

Platelet Count as a Reliable Test in the Prognosis of Chronic Hepatitis C Virus Patients

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Abstract: Hepatitis C virus had been found to be a major cause of chronic liver disease and is usually accompanied with thrombocytopenia which worsen the progression of liver disease. The cause of thrombocytopenia is uncertain therefore ten controls and twenty three hepatitis C virus patients of both sex are participated in the present study to detect antiplatelet antibodies and to assess the severity of hepatic and extrahepatic parameters associated with thrombocytopenia. The patient groups are subdivided according to the platelet count into non thrombocytopenic and thrombocytopenic virus C. The diagnosis of virus C infection was first performed by HCV Ab with ELISA and qualitative polymerase Chain Reaction (PCR), thereafter blood picture, liver function tests, partial thromboplastin time (PTT), lactic dehydrogenase (LDH), catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), interleukine-2 (IL-2), alpha-tumor necrosis factors (α -TNF) and antiplatelet antibodies were determined.

Although there is no detection of the antiplatelet antibodies in all tested groups the results revealed that there is much significant changes in thrombocytopenic groups compared to controls and non thrombocytopenic ones. Therefore we may conclude that platelets exert a role in hepatic and extrahepatic parameters associated with liver damage in hepatitis C virus patients and platelet count can be considered a useful test in monitoring the prognosis of chronic uncomplicated hepatitis C virus.

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1. Introduction:

Viral hepatitis is a systemic infection which predominantly affects the liver. Hepatitis C virus, first cloned in 1989, had been found to be the major cause of chronic liver disease with around 130 millions infected worldwide (Baldo et al, 2008) accounting for an estimated 27% of cirrhosis cases and 25% of primary hepatocellular carcinoma cases (Alter, 2007). Hepatitis C virus is encountered worldwide distribution with relatively high prevalences in Japan, the Southern part of USA, the Mediterranean countries of Europe, Africa and Middle East (McOmish et al., 1994 and Baldo et al., 2008). The incidence of hepatitis C viral infection among adult Egyptians showed wide variation. In some studies Kassab 2003, found that the prevalence of HCV in Big Cairo governorate is 21 %.

Infection with HCV lead to chronicity in 50% to 90% of adult patients despite evidence of active antiviral immunologic response (You and Kathleen, 2002). Chen and Morgan, 2006, stated that although the incidence of hepatitis C virus infection has dramatically decreased during the past decade, the worldwide reservoir of chronically infected persons is estimated at 170 million, 3 % of global population

.The reason for persistence of HCV in most cases of acute infection by hepatitis C virus that progress to chronic infection is uncertain but viral clearance in acute infection has been attributed to the reactivity of cytotoxic T lymphocytes, with an inefficient and poor humoral response to HCV (Bolacchi et al., 2006).

In some patients suffering from chronic hepatitis C virus there is accompanying thrombocytopenia. Thrombocytopenia is one of the most frequent haematological manifestation of HCV infection, which typically worsens with progression of the liver disease and can become a major clinical complication (Aref et al., 2009). The cause of this thrombocytopenia is a matter of dispute whether it is due to liver disease, inflammation or /and it is related to immune mechanisms.

The aim of the present study is to detect the presence of antiplatelets antibodies in male and female hepatitis C virus patients and to assess the severity of hepatic and extrahepatic parameters related to thrombocytopenia in those patients.

2. Subjects and Methods

After their permission Twenty three patients

suffering from chronic hepatitis C virus and ten controls were participated in this study. The patients and the controls were selected from El Sahel Teaching Hospital, Shoubra, North Cairo, Egypt. Prior to the experiment all volunteers were examined ; the controls were clinically and laboratory free, they were five males (41-52 y) and five females (43-58y). As regard patients , they were Bilharzia Ab negative (performed by indirect haemagglutination technique) HBs Ag negative and HCV Ab positive; both virus B and C were detected by ELISA fourth generation produced by Axiom Diagnostic Germany, the infection with virus C was confirmed by qualitative PCR (Roche Diagnostics USA by using COBAS AMPLICOR) . All patients were clinically free from ascites, edema of lower limbs or / and splenomegaly.

