, and one may reasonably speculate that its mechanism involves the hydrostatic

displacement of intrasinusoidal red cells into the slow-flowing cordal passages by forcing them through the basement membrane or by retarding their return to the sinuses.

Experimental studies also have shown that in those patients with hypersplenism the splenic sequestration is associated with decreased survival of blood cells to certain extent.

Stasis of cells in the splenic cords leads to metabolic stress as they are tailored for normal circulation period in the spleen otherwise.

Another important situation is with reticulocytes. These immature red cells, particularly the youngest of them, are somewhat selectively concentrated in the spleen, probably by virtue of their larger size and their sticky surfaces. The same may hold good in case of platelets also.

Penny et al. harvested all the platelets they could from operative spleens by various perfusion methods; their direct counts of platelet numbers and Aster's kinetic studies agree rather closely with respect to the magnitude of platelet pooling, and show that the sum of circulating and splenic platelets in hypersplenic thrombocytopenia usually represents a normal total platelet mass.

From the foregoing it is clear that in man the normal spleen has little reservoir function with respect to red cells and granulocytes, but does contain a relatively large, moving pool of platelets, about 25-30% of the total, and perhaps one-third of the total lymphocyte population. In many animals, and possibly in man, the platelet pool enlarges during sleep, and discharges during excitation. From the standpoint of maintaining blood fluidity during the slowed circulation of sleep, a lowering of blood hematocrit, platelet levels and plasma coagulability through splenic pooling presumably serves a valuable physiological purpose.

Hypersplenism, for the most part, represents an exaggeration of the actual or potential capacity of the spleen to pool blood elements.

The thrombocytopenia which results from extensive pooling may heighten the hazard of massive bleeding and may potentiate other haemostatic defects, but it alone is not usually a cause of dangerous purpura and the spleen pool presumably does contract during bleeding so as to make available the functional platelets therein.

However there are certain findings which argue against this theory i.e even after splenectomy thrombocytopenia is not completely reversed in some cases, so also with splenic artery embolisation. 25% of cirrhotic patients with normal spleen size have low platelet count and conversely, 19%–29% of cirrhotic patients with splenomegaly were found to have normal platelet counts. So, splenic pooling alone can't explain thrombocytopenia in cirrhotic patients.

Decreased thrombopoietin production

Thrombopoietin is a glycoprotein hormone produced by liver mostly which acts as a growth factor on megakaryocytes to regulate platelet production. In advanced liver disease the synthetic functions of liver are compromised so also thrombopoietin production. This is supported by the fact that TPO expression in liver is constitutive and is not regulated at the level of transcription.

Platelet count regulates plasma TPO levels and is inversely correlated[34]. A recent study has shown that platelets which are old and about to get destroyed stimulates thrombopoietin production and in turn replenishes the platelet pool.

In patients with low platelet count with normal liver function, TPO level ranges from 600-2900 pg/ml. In patients with advanced liver dysfunction have significantly low TPO levels in plasma.

The evidence in favor of this has come from those who undergo liver transplantation. In patients with liver transplantation plasma TPO level and platelet count significantly increases by post op day 14. Similar findings are not seen in patients who undergo TIPSS for portal hypertension

complications even though HVPG decreases significantly. These two findings suggest that deficiency of TPO drives thrombocytopenia. However other cytopenias seen in significant size of patients can't be explained with TPO deficiency alone.

Initial findings from some studies have shown 30-40% reduction in thrombopoietin mRNA content in the liver in patients with liver disease compared to those without liver disease.

Beside a decreased TPO production, there is also an increased TPO breakdown in cirrhosis. There are certain studies with conflicting results in relation to TPO level in patients with cirrhosis.

Suppression of bone marrow platelet production

Decreased bone marrow production is supposed to be one of the cause for thrombocytopenia in patients with cirrhosis. It may be due to the etiology of liver disease like in those with ALD and in hepatitis C related cirrhosis as shown in certain studies or it may due to idiopathic bone marrow dysfunction or exhausted bone marrow secondary to long standing liver disease.

G Stiegler et al looked at TPO level, platelet count and reticulated platelets in patients with liver cirrhosis pre and post LTx and found that all patients except one were thrombocytopenic and TPO level was in the normal range prior to transplantation and it increased by 5 fold on post transplant day 5. Platelet count decreased till day 5 even though reticulated platelets increased from day 2 post LTx till day 6. When platelet count got normalized reticulated platelet count was also normal. This provided evidence for an augmentation in de novo platelet production post liver transplant.

However bone marrow suppression may have only minor contribution towards thrombocytopenia in cirrhotic patients.

Hemostasis rebalances in liver disease

Hemostatic system changes are frequently encountered in liver disease patients. Common changes encountered are low platelet count, functional defects of platelets, decreased coagulation factors and inhibitors and decreased fibrinolytic proteins. As a consequence routine tests of coagulation like PT, aPTT and platelet count are abnormal frequently. Usually in normal tests abnormalities of these tests would ofte due to complex hemostatic changes.

In recent years, the traditional concepts of hemostatic disorders and its clinical consequences have dramatically changed. In particular, the concept of hypocoagulability in liver disease has changed. Abnormal routine coagulation tests may not always indicate increased bleeding risk and these patients in contradiction may be prone to thrombotic disorders.

For example, platelet number and function defects have been balanced by vWf- ADAMTS13 imbalance. The net result is enhanced platelet clumping in invitro testing. Endothelial changes, which have been not studied in detail in liver disease may play central role in hemostatic rebalance. This aspect needs further exploration.

Anticoagulants

Procoagulants
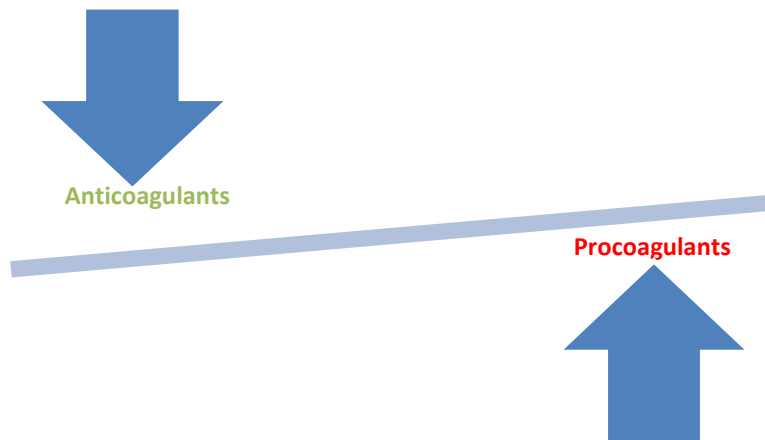
Factors causing hemostatic rebalance in patients with liver cirrhosis[40]

Changes-antithrombotic	Changes –prothrombotic
Low platelet count	Elevated levels of vWf
Defective platelet function	Decreased levels of ADAMTS13
increased production of PG and NO	Increased factor VIII level
Decreased coagulation factor levels	Low protein C and S, antithrombin III level
Deficiency of vitamin K	
Decreased fibrinogen level	Decreased plasminogen level
Elevated t-PA levels	

vWf- von Willebrand factor, t-PA- tissue plasminogen activator, PG- prostaglandin, NO- nitric oxide,

Endothelial dysfunction in liver disease

Endothelial dysfunction plays very important role in portal hypertension and liver disease pathogenesis. In portal hypertension role of LSEC dysfunction and stellate cell activation has been explained in detail in previous sections of the review.

Next much less explored important component is ADAMTS13 and von Willebrand factor imbalance. Whether it has causative role in liver disease primarily or is a secondary outcome of endothelial dysfunction is not yet well understood. However, of late many studies have tried to understand the role and/ or effect of this imbalance in patients with liver disease.

ADAMTS13 is a metalloproteinase it specifically cleaves large VWF between two residues (Tyr1605 and Met1606). Absence of ADAMTS13 activity leads to defective cleaving of ULVWFMs which are released from vascular ECs leading to accumulation of ULVWFMs which can induce platelet micro thrombi formation under shear stress.[3]

Currently, a severe deficiency of ADAMTS13 activity is noted in those with genetic mutations of ADAMTS13 gene, as in Upshaw- Schulman syndrome [41] or due to autoantibodies produced against ADAMTS13[42] as in TTP. Analysis has shown that ADAMTS13 mRNA is highly expressed in the liver[43] is produced exclusively in HSCs. Even though Platelets, vascular endothelial cells, and podocytes of kidneys have been implicated as ADAMTS13-producing cells, the amount produced by them appears to be far less than that by HSCs.

ADAMTS 13 levels have been noted to be significantly low in patients with alcoholic hepatitis, veno occlusive disease, advance liver cirrhosis, non cirrhotic intrahepatic portal hypertension, those undergoing LDLT and post partial hepatectomy.

Vascular endothelial cells play central role in hemostasis and thrombosis[41]. Von Willebrand factor, is a marker of endothelial cell damge/ activation. In injured liver due to necro-inflammatory process SCEs shows positivity to vWf which otherwise is not expressed in normal liver cells. Along with this capillarization of hepatic sinusoids is also noted[44]

Subsequently, increased levels of UL-VWFM mediates platelets adherence to to subendothelial tissue . Normally ADAMTS13 then cleaves it into smaller VWF multimers [45], essentially the initial step in hemostasis.

In patients with liver dysfunction, plasma VWF levels are high [46]. In an autopsy series one half of the patients had shown microthrombi in one or multiple organs [47] Such a imbalance leading to hypercoagulable state in liver diseases may lead to hepatic parenchymal extinction and may accelerate liver fibrosis and in turn disease progression. Subsequently it will end up in complications like HRS, HPS, port pulmonary hypertension, and SBP which are the main causes of mortality in advanced liver disease.

As ADAMTS13 is synthesized in hepatic stellate cells and its substrate, UL-VWFM in the transformed SEC as a result of liver injury, ADAMTS13/vWf imbalance cause sinusoidal microcirculatory disturbances, add to subsequent progression of liver diseases and terminates in multiorgan failure.

Relation between thrombocytopenia and ADAMTS 13 and vWf imbalance

It is well accepted that thrombocytopenia progresses as with advancing liver dysfunction.[2] Thrombocytopenia in liver disease is caused by multiple factors as discussed in detail in above section.

Recent studies have provided insight in this aspect. In patients with advanced cirrhosis, increased ULVWFM enhances platelet aggregation and it results in thrombocytopenia.[48]

Platelets have recently been shown as a driver of liver injury in animal models of viral hepatitis. It can promote liver regeneration through intra-platelet serotonin. In spite of their emerging role in those with inflammatory liver disease, not much is known about the mechanisms by which thrombocytes bind to the hepatic vasculature.

Recently it has become apparent that platelets can, under some circumstances, bind to endothelial cells, where they can support leukocyte recruitment to the vessel wall [48]

In L-selectin- deficient mice, activated platelets can reconstitute P-selectin-dependent lymphocyte homing to lymph node high endothelial venules [49]. Hepatic endothelial cells fail to express P-selectin even when inflamed, and a similar mechanism could provide a P-selectin substrate within hepatic sinusoids in the absence of endothelial P-selectin[51]. Platelets are sequestered in the liver following experimental transplantation [51] and correlate with graft survival. Similarly increased binding of platelets to sinusoidal endothelium and enhanced neutrophil recruitment is observed in mice exposed to LPS and following I/R injury, and serum from cirrhotic patients contains elevated levels of von Willebrand factor (vWF) and promotes platelet binding to collagen.

Mechanism of decreased ADAMTS 13 levels in liver cirrhosis

The mechanism responsible for the decrease in ADAMTS13 in advanced liver disease is not clear. Causes may include enhanced consumption by vWf degradation, inflammatory ADAMTS13 plasma inhibitor or cytokines released secondary to inflammation[52]

It is controversial whether decreased ADAMTS13 level is due to decreased production in the liver or due to liver dysfunction. Alternatively, some studies have shown role of cytokinemia[53,54] and endotoxemia as additional potential candidates. Investigations have demonstrated that IL-6 inhibited the action of ADAMTS13 under flow conditions and both IL-8 and TNF- stimulated the release of UL-VWFM in human umbilical vein endothelial cells in vitro. In addition, ADAMTS13 deficiency associated with inflammation promoted formation of UL-VWFM , and intravenous infusion of endotoxin to healthy volunteers caused a decrease in plasma ADAMTS13 together with the appearance of UL-VWFM.

As systemic inflammation is an important drive for ADAMTS 13 and vWf imbalance which may promote platelet adhesion to endothelium leading to microvascular thrombosis and multi organ dysfunction with very grave prognosis.

We studied vWf level in patients with ACLF, a condition with high systemic inflammatory state with high short term mortality rate.

Acute-on-chronic liver failure (ACLF)

Liver failure is a common condition and recently its incidence is increasing with increasing use of alcohol and increasing incidence of obesity and diabetes. It has a spectrum of presentation. It can be ALF if there is no preexisting liver disease, ACLF, acute deterioration in those with known or unknown prior liver disease, or as an acute decompensation of ESLD.

ACLF is a syndrome in which acute and severe hepatic derangement results from various insults. This term was first came to use in 1995 to describe a condition in which two insults were operating in liver simultaneously. One being chronic which is ongoing and the other acute. These patients are uniquely different from acute worsening of DCLD in which liver failure is central as against ACLF in which extrahepatic organ dysfunction plays central role.[9]

Definition of ACLF

APASL consensus statement defines ACLF as an acute hepatic insult manifesting as jaundice (serum bilirubin 5 mg/dl) and coagulopathy (INR   1.5 or prothrombin activity <40 %) complicated within 4 weeks by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease/ cirrhosis, and is associated with a high 28-day mortality.[9]

However there are many more definitions for ACLF. One of the important among them is EASL-AASLD consortium definition which defines ACLF as acute deterioration of pre-existing, CLD, usually related to an acute precipitating event which is associated with increased mortality at 390 days due to MOF.

Epidemiology

In Asian countries the common acute events are flare of hepatitis B, acute hepatitis E, alcohol, drug induced specifically ATT. Underlying chronic liver diseases are ethanol or hepatitis B related and then cryptogenic liver disease. In western countries this varies. Bacterial infection, variceal bleed, alcohol are the important acute precipitating factors. Hepatitis C and ethanol related liver disease are important causes for underlying chronic liver disease.

ACLF flow diagram[9]
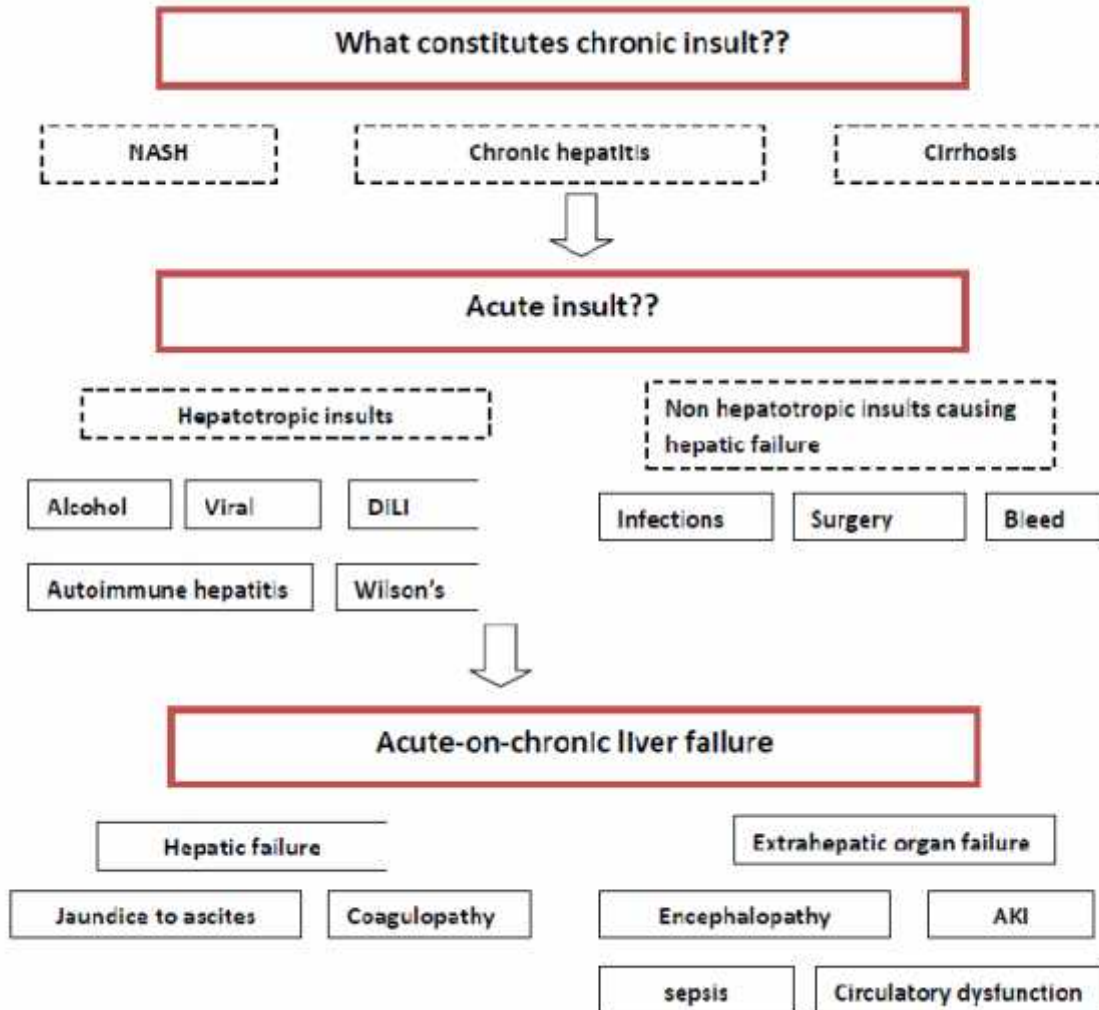
Sepsis is important in patients with ACLF. It plays important role in inflammation and imbalance of innate & adaptive immune responses in ACLF. It is very difficult to differentiate SIRS and early sepsis in cirrhotic patients. Identifying infections in cirrhotic patients at the earliest and initiation of appropriate antibiotics is helpful in treating sepsis, organ failure, and inturn mortality. Sepsis in a patient of ACLF has very high mortality due to MOD.

PIRO concept in acute on chronic liver cell failure- pathophysiology

ACLF carries very high short term mortality to the tune of 46- 89%. However, Pathophysiology is not well understood. The main cause for high mortality rate has been shown to be associated organ dysfunction in ACLF. Understanding Pathophysiology is key to improve survival in these patients.[56]

The pathophysiology of ACLF can be explained using the PIRO concept. It was initially developed for use in the sepsis setting. Using this concept in ACLF, in which 'P' stands for predisposition: predisposing factors which make a cirrhotic individual more likely to develop ACLF and organ failure. 'I' stands for acute insult or precipitating event, 'R' stands for inflammatory/immune response, which is a consequence of the acute insult. 'O' signifies organ dysfunction, which is the final sequelae of the inflammatory response generated following the acute insult. These four could represent the most important factors determining outcome in ACLF.

The PIRO concept of acute on chronic liver failure | Assessment

Predisposition → Severity of Cirrhosis →	Aetiology, Pulgh Score, MELD, Biomarkers
Injury → Precipitating Event →	Hepatic, Extra Hepatic, Therapies
Response → Inflammation →	SOFA, APACHE II (Biomarkers)
Organ → Organ Failure →	Inflammation, Immune failure (Biomarkers)
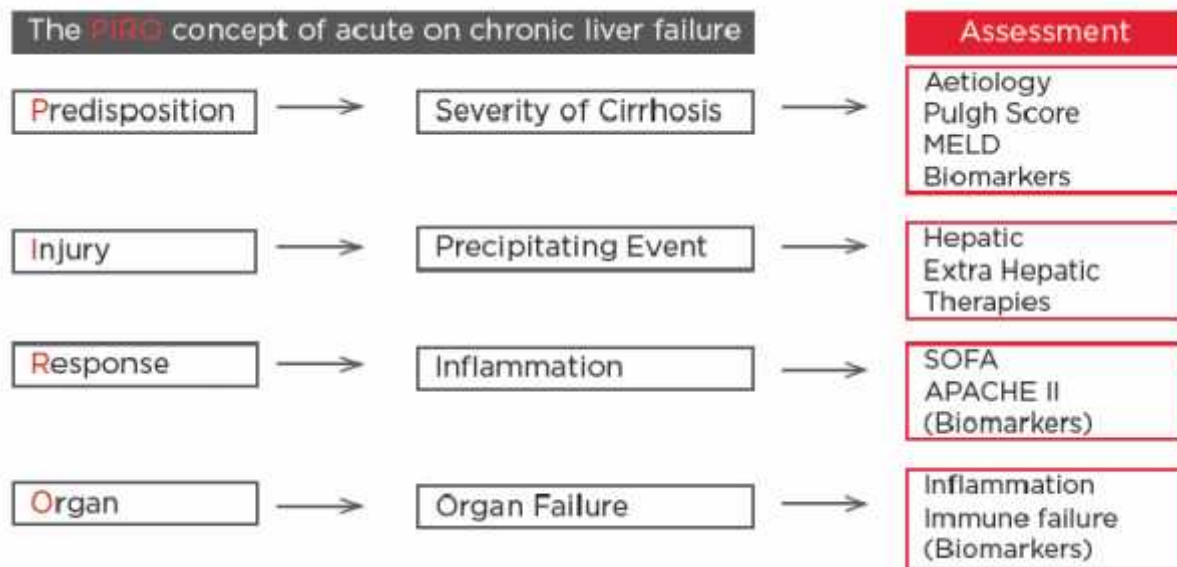
As determined by various prospective studies the important predisposing factors determined are

Male gender

Higher bilirubin level

Lower albumin level

Higher Child-Pugh (CTP) and MELD score

Hospital admission in the preceding six months with hepatic decompensation.

However the above mentioned factors may not help in predicting survival in ACLF one organ failure develops. The literature suggests that once extrahepatic organ failure sets in, then organ failure scores [55] such as the Sequential Organ Failure Assessment (SOFA) or the Acute Physiology, Age, and Chronic Health Evaluation (APACHE) may be more useful in predicting outcome and survival.

In the majority of patients, ACLF usually develops following an identifiable precipitating event. These precipitants may directly affect the liver or may be a consequence of an extrahepatic

insult. The most common precipitating event in ACLF is infection. According to the literature, it accounts for up to 47% of all precipitating events.

Precipitants may be hepatic, like- alcohol, acute viral hepatitis A,B,E, drug, portal vein thrombosis, ischemia hepatitis, non hepatotropic viral or bacterial infections or Extrahepatic, like- surgery, trauma.

Sepsis and variceal bleed are also considered as acute insult in certain definitions.

Inflammatory response follow the initial insult, there is an altered host response to injury, resulting in excessive systemic inflammatory response. This altered response induces tissue damage and subsequent organ failure. Dysregulated inflammation is considered a hallmark of ACLF and the mechanism by which this arises is multifactorial. Hyperdynamic circulation is a common aspect in patients with systemic inflammatory response syndrome. In these patients with enhanced cytokine production, there are severe disturbances of the cardiovascular system: the circulation becomes hyperdynamic, cardiac output increases, and both blood pressure and systemic vascular resistance decrease. In cirrhotic patients, in addition to the precipitating insult there is increased bacterial translocation, secondary to increased intestinal permeability and changes in intestinal microflora which add to the inflammatory response. Despite the insult ACLF patients fail to generate adequate immune response due to defect in the innate immune system. In patients with ACLF, the Kupffer cells are bypassed due to presence of both intra and extrahepatic shunts, leading to defective clearance of endotoxins. In addition,reduced protein synthesis in cirrhosis results in defective complement production. This leads to decreased opsonisation capacity of the Kupffer cells and thus, bacterial phagocytosis is impaired.3 Another contributing factor to altered host response in ACLF is a phenomenon known as "immune paralysis", as in sepsis, and is associated with a high mortality.

In ACLF patients, reduced HLA-DR (antigen-presenting receptor complex on peripheral monocytes) expression and decreased TNF- production following stimulation with LPS is noted.

Dysregulated immune system in ACLF following an initial response shows an exaggerated host response with release of proinflammatory cytokines in to the circulation. This leads on to systematic inflammatory response syndrome (SIRS). Increased incidence of organ dysfunction (hepatic encephalopathy, acute kidney injury and bacterial infection) is noted in patients with SIRS. Presence of SIRS predicts poor survival as studies have shown that in ACLF, SIRS incidence was high among those who did not survive an episode of ACLF than those who survived.

Along with micro circulatory dysfunction dysregulated innate immune system promotes macro circulatory dysfunction also. Hemodynamic changes in ACLF leads to hyper dynamic circulation and decreased systemic vascular resistance leading to end organ hypo perfusion, SIRS along with end organ hypo perfusion lead to multi organ dysfunction.

Approximately 33% of patients with ACLF develop organ dysfunction and it has an important role in the natural history, morality/morbidity. Following the development of organ failure, mortality steeply increases. Other than liver, kidneys, brain, adrenals and circulatory system are also commonly affected.

Endothelial dysfunction in ACLF

As mentioned above deregulated inflammatory response is characteristic in the setting of ACLF. Severe inflammation is an important stimulant for stellate cells and liver sinusoidal endothelial cells (LSEC's). Deregulated inflammatory response may act as an important drive for vWf secretion in the endothelial cells. As mentioned above in the pathogenesis of portal hypertension

and thrombocytopenia patients with liver cirrhosis are known to have low ADAMTS 13 level and inflammation further inhibits secretion of the same. In the background of decreased ADAMTS 13 level, increased vWf levels may prove detrimental leading to microcirculatory thrombus in which platelets may play important role and hence organ dysfunction, a form of low grade TTP. There is some indirect evidence for this hypothesis. Correlation has been established between severity of inflammation, organ dysfunction and survival in recent studies.

In a recently published abstract by R Garcia Martinez et al[82] endothelial dysfunction was studied by measuring vWf and NO levels and a good correlation was found between the level of these markers of endothelial dysfunction and organ dysfunction and so also decrease in the level of these markers with albumin infusion as the organ dysfunction improved.

Defining liver failure in ACLF:[9]

The two main variables in defining liver failure are bilirubin and coagulopathy. Bilirubin of >5mg/dl is considered as cutoff in APASL guideline and 12 mg/dl in CANONIC study. Coagulopathy is an important hallmark of liver failure. INR cutoff to define liver failure in ACLF is taken as >1.5 by APASL and >2.5 in CANONIC group. It has been reported that platelet count inversely correlates with ACLF grade.

Conventionally hepatic failure is defined by the onset of clinical ascites and/or encephalopathy. However, both are not commonly seen patients with ACLF, and hence, the presence of one of them can define ACLF.

 SOFA appears to be good prognostic model in ACLF patients in ICU setting with critical illness.

These patients with ACLF have very high 28 day and 90 day mortality. Most of the studies have shown about 30%-35% 28 day mortality and 50%-60% 90 day mortality. Hence it is very

important to recognize this at the earliest and start appropriate therapy and to prevent organ dysfunction and intern mortality.

ACLF outcome

As per CLIF-EASL consortium study mortality it varied according to the grade in turn the number of organ dysfunction. In those with no organ dysfunction 28 day mortality was 4.7%, 90 day mortality was 14%. With grade 1, 28 day mortality was 22.1% and at 90 day it was 40.7%. with grade 2, 28 day mortality was 32% and at 90 day 52.3%. in grade 3, 28 and 90 day mortality was7 6.1% & 79%.[11]

Overall in ACLF short term mortality varies from 30-90%. In hospital mortality is up to 53%.[90]

Treatment of ACLF [9]

Treatment of ACLF will be based on the underlying cause. In those with hepatitis B flare oral antivirals are effective. Urgent liver transplantation to be considered in those with severe liver failure, i.e MELD >30. Organ failure by itself should not be a contraindication for liver transplantation. Only if high cardiac or pulmonary support is needed or if they have rapidly progressing organ failure at day 4 or 7 liver transplant may be a contraindication.

Liver dialysis like MARS and PROMETHEUS has been attempted with some success in some studies, but needs further studies before being routinely used.

Immature platelet fraction (IPF)

Immature platelet fraction is a novel parameter which measures reticulated, young platelets in the peripheral blood. IPF increases as the production of platelets increases in the bone marrow. It is a simple, indirect measure of bone marrow productive function in thmbocytopenic patients, in a similar way to how reticulocyte count provides measurement of red blood cells production.[57]

Measurement of IPF may be a useful in distinguishing causes of thrombocytopenia. In case of peripheral platelet destruction as in immune thrombocytopenic purpura, hypersplenism and thrombotic thrombocytopenic purpura where in IPF will be increased in comparison to those with bone marrow suppression or failure like aplastic anemia or chemotherapy induced bone marrow suppression where in IPF will be low. This may even be used as a guide for platelet transfusion requirements in certain clinical situations.

Circulating immature platelets, immature platelet fraction (IPF), is the term that defines much larger platelets that have been recently released from the bone marrow. IPF measurement is based on fluorescent flowcytometry principle, IPF have a much greater RNA content, and will be measured by automated analyzers which has a reticulocyte detection channel, and then are reported as percentage of the total platelet count (%-IPF).

Various studies have shown normal range as 0.5 to 5.2%, 1.1 to 6.1%, 0.5 to 3.2 %. Normal ranges as per an Indian study were 0.7- 4.3%.

Thrombocytopenia in liver disease is caused by multiple factors. One among them is hypersplenism. In this case the IPF is expected to be higher in comparison to healthy controls as bone marrow production will be normal with excessive peripheral destruction as in immune thrombocytopenic purpura.[39]

In the setting of sepsis studies have shown that IPF can predict onset of sepsis and so also the severity of sepsis, so it can be a better, cost-effective useful marker of sepsis which can be easily done in all settings.

Constitutional macrothrombocytopenia (CMT)

Constitutional macrothrombocytopenia is an uncommon disorder characterized by thrombocytopenia, giant platelets, with normal platelet function and absence of any bleeding symptom**.** It is also called Harris platelet syndrome. It is an autosomal dominant inherited disorder. Till date few studies have been published in this disorder and it shows typical geographic distribution of this disorder. It is common in the north eastern India, Nepal, Bhutan and Bangladesh. An SNP of a gene with similar functions as MYH9A has been detected by some investigators and it has been considered as a causative factor.[89]

Mediterranean macrothrombocytopenia is documented among the northern and Mediterranean origin European population which is a similar condition. It is described as a disorder with large platelets without any specific diagnostic clinical feature. It has been attributed to a defect in the demarcatory membrane system of megakaryocytes by which the megakaryocytic cytoplasm divides and forms a few larger blushed platelets preserving the overall platelet mass almost normal.[88]

In a publication by our institution including healthy blood donors, it was noted that patients specifically from West Bengal had macro thrombocytopenia and were asymptomatic and their bleeding and clotting factors were normal. Total 78 patients from WB were included in this study and 2.6% of them had severe thrombocytopenia, 11% had moderate thrombocytopenia and 18.8% had mild thrombocytopenia. When compared to healthy donors from Tamil Nadu, 80% of the West Bengal donors had giant platelets (MPV-10 fL (7.5-16.8 fL)). Where in Tamil Nadu no patient had this.[8]

Aim:

Aim: Analysis of thrombocytopenia and endothelial dysfunction in patients with portal hypertension

Objective 1:

To analyze thrombocytopenia in patients with portal hypertension and in control groups of patients (with thrombocytopenia due to other causes) by

   a. **Study of the difference between manual and coulter platelet counts in study subjects**

This was a prospective observational study. The patients were recruited in the study from November 2013 to January 2014. Patients with cryptogenic chronic liver disease CLD with portal hypertension including non cirrhotic Intrahepatic portal hypertension (NCIPH) were included as cases. Control groups had patients with hepatitis B or C related CLD with portal hypertension, extrahepatic portal vein obstruction (EHPVO) with portal hypertension, aplastic anemia, idiopathic thrombocytopenic purpura (ITP), constitutional macrothrombocytopenia (CMT) and healthy volunteers.

The study was approved by Institutional Review Board (IRB) of Christian Medical College, Vellore. Patients with age less than 18 years and those who did not provide consent for the study were excluded from this study.

Cases and controls with liver disease were recruited from Liver clinic or Hepatology inpatient.

Diagnosis:

Cryptogenic cirrhosis: Cryptogenic CLD was diagnosed when the etiological workup of chronic liver disease (ANA, serum ceruloplasmin, 24 hr urinary copper level, viral serology, iron studies) was negative

Non Cirrhotic Intrahepatic Portal Hypertension (NCIPH) was diagnosed as per following criteria [4]

- Portal hypertension (as evidenced by gastro-esophageal varices on upper GI endoscopy)
- Patent portal vein and hepatic venous outflow tract
- Absence of advanced fibrosis (cirrhosis/ bridging fibrosis) on liver biopsy
- No evident etiology of chronic liver disease (e.g. alcohol, Hepatitis B/C)
- No lesion which can mimic NCIPH on biopsy (e.g. sarcoidoisis, congenital hepatic fibrosis)

Portal hypertension was documented in all cases and appropriate controls in the presence of esophageal and/or gastric varices on esophagogastroduodenoscopy (OGD).

Hep B and or C related CLD with portal hypertension: CLD as per the clinical, radiological and biochemical features and portal hypertension as evidenced by gastro-esophageal varices on upper GI endoscopy

Idiopathic Thrombocytopenic Purpura (ITP): was diagnosed based on history**:** Isolated bleeding symptoms consistent with thrombocytopenia without constitutional symptoms, Physical examination: Bleeding symptoms in the absence of hepatosplenomegaly, lymphadenopathy, or

stigmata of congenital conditions Complete blood count: Isolated thrombocytopenia (platelet count <100 x 109/L). Anemia only if due to Significant bleeding—otherwise normal red cell indices, white blood cell count and differential. Peripheral blood smear**:** platelets normal to large in size. Red and white blood cell with normal morphology[6]

Aplastic anemia: was defined as pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin[7]

Patients with ITP and aplastic anemia were recruited from Hematology department who attended OPD for evaluation

Constitutional macrothrombocytopenia: asymptomatic mild to moderate thrombocytopenia i.e platelet count 50-150x 10^9 /L with increased mean platelet volume (MPV>13) found during routine evaluation in the absence of any significant disease[8]

Extrahepatic portal vein obstruction: extrahepatic portal vein obstruction was diagnosed on colour doppler which showed chronic occlusion of the extrahepatic portal vein with or without involvement of splenic vein, superior mesenteric vein with or without portal cavernoma with esophageal and /or gastric varices documented on OGD

Platelet count and clumping:

Two blood samples, one with EDTA and another citrated were collected from the cases and controls. Platelet counts, both manual and coulter, mean platelet volume (MPV) was done in all these samples within four hours of collecting samples.

Platelet clump counting was also done by using the smear prepared from both samples. Number of platelet clumps per HPF was noted.

Manual Platelet Count:

The test was performed on a venous blood sample collected using evacuated tube system and anticoagulated with K2EDTA & citrate. The Platelet diluting fluid used was Fromal citrate solution (1% Formalin in a 3% solution of trisodium citrate to which is added 1-2 drops of 1% Brilliant cresyl blue stain). The diluting fluid was stored in a refrigerator prior to use. 20uL of well mixed venous blood was diluted with 1.98 mL of diluting fluid to give a 1:100 dilution. This sample was mixed well for 10 minutes. The improved Neubaeur chamber was then charged and kept covered in a moist chamber for 10 minutes prior to performing the count.

The platelets were counted with a high dry objective (40x) and 10X eyepiece (combined magnification of 400x) where the platelets are seen as refractile particles in the chamber. All platelets in the large central square were counted. At least 100 platelets were counted. If the central square did not yield 100 platelets another additional large square was counted.

Calculation:

Platelets/uL = $\dfrac{\text{No of platelets counted x dilution x depth}}{\text{Area}}$

Usual dilution: 1:100

Standard depth: 1/10 mm

Area counted: 4 sq mm (4 corner WBC squares)

Automated Platelet count - Impedance method (coulter count)

Automated platelet count was performed using the Beckman Coulter DXH 800 (Beckman Coulter Inc, Hilaeah, FL, USA). Peripheral venous blood anticoagulated with K2EDTA& citrate was used for this analysis and the samples were analyzed in the primary mode (samples aspirated by cap piercing method after positive identification by the analyzer) and the platelets were enumerated using the impedance principle where the resistance created by the platelets passing through an aperture in an isoelectric medium is converted into pulses that are directly proportional to their size. The numbers of platelets were derived from the transformed data resulting from the count of pulses generated in the impedance channel using the DXH Software. Mean platelet volume was also obtained with the same machine in the same samples.

Corrected difference in platelet count (CDPC)

It was calculated using formula, CDPC=manual platelet count-coulter platelet count/coulter platelet count. This was to avoid inadvertent difference between manual and coulter platelet count due to difference in total platelet count in different groups, specifically healthy controls who had normal platelet count.

The platelet counts were performed by 2 experienced Technicians in department of Transfusion Medicine, who were not aware of the clinical diagnosis of each study subject.

Objective 1:

b. Study of platelet clumping and immature platelet fraction in study subjects

During manual platelet counting platelet clumps were looked for in both EDTA and citrate samples. The method for manual counting has been explained above in objective 1.

To study the immature platelet fraction in different study groups

Immature platelet fraction was studied using five groups of patients. This was done prospectively from January 2015 to March 2015. Cases included those with cryptogenic liver disease with portal hypertension and controls included hepatitis B or C related chronic liver disease with portal hypertension and healthy controls. Patients with aplastic anemia and ITP were also included in the study but the number was very low.

We recruited 23 cases of cryptogenic CLD with portal hypertension, 14 HBV/HCV CLD with portal hypertension and 19 healthy controls. 2 cases each of aplastic anemia and ITP were also included after taking consent. All the cases and controls were defined as explained in objective 1

From each study subject 2ml blood was collected with vaccutainer in EDTA tubes and was transferred to clinical pathology within 2 hours at room temperature.

IPF was measured using the Sysmex XN-9000 (Sysmex). It is a fully-automated hematology analyzer employing flow cytometry and a semi-conductor diode laser system to analyze leukocytes, nucleated red cells, and reticulocytes (RET channel) in the RET channel. Two fluorescent dyes (polymethine and oxazine) in the RET-SEARCH (II) reagent penetrate into the cells and stain DNA/RNA. The stained cells are passed through a semiconductor diode laser beam and the resulting forward scatter light (cell volume)and fluorescence intensity (RNA content) are measured. The mature and immature platelet fractions are identified on the basis of their fluorescence intensity using the XN Software. The IPF is expressed as a proportional value (IPF%) of the total optical platelet count to indicate the rate of platelet production. Peripheral blood samples (2 mL) anticoagulated with K2EDTA were analyzed for IPF% and the results

were recorded. All samples were kept at room temperature until analysis and were analyzed within8 hr after collection.

Objective 2:

To analyze the impact of thrombocytopenia and of plasma von Willebrand factor (vWf) levels on in-hospital survival in patients with acute on chronic liver failure (ACLF).

Patients with ACLF as per Asia Pacific Association for Study of Liver (APASL) criteria were enrolled prospectively from October 2014 to March 2015. In patients diagnosed with ACLF after detailed clinical examination liver function test (LFT), complete blood count (CBC), arterial blood gas (ABG), creatinine, prothrombin time PT/INR and plasma von Willebrand factor levels were done (vWf) were done on day of admission. vWf level and the above mentioned biochemical tests except ABG were repeated on 3rd day of hospital admission. SOFA (sequential organ failure assessment), MELD (Model for End stage Liver Disease) and CTP (Child-Pugh-Turcotte) scores were calculated on day of admission.

Presence of sepsis/SIRS (systemic inflammatory response syndrome), and new onset organ failure (acute kidney injury or hepatic encephalopathy) was documented during hospitalization. The end point noted was the in-hospital outcomes i.e. discharge, death, discharge in terminal condition (DTC) or liver transplantation.

Definitions:

Acute-on-Chronic Liver Failure[9]:

Patients with ACLF were diagnosed based on APASL criteria i.e. acute hepatic insult manifesting as jaundice and coagulopathy, complicated within 4 weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease.

Acute insults: Hepatotropic (HAV/HEV) and non-hepatotropic viruses, reactivation of Hepatitis B (overt or occult) or Hepatitis C, Other infectious agents afflicting the liver. Alcohol: active drinking within the last four weeks, use of hepatotoxic drugs, herbs, flare of autoimmune hepatitis or Wilson's disease. Surgical intervention and variceal bleed.

Underlying CLD will be diagnosed based on clinical, biochemical, radiological and/or histological features. Will include compensated cirrhosis of any etiology, Chronic hepatitis, Nonalcoholic steatohepatitis (NASH), Cholestatic liver disease and Metabolic liver disease.

Defining the liver failure in ACLF: Jaundice (serum bilirubin 5 mg/dL) and coagulopathy (INR 1.5) Ascites and/or encephalopathy as determined by physical examination

Sepsis:

Sepsis is the clinical syndrome that results from a dysregulated inflammatory response to an infection. Sepsis is defined as the presence (probable or documented) of infection together with systemic manifestations of infection.

SIRS (systemic inflammatory response syndrome); SIRS defined as fulfilling at least two of the following four criteria:

- Fever >38.0°C or hypothermia <36.0°C,
- Tachycardia >90 beats/minute,
- Tachypnea >20 breaths/ minute,
- Leucocytosis >12x10^9/l or leucopoenia <4x10^9/l.

Organ Dysfunction: SOFA (sequential organ failure assessment)[10]

Points	1	2	3	4
Respiratory				
PaO2/FiO2	<400	<300	<200	<100
			With support	Without support
Cardiovascular				
Hypotension	MAP<70mmHg	Dopamine <5	Dopamine>5	Dopamine>15
		Dobutamine	Adr < 0.1 or	Adr >0.1 or
			NE <0.1	Nor adr >0.1
Liver				
Bilirubin mg/dl	1.2-1.9	2-5.9	6-11.9	>12
Renal				
Creatinine mg/dl	1.2-1.9	2-3.4	3.5-4.9	5
Urine output			Or<500ml/d	<200ml/day
Coagulation				
Platelets x103/μl	<150	<100	<50	<25
CNS				
GCS	13-14	10-12	6-9	<6

Exclusion criteria

Those with age<18 years and those who didn't give consent were excluded from study

ACLF grading

Acute-on- chronic liver failure was graded as in EASL-CLIF consortium definition [11]

ACLF Grade 0. Comprises of 3 subgroups: first those with no organ failure. Second, those with single organ failure other than kidney (liver, coagulation, circulation, or respiratory) with serum Cr level <1.5 mg/dL and no HE. Third, those with single cerebral failure with serum Cr <1.5 mg/dL.

ACLF grade 1. It includes 3 subgroups: first, those with only kidney failure. Second, those with only liver, coagulation, circulation, or respiratory failure with serum Cr level 1.5-1.9 mg/dL and/or mild to moderate HE. Third, those with only cerebral failure with serum Cr of 1.5-1.9 mg/dL.

ACLF grade 2. Patients with 2 organ failures are included in this group

ACLF grade 3. Patients with 3 or more organ failures are included under this

Assay for VWF

Plasma VWF antigen (VWF:Ag) was measured using an ELISA kit (quantitiative ELISA) as per manufacturer's instructions. In short, diluted plasma was introduced into a microwell coated with a polyclonal antibody specific for human vWF, which binds to the protein in plasma. Following a washing step, a polyclonal antibody for vWF coupled to horse radish peroxidase (HRP) was

introduced, which binds to free epitopes of immobilized vWF. Following a washing step, the peroxidase substrate, 3,3',5,5' – Tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H2O2), was introduced and was observed for blue colour development. When the reaction was stopped with Sulfuric Acid, a yellow colour was obtained. The amount of colour developed was directly proportional to the concentration of human vWF in the sample. For VWF collagen binding (VWF:CB) activity, a similar method using microwells coated with fibrillary collagen instead of anti-vWF antibody was used. The levels were obtained after analysis of the graphs shown below.

Graph 1: Standard curve from which the vWf value was calculated

vWF Activity CBA

$y = -8E\text{-}05x^2 + 0.027x - 0.000$

$R^2 = 0.999$
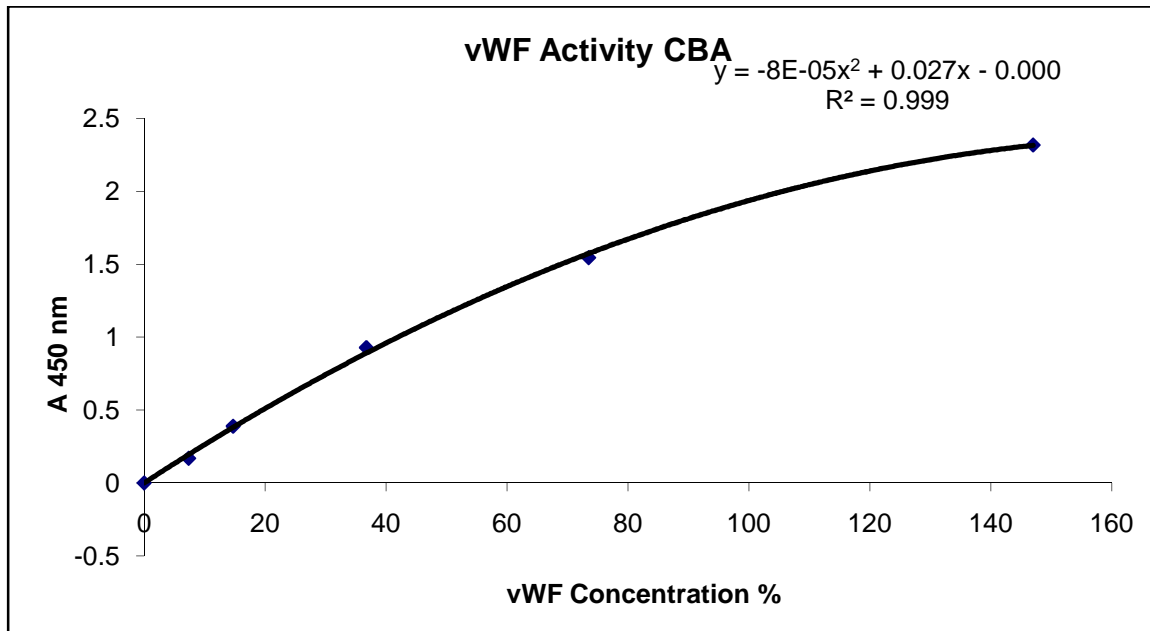
Statistical methods:

The continuous variables were expressed as mean, median, standard deviation and range. The discrete variables were expressed as numbers and percentage.