After confirming the diagnosis of uncomplicated chronic virus C, the patients and the controls were subjected to:

- Complete blood picture analyzed by automated hematological analyzer called Sysmex K-1000 from Roche Diagnostics (Germany).

- Liver Function Tests: total and direct bilirubin (Spectrum Germany), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (Alk. ph.) (BioSystems Spain), gamma glutamyl transferase (γ -GT) (ProDia International Germany), total protein and albumin (Spectrum Germany), and prothrombin time (PT) (DiaMed, Switzerland).

-Partial thromboplastin time (PTT) (DiaMed, Switzerland).

Lactic dehydrogenase (LDH) (Pro ia International Germany) .

- Malondialdehyde(MDA), superoxide dismutase (SOD) and catalase enzymes(Biodiagnostic, Egypt).

These parameters and the liver function tests were performed by RA-50 chemistry analyzer from Bayer Diagnostics (Ireland) except PT and PTT by Fibrinmeter (Behring).

- Interleukin- 2 and α - tumor necrosis factor by ELISA using kits from Diaclone France.

- Antiplatelet antibodies were detected by indirect immunofluorescence technique.

According to the number of platelets, patients were divided into:

*Non thrombocytopenic virus C (platelet count more than $150 \times 10^3/\mu\text{l}$)

male (n=5) and female (n=5) their ages (45-55y) and (43-54y) respectively.

*Thrombocytopenic virus C (have platelet count less than $150 \times 10^3/\mu\text{l}$)

male (n=8) and female (n=5) their ages (40-60y) and (50-65y) respectively.

Statistical analysis of the results was performed using version 10 SPSS / PC computer programs (SPSS Inc. Chicago). Simple t- test and analysis of variance (ANOVA) for comparison of means and simple correlation was done according to Hirsh and Riegl. All reported P- values were two-tailed, value <0.05 was considered significant.

3. Results

Table (1) analyzed the data of controls, non thrombocytopenic and thrombocytopenic male and female groups. The haemoglobin (HB), red blood corpuscles (RBCs), total leukocytic count (TLC) and absolute number of neutrophils showed significant decrease ($p < 0.05$) in thrombocytopenic groups compared to controls , while there is insignificant changes between non thrombocytopenic virus C groups and both thrombocytopenic virus C and controls. Regarding absolute number of lymphocytes no statistical changes were observed, to the contrary there is significant decrease in platelet count between thrombocytopenic and non

Thrombocytopenic virus C groups and between both and the control one ($P < 0.01$). In female groups the significant changes were much less observed ; no significant changes between the three groups in erythroid parameters.(HB) and (RBCs) , similarly in the (TLC) and lymphocytic count, while the neutrophils and platelets experienced significant increase and decrease respectively in thrombocytopenic group compared to the control one (Table 1) .

Table (2) showed the difference in liver function tests and PTT between controls and two groups of HCV patients. With the exception of total protein, albumin and partial thromboplastin time all liver function parameters tested in this study showed significant increase in thrombocytopenic group compared to controls. The albumin, on the other hand showed significant decrease ($p < 0.01$) and insignificant changes were observed in total protein and partial thromboplastin time. At the same time the liver enzymes ALT, AST and γ -GT together with globulin experienced significant increase in thrombocytopenic group compared to non thrombocytopenic one while significant decrease was detected in albumin parameter. In females, the total and direct bilirubin, ALT, total protein, and PTT were insignificantly changed in the three groups. The non thrombocytopenic group showed significant increase of AST, alkaline phosphatase and γ -GT compared to the controls, while AST and PT showed significant increase in thrombocytopenic group compared to the control. Albumin, globulin showed significant decrease and increase

respectively in the last group compared to the controls ($p < 0.01$).

Table (3) showed the difference in LDH, MDA, SOD, catalase, IL-2 and α -TNF between controls and patients. LDH, MDA and SOD showed insignificant changes in the three groups although these parameters tend to be increased in the third group while both patient groups showed significant decrease in catalase enzyme ($p < 0.01$). α -TNF showed insignificant increase in thrombocytopenic group compared to non thrombocytopenic one, while

IL-2 showed insignificant increase in both patient groups. No significant changes in the immunological parameters (IL- 2 and α - TNF) or LDH in female in the three groups (Table 3). As regards the oxidant antioxidant parameters (SOD, MDA and catalase enzymes) only MDA showed significant increase in both patient groups.