The continuous variables were compared by Mann Whitney U test and a p-value of <0.05 was considered as significant.

Discrete variables were compared using chi square test or Fischer's exact test. Bivariate correlation was assessed by Pearson correlation coefficient.

Multivariate logistic regression was done to assess independent factors affecting survival in patients with ACLF.

Receiver operating characteristic curve (ROC) was used to assess the sensitivity and specificity of plasma vWF as predictor of primary outcome.

Base line characteristics in the study groups

The mean age of the cases was 48 years, in healthy controls it was 32 yrs, 47 years in those with HBV or HCV related CLD with portal hypertension (disease controls), 29 yrs in those with EHPVO, 34 years in hematology controls (aplastic anemia or ITP) and 49 yrs in patients with constitutional macro thrombocytopenia.

Males were predominant among cases (82%), disease controls (84%), EHPVO (69%) and CMT (80%). It was equal in healthy controls group (50%) and female predominant in hematology group (53%).

Table 1; Baseline characteristics of different study groups

Characteristics	Cases (n=50)	HBV/HCV CLD (n=44)	Healthy controls (n=20)	EHPVO (n=13)	ITP/Aplastic anemia (n=39)	CMT (n=20)
Age [Median(range)]	44.7(10-73)	46.6(23-74)	32.3(21-63)	28.5(13-55)	33.6(6-69)	48.5(22-68)
Male: Female	41:9	37:7	10:10	9:4	18:21	16:4

HBV/HCV-Hepatitis B and /or C related cirrhosis with portal hypertension, CMT-Constitutional Macro Thrombocytopenia, EHPVO-Extrahepatic Portal Vein Obstruction, ITP- Idiopathic Thrombocytopenia Purpura

Liver disease severity in the different study groups

In this study three groups with liver disease were included. Cases with cryptogenic CLD including NCIPH, those with HBV or HCV related CLD with portal hypertension and extrahepatic portal vein obstruction as controls.

Splenomegaly was noted in majority of patients with portal hypertension and the mean spleen size in cases was 16.03cm, 14.23cm among those with HBV or HCV related CLD with portal hypertension and it was 17.35cm, slightly more in comparison to other groups in EHPVO group.

Majority of cases (76%) and extrahepatic portal vein obstruction group were in CTP A class and in those with HBV or HCV related CLD CTP A class constituted 47% of the total, CTP B and C constituted 25% and 27% respectively.

Table2: Spleen size and CTP distribution in different liver disease groups

Characteristics	Cases (n=50)	HBV/HCV (n=44)	EHPVO (n=13)
Spleen size (cm)	16.03(9.2-26.5)	14.23(8.9-21.4)	17.35(13-23.5)
CTP (A:B:C)	38:7:5	21:11:12	12:1:0

EHPVO- extrahepaticportal vein obstruction, HBV/HCV- hepatitis B/ C, Spleen size as per ultrasound abdomen, CTP- Child-Pugh-Turcott's score

Platelet count in the different study groups

When platelet counts were done using two anticoagulants by coulter and manual methods the difference was noted between manual and coulter platelet counts. This difference was present in the cases and all the controls groups studied as shown in Table 3.

Table 3: Manual and Coulter platelet counts with different anticoagulants in study groups

Groups	EDTA			Citrate		
	Manual $(x10^3/\mu L)$	Coulter $(x10^3/\mu L)$	Difference $(x10^3/\mu L)$	Manual $(x10^3/\mu L)$	Coulter $(x10^3/\mu L)$	Difference $(x10^3/\mu L)$
Cases (n=50)	82(15-240)	67(5-179)	15(19-61)	60(8-163)	47(5-110)	12(27-138)
HBV/HCV CLD controls(n=44)	88(16-194)	76(16-180)	12(22-63)	72(18-186)	59(4-159)	12(22-72)
Healthy controls (n=20)	258(124-348)	239(124-364)	19(25-64)	208(86-357)	181(81-318)	26(40-108)
EHPVO(n=13)	78(26-158)	68(14-147)	10(22-33)	64(18-135)	53(8-117)	10(9-28)
ITP & Aplastic anemia(n=39)	30(5-165)	24(1-152)	6(28-76)	22(4-128)	18(2-120)	4(15-79)
CMT(n=20)	125(58-185)	74(38-97)	52(16-93)	----	---	----

EHPVO-extrahepatic portal vein obstruction, ITP-idiopathic thrombocytopenic purpura, CMT-constitutional macrothrombocytopenia, EDTA-ethylenediaminetetraaceticacid

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Mean platelet volume in the study groups

The mean platelet volume was significantly higher in the all the study groups in comparison to healthy controls both in EDTA and citrate samples. Constitutional macro thrombocytopenia had significantly higher MPV in comparison to cases (as expected as the cut off for CMT was 13fL). However the mean platelet volume difference was not significant in cases when compared to other control groups (HBV/HCV related CLD with portal hypertension, EHPVO and hematology controls).

Table 4: Mean Platelet Volume in study groups

	Cases (n=50)	HBV/HCV controls (n=44)	Healthy controls (20)	EHPVO (n=13)	ITP/Aplastic anemia (n=39)	CMT (n=20)
MPV EDTA (fL)	10(7-16)	9.9(7.4-12.6)	8.7(6.9-14.2)	10.1(8.3-12.4)	10(4.8-13.6)	14.1(13-16.2)
P value*	-----	0.701	**0.001**	0.424	0.238	**<0.001**
MPV Citrate (fL)	9(7-13)	9.2(6-13)	8.2(6-13)	9(7-13)	9.4(3-15)	-----
P value*	-----	0.897	**<0.001**	0.872	0.931	-----

EHPVO-extrahepatic portal vein obstruction, ITP-idiopathic thrombocytopenic purpura, CMT-constitutional macrothrombocytopenia, MPV-mean platelet volume, *All p values as compared to cases

Corrected difference in platelet count in the study groups

In EDTA sample, in different groups

Platelet count difference was corrected for the total platelet counts as different study groups had different numbers and the total count may affect the difference. The mean corrected difference in platelet count-EDTA (CDPC=manual platelet count-coulter platelet count/coulter platelet count) in cases [0.331(-0.22-2.0)] was significantly different in comparison to healthy controls [0.091(-0.13-0.52); P 0.002]. However, it was (? Not) significantly different in citrate sample [0.492(-0.50-11.5); 0.173(-0.18-0.93); P 0.330].

Table 5; Corrected difference in platelet count in different study groups with EDTA sample

Groups	CDPC –EDTA	P value
Cases	0.331(-0.22-2.0)	
HBV/HCV controls	0.282(-0.20-1.94)	0.290
Healthy controls	0.091(-0.13-0.52)	**0.002**
EHPVO	0.258(-0.15-0.86)	0.519
ITP & Aplastic anemia	1.247(-0.45-19)	0.383
CMT	0.701(0.19-1.02)	**0.000**

CDPC-corrected difference in platelet count, EHPVO-extrahepatic portal vein obstruction, ITP-idiopathic thrombocytopenic purpura, CMT-constitutional macrothrombocytopenia

Graph 1: Graph depicting the difference in corrected platelet counts in different study groups with EDTA as anticoagulant
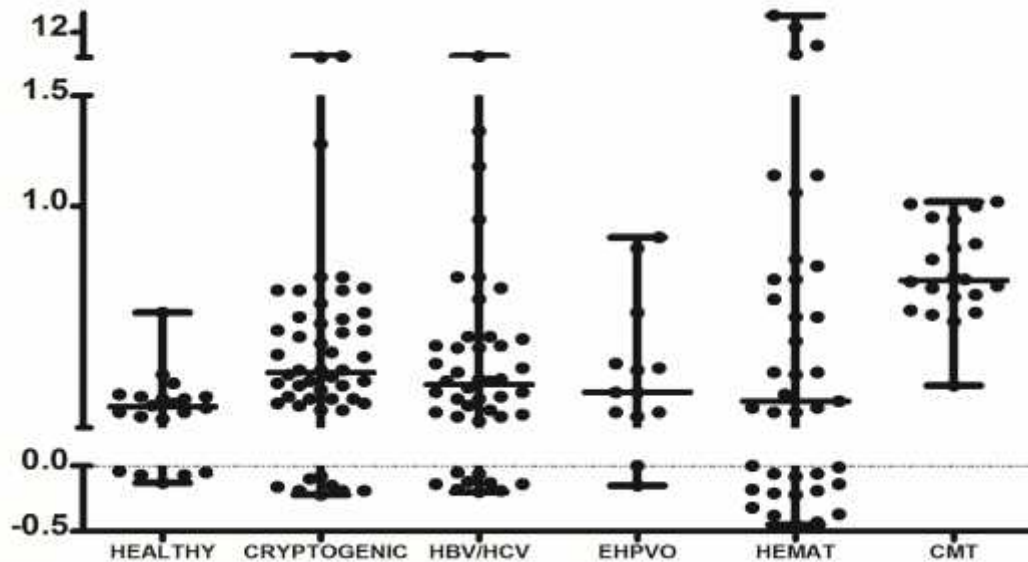
*Hemat- Aplastic anemia & ITP

In Citrate samples, in different groups

Mean CDPC –citrate was significantly different in hematology controls [0.988(-0.44-7.18)] in comparison to cases [0.492(-0.50-11.5); P 0.010]. But, in EDTA sample the difference was not significant [1.247(-0.45-19); 0.331(-0.22-2.0); P 0.383].

The mean CDPC-EDTA was significantly higher in patients with constitutional macro thrombocytopenia [0.701(0.19-1.02)] in comparison to cases [0.331(-0.22-2.0); P 0.000]

Table 6: Corrected platelet count in different groups in citrated sample

Groups	CDPC-Citrate	P value
Cases	0.492(-0.50-11.5)	----
HBV/HCV controls	0.372(-0.40-4.0)	0.562
Healthy controls	0.173(-0.18-0.93)	0.330
EHPVO	0.334(-0.29-1.25)	0.604
ITP & Aplastic anemia	0.988(-0.44-7.18)	0.010
CMT	---	----

CDPC-corrected difference in platelet count, EHPVO-extrahepatic portal vein obstruction, ITP-idiopathic thrombocytopenic purpura, CMT-constitutional macrothrombocytopenia

Graph 2: Graph depicting the difference in corrected platelet counts in different study groups with citrate as anticoagulant
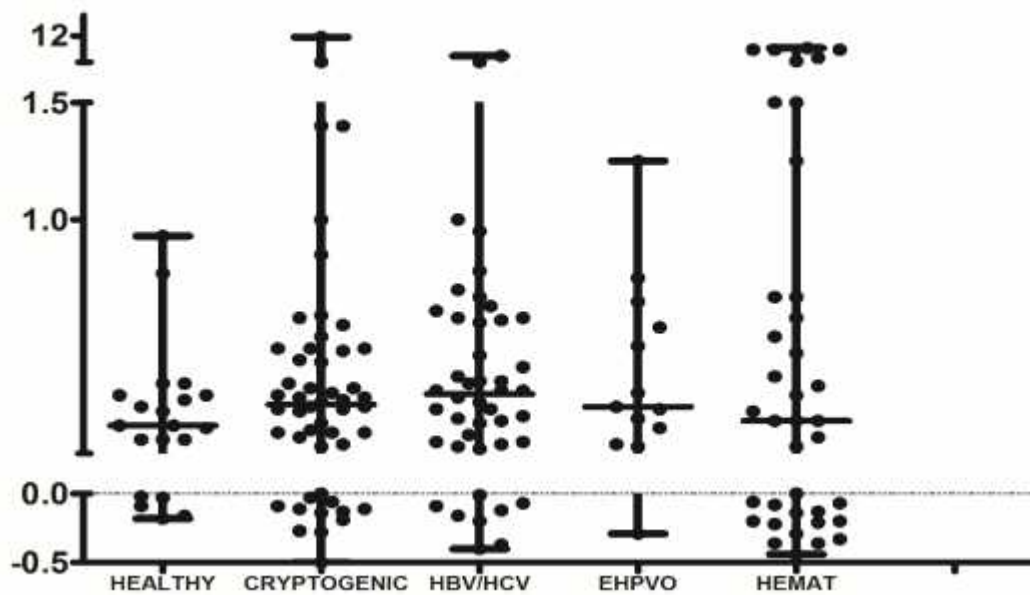
Hemat- Aplastic anemia & ITP

Platelet clumping

Platelet clumping was noted in 9(18%) cases, 5(11.4%) HBV/HCV CLD controls, 2(10%) healthy controls and 3 (15%) constitutional macrothrombocytopenia patients in the peripheral smear. In patients with EHPVO, aplastic anemia and ITP clumping was not noted in the peripheral smear.

There was no difference noted in the platelet clumping in EDTA and citrate samples. Both the anticoagulants had similar clumping profile.

Table 7: Platelet clumping in study groups

	Cases (n=50)	HBV/HCV controls (n=44)	Healthy controls (n=20)	EHPVO (n=13)	ITP & Aplastic anemia (n=39)	CMT (n=20)
Platelet clumps-EDTA n(%)	9 (18)	5 (11.4)	2 (10)	0 (0)	0 (0)	3 (15)
	-----	0.400	0.500	0.200	0.004	1.00
Platelet clumps Citrate n(%)	9 (18)	5 (11.4)	2 (10)	0 (0)	0 (0)	3 (15)
	----	0.400	0.500	0.200	0.004	1.00

EHPVO-extrahepatic portal vein obstruction, ITP-idiopathic thrombocytopenic purpura, CMT-constitutional macrothrombocytopenia

Graph 3: Graph depicting the platelet clumping in different study groups

Platelet clumps

	Cases	Disease controls	Healthy controls	EHPVO	ITP & Aplastic anemia	CMT
■ Platelet clumps	18	11.4	10	0	0	15
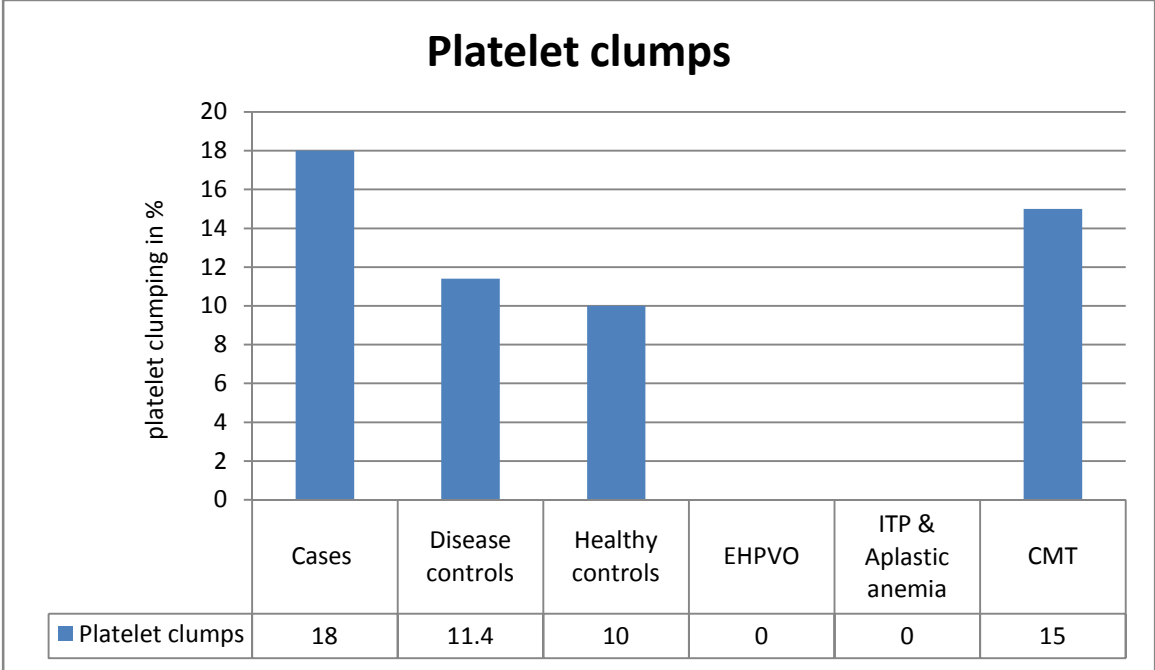
Immature platelet fraction in study groups

We studied immature platelet fraction in 23 cases (cryptogenic cirrhosis with portal hypertension including NCIPH), 14 disease controls (HBV/HCV related chronic liver disease with portal hypertension), 19 healthy controls, 2 patients with aplastic anemia and ITP each.

The mean age in the cases was 46.6 (24-81) years; it was (51.534-64) yrs in disease controls, 31.2 (23-42) years in healthy controls, 32(23-41) yrs in aplastic anemia group and 32.5 (23-42) years in those with ITP.

Male and female were almost equal in number in cases and healthy controls (11:2, 10:9; M:F). Disease control group was female predominant (10:4). In hematology patients aplastic anemia patients were males and ITP were females.

The mean platelet count and mean platelet volume was low among the study groups except in healthy controls. Patients with HBV/HCV related chronic liver disease patients had lower platelet count compared to cases.

Mean spleen size was more in both the liver disease groups, it was more so in cases in comparison to HBV/HCV CLD controls.

Immature platelet fraction was elevated in liver disease group and ITP patients. Those with HBV/HCV CLD group (7.8) had higher IPF compared to cryptogenic CLD group (6.3). IPF was significantly higher in both the patients of ITP and was with in lower range in aplastic anemia group. Healthy controls had mean IPF of 3.1

Table 8: Platelet count, mean platelet volume, immature platelet fraction, spleen size and CTP in the study groups

Characteristics	Cryptogenic chronic liver disease(n=23)	HBV/HCV-chronic liver disease(n=14)	Healthy controls(19)	Aplastic anemia(n=2)	ITP(n=2)
Platelet count(x10³/μL)	142.7(20-698)	84.21(15-143)	322(219-426)	28.5(5-52)	43(14-72)
Mean platelet volume(fL)	11.9(10-14)	12.92(10-14)	10.38(9-12)	12.25(11-14)	14(14-14)
IPF (%)	6.3(1.2-28.3)	7.8(2.9-17.3)	3.1(0.8-6.1)	2.2(2.2-2.3)	19.3(13.4-25.2)
Spleen size (cm)	16.5(10-26)	13.6(10-20)	----	----	----
CTP (A:B:C)	11:7:5	3:4:7	-----	----	----

IPF-immature platelet fraction, HBV/HCV- hepatitis B and hepatitis C, ITP-idiopathic thrombocytopenic purpura, CTP- child pugh turcot's

Comparison of platelet factors in different groups

Comparison between cryptogenic and HBV/HCV chronic liver disease

When the platelet count, mean platelet volume, spleen size and IPF was compared in cryptogenic chronic liver disease group with those with HB/HCV chronic liver disease group the difference in MPV and spleen size was significantly different. However, the difference in platelet count and IPF was not statistically significant

Comparison between cryptogenic chronic liver disease and healthy controls

When mean platelet count, mean platelet volume and immature platelet fraction was compared between cryptogenic chronic liver disease and healthy controls the difference in all the three groups were statistically significant. Platelet count was significantly low, mean platelet volume was higher and IPF was higher in cryptogenic CLD group compared to healthy controls

Table 9: Comparison of platelet count, mean platelet volume and immature platelet fraction among cryptogenic chronic liver disease and healthy controls and HBV/HCV controls

	Cryptogenic chronic liver disease(n=23)	Healthy controls(n=19)	HBV/HCV-chronic liver disease(n=14)	P value cases/healthy controls	P value Cases Vs HBV/HCV controls
Platelet count (x10³/µL)	142.7(20-698)	322(219-426)	84.21(15-143)	**0.000**	0.394
MPV(fL)	11.9(10-14)	10.38(9-12)	12.92(10-14)	**0.001**	**0.042**
IPF (%)	6.3(1.2-28.3)	3.1(0.8-6.1)	7.8(2.9-17.3)	**0.002**	0.284
Spleen size (cm)	16.5 (10-26)	------	13.6(10-20)	**-----**	**0.039**

IPF-immature platelet fraction, HBV/HCV- hepatitis B and hepatitis C

Graph 4: Immature platelet fraction in different study groups
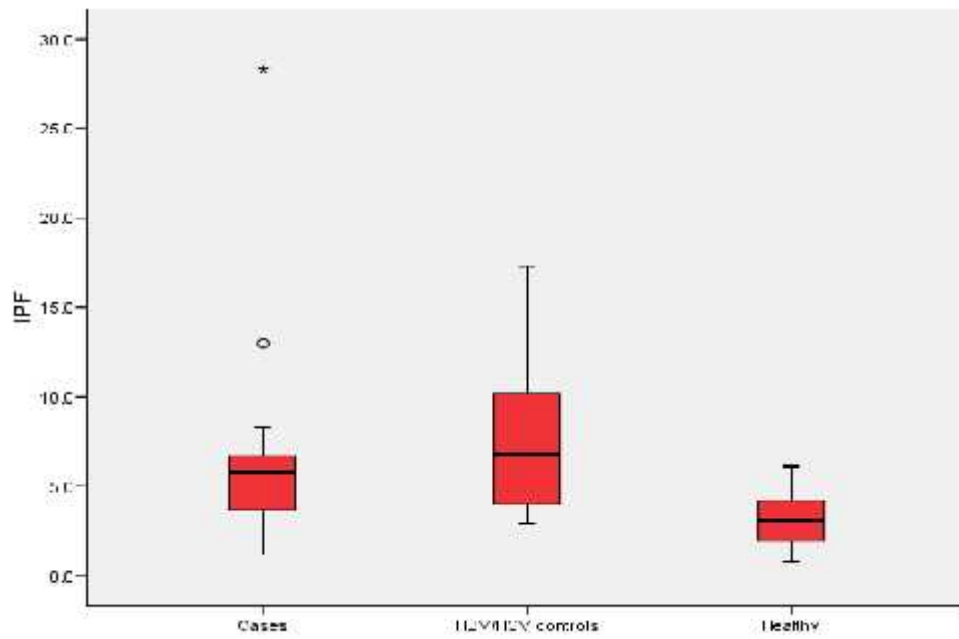
Correlation between IPF and mean platelet volume

When linear correlation between MPV and IPF was analyzed using Pearson's correlation coefficient, a statistically significant (P 0.000) strong correlation (r 0.718) was found between mean platelet volume and immature platelet fraction.

In chronic liver disease group

When the correlation was separately analyzed for those with liver disease (cryptogenic CLD and HBV/HCV CLD) with healthy controls there was statistically significant(P 0.000) good correlation (r 0.642)between MPV and IPF.

Graph 5: Correlation between mean platelet volume and immature platelet fraction in liver disease
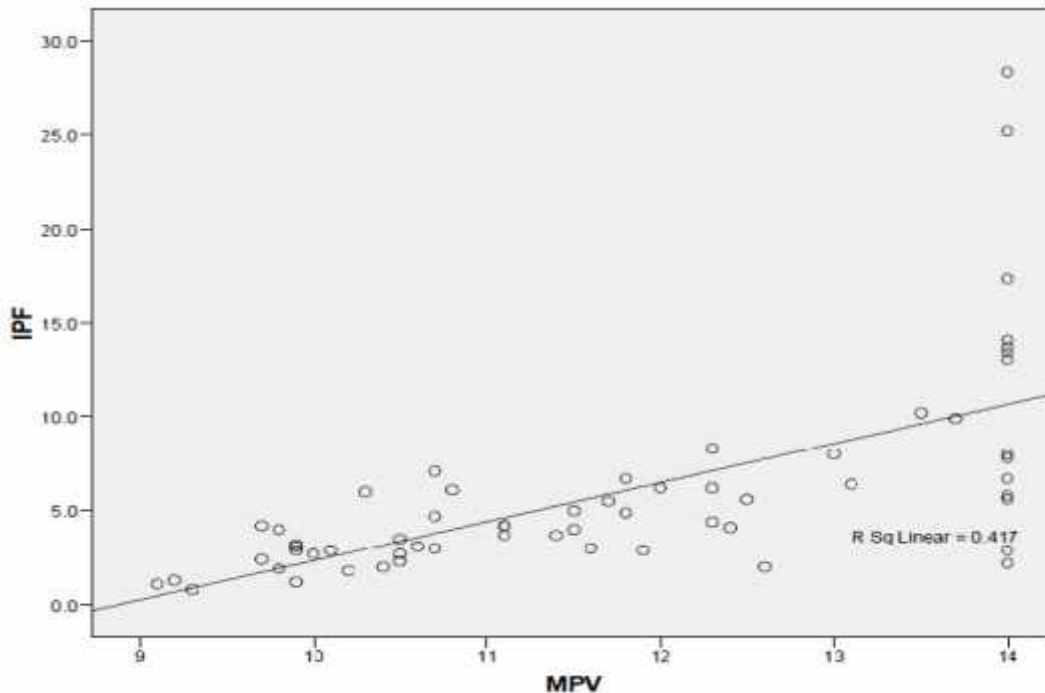
In healthy controls

There was a strong (r 0.894) and statistically significant (p <0.001) correlation between mean

platelet volume and immature platelet fraction in healthy controls

Graph 6: Correlation between mean platelet volume and immature platelet fraction in

healthy control group
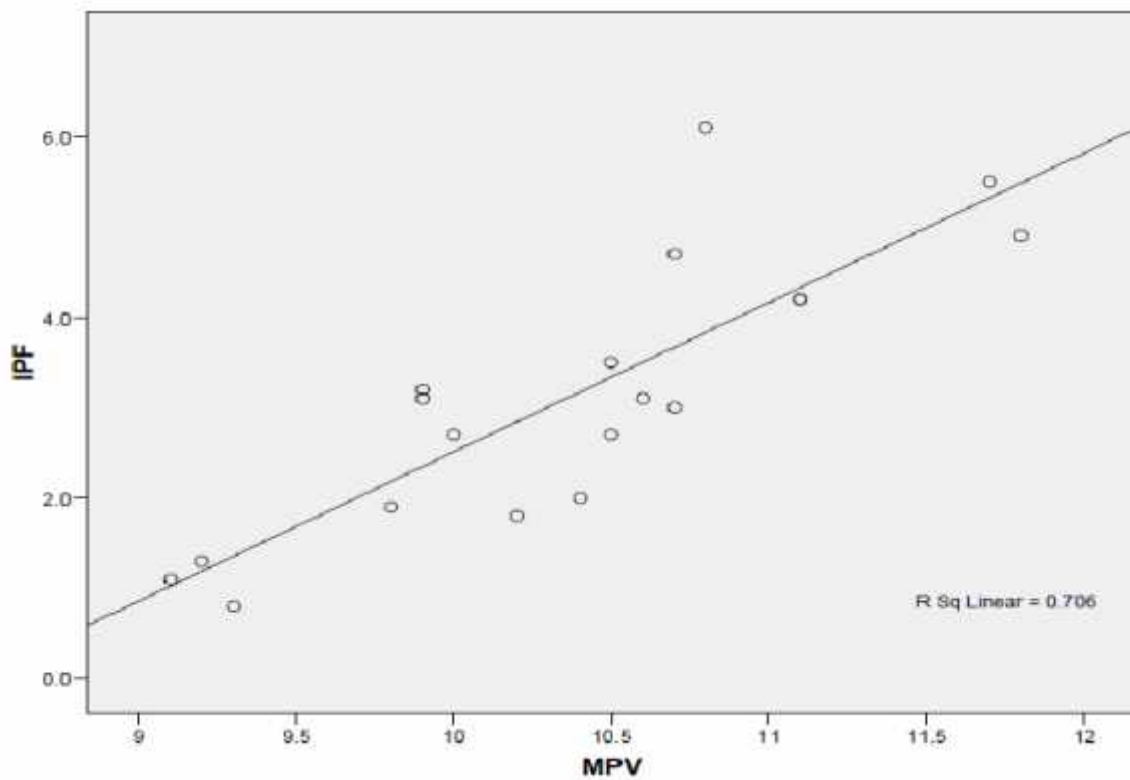
Correlation matrix

In the study group as a whole

When correlation was analyzed in relation to IPF, MPV, spleen size and platelet count in the study group a statistically significant good positive correlation was noted between IPF and MPV. A statistically significant good negative correlation was noted between MPV, IPF and platelet count. This correlation has been depicted in above graph

Table 10: Correlation matrix between platelet counts, mean platelet volume, immature platelet fraction and spleen size in study group

		IPF %	MPV (fl)	Spleen size (cm)	Platelet count (x10³/µL)
IPF %	Pearson CC	1	**0.640**	0.015	**-0.394**
	P value		**0.000**	0.933	**0.002**
MPV(fl)	Pearson CC	**0.646**	1	0.279	**-0.632**
	P value	**0.000**		0.104	**0.000**
Spleen size(cm)	Pearson CC	0.015	0.279	1	-0.193
	P value	0.933	0.104		0.268
Platelet count (x10³/µL)	Pearson CC	**-0.394**	**-0.632**	-0.193	1
	P value	**0.002**	**0.000**	0.268	

IPF-immature platelet fraction, MPV-mean platelet volume, Pearson CC- Pearson correlation coefficient

Graph 7: Correlation matrix of platelet counts, mean platelet volume, immature platelet fraction and spleen size in study group
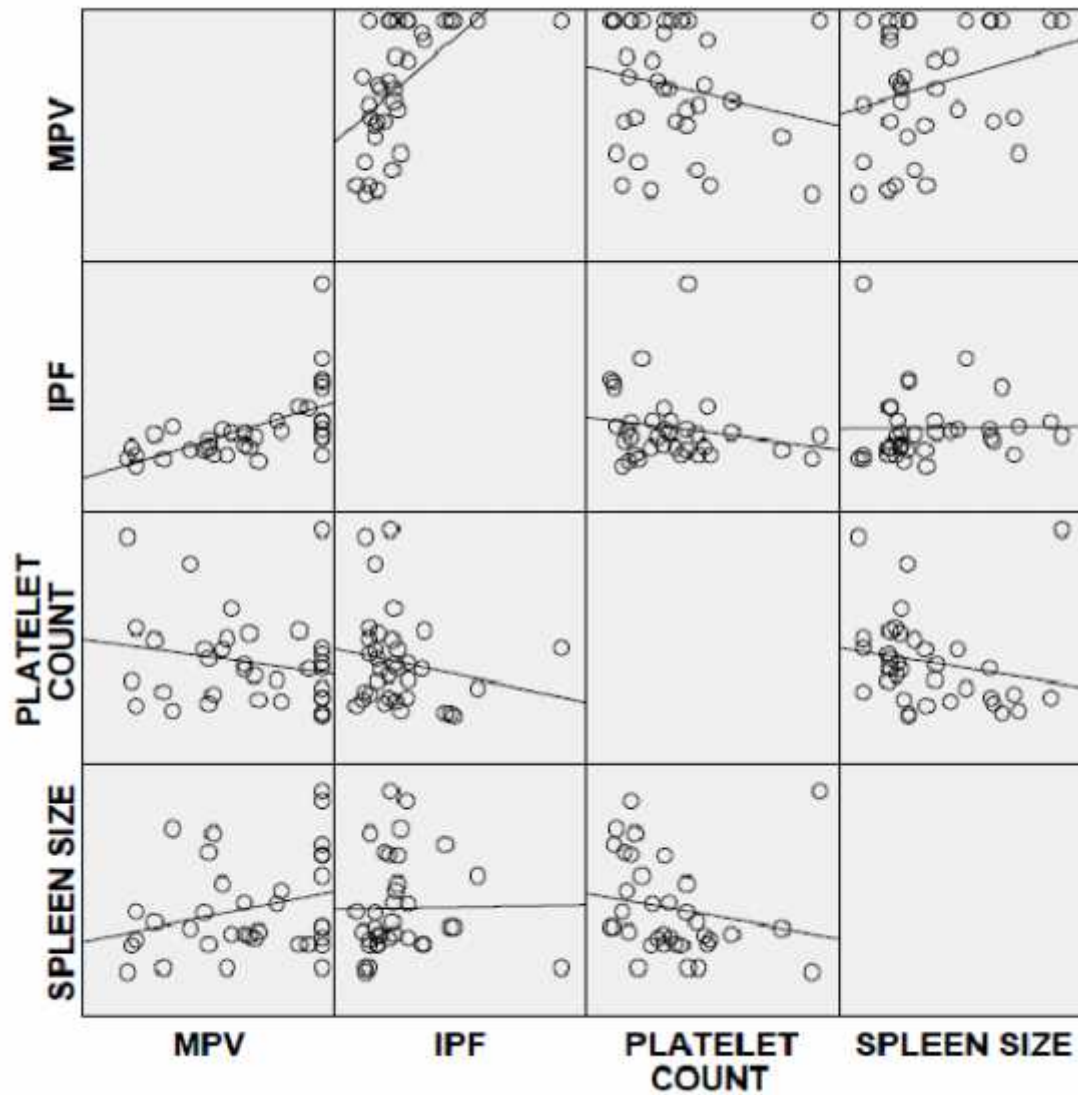
Correlation matrix in liver disease

When the correlation was analyzed in the liver disease group a statistically significant positive correlation was noted between immature platelet fraction and mean platelet volume. A Statistically significant negative correlation between platelet count and mean platelet volume was also noted

Table 10: Correlation matrix between mean platelet volume, immature platelet fraction , spleen size and platelet count in liver disease group (cryptogenic, HBV and HCV- chronic liver disease)

		IPF %	MPV (fl)	Spleen size (cm)	Platelet count (x10^3/µL)
IPF %	Pearson CC	1	**0.573**	0.018	**-0.170**
	P value		**0.000**	0.919	**0.315**
MPV(fl)	Pearson CC	**0.573**	1	0.244	**-0.356**
	P value	**0.000**		0.164	**0.031**
Spleen size(cm)	Pearson CC	-0.018	0.244	1	-0.223
	P value	0.919	0.164		0.206
Platelet count (x10^3/µL)	Pearson CC	**-0.170**	**-0.356**	-0.223	1
	P value	**0.135**	**0.031**	0.206	

IPF-immature platelet fraction, MPV-mean platelet volume, Pearson CC- Pearson correlation coefficient

.

Graph 8: Correlation matrix of mean platelet volume, immature platelet fraction , spleen size and platelet count in liver disease group (cryptogenic, HBV and HCV- chronic liver disease)
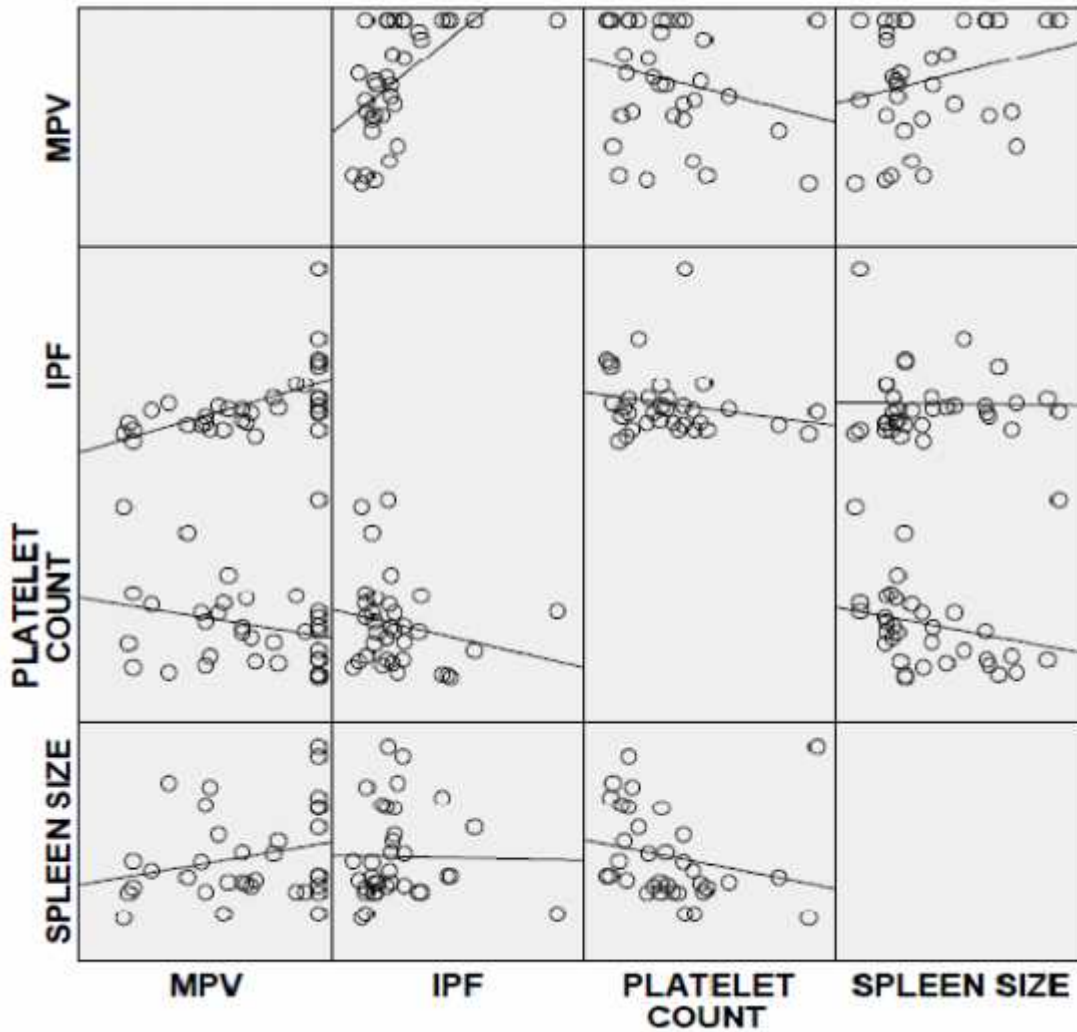
Correlation matrix in healthy controls

When correlation was analyzed between immature platelet fraction, mean platelet volume and platelet count in healthy controls a statistically significant good positive correlation was noted between immature platelet fraction and MPV and a statistically significant good negative correlation was noted between platelet count and immature platelet fraction, so also platelet count and mean platelet volume.

Table 12: Correlation matrix between mean platelet volume, immature platelet fraction, and platelet count in healthy control group

		IPF %	MPV (fl)	Platelet count (x10^3/µL)
IPF %	Pearson CC	1	**0.840**	**-0.722**
	P value		**0.000**	**0.000**
MPV(fl)	Pearson CC	**0.840**	1	**-0.735**
	P value	**0.000**		**0.000**
Platelet count (x10^3/µL)	Pearson CC	**-0.722**	**-0.735**	1
	P value	**0.000**	**0.000**	

IPF-immature platelet fraction, MPV-mean platelet volume, Pearson CC- Pearson correlation coefficient

Graph 9: Correlation matrix of mean platelet volume, immature platelet fraction, and platelet count in healthy control group
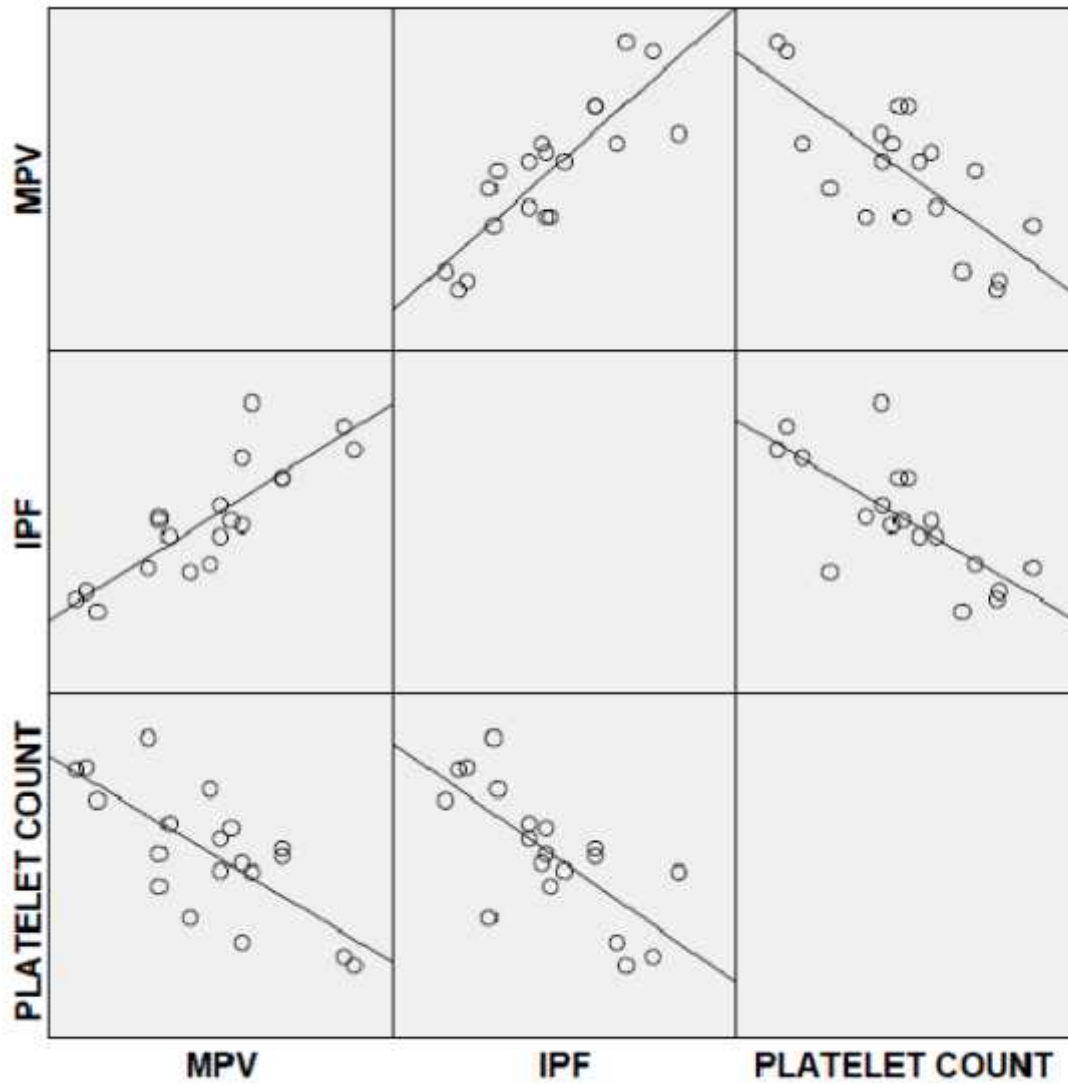
Results of Objective 2:

Characteristics in patients with Acute-on-Chronic Liver Failure group

We recruited 50 patients with ACLF as per APASL criteria for the study. The mean age in the study group was 43.5 years. Males predominated the study (90%), ethanol was the most common cause (80%) in the study followed by cryptogenic liver disease and hepatitis B.

Table 13: Characteristics in patients with Acute-on-Chronic Liver Failure group

Characteristics	Median (Range)- n=50
Age (years)	43.5 (28-64)
Male: Female	45:5
Etiology **Ethanol**	40(80%)
HBV/HCV	4(8%)
Cryptogenic	5(10%)
Others	1(2%)

Severity of liver disease in ACLF

Mean MELD score in the study group was 30 and SOFA 7.6 indicating the severity with which they presented. 16 (32%) patients did not have any major organ injury in this study and constituted ACLF grade 0, 13 (26%) each in ACLF grade 1 and 2 and 8 (16%) had 3 organ

injury constituting ACLF grade 3. 41 (82%) of patients with ACLF had features of SIRS at admission

Duration and outcome of hospital stay: Among 50 patients, 29 got discharged, average hospital stay was 8.2 (3-28)days, 10 died, average hospital stay was 6.5 (2-12) days, 9 were discharged in terminal condition, average hospital stay was 5.7 (2-11) days and 2 underwent liver transplantation, average number of hospital stay prior to LTx was 8 (5-11) days.

Table 14: Severity of liver disease in patients with acute-on-chronic liver failure

MELD	30 (17-49)
SOFA- Day 1	7.62(4-14)
ACLF grading (Grade 1:2:3)	16:13:13:8
Bilirubin (mg/dl)	17.3 (5-37)
SIRS n(%)	41/50 (82)
INR	2.3 (1.5-10)
Albumin (g/dl)	2.35 (1.5-3.4)
Platelet count (x10^3/µL)	110 (30-243)
Hospital stay (no. of days)	7.3 (2-28)

Plasma von Willebrand factor antigen and activity levels

In patients with ACLF the mean plasma vWf Ag level on day 1 was 756 and on day 3 it was 712.

vWf activity on day 1 was 570 and on day 3 it was 562 as shown in Table 15.

Table 15: Plasma von Willebrand antigen and von Willebrand factor activity in patients with acute on chronic liver failure on day 1 and day 3

Parameters		Median (Range)
vWf Ag	day 1 % (n=46)	756 (212-1346)
	day 3 % (n=42)	712 (278-1411)
vWf activity	day 1% ()	570 (97.3-1156)
	day 3	562 (100-1300)

Difference in patient characteristics in relation to in hospital outcome

MELD was significantly different in those who did not survive compared to those who survived. Mean MELD in those who died was 33.9 and 27.2 in those who got discharged. SOFA score and bilirubin on day of admission was also significantly different.