In all groups there is no detection of antiplatelet antibodies by the indirect immunofluorescence technique

Table (1) :Complete Blood Picture in male and female controls and patient groups

Groups Parameters	Controls		Non thrombocytopenic virus C		Thrombocytopenic virus C	
	Male n=5	Female n=5	Male n=5	Female n=5	Male n=8	Female n=5
HB g/dl	a	a	ab	a	b*	a
Mean \pm S.E.	14.68 \pm 0.22	12.30 \pm 0.16	13.10 \pm 0.64	12.52 \pm 0.21	12.89 \pm 0.37	12.86 \pm 0.43
% of change			- 11.0 %	2.0 %	- 12.0 %	5.0 %
RBCs x 10⁶ / μl	a	a	ab	a	b	a
Mean \pm S.E.	5.07 \pm 0.16	4.35 \pm 0.12	4.67 \pm 0.49	4.26 \pm 0.05	4.15 \pm 0.19	4.66 \pm 0.26
% of change			- 8.0 %	- 2.0 %	- 18.0 %	7.0 %
Platelets x 10³ / μl	a	a	b*	b*	c*	c*
Mean \pm S.E.	335.00 \pm 10.35	268.20 \pm 23.37	214.00 \pm 20.05	183.00 \pm 6.47	93.38 \pm 12.40	66.20 \pm 13.62
% of change			- 36.0%	- 32.0 %	- 72.0 %	- 75.0 %
Total leukocytic count x 10³ / μl	a	a	ab	a	b*	a
Mean \pm S.E.	7.12 \pm 0.25	5.20 \pm 0.23	6.26 \pm 1.40	5.42 \pm 1.07	5.08 \pm 0.51	5.95 \pm 0.30
% of change			- 12.0 %	4.0 %	- 29.0 %	14.0 %
Absolute number of lymphocytes x 10³ / μl	a	a	a	a	a*	a
Mean \pm S.E.	2.12 \pm 0.28	1.86 \pm 0.14	1.72 \pm 0.038	1.79 \pm 0.16	1.83 \pm 0.28	1.38 \pm 0.28
% of change			- 19.0 %	- 4.0 %	- 14.0 %	- 26.0 %
Absolute number of neutrophils x 10³ / μl	a	b	a	ab	b	a
Mean \pm S.E.	3.82 \pm 0.52	2.56 \pm 0.31	4.14 \pm 0.99	3.21 \pm 0.94	2.47 \pm 0.30	4.22 \pm 0.40
% of change			9.0%	25.0 %	- 35.0 %	65.0 %

Different superscripts in the same row are significant $P < 0.05$

Same superscripts in the same row are insignificant $P > 0.05$

* $P < 0.01$

Table (2): Liver Function Tests and partial thromboplastin in male and female control and patient groups