Higher number of patients in group 1 (composite poor in-hospital outcome-death/DAMA/LTx) had high creatinine compared to group 2 (discharge). There was no significant difference in SIRS, lactate, platelet count and albumin in both the groups. vWf activity on day 1 was significantly different in group 1 compared to group 2. vWf Ag D1, D3 and vWf activity on D3 was almost similar in both the groups.

On multivariate logistic regression analysis controlling for MELD score vWf activity at admission (p 0.102) had a trend towards prediction of mortality [AOR 1.002; 95%CI (1-1.005)]

Table 16: Difference in base line characteristics in those who survived and those who did not

Characteristics	Composite poor outcome (n=21)	Discharged in stable condition (n=29)	P value
Age (years)	40 (30-58)	43 (28-64)	0.687
MELD	35 (22-47)	26 (17-49)	**0.002**
SOFA score	8 (4-14)	6 (4-14)	**0.007**
Bilirubin (mg/dl)	21.7 (5.1-37)	11.6 (5-33.4)	**0.013**
Albumin (gm/dl)	2.3 (1.8-3.3)	2.3 (1.5-3.4)	0.760
INR	2.17(1.5-10)	1.9 (1.5-3.4)	0.293
Platelet count (x10^3/ μL)	96(30-353)	92(30-240)	0.969
Creatinine	2.2 (1-6)	1 (1-6)	**0.005**
Lactate	1.9 (1-7)	1.5(1-16)	0.113
SIRS	17/21	24/29	1.000
vWf Ag day 1	742(264-1347)	699 (212-1249)	0.135
vWf Ag day 3	746(326-1157)	689 (279-1411)	0.600
vWf activity day 1	632 (119-1157)	490 (97-986)	**0.025**
vWf activity day 3	457(244-1334)	497 (100-1304)	0.916

ACLF grades and relation to in-hospital outcome

When the data was analyzed as per the grade of ACLF, MELD was significantly different in different grades. As the grade of ACLF increased MELD also increased.

vWf activity was also significantly increasing as the grade of ACLF increased from grade 0 to 3.

In-hospital mortality also significantly increased with increasing grade.

However vWf Ag on D1 and 3, vWf activity on D3 was not significantly different in different grades.

Table 17: ACLF grades and its relation to in-hospital outcome

	ACLF grades				P value
	Grade 0 (n=16)	Grade 1(n=13)	Grade 2 (n=13)	Grade 3 (n=8)	
Age(years)	41 (30-63)	49 (30-62)	41 (28-64)	40.5 (35-58)	1.140
MELD	23 (19-27)	29 (17-36)	37 (26-47)	41 (30-49)	**<0.001**
vWf Ag D1 %	690 (264-1082)	700 (212-1347)	686 (515-1338)	986 (742-1344)	0.107
vWf Ag D3 %	654 (301-1169)	640 (279-1411)	594 (334-998)	885 (702-1054)	0.184
vWf activity D1 %	491 (97-949)	468 (116-835)	532 (221-1090)	882 (676-11157)	**0.005**
vWf activity D3 %	466 (100-1247)	447 (132-1218)	441 (277-1304)	766 (713-904)	0.081
Platelet (x103/μL)	99 (39-243)	71 (30-225)	121 (30-353)	94 (35-198)	0.549
In hospital outcome Death (%)	1(6)	5 (38.5)	10 (76.9)	5 (62.5)	**<0.001**

Graph 10: Depiction of vWf Ag on day 1 in different study group
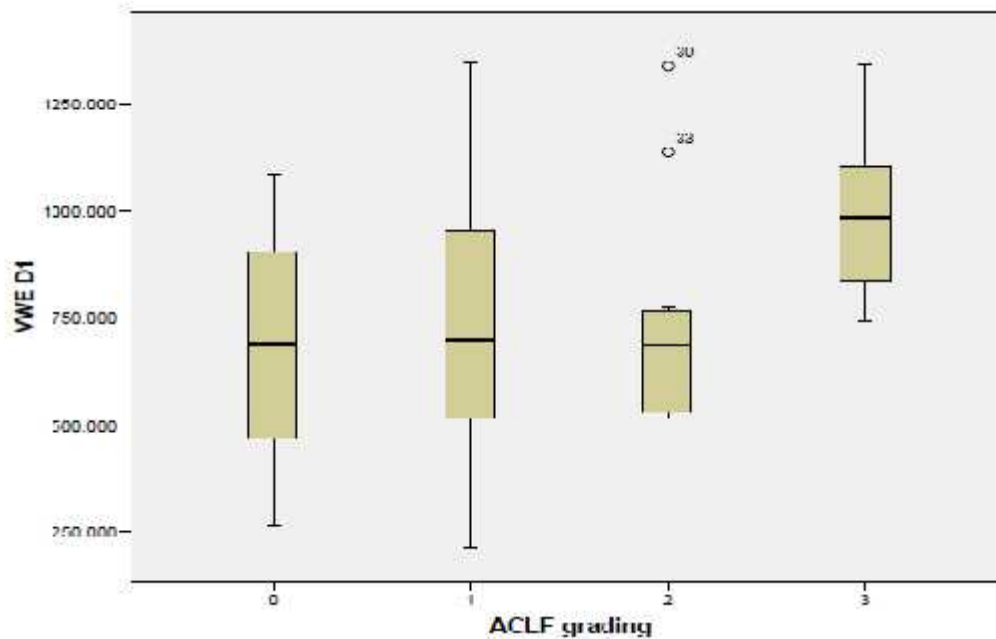
Graph 11: Depiction of vWf activity on day 1 in different study group
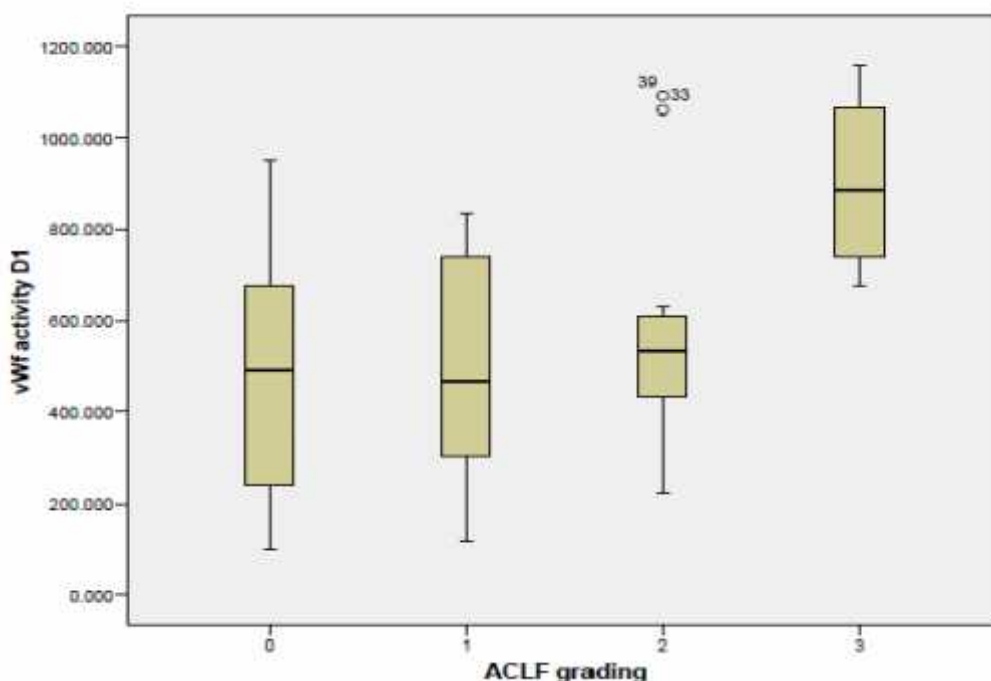
ROC for MELD, vWF Ag and vWf activity

ROC was plotted for MELD, vWf Ag on day1 and vWF activity on D1. The sensitivity and specificity for different cut off are given in the table below.

AUROC for MELD was 0.744 (95% CI; 0.599-0.888), AUROC for vWf Ag D1 was 0.631 (95% CI; 0.465-0.796) and AUROC for vWf activity D1 was 0.681 (95% CI; 0.521-0.842).

A MELD cut off of 28 had 74% sensitivity and 63% specificity, vWf Ag cut off of 529 had 84% sensitivity and 33% specificity and vWf activity cut off of 402 had 79% sensitivity and 33% specificity.

Graph 12; Receiver Operating Characteristic curve for MELD, vWf Ag and vWf acticity at admission to predict survival
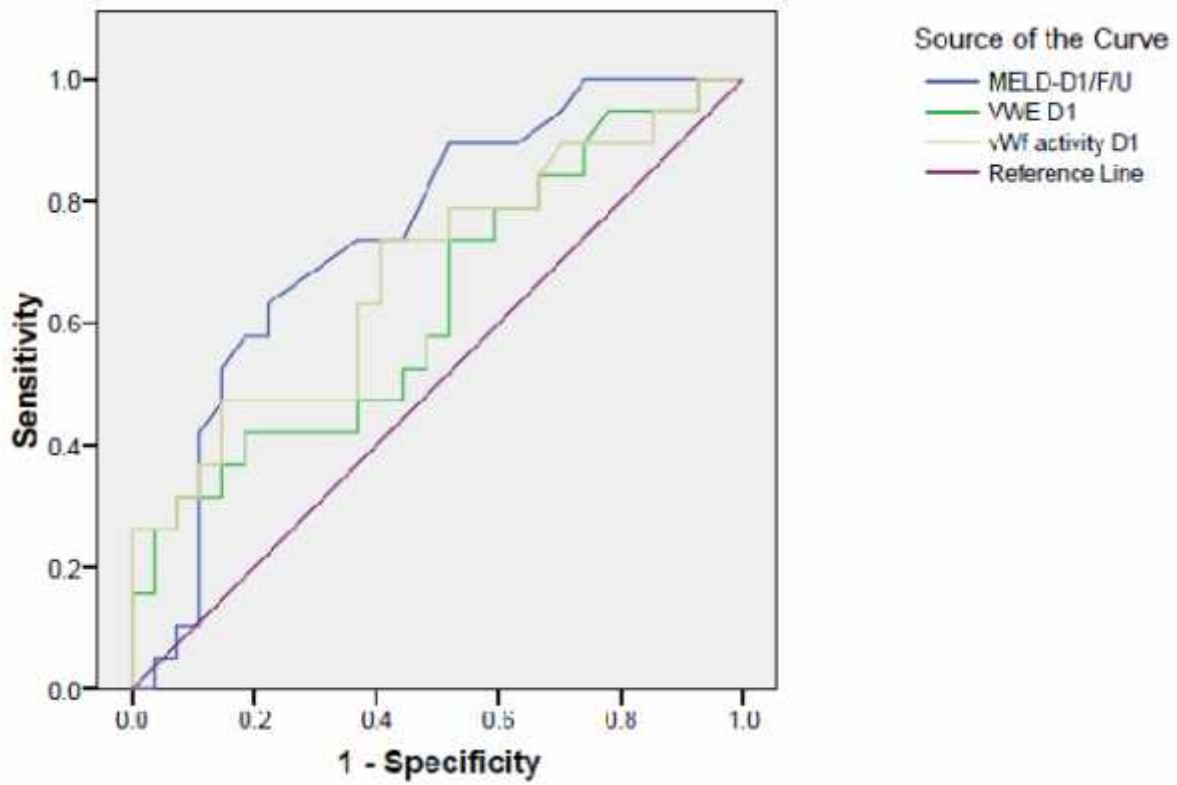
	Area	P value	95% confidence interval	
			Lower bound	Upper bound
MELD	0.744	**0.005**	0.599	0.888
vWf Ag D1	0.631	0.138	0.465	0.796
vWf Activity D1	0.681	**0.038**	0.521	0.842

Table 18: Area under receiver operating curve for vWf Ag and vWf activity on Day 1 of admission in acute-on-chronic liver failure patients

	Cut-off value	Sensitivity (%)	Specificity (%)
MELD	22	100	26
	26	90	48
	28	74	63
	31	63	78
vWf Ag D1	492	95	22
	529	84	33
	685	74	48
	865	42	70
vWf Activity D1	291	90	26
	402	79	33
	507	74	56
	621	53	63

Correlation matrix for MELD, vWf Ag, vWf activity and platelet count

On correlation matrix analysis a significant strong positive correlation was noted between vWf Ag and activity.

A significant weak positive correlation was noted between MELD and vWf activity D1.

A weak negative correlation was noted between vWf Ag and vWf activity with platelet count. However, it was not statistically significant.

Table 19: Correlation matrix for MELD, von Willebrand factor antigen, von Willebrand factor activity and platelet count

		MELD	vWf Ag D1	vWf activity D1	Platelet count
MELD	Pearson CC P	1	0.249 0.095	**0.321** **0.023**	0.031 0.832
vWf Ag D1	Pearson CC P	0.249 0.095	**1**	**0.831** **<0.001**	**-0.136** 0.372
vWf activity D1	Pearson CC P	**0.321** **0.023**	**0.831** **<0.001**	1	**-0.165** 0.253
Platelet count	Pearson CC P	0.031 0.832	**-0.135** 0.372	**-0.165** 0.253	1

Pearson CC- Pearson correlation coefficient

Graph 13; Correlation matrix for MELD, von Wilebrand factor antigen, von Willebrand factor activity and platelet count
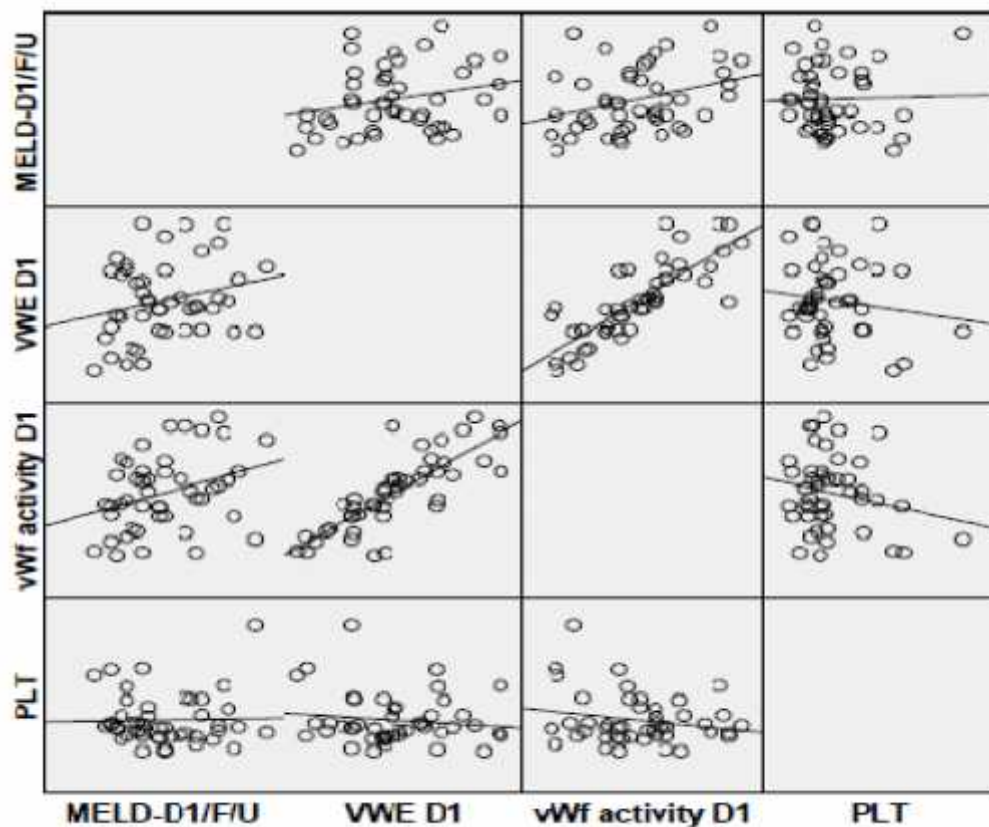
This was a prospective observational study done to analyze platelet clumping in patients with portal hypertension. Various control groups as mentioned in methodology were included to compare the results with cases (cryptogenic cirrhosis with portal hypertension including non-cirrhotic intrahepatic portal hypertension).

In our study difference between manual and coulter platelet count was noted in the study groups along with those with liver disease. This difference was significant in cases when compared to healthy controls. This difference is most probably due to increased mean platelet volume which was significantly higher in cases compared to healthy controls and the MPV was significantly correlating with immature platelet fraction indicating increased large immature platelets in the circulation. In our study one of the reasons for increased immature platelets in the circulation secondary to increased peripheral destruction/ sequestration in the setting of liver disease was studied. vWf Ag and activity was increased in patients with ACLF and a negative correlation was also noted between vWf activity and platelet count suggesting role of vWf in peripheral platelet destruction/sequestration.

We observed difference between manual and automated coulter platelet count in all the groups including healthy controls and the difference was significantly different in cases (cryptogenic cirrhosis with portal hypertension including non-cirrhotic Intrahepatic portal hypertension) in comparison to healthy controls in EDTA sample but not in citrate sample. Similar significant difference was noted when compared with citrated sample in hematology controls (aplastic anemia and ITP), so also in comparison to constitutional macrothrombocytopenia.

The difference noted in CMT group was significantly higher in comparison to cases. The differences noted are specifically seen in either EDTA or citrate and the results are not consistent with different anticoagulants in same group. This may point towards anticoagulant related spurious platelet count results.

In a study by M Zandecki et al[1] it was concluded that EDTA dependent thrombocytopenia is one of the frequent anomalies associated with spurious counts on automated counters. It was attributed to platelet clump formation due to EDTA. However in our study this anomaly is unlikely as the study was designed to avoid this and there was no increased clumping noted in EDTA samples compared to citrate samples.

Anton H. Sutor et al[2] has shown in their study that automated counter can't be relied up on in cases of thrombocytopenia especially if count was lower than 20000 as the average difference in automated and manual count was 7000/ µL, which in our study was not common. Cases and all the control groups except the hematology controls had mean platelet count >50,000/µL.

However other causes for spurious low platelet count in automated platelet counts like platelet satellitism, platelet-neutrophil agglutination, fragmented RBC's, cytoplasmic fragments, cryoglobulins, bacteria and coagulation within sample listed by M Zandecki et al[1] were not looked into in our study.

One more important cause of spurious low platelet count in automated platelet count is abnormal platelet size. In our study the mean platelet volume was measured in all cases and control groups and was noted that, MPV in cases was significantly higher in comparison to healthy controls. This difference in mean platelet volume may explain the difference in platelet count noted between manual and coulter platelet counts.

Increased mean platelet volume was noted in all the study groups in comparison to healthy controls so also the difference in corrected platelet counts. Hence increased MPV is the likely explanation for the difference in manual and coulter platelet count.

Our hypothesis to start with was that, the difference in manual and coulter platelet count is due to platelet clumping and this platelet clump leads to portal venular occlusion leading to obliterative portal venopathy. However we did not find platelet clumping in the peripheral circulation which was significantly different from other study groups and the difference in platelet count was noted in other study groups also.

Platelet clumps in peripheral circulation was noted in 18% of cases, 11.4% HBV/HCV related portal hypertension, 15% CMT patients and 10% of healthy controls also. So, platelet clumping may not be the cause of this difference as hypothesized. However platelet clumping at microvascular level as cause of oblitrative portal venopathy cannot be ruled out with this study.

Increased mean platelet volume may be due to immature platelets in the circulation or due to activated platelets in the circulation. To understand this better we did immature platelet fraction estimation in patients with liver disease and compared with healthy controls.

Further, when the difference in manual and coulter platelet count was noted in all the study groups along with increased mean platelet volume when compared to healthy individuals it was suggesting that the difference noted was most probably due to increased platelet volume. Increased platelet volume means either activated platelets or immature platelets. Immature platelets are released into peripheral circulation when there is high turnover due to increased peripheral destruction.

In our study those patients with HBV/HCV related chronic liver disease with portal hypertension had lower platelet count compared to cryptogenic chronic liver disease with portal hypertension. This can be explained with the higher number of CTP C patients in the former group which reflects more severe liver disease.

Studies [3,4] have shown that immature platelet fraction measurement based on flowcytometry principle using fluorescent dye is a good test to measure the immature platelet fraction in the peripheral circulation. So, we did IPF measurement in total 60 patients.

In a study by Sehgal K et al[5] it was found that the normal IPF% in healthy individuals was 0.7-4.3%. In another study of Carlos Brigg's et al[3] in western healthy population the IPF% varied from 1.1-6.1%. Carlos Brigg's et al studied IPF% in different study groups and they found that in patients with ITP IPF varied from 2.3-52.1% and in TTP it varied from 11.2-30.9%.

In our study the IPF% varied from 0.8-6.1% in healthy individuals and was in line with the above mentioned studies. Similarly in aplastic anemia and ITP patients also the results were in line with the other studies. ITP patients had significantly higher IPF% as they have normal bone marrow production with peripheral destruction. Whereas in aplastic anemia as bone marrow production is abnormal as expected IPF% was low.

In a study by T Nomura et al[6] it was noted that patients with liver disease have higher IPF fraction compared to healthy controls [5.4(3-9.5); 3(0.7-7.3)]. Similarly in a study by Issac Pons et al[7] it was shown that patients with liver cirrhosis (HCV/HBV and cryptogenic cirrhosis) with splenomegaly had significantly high IPF% compared to healthy controls.

In our study we noted that patients with portal hypertension had higher IPF% compared to healthy controls. When cryptogenic chronic liver disease with portal hypertension group was

compared with healthy controls IPF% was significantly higher in the cryptogenic chronic liver disease group (p 002).

In our study a significant strong (p <0.001, r 0.718) linear correlation was noted between mean platelet volume and immature platelet fraction. As expected, thrombocytopenic patients had higher MPV and IPF in the circulation suggesting that mean platelet volume can be considered as an indirect measure of immature platelets.

Further on analysis a statistically significant strong negative correlation was found between platelet count and immature platelet fraction when cryptogenic chronic liver disease with portal hypertension patients were compared with healthy controls (p <0.001; r 0.722). This suggested increased peripheral destruction of the platelets in patients with portal hypertension. This finding supports the fact that in liver disease hypersplenism is an important cause of thrombocytopenia. This finding suggests that the bone marrow function is normal in patients with chronic liver disease with portal hypertension as against some studies which suggested suppressed bone marrow function as a cause of thrombocytopenia in chronic liver disease with portal hypertension. However, when the correlation between spleen size and IPF and platelet count was analyzed, no significant correlation was noted which may be because the spleen size measurement in ultrasound abdomen may not be the accurate measurement of actual spleen size and also it is known fact that hypersplenism is not related to spleen size[8].

In our study it was also noted that there was significant strong negative correlation between platelet count and mean platelet volume in patients with cryptogenic chronic liver disease with portal hypertension when compared to healthy controls. This finding is in line with findings in first group of our study as described above. This negative correlation is mostly because the

automated platelet count is lower when the MPV increases as the impedance technique may miss giant platelets while counting.

To explore the cause of peripheral platelet destruction/sequestration we studied vWf in patients with ACLF. In our study increased vWf Ag and activity was noted in those who had poor in-hospital outcome. There was significant increase in vWf activity with increasing grades of ACLF and a negative correlation of vWf activity with platelet count.

In CANONIC study[11], a study in the western population the mean age in ACLF patients was 56±11 years and was male predominant (64.4%). In a study by Khot A A et al[9] from Mumbai the mean age was 43.1 years and was predominantly males (54%). In another study from Jaipur[10] the mean age was 38.6±16.7 years and was also male predominant (78.8%). Garg H et al[11] studied hemodynamics in 57 patients with ACLF and the mean age group in the study patients was 41 (15-67) yrs. Swastik A et al[12] from Chandigarh studied 106 patients with ACLF and the mean age was 45±11 years with male predominance (87%). In our study the mean age in the study group was 43.5 (28-64) years and was predominantly males (90%). ACLF affects relatively younger age group as shown in above mentioned studies. More so in the Asian countries as the main cause in the Asian countries is viral or alcoholic.

In ACLF study by Moreau R et al[11] the main cause of underlying cause of cirrhosis was alcohol (49.2%) followed by chronic hepatitis C (21.4%), most common acute insult was sepsis (21.4%) followed by variceal bleed (17.3%). In a study from New Delhi by Garg V et al[13] the most common cause of cirrhosis was ethanol (74%) followed by hepatitis B (17%) and cryptogenic

(9%). So also the common acute insult alcohol (65%) followed by hepatitis B flare (17%) and anti tubercular drugs (9%). In a study from Jaipur by AK Jha et al[10] the most common cause of cirrhosis was cryptogenic (30.7%) followed by ethanol (28.8%) and hepatitis B (19.3%). In another study from Mumbai by A A Khot et al[9] the most common cause of underlying CLD was hepatitis B (29.6%) followed by cryptogenic (27.7%) and ALD (14.8%). Most common acute insult in this study was acute hepatitis followed by sepsis. One more study by Swastik A et al[12] from PGI Chandigarh has shown alcohol as most common cause of CLD (66%) followed by cryptogenic CLD (11%) and hepatitis B related CLD (8%)

In our study the most common cause of underlying CLD was alcoholic liver disease (80%) followed by cryptogenic CLD (10%) and hepatitis B (8%). This finding is in line with studies from western countries and Indian studies, Garg V et al[13]. and Swastik A et al[12]. However the etiological profile has been different from other studies mentioned above.

The mean MELD score in the study subjects in CANONIC study[11] was 18.8±7.5 and 75.7% of the patients had Grade 0 ACLF, 13.7% grade1, 7.4% grade 2 and 3.2% grade 3 ACLF. In a study by Swastik et al[12] from PGI Chandigarh the MELD score was 33±9 and the SOFA score was 8 (3-20). When the patients in this study were classified as per ACLF grades Grade 0 had 19.8%%, Grade 1 had 25.5%, grade 2 had 22.6% and grade 3 had 32% of patients. In study by Garg V et al[13] from Delhi the MELD score was 29 (21-40) and SOFA score was 5 (4-9).

In our study the mean MELD score was 30 (17-49) and SOFA score was 7.6 (4-14). These findings were in line with the above mentioned Indian studies from different centers, however, in comparison to CANONIC study MELD score was significantly higher in all these Indian studies including ours. This suggests more severe disease at presentation in our study population. When

our study population was divided based on ACLF grades as in CANONIC study grade 0 had 32%, grade 1 and 2 had 26% each and grade 3 had 16% of patients. This finding contradicts the results from the above mentioned Indian study where grade 3 had highest number of patients and that of CANONIC study which had highest number in grade 0 ACLF. Our study had only 50 patients where as in other studies numbers of patients were much higher. The difference in results we got may be because of the small study population or because of the referral bias, in our study and one from Chandigarh as both are tertiary referral hospitals we may be getting more sick patients.

In Swastik A et al[12] study the overall in hospital mortality was 48% and when divided according to ACLF grades grade 0 had 9.5%, grade1 had 29.6%, grade 2 had 58.3% and grade 3 had 79.4% mortality. In Moreau R et al[11] study no in hospital data was available but 28 and 90 day mortality was correlating with the grades of ACLF, higher the grade more the mortality was. In A K Jha et al[10] study from Jaipur the in-hospital mortality was 61.4% and the mean number of days of hospital stay was 4.9 days. In a study from Mumbai by A A Khot et al[9] in-hospital mortality was 62.9%.

In our study in-hospital mortality was 42% and mean number of days of hospital stay was 7.3 days. This finding is in line with study from Chandigarh which had a comparable study population. However it contradicts other Indian study data, it may be because of difference in the study population.

Further when our study group in-hospital mortality was subdivided as per the ACLF grading grade 0 had 6%, grade 1 38.5%, grade 2 76.9% and grade 3 had 62.5% mortality. This finding was almost in line with above mentioned study by Swastik et al[12] except for difference in grade 2

and 3 mortality rates. Grade 3 had lower mortality rates in contradiction to expected higher mortality.

The mean platelet count in CANONIC study[11] was $100 \times 10^9/L$ and when it was compared with those who did not have ACLF it was significantly different ($110 \times 10^9/L$; p 0.02), further when it was analyzed in relation to grades of ACLF, platelet count significantly decreased with increasing ACLF grade. In Swastik et al[12] study the mean platelet count was $99.5 \times 10^3/mm^3$. There was no data available in this study regarding platelet count in different grades of ACLF. In our study the mean platelet count was $110 \times 10^3/\mu L$. and when it was analyzed in relation to different grades of ACLF there was no significant difference noted. Even when it was compared with those who survived and those who did not there was not much difference in platelet count.

The finding of CANONIC study[11] support the hypothesis that increased vWf in ACLF leads to thrombus formation in the microcirculation leading to TMA which in peripheral circulation will be reflected as thrombocytopenia when analyzed in terms of platelet count alone as thrombocytopenia increases with increase in number of organ failure, however vWf was not measured in that study and this study had good large study population in its favor which we lack.

If patients with ACLF develops TMA as hypothesized will consume platelets as in TTP and as number of platelets is the primary drive for the bone marrow to sense and produce platelets, this increased consumption of platelets at the microcirculatory level can stimulate bone marrow and produce more platelets as a compensatory response which can explain relatively normal platelet count.

To best of our knowledge this is the first study of vWf in ACLF patients. vWf ADAMTS 13 imbalance has been studied in liver cirrhosis, alcoholic hepatitis, sepsis and acute liver failure setting but not in ACLF.

In a study by J A Kremer Hovinga et al[14] from Switzerland ADAMTS 13 and vWf Ag and activity was studied in patients with severe sepsis and septic shock. It was found that those with sepsis had significantly higher levels of vWF (373 Vs 89; p <0.001), vWf activity (344 Vs 118; p <0.001) and low ADAMTS 13 levels (60 Vs 110; p <0.001) in comparison to healthy controls.

In another study by J J J Hulstein et al[15] from Netherlands small number of preeclampsia and HELLP syndrome along with healthy pregnant women were studied. They found that patients those with HELLP had significantly low platelet count in comparison to preeclampsia patients (70 Vs 251 x10^3/µL). When vWf level was measured those with preeclampsia had significantly higher level in comparison to healthy pregnant women but was not significantly different from those with HELLP. But, vWf activity was significantly higher in those with HELLP compared to healthy and preeclampsia women. The author's concluded acute activation of the endothelial cell layer in HELLP syndrome results in increased vWf antigen and vWf pro-peptide levels.

In a study by Ton Lisman et al[16] from Netherlands it was shown that vWf level was significantly elevated in patients with cirrhosis along with significantly decreased ADAMTS13 levels and further they showed that adhesion of either normal or patient platelets to a collagen surface was substantially increased when these platelets were resuspended in plasma of patients with cirrhosis, as compared with control plasma. The authors of this study concluded that highly elevated levels of VWF in patients with cirrhosis contribute to the induction of primary

hemostasis despite reduced functional properties of the molecule. This phenomenon might compensate for defects in platelet number and function in patients with cirrhosis.

In our study both vWf Ag and vWf activity (collagen binding assay) was measured. And it was found that in patients with ACLF both vWf Ag and vWf activity was significantly elevated and vWf activity at admission was significantly higher in those who did not survive when compared those who did well and was discharged in stable condition. This significant difference was preserved even when it was analyzed in terms of ACLF grades. As the grade of ACLF increased vWf activity also increased suggesting its role in organ dysfunction.

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When the level of vWf Ag and activity was repeated on day 3 in our study there was no significant difference. In a study by R A Reiter et al[17] from Vienna it was demonstrated that in healthy individuals when endotoxin was injected inflammation was induced and the vWf Ag level was elevated significantly which got normalized only by day 7. They had checked the levels on day 3 but it remained elevated then.

In our study no significant difference was noted in day 1 and day 3 vWf Ag or activity levels. and was not predicting in-hospital survival

The vWf Ag, activity and ADAMTS13 levels along with platelet levels in different studies with variable population and disease settings in the background of liver disease and inflammation has been listed in the below table

Table 1: Different studies in various liver diseases in relation to vWf

Study	Cases (n) Controls (n)	vWf Ag%	vWf activity%	ADAMTS13%	Platelet count
JJJ Hulstein et al[15]	HELLP syndrome (14)	339	253	----------	70
	Healthy (9)	272	151		251
M Ferlitsch et al[12]	Compensated CLD (145)	394	----------	----------	----------
	Decompensated CLD (141)	264			
Greg C. G. Hugenholtz et al [18]	ALF (50)	547	97	28	
	Healthy (40)	107	105	92	
Ashish G et al[4]	Cryptogenic CLD (22)	216	-----------	36	33
	Healthy (17)	73	----------	90	---
CL Bockmeyer et al[19]	Sepsis non survivor (6)	475	-----------	20	99
	ICU control(24)	175		41	166
R A Reiter et al[17]	Endotoxin injected healthy	259	------------	64	81
	Healthy	86		108	239
T Matsuyama et al[20]	Severe AH	806	--------	24	110
		100	--------	107	
Our study		**756**	**570**	----------	110

Platelet count expressed inx103/µL, AH- alcoholic hepatitis

In a study by Reuken et al[21] when correlation matrix analysis was done a negative correlation between platelet count and vWf Ag (-0.067) level and vWf activity (r -0.157) but was not strong and statistically significant. In our study also there was a negative correlation noted

between platelet count and vWfAg (-0.136) and vWf activity (-0.165) but was not strong and significant.

There was a strong significant positive correlation noted between vWf Ag and vWf activity in this study.

When ROC was plotted for MELD, vWf Ag D1 and vWf activity D1 AROC was fair for MELD (0.744) and was poor for vWf Ag and activity (0.631 & 0.681). AROC was statistically significant for MELD and vWF activity.

In a study by Monika Ferlitsch et al[12] they studied vWf level as a predictor of survival in patients with portal hypertension. In their study vWf Ag level predicted PHTN and CSPHTN independent of CTP scores. A vWf cutoff value of 241% predicted CSPH. A cut off value of 315 significantly predicted decompensation and complications including mortality and was as good as MELD in predicting mortality.

In a study by Vincenzo La Mura et al[22] a study done to analyze vWf as predictor of clinical outcome in patients with liver cirrhosis and portal hypertension. In patients with cirrhosis and PHTN plasma and hepatic levels of vWF correlated with liver functions and HVPG. Elevated plasma levels of vWF predicted the prognosis of patients with liver cirrhosis, independently of liver function and PHTN. A vWF value of 216 U/dl is a possible cutoff to differentiate two different groups of patients with cirrhosis with a different probability of survival free of PHTN-related complications/events and of liver transplantation.

They concluded that vWF is a promising endothelial-derived molecule to be taken into account to improve prognostic models in patients with cirrhosis and PHTN.

In our study when ROC curve was analyzed vWf activity was significantly predicting the survival and vWf Ag had trend towards the same when compared to MELD.

A vWf Ag cut off of 529 had 84% sensitive and 33% specific and vWf activity cut off value of 402 had 79% sensitivity and 33% specificity.

Summary and conclusions for study objective 1.

➢ Coulter platelet count was lesser than manual platelet counts in all different study groups

➢ Corrected difference in platelet count in cryptogenic CLD (including NCIPH) with portal hypertension patients was significantly higher compared to healthy controls

➢ Mean platelet volume was increased in all study groups and was significantly higher in cryptogenic CLD (including NCIPH) with portal hypertension patients compared to healthy controls

➢ But for 3 study groups (EHPVO, aplastic anemia and ITP), platelet clumping was noted in all other study groups (in 11% – 18% of subjects) (including in 10% of healthy controls). There was no significant difference noted in platelet clumping in cryptogenic CLD with portal hypertension (including NCIPH) compared to other groups

➢ Immature platelet fraction was significantly increased in those with liver disease compared to healthy controls and was significantly correlating with mean platelet volume

➢ Significant negative correlation was noted between MPV and platelet count so also IPF and platelet count

To conclude, we found higher manual platelet counts compared to coulter platelet counts in patients with portal hypertension, thrombocytopenic syndromes due to hematological disorders and in healthy controls. The higher manual platelet counts are probably due to increased mean platelet volume (such platelets with increased volume are probably not counted by the coulter as platelets). The increased platelet volume is probably due to release of immature platelets into the circulation. Platelet clumping in the circulation was noted in only 10 – 18% of study subjects. It is possible that in some of the study groups (for example in portal hypertension), platelet

clumping occurs as endothelial sequestration in spleen and in liver (termed hypersplenism) and not as free-floating platelet clumps in circulation. .

Summary and conclusions for study objective 2.

➤ Plasma von-Willebrand factor levels were significantly elevated in patients with acute-on-chronic liver failure. These levels are much higher than that of plasma vWf levels reported in patients with cirrhosis.

➤ Plasma vWf activity on Day 1 was significantly higher in those who had adverse in-hospital outcome compared to those who survived.

➤ Plasma vWf activity on Day 1 correlated significantly with severity of ACLF, with a trend towards predicting survival independent of MELD.

➤ As the number of organ dysfunction increased, plasma vWf activity increased

➤ There was a negative correlation noted between platelet count and vWf activity

To conclude, in patients with acute-on-chronic liver failure, we found markedly increased plasma vWf levels (compared to that reported in patients with cirrhosis in previous studies). Correlation between severity of acute-on-chronic liver failure along with negative correlation with the platelet count with elevated plasma vWF level was noted. In addition, elevated plasma vWf activity level on Day 1 showed a trend to be an independent predictor of in-hospital mortality. It is possible that platelet thrombi formed by platelets adhering to vWf on endothelial surface leads to microcirculatory occlusion and worsening organ failure in liver and in other affected organs in patients with acute on chronic liver failure, thus contributing to mortality in these patients.

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Abbreviations

5-HT- 5 Hydroxy Tryptamine

AASLD- American Association For The Study Of Liver Diseases

Ach- Acetyl Choline

ACLF- Acute-On-Chronic Liver Failure

ADAMTS13- A Disintegrin And Metalloproteinase With A Thrombospondin Type 1 Motif, Member 13

ADP-Adenosine Di Phosphate

AKI- Acute Kidney Injury

ANA- Anti Nuclear Antibodies

APACHE- Acute Physiology And Chronic Health Evaluation

APASL- Asia Pacific Association For The Study Of Liver

aPTT- Activated Partial Thromboplastin Time

AROC- Area under Receiver Operating Characteristic Curve

AT- Angiotensin

ATP- Adenosine Triphosphate

ATT- Anti Tubercular Therapy

CANONIC- CLIF Acute-on-chronic liver failure in Cirrhosis study

CLD- Chronic Liver Disease

CMT- Constitutional Macrothrombocytopenia

CO- Carbon Monoxide

CTP- Child Pugh Turcott

EASL-European Association For The Study Of Liver

EDTA- Ethanolamine Tetradecyl Trisodiumcitrate

EHPVO- Extrahepatic Portal Vein Obstruction

ET- Endothelin

GI- Gastro Intestinal

HBV- Hepatitis B

HCV- Hepatitis C

HE- Hepatic Encephalopathy

HELLP- Hemolysis Elevated Liver enzymes Low platelet

HSC- Hepatic Stellate Cells

IL- Interleukin

INR- International Normalized Ratio

IPF- Immature Platelet Fraction

ITP- Idiopathic Thrombocytopenic Purpura

LPS- Lipopolysccaride

LSEC- Liver Sinusoidal Endothelial Cells

LTX-Liver Transplant

MAO- Mono Amine Oxidase

MARS- Molecular Adsorbent Recycling System

MDA- Mono Dialdehyde

MELD- Model For End Stage Liver Disease

miRNA- Micro Ribo Nucleic Acid

MPV- Mean Platelet Volume

NAFLD- Non Alcoholic Fatty Liver Disease

NCIPH-Noncirrhptic Intrahepatic Portal Hypertension

NE- Nor Epinephrine

NO- Nitric Oxide

PG- Prostaglandin

PHTN-Portal Hypertension

PT- Prothrombin Time

ROC- Receiver Operating Characteristic curve

SEC-Sinusoidal Endothelial Cell

SERT- Serotonin Reuptake Transporter

SIRS- Systemic Inflammatory Response Syndrome

SOFA- Sequential Organ Dysfunction Assessment

TGF- Transforming Growth Factor

TIPSS- Transjugular Intrahepatic Portosystemic Shunt

TLR- Toll Like Receptor

TMA- Thrombotic Micro Angiopathy

TNF- Tumor Necrosis Factor

T-PA- Tissue Plasminogen Activator

TPO- Thrombopoietin

TTP- Thrombotic Thrombocytopenic Purpura

TXA- Thromboxane

UL vWf- Ultra Large Von Willebrand Factor

vWf Ag- von Willebrand factor antigen

vWf- Von Willebrand Factor

Analysis of platelet clumping in portal hypertension

Identification data

Name : _____

ID No: _____

Contact No: _____

Important dates

Date of proforma entry: _____

Date of visit to CMC: _____

Date of liver biopsy: _____

Initial presentation

_____

_____

Present symptoms:

_____

_____

Associated comorbidities

H/O drugs: _____

Cardiac disease: _____

Hematological disease: _____

DM/HTN: _____

Any other: _____

Study participant No: _____

Case/control: _____

Occupation: _____

Height: _____ cms   Weight: _____ Kg

Examination : _____

_____

Upper GI scopy:

Date: _____

Esophageal varices: _____

Gastric varices: _____

Ultrasound/Doppler abdomen

Liver size: _____

Surface/volume: _____

Portal/hepatic veins: _____

Diagnosis/CTP Score

_____

_____

Samples to be sent

- Blood in citrate(3ml) and EDTA(2ml) tubes for platelet count
- Blood in EDTA tube (4ml)

Study of von Willebrand factor as predictor of in-hospital survival in patients with acute on chronic liver cell failure

Study subject No :-----------------------

Name :-------------------------------------

Hospital number: -----------------------

Contact no :------------------------------

Presenting symptoms: -----------------

Alcoholism –significant/not significant

Clinical findings

Temperature :--------------------------

Heart rate: -----------------------------

Respiratory rate: ----------------------

GCS : -------------------------------------

SBP/MAP : ------------------------------

Mottling /capillary refilling; Yes/No

Inotropes – dopamine/epinephrine/nor epinephrine

Urine output : ------------------------

Comorbidity:

DM/HTN: ------------------------------

Any drugs: ----------------------------

Investigations:

Varices: -------------------------------

Ultrasound : ---------------------------

BBVS; HBsAg / HCV/HIV

IgM-HAV/HEV : ---------------------------------------

ABG- PaO2-----, PaCo2-----, lactates-----,P/F-----

GRBS: --

TLC/BF: ---

Hb : ---

Delta platelet : --

PT/INR : ---------/-------; APTT------------

LFT : ---

Creatinine: --

Cultures: blood/urine/ascetic fluid

MELD : --

CTP : A/ B/ C

Sepsis : Yes/ No

FINAL DIAGNOSIS: --------------------------------------

Etiology: ---

Study of von Willebrand factor as predictor of in-hospital survival in patients with acute on chronic liver cell failure

SOFA score:

At admission; ----------

Score points	1	2	3	4
Respiration				
PaO$_2$/FiO$_2$	<400	<300	<200 with respiratory support	<100 with respiratory support
Cardiovascular				
Hypotension*	MAP <70 mmHg	Dopamine ≤5 or dobutamine in any dose	Dopamine >5 or epinephrine ≤0.1 or norepinephrine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Liver				
Bilirubin mg/dl	1.2–1.9	2.0–5.9	6.0–11.9	>12.0
Renal				
Creatinine mg/dl	1.2–1.9	2.0–3.4	3.5–4.9	5.0
or urine output			or <500ml/2 4h	or <200ml/24 h
Coagulation				
Platelets ×10^3/mm^3	< 150	< 100	< 50	< 25
Central nervous system				
Glasgow Coma Scale	13–14	10–12	6–9	<6

* Adrenergic agents administered for at least 1 h (doses are given in µg/kg/min)

H.no.	manual	coulter	MPV	clumps	manual	coulter	MPV
902036B	268000	238000	8.5	0	242000	186000	8
834880B	220000	183000	8.1	0	204000	163000	7.9
279795F	212000	199000	9	0	191000	159000	8.1
260518F	355000	323000	6.9	0	324000	260000	6.5
264634F	281000	248000	6.9	0	259000	199000	6.4
946978c	192000	184000	9.9	0	126000	129000	9.3
079688d	124000	142000	8.5	0	96000	86000	8.3
503938d	292000	235000	7.9	0	168000	158000	7.5
764511f	151000	132000	11	0	86000	81000	10.1
635724f	236000	247000	8.7	0	181000	199000	8.3
763483c	188000	197000	9.1	0	142000	147000	8.6
553297b	253000	272000	8	0	181000	220000	7.4
685843f	188000	124000	14.2	0	191000	99000	13.3
093089f	312000	337000	7.8	1	204000	244000	7.3
853165c	384000	364000	7.6	0	357000	318000	6.9
450073d	370000	323000	8.4	1	254000	215000	7.8
348715a	290000	271000	7.6	0	253000	227000	7.2
307655d	260000	238000	9.8	0	200000	188000	8.9
080473b	300000	263000	8.6	0	248000	140000	7.8
014010a	290000	262000	8.8	0	260000	211000	8.7
019155c	68000	56000	12.1	0	24000	9000	9.1
244405	56000	47000	11.2	0	21000	15000	8
167857d	91000	66000	8.8	0	66000	53000	7.8
313525b	48000	42000	9.3	0	36000	35000	8.8
788009D	142000	126000	9.9	1	94000	88000	9.1
089521F	54000	43000	9.9	0	48000	33000	8.7
089521f	54000	43000	9.9	0	48000	33000	8.7
712575f	115000	71000	11.7	0	82000	57000	10.5
646927f	112000	99000	9.5	0	68000	57000	8.6
693740f	81000	50000	10.3	0	42000	35000	8.8
692695f	165000	125000	7	0	108000	91000	6.5
699699f	68000	62000	7	0	58000	46000	6.6
699356d	131000	93000	8.9	0	102000	94000	8.3
693226f	153000	121000	9.4	0	146000	92000	8.2
648000f	63000	39000	10.8	1	48000	31000	9.5
639590f	62000	50000	8.3	0	46000	36000	7.5
379830f	68000	56000	9.2	0	43000	39000	8.1
428378f	80000	74000	8.7	1	60000	53000	7.6
607318f	108000	92000	8.6	0	78000	61000	8
702248f	85000	69000	11.5	0	68000	55000	10.4
711056f	64000	42000	10.8	0	39000	28000	8.7
064554f	112000	94000	8.9	0	82000	75000	8.1
835371d	76000	84000	8.8	0	39000	48000	8.3
702105f	120000	130000	8.7	1	72000	99000	8.7
714589f	17000	21000	10.6	0	8000	16000	9
718000f	82000	101000	9	0	65000	67000	8.8