Groups Parameters	Controls		Non thrombocytopenic virus C		Thrombocytopenic virus C	
	Male n=5	Female n=5	Male n=5	Female n=5	Male n=8	Female n=5
Total bilirubin mg/dl Mean ± S.E.	b 0.50±0.07	a 0.55±0.02	ab 0.95±0.41	a 0.67±0.06	a* 1.5±0.32	a 1.18±0.26
% of change			90.0 %	22.0 %	202.0 %	115.0 %
Direct bilirubin mg/dl Mean ± S.E.	b 0.15±0.02	a 0.18±0.03	ab 0.42±0.30	a 0.20±0.03	a 0.69±0.18	a 0.46±0.12
% of change			180.0 %	11.0 %	360.0 %	156.0 %
ALT U/L Mean ± S.E.	b 21.60±0.8	a 24.00±3.48	b 25.40±7.34	a 34.40±3.25	a* 69.38±12.82	a 36.00±3.52
% of change			18.0 %	43.0 %	221.0 %	50.0 %
AST U/L Mean ± S.E.	b 25.60±2.4	b 25.40±1.82	b 39.0±17.90	a* 51.40±2.76	a 137.13±39.4	a* 45.80±3.70
% of change			52.0 %	102.0 %	436.0 %	80.0 %
Alk. Phosphatase U/L Mean ± S.E.	b 51.80±14.1	b 52.20±7.70	ab 65.40±14.85	a 91.80±10.84	a 117.75±24.3	ab 74.40±12.89
% of change			27.0 %	76.0 %	127.0 %	43.0 %
GGT U/L Mean ± S.E.	b 30.40±3.27	b 26.60±2.25	b 32.80±3.90	a 52.40±6.81	a* 79.00±13.01	ab 47.60±18.09
% of change			8.0 %	97.0 %	160.0 %	79.0 %
Total protein g/dl Mean ± S.E.	a 7.52±0.16	a 7.30±0.17	a 7.22±0.18	a 7.62±0.27	a 7.81±0.16	a 7.54±0.26
% of change			- 4.0 %	4.0 %	4.0%	3.0 %
Albumin g/dl Mean ± S.E.	a 4.48±0.12	a 4.40±0.04	a 4.14±0.41	ab 4.02±0.16	b* 3.09±0.18	B* 3.30±0.30
% of change			- 8.0 %	- 9.0 %	- 31.0 %	- 25.0 %
Globulin g / dl Mean ± S.E.	b 3.04±0.08	b 2.90±0.20	b 3.08±0.24	ab 3.60±0.26	a* 4.73±0.29	a 4.24±0.36
% of change			1.0 %	24.0 %	56.0 %	46.0 %
Prothrombin time sec Mean ± S.E.	b 14.20±0.06	b 12.88±0.06	ab 16.16±2.37	ab 13.94±0.69	a* 19.48±1.18	a* 18.04±1.35
% of change			14.0 %	8.0%	37.0 %	40.0%
Partial thromboplastin time sec Mean ± S.E.	a 36.94±1.95	a 34.12±0.74	a 37.80±2.34	a 36.28±0.81	a 41.46±5.74	a 41.46±3.31
% of change			2.0 %	6.0 %	12.0 %	22.0 %

Different superscripts in the same row are significant P < 0.05

Same superscripts in the same row are insignificant P > 0.05

* P < 0.01

Table (3): Lactic dehydrogenase, malondialdehyde, superoxide dismutase, catalase, Interleukin- 2 and α -tumor necrosis factor in male and female controls and patient groups.

Group Parameters	Controls		Non thrombocytopenic virus C		Thrombocytopenic virus C	
	Male n=5	Female n=5	Male n=5	Female n=5	Male n=8	Female n=5
Lactic dehydrogenase U / L	a	a	a	a	a	a
Mean \pm S.E.	398.20 \pm 38.9	377.40 \pm 15.47	308.60 \pm 35.54	325.60 \pm 15.5	441.00 \pm 59.5	371.60 \pm 48.71
% of change			-23.0 %	-14.0 %	11.0 %	2.0 %
Malondialdehyde n mole /ml	a	c	a*	b	a*	a*
Mean \pm S.E.	15.42 \pm 3.61	4.58 \pm 0.76	19.06 \pm 2.28	7.54 \pm 0.57	20.06 \pm 2.69	15.40 \pm 2.15
% of change			24.0%	65.0 %	30.0 %	233.0 %
Superoxide dismutase Mean \pm S.E.	a	a	a	a	a	a
	185.95 \pm 4.71	180.40 \pm 19.25	167.50 \pm 57.97	163.43 \pm 7.31	213.29 \pm 26.2	202.54 \pm 34.84
% of change			-10.0 %	-9.0 %	15.0 %	12.0 %
Catalase U/L Mean \pm S.E.	a	a	b*	a	b*	a
	190.67 \pm 13.2	154.82 \pm 14.02	138.45 \pm 5.50	152.58 \pm 17.0	123.46 \pm 15.5	140.83 \pm 12.25
% of change			-27.0 %	-2.0 %	-35.0 %	-9.0 %
Interlukine-2 pg/ml Mean \pm S.E.	a	a	a	a	a	a
	20.10 \pm 2.62	23.80 \pm 4.83	39.00 \pm 9.08	35.00 \pm 3.19	43.75 \pm 12.55	36.80 \pm 8.57
% of change			94.0 %	47.0 %	118.0%	55.0%
α- tumor necrosis factors pg/ml Mean \pm S.E.	a	a	a	a	a	a
	94.40 \pm 11.71	85.00 \pm 13.70	73.80 \pm 3.18	112.30 \pm 6.57	117.81 \pm 12.4	148.00 \pm 20.31
% of change			-22.0 %	32.0 %	25.0 %	74.0 %

Different superscripts in the same raw are significant P < 0.05

Same superscripts in the same raw are insignificant P > 0.05

*P < 0.01

4. Discussion:

Thrombocytopenia is the decrease of platelets less than $150 \times 10^3 / \mu\text{l}$ (Lewis, 2006) therefore the patients of thrombocytopenic groups were chosen according to this reference range. Thrombocytopenic patients showed significant decrease in RBCs, HB, and TLC.