711476f	32000	19000	9.2	0	16000	18000	9.7
385667f	28000	19000	10.4	0	13000	18000	10.6
631215f	62000	38000	11.7	1	58000	29000	11.4
280962F	240000	179000	9.9	1	150000	12000	10
790782F	32000	41000	10.9	0	32000	34000	8.7
394475F	69000	48000	9.9	0	59000	50000	9.6
371462F	108000	96000	7.9	0	94000	90000	7.8
712962F	38000	47000	9.6	0	31000	36000	8.4
212840F	53000	44000	8.6	0	53000	44000	8.6
791656F	70000	82000	11.2	0	76000	70000	10.6
395057D	43000	30000	9	0	48000	26000	8.8
018241F	94000	63000	11.5	1	52000	42000	9.8
301248F	64000	59000	16	0	53000	43000	12.7
492943F	75000	52000	10	0	80000	55000	10.2
607484F	66000	29000	11.1	1	48000	20000	8.9
711476F	32000	19000	9.2	0	16000	18000	9.7
876572C	15000	5000	9.9	0	12000	5000	10.3
003887G	30000	20000	11.4	0	18000	18000	9.6
093892F	39000	15000	11	0	12000	8000	8.7
714416F	90000	107000	10	0	80000	88000	9.1
773439F	70000	45000	10.2	0	50000	41000	9.9
688818D	92000	83000	11.2	0	54000	62000	8.8
607298F	176000	159000	9.4	0	143000	110000	8.5
475356F	228000	172000	12	1	163000	103000	11
659692f	33000	17000	11.8	0	20000	4000	11.7
912383c	99000	85000	11	0	75000	59000	9.8
678972f	138000	113000	8.5	0	142000	88000	8.1
687696F	75000	55000	9.9	1	61000	43000	8.8
674737f	136000	143000	8	0	130000	115000	7.4
697066f	116000	90000	8.2	0	68000	69000	7.4
709572f	79000	58000	8.4	0	56000	44000	7.7
704092f	47000	28000	9.1	0	34000	20000	8.4
725559D	120000	86000	12.5	0	110000	70000	11.9
712821f	56000	41000	9.7	0	52000	33000	8.6
698417f	110000	47000	10.9	0	70000	42000	10.5
704390f	85000	52000	9.8	0	76000	38000	8.7
718088f	64000	60000	10	0	37000	44000	9.2
986857d	42000	49000	11.3	0	36000	41000	11.3
713948f	144000	166000	7.4	0	116000	127000	7.1
184593d	72000	84000	11.3	0	64000	69000	10.2
751084f	194000	153000	10.4	1	186000	114000	10.2
782733f	97000	85000	10.8	0	92000	88000	9.7
729364f	105000	86000	9	0	98000	63000	8.5
753686f	80000	99000	8.9	0	61000	76000	8.3
625442f	62000	44000	11.7	0	46000	35000	10.1
157046f	154000	136000	10.1	1	118000	99000	9.1
139164f	32000	39000	7.9	0	42000	37000	8

945835a	98000	91000	8.2	1	80000	60000	7.9
897252F	85000	70000	10.8	0	65000	50000	9.3
912487F	59000	56000	9.2	0	53000	46000	8.3
751235F	109000	103000	9	0	96000	83000	8.3
249034D	47000	16000	12.6	0	34000	13000	12.7
615107F	44000	38000	10.7	0	27000	25000	9.2
875579F	89000	85000	10.4	0	66000	63000	9.9
418036D	95000	60000	10.7	0	56000	41000	8.8
865156F	48000	22000	9.5	0	39000	20000	8.8
863649F	182000	154000	10	0	152000	147000	9
857190F	138000	126000	8.9	0	94000	79000	8.7
852708F	16000	17000	11.8	0	19000	15000	11
841629F	36000	32000	11.9	0	18000	30000	11.1
854912F	68000	85000	9	0	38000	60000	9
852765F	185000	180000	9	0	166000	159000	8
902911F	41000	34000	7.4	0	26000	21000	5.8
863531D	110000	78000	11	0	84000	64000	10.1
712821f	56000	41000	9.7	0	52000	33000	8.6
841629F	67000	40000	9.9	0	48000	27000	9.9
462820D	40000	32000	10.7	0	33000	27000	9.8
754233F	148000	168000	9.1	1	132000	129000	8.6
872933d	67000	37000	12.4	0	43000	26000	12.6
341343f	37000	32000	9	0	38000	26000	8.4
901002d	158000	125000	8.3	0	102000	98000	8
850023f	26000	14000	11.3	0	18000	8000	9.1
831671f	148000	128000	9.6	0	135000	107000	8.5
400177c	66000	52000	11.6	0	54000	35000	10.9
660437d	48000	48000	9.4	0	42000	35000	8.4
218345F	45000	35000	10.5	0	22000	31000	9.6
000930c	90000	84000	8.8	0	70000	63000	8.2
889741F	135000	129000	10.8	0	110000	96000	8.6
043487G	125000	147000	9.8	0	120000	117000	9.1
035169G	30000	28000	11	0	32000	27000	7.3
920538F	44000	29000	8.6	0	42000	24000	8
021920G	30000	32000	7.3	0	40000	30000	6.7
022382G	15000	7000	12	0	9000	7000	11.4
000827G	15000	9000	10.5	0	7000	9000	9.6
915622F	15000	15000	10	0	4000	6000	8.8
879719F	15000	9000	8.6	0	9000	4000	3.4
686371D	15000	10000	4.8	0	9000	16000	11.2
021539G	75000	67000	8.8	0	62000	60000	7.5
004325G	165000	152000	13.6	0	128000	120000	11.3
016345G	46000	33000	7.6	0	33000	28000	7.1
862676F	80000	4000	10.3	0	6000	4000	8.8
853365F	6000	4000	8.5	0	5000	2000	12.4
037020G	60000	34000	13.1	0	30000	35000	14.9

ID	A	B	C	D	E	F	G
041956G	60000	8000	10.6	0	30000	7000	11.4
035967G	45000	26000	13.3	0	30000	18000	8.6
917994F	15000	7000	8.5	0	15000	2000	11.6
037142G	15000	13000	9.9	0	15000	6000	9.5
038897G	15000	4000	8.4	0	15000	2000	9.5
920047F	15000	14000	8.5	0			
917939F	30000	19000	10.5	0	30000	19000	9.5
041475G	15000	16000	6.1	0	15000	16000	6.8
040913G	15000	1000	12	0	15000	2000	10.4
041081G	15000	14000	9.3	0	15000	5000	11.1
040644G	15000	19000	13.1	0	15000	15000	10
026390G	45000	73000	11.1	0	15000	21000	8.7
027612G	25000	29000	10.6	0	25000	22000	8.4
034874G	78000	63000	10.2	0	14000	22000	8
025104G	23000	40000	8.4	0	14000	22000	8
037502G	10000	8000	7.3	0	90000	11000	6.6
037598G	7000	9000	8.9	0	5000	4000	9.7
917439F	68000	69000	10	0	57000	72000	8.6
923373F	12000	13000	8.5	0	8000	7000	8.7
065033G	14000	17000	10	0	13000	14000	8.2
273580C	12000	11000	13	0	8000	10000	10.2
044061G	12000	19000	12.4	0	8000	10000	9.2
048721G	13000	19000	9.6	0	16000	14000	8
030937G	16000	29000	11.2	0	12000	13000	11.8
718506F	13000	16000	9.6	0	7000	8000	11
010672D	33000	16000	11.7	0	20000	14000	10.8
873369D	5000	4000	12.6	0	5000	3000	11.4
215029M	125000	76000	14.2	0			
344471F	128000	84000	13	0			
985940D	185000	92000	15.8	0			
672929D	153000	91000	13.3	0			
668243D	155000	97000	14.6	0			
028597G	132000	81000	16.2	0			
175809D	128000	70000	14.4	0			
042243G	138000	71000	14.8	0			
991502D	132000	79000	15	0			
036337G	58000	38000	13.5	1			
854954D	96000	58000	13.7	0			
042600G	102000	86000	14.6	0			
044060G	98000	65000	14.5	0			
043059G	103000	51000	13.6	1			
007446G	98000	54000	15.3	0			
044082G	123000	83000	13	0			
045890G	153000	87000	13.1	0			
813361F	128000	64000	13.7	0			
043025G	121000	76000	13.8	1			
914227C	158000	81000	13.6	0			

clumps	PPP serpto	platelet ser	Age	Sex	CTP	GROUP	SPLEEN SIZI
1	11.134	0.774	31	0		1	
0	33.49	1.25	37	0		1	
0	10.436	0.088	29	1		1	
0	6.901	0.505	31	1		1	
0	14.43	7.624	31	1		1	
0			28	0		1	
0			27	0		1	
0			22	0		1	
1			63	1		1	
1			32	1		1	
0			34	0		1	
0			40	1		1	
0			21	1		1	
1			22	1		1	
0			29	0		1	
1			29	0		1	
0			45	0		1	
0			30	1		1	
0			25	1		1	
0			40	0		1	
0	2.227	0.499	60	1	1	2	14.1
0	0.372	0.344	65	0	3	2	13.6
0	0.392	0.16	60	0	1	2	10.5
0	1.875	0.041	36	1	2	2	19.6
1	51.038	2.39	58	1	1	2	11
0			38	1	1	2	17.1
0			38	1	1	2	17
0			42	1	1	2	13.3
0	1.309	1.337	64	1	1	2	11.4
0	1.948	0.167	50	1	3	2	14
0			56	1	1	2	17
0	0.429	0.226	58	1	3	2	15.2
0			47	0	2	2	14.6
0			52	1	1	2	12.8
0			40	1	2	2	23.7
0	0.15	1.698	30	1	1	2	15.4
0			31	1	1	2	16.9
0	0.161	0.251	22	0	1	2	12.3
0	0.106	1.795	25	1	1	2	21.5
0	0.023	0.386	66	0	2	2	16.2
0	0.126	0.188	10	1	1	2	19.5
0	0.081	0.523	48	0	1	2	14.5
0			57	1	3	2	11.6
1			63	1	1	2	19.5
0			19	1	2	2	15.2
0			43	1	2	2	12.2

	0			21	1	1	2	24.6
	0			64	1	3	2	13.8
	1			45	1	1	2	14.5
1				54	1	1	2	12.5
0				46	1	1	2	12
0				20	1	1	2	19.3
0				73	1	1	2	10.8
0				59	1	1	2	18.5
0				20	1	1	2	23
	0			66	0	1	2	12.5
	0			45	1	1	2	23.5
	1			43	1	1	2	15.2
	0			38	0	1	2	16.6
	0			55	1	1	2	20
	1			26	1	1	2	17.4
	0			23	1	1	2	24.6
	0			30	1	1	2	10.9
	0			38	1	1	2	26.5
	0			43	1	1	2	21
	0			62	1	1	2	11.5
	0			50	1	2	2	14.3
	0			50	0	1	2	16.9
	0			51	1	1	2	12.8
	1			36	1	1	2	9.2
	0	0.786	0.103	24	1	1	3	21
	0	0.454	0.267	57	1	1	3	9.9
	0	7.003	3.059	37	1	1	3	15.2
	0	11.317	1.535	33	1	2	3	12.3
	0	0.494	0.062	64	1	3	3	8.9
	0			44	0	1	3	14
	0	0.617	2.138	43	1	3	3	15.2
	0	0.011	0.05	55	0	3	3	16
	0			60	1	1	3	10
	0			46	1	1	3	21.4
	0			29	1	2	3	16.7
	0			37	1	2	3	15.5
	0			56	0	2	3	14.4
	0			40	1	2	3	15
	0			54	1	3	3	11.9
	0			48	1	1	3	12.8
	0			45	1	2	3	14.2
	0			60	1	3	3	13
	0			49	1	2	3	13.3
	0			49	1	2	3	18
	0			54	0	2	3	16
	1			51	1	1	3	10.7
	0			47	0	3	3	12.9

	1			57	0	3	3	13.2
	0			38	1	1	3	13
	0			74	1	3	3	10.6
	0			47	1	1	3	15
	0			52	1	1	3	18.6
	0			57	1	1	3	12.3
	0			38	1	2	3	15.7
	0			48	1	1	3	14.6
	0			41	1	1	3	18
	0			50	1	1	3	9
	0			51	1	1	3	14
	0			41	1	2	3	13
	0			45	1	3	3	12.8
	0			24	1	1	3	14.3
	0			41	1	3	3	12.8
	0			53	0	3	3	13.2
	0	4.472	1.474	23	1	1	3	13.2
	0			46	1	1	3	21.4
0				46	1	1	3	12.7
0				33	1	1	3	20
1				65	1	3	3	10.8
	0	1.779	0.279	38	1	1	4	18.6
	0	3.851	0.257	34	1	1	4	20.9
	0	10.7	0.652	33	0	1	4	14.9
	0			20	0	1	4	20.9
	0			13	1	1	4	14
	0			20	1	1	4	13
	0			18	1	1	4	17.4
	0			20	0	1	4	17
	0			41	0	1	4	14.3
	0			55	1	2	4	13.9
	0			34	1	1	4	17
	0			18	1	1	4	20.2
	0			27	1	1	4	23.5
	0			43	1		5	
	0			7	0		5	
	0			64	1		5	
	0			10	0		5	
	0			14	0		5	
	0			9	0		5	
	0			47	0		5	
	0			30	0		5	
	0			51	1		5	
	0			56	1		5	
	0			24	1		5	
	0			33	1		5	

0	23	1	5
0	18	1	5
0	35	0	5
0	40	0	5
0	23	0	5
	37	0	5
0	27	1	5
0	50	0	5
0	28	0	5
0	59	0	5
0	6	0	5
0	28	1	5
0	16	1	5
0	40	0	5
0	36	1	5
0	69	1	5
0	19	0	5
0	37	1	5
0	38	0	5
0	49	0	5
0	18	0	5
0	18	1	5
0	29	1	5
0	59	1	5
0	38	0	5
0	58	1	5
0	24	0	5
	40	0	6
	68	0	6
	51	1	6
	47	1	6
	61	1	6
	54	1	6
	40	1	6
	22	0	6
	64	1	6
	66	1	6
	66	1	6
	58	1	6
	46	0	6
	58	1	6
	49	1	6
	25	1	6
	37	1	6
	60	1	6
	30	1	6
	28	1	6

HOSP NO	PLATELET COUNT	MPV	IPF	SPLEEN SIZ	GROUP(1-c	AGE	SEX
946690F	176000	12	6.2	13	1	63	0
165928G	87000		6.7	20	1	38	1
945910F	116000	11.4	3.7	14.9	1	36	1
146759D	52000	10.5	2.3	10	4	41	1
163102G	48000	11.6	3	22	1	50	1
947119F	95000		8	12.7	2	54	1
445840F	139000	12.4	4.1	12.9	2	64	1
487116F	129000	10.3	6	14.1	1	27	1
297798B	293000		5.8	25.8	1	47	0
125348D	57000		17.3	18.2	2	57	1
112986F	85000	12.3	4.4	13	1	55	1
074055G	43000		7.8	24.9	1	28	1
857347C	109000		2.9	12	2	62	0
158225G	40000	12.6	2	13.2	1	51	1
945677F	15000		14.1	13.6	2	43	0
932013D	698000	9.7	4.2		1	33	1
945334F	147000	9.9	2.9	12.5	1	81	0
335866F	143000	13.5	10.2	12.1	2	60	1
946321F	116000	11.8	6.7	17.5	1	55	1
945677F	19000		13.7	13.6	2	43	0
942635F	93000	12.3	6.2	15.8	1	24	0
148636G	68000	9.8	4	12	2	63	1
946090F	282000	9.7	2.4	9.6	1	68	0
948001F	127000	10.1	2.9		2	51	1
915029F	102000	11.5	4	12.1	1	26	0
156193G	23000	10.7	7.1	22.4	1	41	0
756752F	31000	9.9	1.2	15	1	54	0
279795F	240000	10.7	4.7		3	30	1
417136A	318000	11.1	4.2		3	30	0
291751B	262000	10.2	1.8		3	40	0
114589F	399000	9.2	1.3		3	24	0
496784D	304000	10.8	6.1		3	32	1
440682C	320000	9.9	3.1		3	27	1
249658A	369000	9.3	0.8		3	33	0
715181D	348000	10	2.7		3	28	0
843237A	291000	9.9	3.2		3	42	0
211606D	334000	10.5	2.7		3	36	1
208797C	344000	10.6	3.1		3	33	0
445175B	379000	10.4	2		3	38	0
268017D	312000	10.7	3		3	23	1
307655D	325000	11.1	4.2		3	28	1
364685F	37000	13.1	6.4	16.9	1	39	1
561488D	242000	11.1	3.7	13.5	1	53	0
607223F	397000	9.1	1.1		3	29	1
625631B	305000	10.5	3.5		3	35	1
816713D	86000	13.7	9.9	12.1	2	49	0

015027G	426000	9.8	1.9		3	29	0
015108G	227000	11.7	5.5		3	27	1
015204G	219000	11.8	4.9		3	29	1
926801F	131000	11.9	2.9	10	2	51	1
005414G	20000		13	21	1	49	0
166723G	5000		2.2		4	23	1
163372G	14000		25.2		5	23	0
506210C	43000		5.6	20.1	2	40	1
715009D	70000	13	8	15.7	2	34	1
167442G	322000	12.3	8.3		1	54	0
024841G	77000	12.5	5.6	12.6	2	50	1
740531F	34000	11.5	5	20.3	1	47	0
775066F	118000		28.3	10	1	54	1
947291F	72000		13.4		5	42	0

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NAME	H.NO	AGE	SEX(M-	CTP	MELD-D1/F/	VWE D1	VWF D3	vWf activity
THIRUMALAI	053449G	45	1	3	17			
RAJA GOPAL REDDY	923890F	49	1	3	29			
MD IBRAHIM	071755G	50	1	3	30			
RAJMANI SINGH	070180G	45	1	3	30			
SURESH CHANDRA SIN	066983G	63	1	3	23			
MANSOOR	926237F	39	1	3	20			
MAHINDRA AGRWAL	646620F	31	1	3	22			
SARAVANAN	926645F	48	1	3	39			
RAMESHWAR ORAN	928179F	45	1	3	27			
LAXMI BALA DAS	928353F	52	0	3	23			
VADIVEL	079658G	38	1	3	35			
SIVAKUMAR	928429F	32	1	3	25			
HIRAK MONDAL	082366G	36	1	3	23			
LAVAKUMAR	928956F	41	1	3	26			
ANIL KUMAR SINGH	929379F	45	1	3	21			
THIRUMALESH	929531F	34	1	3	33			
BALARAMAN	929661F	51	1	3	29			
THOMAS MV	092111G	60	1	3	24			
VENKATAIYA	097517G	30	1	3	36			
LALAN YADAV	925391F	38	1	3	40			
MANJUNATH RAJU	931210F	28	1	3	27			
MD AQBAL	929139F	55	1	3	37			
SACHIN NASKAR	931488F	57	1	3	40			
SYED MARIAM	654424C	30	0	3	26			
RANJITA SHERPA	932212F	41	0	3	42			
DURGA ROY	941455C	43	1	3	26			
MURALIDHAR	327546D	39	1	3	36			
BISHNUKUMAR GUPT/	104833G	46	1	3	20			
BALARAMAN	929661F	51	1	3	31			
SIVAKUMAR	928429F	33	1	3	26			
RAJESH SHARMA	117134G	38	1	3	37			
SARAVANA BALAJI	853822F	35	1	3	49			
SURIYA CHANDRA RAC	753762F	55	1	3	20			
THIRUMURUGAN	937832F	37	1	3	19			
SHANKAR NAIK	939146F	40	1	3	41			
VISHAL SHARMA	939341F	35	1	3	44			
KUBER JAISWAL	497313D	58	1	3	30			
TINKU RAJGADIA	093001G	32	1	3	21			
JAGIR SINGH	725162F	47	1	3	22			
ANANDI	158394g	40	0	3	47			
MANOJ SAHU	157060G	33	1	3	43			
JAYAPAL	717195D	62	1	3	30			
ARUMUGAM	947100F	58	1	3	26			
PRASAD	945910F	36	1	3	22			
DEBDAS ROY	165928G	38	1	3	25			
PRABHAKAR	947211F	31	1	3	39			

RAVI	946610F	43	1	3	34
SELVAM	947428F	46	1	3	26
NAGENDRA PRASAD S	948001F	52	1	3	34
Bhawari devi	948206F	64	0	3	29

vWf activity	ACLF grading	lactate	SOFA	TLC	PLT	HB	INR-D1	INR-F/U
	1	16	6	13500	225000	9.9	1.51	1.68
	1	2.1	8	7000	64000	13	1.74	
	1	2.2	10	12100	30000	10.3	2.03	1.9
	3	1.9	13	12800	35000	8.9	1.82	2.32
	0	1.3	4	6700	195000	6.3	1.7	1.5
	0	3.2	5	23900	240000	10.6	1.52	
	1	2.3	7	26500	122000	8.6	1.5	1.8
	2	4.2	10	5800	59000	8.6	2.19	3.79
	0	1.7	5	40200	119000	10.1	2.43	2.05
	1	1.2	6	8350	69000	11.7	2.1	3.4
	2	3.7	7	15700	164000	10	2.01	2.14
	0	1.9	6	19700	81000	8.7	2.3	
	1	3.5	5	18400	161000	10.7	1.67	
	2	1.2	9	8000	30000	8.2	2.52	
	0	1.7	7	6600	88000	10	1.89	
	1	1.2	9	42400	71000	6.9	2.34	1.83
	1	11	6	14900	90000	6.2	2.44	1.89
	1	1.2	5	13100	103000	14.1	1.9	
	1	1	8	25700	67000	9.1	2.47	
	3	1.4	14	13600	96000	9.5	3.19	
	2	1.3	6	12300	144000	12.4	2.16	
	2	2.4	9	16900	123000	8.5	1.83	
	2	6.5	9	9100	83000	8.3	2.2	
	0	1.7	4	6700	243000	10.7	2.16	
	3	1.9	9	55400	139000	9.5	3.63	1.83
	0	1.4	8	7400	82000	11.7	1.99	
	1	2.7	7	6300	49000	5.3	4.5	2.38
	0	1.5	7	9400	98000	8.7	1.67	
	3	1.4	12	39300	68000	7.1	1.78	
	0	0.9	7	3700	39000	7.2	1.93	
	2	1.7	7	18100	164000	7.7	2.17	
	3	9.8	12	26400	78000	6.7	3.69	1.98
	0	0.8	5	11600	102000	9.5	1.75	
	0	4.3	6	11000	94000	12.1	1.59	
	3	1.6	11	24800	198000	8.3	2.18	
	3	1.2	14	14600	92000	10.1	2.33	1.59
	3	7.2	7	17500	120000	5.3	2.5	
	0	3	6	18500	91000	8.9	1.99	1.68
	0	1.8	8	14600	61000	8.4	1.55	
	2	2	5	13300	353000	9.2	10	
	2	1.8	14	15500	37000	7.9	2.21	
	1	1.5	7	15000	90000	6.7	1.95	
	1	1.9	8	12200	70000	8.2	1.99	
	0	1.2	4	8400	150000	9.5	1.57	
	0	1.3	5	5000	103000	9.8	1.62	
	2	1.3	9	12200	121000	12.3	1.68	

2	1.4	6	39600	167000	8.5	1.97
0	1.2	5	8200	100000	12.2	2.02
2	3.5	7	10500	76000	10.1	2.77
2	1.7	7	13200	65000	13.4	2.42

BILI-D1	BILI-F/U	Cr-D1	Cr - F/U	AST-D1	AST-F/U	ALT-D1	ALT-F/U	ALP
5.3	4.9	1	0.58	75	83	43	62	180
37	36	1.3	3.3	512		102		119
11.4	14	2.05	0.73	40		20		59
21.7	26.7	1.84	0.94	158	117	41	129	255
7.3		1.4		64		17		185
10.8		0.74		83		10		213
21	20	0.5	1.6	168		108		111
25.6		3.7	5.4	66		23		53
11.6		1.23	0.9	55		19		154
8	23	0.93	1	976		333		173
35.7	52	2.2	2.75	154	137	28	37	103
8.4	5.8	1.15	1.6	47		17		110
13	9.6	1.1	2.14	183		54		85
12		0.59		40		15		114
7.2		0.5		74		27		100
17.3	17	1.92	1.21	7		30		158
5		1.98	1.2	48		16		65
15.6		0.67		121		58		152
30.3		1.89		90		29		62
30.7		4.1		130		50		103
23.9		1.03		226		39		149
8.8		4.95		71		30		193
29.4		3.57		701		189		124
21.4		0.81		1485		477		102
20.9	22.57	2.64	0.71	81	99	20	12	113
11.9		1.35		111		18		81
8.8		1.59	0.91	85		30		86
8.2		0.84		153		82		149
7.4		2.87	1.52	70		36		92
5		0.66		37		10		65
29		2.78	2.38	170		14		96
33.4		4.8	1.4	141		24		136
7.4		0.71		98		41		175
7.1		0.86		139		52		166
29.3		4.1		281		68		118
21.8	34.9	5.5	2.4	116	102	18	28	101
5.1		2.1		81		36		44
6.3	8.5	0.56		155		71		124
15.7		0.73		63		26		208
26.8		1.57		317		175		133
21.1		5.5		113		26		83
11.9		2.08		53		18		111
10.8		1.41		126		80		213
17		0.69		55		37		145
31.2		0.9		224		75		102
29.6		4.3		317		46		202

20.6	2.48	77	8	244
22.5	0.8	227	55	135
7.2	2.53	171	23	117
31.5	0.92	95	51	100

ALBUMIN-I	ALB-F/U	SIRS(Y-1/N	ETIOLOGY(ETH	NEW ONSE	NEW ONSE	BLOOD CUl	URINE CUL	SBP
1.7	2.2	1	1	0	0	0	0	0
2.7		0	1	1	0	0	0	0
2.4		1	2	0	1	1	0	0
2.3	2.7	1	1	0	1	0	0	0
2.8		0	1	0	0	0	0	0
2.3		1	1	0	0	0	0	0
2.3		1	1	1	1	0	0	1
2.4		1	1	0	0	0	0	1
2.2		1	1	0	0	0	0	0
2		1	3	0	0	0	1	0
2.2	3.6	1	1	0	0	0	0	0
2		1	1	1	0	1	0	1
2.3		1	1	1	0	0	0	0
2		0	1	0	0	0	1	0
2.1		1	1	0	0	1	0	1
2.6	3	1	1	0	0	0	1	1
1.6		1	1	0	0	0	0	0
2.7		1	3	0	0	0	1	0
2.3		1	1	0	0	0	0	0
2.4		1	1	0	0	0	0	0
2.3		1	2	0	0	0	0	1
1.9		1	1	0	0	0	0	0
2		1	3	0	0	1	0	0
3		0	4	0	0	0	0	0
3.4	3.5	1	1	0	0	0	1	0
2.4		1	1	0	0	0	0	0
1.5		1	1	0	0	0	0	1
1.9		1	2	0	0	0	0	1
2.6		1	1	0	0	1	1	1
2.5		1	1	0	0	0	0	1
1.8		1	1	0	0	0	0	0
2.8		1	1	0	0	0	0	0
2.4		0	1	0	0	0	0	0
3.4		1	1	0	0	0	0	0
2.3		1	1	0	0	0	0	0
2.4	3	1	1	0	0	0	0	0
2.1		1	1	0	0	0	0	0
2		1	1	0	0	0	0	1
2		1	1	0	0	1	0	1
2.7		1	3	1	1	0	0	0
2.5		1	1	1	1	0	0	0
2.1		1	1	0	0	0	0	1
2.1		1	1	1	1	0	0	0
2.8		0	1	0	0	0	1	1
2.8		0	1	0	0	0	0	0
3.3		1	1	0	0	0	0	0

2.3	1	1	0	0	0	0	0
2.1	0	1	0	0	0	0	0
1.8	0	2	0	0	0	0	0
3.3	1	3	0	0	0	0	0

AF CULTURE	HAV	HEV	OGD	SPLEEN SIZE	HSA NO OF	OUTCOME	FFP Y/N(1/0)	REMARKS
0	0	0	0	10.7	1	3	0	
0	0	0	POST EVL U	22	1	2	1	
0	0	0		17.8	1	3	0	
0				16.1	1	1	0	
0	0	0	ESO VX	10	0	3	0	
0	0	0		10	1	3	0	
1	0	0		16.5	1	1	0	
1	0	0		11.8	1	1	1	AFTER 5 DAYS
0	0	1		9.8	0	3	0	
0	0	0		12.4	1	3	1	AFTER 9 DAYS
0	0	0		13	1	1	0	
0	0	0		19.5	1	3	0	
0	0	0		15.7	1	2	0	AKI RESOLVED
0	0	0		18.1	1	2	0	
0	0	0		10.7	1	3	0	
0	0	0		15	1	3	1	FFP GIVEN FOR
0	0	0		12	1	3	1	FFP GIVEN FOR
0	0	0		14.6	0	3	0	
0	0	0			1	1	0	
0	0	0			1	2	0	
0	0	0		11.4	1	3	0	
0	0	0		14.3	1	1	0	
0	0	0			1	1	0	FFP GIVEN
0	0	0		11.9	0	4	6	FFP GIVEN FRO
0	0	0		13.4	1	3	8	RECEIVED FFP
0	0	0		14.3	0	3	0	
1	0	0		16	1	3	8	RECEIVED ON
0	0	0		14.3	1	3	0	10 in second a
0	0	0		12	1	1	6	FROM D1 but
0	0	0		19.5	1	3	0	
0	0	0		14.7	1	2	0	
0	0	0		14.8	1	3	0	
0	0	0		11.4	1	3	4	AFETR 5 DAYS
0	0	0		11.7	0	3	0	
0	0	0			1	1	0	
0	0	1	eso vx	14.7	1	3	0	
0	0	0		10.1	1	1	4	ON DAY OF AD
1	0	0		10	1	3	0	
1	0	0	ESO VX	17.4	1	3	0	
0	0	0		12.5	1	4	0	
0	0	0			1	2	5	SECOND DAY (
0	0	0		20.6	1	3	0	
0	0	0		9.8	1	2	0	
0	0	0		14.9	1	3	0	
0	0	0		20	1	3	0	
0	0	0		16	1	2	0	

0	0	0	14	1	3	0
0	0	0	15	0	3	0
0	0	0		1	2	0
0	0	0	13.2	0	3	0

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NAME	H.NO	AGE	SEX(M-1,F-	CTP	MELD-D1/F	VWE D1	VWF D3	vWf activity
THIRUMAL	053449G	45	1	3	17	212.11	544.63	128.60
RAJA GOPA	923890F	49	1	3	29	684.99	722.62	396.55
MD IBRAHI	071755G	50	1	3	30	1249.44	1411.33	819.54
RAJMANI S	070180G	45	1	3	30	985.85	805.27	736.29
SURESH CH	066983G	63	1	3	23	990.86	1137.89	521.98
MANSOOR	926237F	39	1	3	20	982.52	721.90	477.74
MAHINDRA	646620F	31	1	3	22	955.22	1156.73	835.43
SARAVANA	926645F	48	1	3	39	693.43	748.72	610.57
RAMESHW	928179F	45	1	3	27	739.97	959.34	680.29
LAXMI BAL	928353F	52	0	3	23	259.59	278.80	239.94
VADIVEL	079658G	38	1	3	35	687.09	589.52	590.83
SIVAKUMA	928429F	32	1	3	25	369.18	410.90	281.03
HIRAK MON	082366G	36	1	3	23	1029.68	746.53	810.07
LAVAKUMA	928956F	41	1	3	26	634.46	597.69	478.03
ANIL KUMA	929379F	45	1	3	21	639.51	586.98	97.37
THIRUMAL	929531F	34	1	3	33	779.15	739.93	683.50
BALARAMA	929661F	51	1	3	29	525.67	488.76	468.55
THOMAS N	092111G	60	1	3	24	387.79	412.52	300.00
VENKATAIY	097517G	30	1	3	36	710.60		536.72
LALAN YAD	925391F	38	1	3	40	1200.82		1156.92
MANJUNAT	931210F	28	1	3	27	761.35	730.15	589.31
MD AQBAL	929139F	55	1	3	37	1137.37		1060.04
SACHIN NA	931488F	57	1	3	40	776.00		631.64
SYED MARI	654424C	30	0	3	26	264.07	326.28	119.37
RANJITA SH	932212F	41	0	3	42	750.78	702.15	675.65
DURGA RO	941455C	43	1	3	26	817.88	843.89	667.90
MURALIDH	327546D	39	1	3	36	699.17	558.07	116.17
BISHNUKUI	104833G	46	1	3	20	551.44	582.97	408.16
BALARAMA	929661F	51	1	3	31	742.30	798.64	1089.53
SIVAKUMA	928429F	33	1	3	26			150.03
RAJESH SH	117134G	38	1	3	37	531.77	334.46	532.01
SARAVANA	853822F	35	1	3	49	1013.00	963.35	986.27
SURIYA CH	753762F	55	1	3	20	310.73	301.38	201.29
THIRUMUR	937832F	37	1	3	19	468.19	411.53	490.25
SHANKAR N	939146F	40	1	3	41	1344.39	1053.80	1038.32
VISHAL SH	939341F	35	1	3	44	918.37	1032.85	740.25
KUBER JAIS	497313D	58	1	3	30			778.70
TINKU RAJ	093001G	32	1	3	21	1081.97	959.92	711.60
JAGIR SING	725162F	47	1	3	22	629.51	461.76	492.44
ANANDI	158394g	40	0	3	47	516.98	537.86	220.68
MANOJ SAI	157060G	33	1	3	43	515.49	510.27	395.65
JAYAPAL	717195D	62	1	3	30	515.49	510.27	395.65
ARUMUGA	947100F	58	1	3	26	1346.67	791.59	739.03
PRASAD	945910F	36	1	3	22			526.66
DEBDAS RC	165928G	38	1	3	25	893.24	1005.81	684.41
PRABHAKA	947211F	31	1	3	39			433.95

RAVI	946610F	43	1	3	34	526.68	585.56	269.68
SELVAM	947428F	46	1	3	26	905.10	1169.84	949.11
NAGENDRA	948001F	52	1	3	34	1337.84	997.67	1090.16
Bhawari de	948206F	64	0	3	29	684.58	689.78	467.98

vWf activity	ACLF grading	lactate	SOFA	TLC	PLT	HB	INR-D1	INR-F/U
468.64	1	16	6	13500	225000	9.9	1.51	1.68
243.93	1	2.1	8	7000	64000	13	1.74	
1217.77	1	2.2	10	12100	30000	10.3	2.03	1.9
773.52	3	1.9	13	12800	35000	8.9	1.82	2.32
679.57	0	1.3	4	6700	195000	6.3	1.7	1.5
257.01	0	3.2	5	23900	240000	10.6	1.52	
1048.37	1	2.3	7	26500	122000	8.6	1.5	1.8
633.41	2	4.2	10	5800	59000	8.6	2.19	3.79
1005.12	0	1.7	5	40200	119000	10.1	2.43	2.05
260.22	1	1.2	6	8350	69000	11.7	2.1	3.4
331.70	2	3.7	7	15700	164000	10	2.01	2.14
369.01	0	1.9	6	19700	81000	8.7	2.3	
511.85	1	3.5	5	18400	161000	10.7	1.67	
457.41	2	1.2	9	8000	30000	8.2	2.52	
100.30	0	1.7	7	6600	88000	10	1.89	
525.00	1	1.2	9	42400	71000	6.9	2.34	1.83
471.50	1	11	6	14900	90000	6.2	2.44	1.89
297.19	1	1.2	5	13100	103000	14.1	1.9	
	1	1	8	25700	67000	9.1	2.47	
	3	1.4	14	13600	96000	9.5	3.19	
479.22	2	1.3	6	12300	144000	12.4	2.16	
	2	2.4	9	16900	123000	8.5	1.83	
	2	6.5	9	9100	83000	8.3	2.2	
295.32	0	1.7	4	6700	243000	10.7	2.16	
713.09	3	1.9	9	55400	139000	9.5	3.63	1.83
678.52	0	1.4	8	7400	82000	11.7	1.99	
132.02	1	2.7	7	6300	49000	5.3	4.5	2.38
544.53	0	1.5	7	9400	98000	8.7	1.67	
903.23	3	1.4	12	39300	68000	7.1	1.78	
	0	0.9	7	3700	39000	7.2	1.93	
277.33	2	1.7	7	18100	164000	7.7	2.17	
758.45	3	9.8	12	26400	78000	6.7	3.69	1.98
213.64	0	0.8	5	11600	102000	9.5	1.75	
438.51	0	4.3	6	11000	94000	12.1	1.59	
904.24	3	1.6	11	24800	198000	8.3	2.18	
749.32	3	1.2	14	14600	92000	10.1	2.33	1.59
	3	7.2	7	17500	120000	5.3	2.5	
727.64	0	3	6	18500	91000	8.9	1.99	1.68
224.49	0	1.8	8	14600	61000	8.4	1.55	
280.03	2	2	5	13300	353000	9.2	10	
424.87	2	1.8	14	15500	37000	7.9	2.21	
424.87	1	1.5	7	15000	90000	6.7	1.95	
312.03	1	1.9	8	12200	70000	8.2	1.99	
	0	1.2	4	8400	150000	9.5	1.57	
492.44	0	1.3	5	5000	103000	9.8	1.62	
	2	1.3	9	12200	121000	12.3	1.68	

1304.39	2	1.4	6	39600	167000	8.5	1.97
1246.62	0	1.2	5	8200	100000	12.2	2.02
1133.87	2	3.5	7	10500	76000	10.1	2.77
313.05	2	1.7	7	13200	65000	13.4	2.42

BILI-D1	BILI-F/U	Cr-D1	Cr - F/U	AST-D1	AST-F/U	ALT-D1	ALT-F/U	ALP
5.3	4.9	1	0.58	75	83	43	62	180
37	36	1.3	3.3	512		102		119
11.4	14	2.05	0.73	40		20		59
21.7	26.7	1.84	0.94	158	117	41	129	255
7.3		1.4		64		17		185
10.8		0.74		83		10		213
21	20	0.5	1.6	168		108		111
25.6		3.7	5.4	66		23		53
11.6		1.23	0.9	55		19		154
8	23	0.93	1	976		333		173
35.7	52	2.2	2.75	154	137	28	37	103
8.4	5.8	1.15	1.6	47		17		110
13	9.6	1.1	2.14	183		54		85
12		0.59		40		15		114
7.2		0.5		74		27		100
17.3	17	1.92	1.21	7		30		158
5		1.98	1.2	48		16		65
15.6		0.67		121		58		152
30.3		1.89		90		29		62
30.7		4.1		130		50		103
23.9		1.03		226		39		149
8.8		4.95		71		30		193
29.4		3.57		701		189		124
21.4		0.81		1485		477		102
20.9	22.57	2.64	0.71	81	99	20	12	113
11.9		1.35		111		18		81
8.8		1.59	0.91	85		30		86
8.2		0.84		153		82		149
7.4		2.87	1.52	70		36		92
5		0.66		37		10		65
29		2.78	2.38	170		14		96
33.4		4.8	1.4	141		24		136
7.4		0.71		98		41		175
7.1		0.86		139		52		166
29.3		4.1		281		68		118
21.8	34.9	5.5	2.4	116	102	18	28	101
5.1		2.1		81		36		44
6.3	8.5	0.56		155		71		124
15.7		0.73		63		26		208
26.8		1.57		317		175		133
21.1		5.5		113		26		83
11.9		2.08		53		18		111
10.8		1.41		126		80		213
17		0.69		55		37		145
31.2		0.9		224		75		102
29.6		4.3		317		46		202

20.6	2.48	77	8	244
22.5	0.8	227	55	135
7.2	2.53	171	23	117
31.5	0.92	95	51	100

ALBUMIN-I	ALB-F/U	SIRS(Y-1/N	ETIOLOGY(	NEW ONSE	NEW ONSE	BLOOD CUI	URINE CUL	SBP
1.7	2.2	1	1	0	0	0	0	0
2.7		0	1	1	0	0	0	0
2.4		1	2	0	1	1	0	0
2.3	2.7	1	1	0	1	0	0	0
2.8		0	1	0	0	0	0	0
2.3		1	1	0	0	0	0	0
2.3		1	1	1	1	0	0	1
2.4		1	1	0	0	0	0	1
2.2		1	1	0	0	0	0	0
2		1	3	0	0	0	1	0
2.2	3.6	1	1	0	0	0	0	0
2		1	1	1	0	1	0	1
2.3		1	1	1	0	0	0	0
2		0	1	0	0	0	1	0
2.1		1	1	0	0	1	0	1
2.6	3	1	1	0	0	0	1	1
1.6		1	1	0	0	0	0	0
2.7		1	3	0	0	0	1	0
2.3		1	1	0	0	0	0	0
2.4		1	1	0	0	0	0	0
2.3		1	2	0	0	0	0	1
1.9		1	1	0	0	0	0	0
2		1	3	0	0	1	0	0
3		0	4	0	0	0	0	0
3.4	3.5	1	1	0	0	0	1	0
2.4		1	1	0	0	0	0	0
1.5		1	1	0	0	0	0	1
1.9		1	2	0	0	0	0	1
2.6		1	1	0	0	1	1	1
2.5		1	1	0	0	0	0	1
1.8		1	1	0	0	0	0	0
2.8		1	1	0	0	0	0	0
2.4		0	1	0	0	0	0	0
3.4		1	1	0	0	0	0	0
2.3		1	1	0	0	0	0	0
2.4	3	1	1	0	0	0	0	0
2.1		1	1	0	0	0	0	0
2		1	1	0	0	0	0	1
2		1	1	0	0	1	0	1
2.7		1	3	1	1	0	0	0
2.5		1	1	1	1	0	0	0
2.1		1	1	0	0	0	0	1
2.1		1	1	1	1	0	0	0
2.8		0	1	0	0	0	1	1
2.8		0	1	0	0	0	0	0
3.3		1	1	0	0	0	0	0

2.3	1	1	0	0	0	0	0
2.1	0	1	0	0	0	0	0
1.8	0	2	0	0	0	0	0
3.3	1	3	0	0	0	0	0

AF CULTURE	HAV	HEV	OGD	SPLEEN SIZE	HSA	NO OF OUTCOME	FFP Y/N(1/0)	REMARKS
0	0	0	0	10.7	1	3	0	
0	0	0	POST EVL U	22	1	2	1	
0	0	0		17.8	1	3	0	
0				16.1	1	1	0	
0	0	0	ESO VX	10	0	3	0	
0	0	0		10	1	3	0	
1	0	0		16.5	1	1	0	
1	0	0		11.8	1	1	1	AFTER 5 DA
0	0	1		9.8	0	3	0	
0	0	0		12.4	1	3	1	AFTER 9 DA
0	0	0		13	1	1	0	
0	0	0		19.5	1	3	0	
0	0	0		15.7	1	2	0	AKI RESOLV
0	0	0		18.1	1	2	0	
0	0	0		10.7	1	3	0	
0	0	0		15	1	3	1	FFP GIVEN
0	0	0		12	1	3	1	FFP GIVEN
0	0	0		14.6	0	3	0	
0	0	0			1	1	0	
0	0	0			1	2	0	
0	0	0		11.4	1	3	0	
0	0	0		14.3	1	1	0	
0	0	0			1	1	0	FFP GIVEN
0	0	0		11.9	0	4	6	FFP GIVEN
0	0	0		13.4	1	3	8	RECEIVED F
0	0	0		14.3	0	3	0	
1	0	0		16	1	3	8	RECEIVED C
0	0	0		14.3	1	3	0	10 in secon
0	0	0		12	1	1	6	FROM D1 b
0	0	0		19.5	1	3	0	
0	0	0		14.7	1	2	0	
0	0	0		14.8	1	3	0	
0	0	0		11.4	1	3	4	AFETR 5 DA
0	0	0		11.7	0	3	0	
0	0	0			1	1	0	
0	0	1	eso vx	14.7	1	3	0	
0	0	0		10.1	1	1	4	ON DAY OF
1	0	0		10	1	3	0	
1	0	0	ESO VX	17.4	1	3	0	
0	0	0		12.5	1	4	0	
0	0	0			1	2	5	SECOND DA
0	0	0		20.6	1	3	0	
0	0	0		9.8	1	2	0	
0	0	0		14.9	1	3	0	
0	0	0		20	1	3	0	
0	0	0		16	1	2	0	

0	0	0	14	1	3	0
0	0	0	15	0	3	0
0	0	0		1	2	0
0	0	0	13.2	0	3	0

NO OF DAY
6
3
28
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