Low platelet count is caused by decreased platelet production, increased platelet sequestration and / or increased peripheral platelet destruction. Decreased platelets production in bone marrow is characterized by decreased or absent megakaryocytes. This condition may occur in cytotoxic chemotherapy, malignant diseases, aplasia, infection, etc (Rinder, 2004). Generally speaking the decrease of megakaryocytes is usually accompanied by decrease in all bone marrow blood elements reflecting on normal other cellular elements in the peripheral blood. Although there is significant decrease in some blood components in male groups the relatively

normal blood picture in the present study exclude decreased platelets production as main cause of thrombocytopenia.

Up to 30 % of circulating platelets are normally contained within the spleen at any given time. Conditions that lead to splenomegaly or hypersplenism cause increased platelet sequestration which is common in advanced liver disease (Rinder, 2004). It seems that increased platelet sequestration due to liver disease plays a role in thrombocytopenia in the present study ; in favour of this view the significant decrease of platelet in the third group (uncomplicated non thrombocytopenic chronic HCV) compared to control .

Increased peripheral platelet destruction caused by immune or non immune mechanisms commonly leads to thrombocytopenia. Autoimmune thrombocytopenia may present as primary immune disorder directed only at platelets or as a secondary complication of another autoimmune disease. The

pathophysiology of immune platelet destruction involves increased levels of polyclonal antiplatelet antibodies in the circulation (Rinder, 2004). In the present study the absence of antiplatelet antibody in the three groups exclude autoimmune platelet destruction as a cause of thrombocytopenia. The present work, however, disagreed with that of Aref. et al., 2009. They detected antiplatelet antibodies in chronic hepatitis C virus patients and concluded that platelet associated glycoprotein specific antibodies represent a common mechanism inducing thrombocytopenia in patients with chronic HCV infection. This contradiction may be due to method of detection and type of the detected antibodies. In their study they evaluated platelet associated glycoprotein specific antibodies by flow cytometry and confirmed by quantitative monoclonal immobilization of platelet antibodies.

Therefore we may conclude that the decrease of platelet count in the present study is most probably due to platelet sequestration as a result of liver disease. Decreased platelet production and increased platelet destruction, if present, had negligible role. Thrombocytopenia, as we noticed, is not only a result of liver disease but also affect many parameters in uncomplicated male and female hepatitis C virus patients. This lead to the suggestion that either thrombocytopenia play a primary role in the prognosis of liver diseases or increase sequestration of platelet affect other hepatic and extrahepatic parameters.

Chronic hepatitis C virus infection has been associated with numerous extrahepatic manifestation (Manns and Rambusch, 1999 and El- Serag et al., 2002). These manifestations can involve multiple organ systems include haematologic system (El-Serag et al., 2002). These haematological manifestations are thought to be induced by the infection of haematopoietic cells with the hepatitis C virus, this may be the cause of thrombocytopenia. In the present study the changes in blood picture especially in male patients point to the suggestion that chronic liver diseases affect haematopoietic cells and agreed with the work of Manns and Rambusch, 1999 and El- Serag et al., 2002.

Concerning the nature of liver enzymes elevated in the present study and the deterioration of liver confirmed by clinical examination it is reasonable to expect increase in their levels. The interesting finding, however, is the significant increase in their value in thrombocytopenic male and female patients; as mentioned earlier in subjects and methods the patients in the present study had the same clinical picture, no ascitis, oedema of lower limbs or splenomegaly and they were divided into two groups according to their platelet count only. Therefore we

can state that there is an intermediate stage in the progression of chronic hepatitis C virus which is clinically undiagnosed but well viewed by the biochemical tests and platelet count. Increase liver toxins, hypersplenism and / or antiplatelet antibodies may be secondary causes for thrombocytopenia in this stage; as regards the first and second causes, there is no concomitant decrease in the other components of the peripheral blood especially in female patients. Moreover the percent of change in platelets is much higher than other elements. Similarly the failure of detection of antiplatelet antibodies in the present study can exclude the third cause. However and irrespective of the cause of thrombocytopenia whether primary or secondary we can state that the presence of thrombocytopenia in early uncomplicated stage of liver hepatitis C affection may worsen the liver progression of liver disease and can be useful test in monitoring liver prognosis.

Patients with mild to moderate liver disease have prolongation of the prothrombin time (PT) and usually normal partial thromboplastin time (PTT) values. Therefore the PT is a sensitive measure of liver function and becomes elevated in patients with even mild liver disorders; this elevation precedes a significant decrease in the albumin or prealbumin levels and is usually coincident with transaminase changes (Rinder, 2004). In the present study the significant decrease of albumin and increase of PT in the thrombocytopenic male and female patients is another guideline to the worst condition of the liver and the association of thrombocytopenia with this progress.

Lactic dehydrogenase (LDH) is an essential enzyme involved in anaerobic glycolysis and is responsible for the anaerobic transformation of pyruvate to lactate. Although LDH is recognized as an enzyme released in liver injury as are AST and ALT it is common to regard monitoring serum LDH as of little value because it is produced in various organs and the specificity for liver disease is low, moreover the production of LDH increases in hypoxic conditions. Another consideration regarding serum LDH in liver disease is its more rapid decline than ALT, because of its shorter half-life in serum. Concerning liver disease, it is well known that dominant elevation of serum

LDH is observed in hypoxic hepatitis caused by shock or heart failure. The elevation of LDH activity has been simply supposed to be enzyme leakage through damaged hepatocyte membranes (Kotoh et al., 2008). Thus, in the present study the insignificant changes of LDH may be attributed to its shorter half-life or / and absence of anaerobic conditions.

Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase were analyzed in the present study to evaluate the oxidant- antioxidant status accompanying viral liver disease. As expected like other diseases there is an oxidative stress manifested in the present study by the non significant increase of MDA with the decrease of catalase whether significant or insignificant. We notice that there is no much difference between the two patient groups in these parameters (MDA, catalase and SOD) compared to that observed in the liver function tests leading to the conclusion that hepatic changes accompanying thrombocytopenia is more vulnerable than extrahepatic ones.

Many authors stated that the severity of hepatitis and the perpetuation of chronic hepatitis C virus infection were related to T-cell activation in the liver and cytokine production by activated T-lymphocytes and liver disease progression is associated with an imbalance between T-helper1 and T-helper2 cytokines (Jacobson and Neuman ,2001 ; Budhu and Wang , 2006 and Ana Teresa et al. , 2007) .

Cytokines are small secreted proteins that regulate immunity, inflammation and haematopoiesis. During viral infection various cytokines play a role both in viral clearance and tissue damage mechanisms. Viruses may interfere with the normal function of this complex cytokine as an escape route to avoid destruction (Larrubia et al., 2009 and Zekri et al., 2010).

In the present study there is insignificant increase of interleukin-2 and α -TNF in both patient groups, according to Larrubia et al. , 2009, when specific immune responses fails to control viral replication non-specific inflammatory infiltrate are recruited into the liver that are responsible for liver damage. The recruitment of persistent mononuclear infiltrates lead to the development of chronic inflammation, which results in sustained liver damage. Finally, chronic inflammation induces regenerating mechanisms in the liver parenchyma. Several factors influence this process, including cytokines such as interleukin-6 and α -TNF.

The present study agrees to some extent with that of Zekri et al., 2010 and other references stated that circulating α -TNF level increases during hepatitis C virus (HCV) infection and is correlated with the severity of hepatic inflammation, fibrosis and tissue injury (Chuang et al., 2004 ; Elsammak et al., 2005 ; Falasca, et al., 2006 and Kamal, et al., 2006) .

From the previous results we may conclude that the severity of liver affection manifested by the deterioration of hepatic and extrahepatic biochemical parameters are closely related to the platelet count to

the extent that we can consider platelet count as a reliable test in the prognosis of liver disease.

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