Age, Platelets Count, Serum Cholesterol and Gamma Glutamyl Transpeptidase, as Non Invasive Markers for Liver Fibrosis in Chronic HCV Patients

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Abstract: The prognosis and management of chronic liver disease greatly depends on the degree and progression of liver fibrosis. Until recently, liver biopsy was the only way to evaluate fibrosis. However liver biopsy is an invasive and painful procedures which has rare but potentially life-threatening complications. Liver histology, especially, fibrosis staging provide prognostic information and may be useful in deciding therapeutic strategies in individual cases. However, liver biopsy may cause undesirable events, such as pain in 20% - 30% of cases, major complications in 0.5% e.g. bleeding and even death. Other than the complication derived from the procedure their is frequent poor patient acceptance also the direct cost of such procedure is high. In this study a total of 101 patients will be studied for their liver biopsy histopathology staging (metavir- staging), for their serum cholesterol, GGT, platelets count and age, and By using a, formula constructed by Forns and colleagues a cutoff value will be determined to discriminate between the significant and non significant fibrosis to avoid unnecessary liver biopsy for HCV patients recommended for interferon therapy. The area under the R O C curve 83.8% sensitivity and 71.4% specificity for the validation group and of 0.751 accuracy, using the best cut off score (5.42) according to the statistical analysis results, presence of significant fibrosis (F2,F3,F4) could be excluded i.e. had high negative predictive value for excluding significant fibrosis, where it has a 66% positive predictive value for diagnosis significant fibrosis i.e. score >5.42., Multivariate analysis identified these factors of the age, gamma glutamyl trans peptidase (GGT), cholesterol and platelets count as an independent predictors of fibrosis.
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1. Introduction:

In our geographic area 15 %-17% of the general population is chronically infected with hepatitis C.(1) Chronic hepatitis C is the most prevalent disease in hepatology clinics,accounting for more than 80% of visits, In general it is accepted that the diagnostic protocol of chronic hepatitis C includes a liver biopsy,particularly in patients with elevated amino transferase levels.(2,5)

Nowadays chronic hepatitis C is often recognized at an early stage of the disease,and liver fibrosis is mild or absent in about 70% of the patients undergoing a liver biopsy.(8), However liver biopsy is an invasive and painful procedures which has rare but potentially life- threatening complication.(2)

The prognosis and management of chronic liver disease greatly depends on the degree and progression of liver fibrosis. Until recently, liver biopsy was the only way to evaluate fibrosis. (1). Liver histology, especially, fibrosis staging provide prognostic information (6, 7) and may be useful in deciding the therapeutic strategies in individual cases (2,3). However, liver biopsy is an invasive procedure that may cause undesirable events, such as pain in 20% to 30% of cases, major complication in 0.5% and even death (8). Other than the complication derived from the procedure such as frequent poor patient acceptance,the direct cost of such procedure is high.(2,3),Thus, many patients are reluctant to undergo liver biopsy,and patients with chronic hepatitis C may be discouraged from starting therapy for this reason.(2).

The accuracy of liver biopsy in assessing fibrosis has also been questioned, especially for sampling errors and intra –and inter observer variability that may lead to over or under staging. (3),even when an experienced physician performs liver biopsy and an expert pathologist reads and interprets finding,up to a 20% error rate in disease staging has been reported.(2,5) Thus, finding of surrogate markers of liver fibrosis /absence of fibrosis would be relevant to reduce the numbers of liver biopsies in patients with chronic hepatitis C.(23)

A variety of surrogate markers of fibrosis have been evaluated for their ability to assess liver fibrosis, primarily in patients with chronic hepatitis C. (23). Other tested parameters include serum hyaluronate.,procollagen III, N-peptide, laminin, type IV collagen matrix, metalloproteinase tissue inhibitory metalloproteinase -1, transforming growth factor –

beta.prothrombin index,platelets count and aspartate aminotransferase (AST), alanin aminotransferase ratio >1, from a clinical standpoint had been used for diagnosis of liver fibrosis (7,9). these markers, when used individually,have limited accuracy for the diagnosis of clinically significant fibrosis i.e. Metavir score F2 or more (the threshold for initiation of antiviral therapy in patients with chronic hepatitis C) (5, 7).

Imbert –Bismuth *et al.* (9) have recently shown that a combination markers of liver fibrosis can be useful to predict the presence or absence of fibrosis and, therefore, to reduce the numbers of liver biopsies in patients with chronic hepatitis C. However,some of these markers,such as alpha 2 macroglobulin's,hapto globulin,or Apo lipoprotein A1 are not routinely used in clinical practice.This is also the case for other proposed predictors for fibrosis,such as hyalo urnidase concentration or III procollagen (10-12).

Forns and colleagues reported a fibrosis index based on Age, Platelet count, Gamma –GT, and Cholesterol levels(6), using variables easily available to clinicians, Forns and colleagues constructed a model and a score system aimed to discriminate patients with substantial fibrosis from those without significant fibrosis. This model may show liver biopsy to be unnecessary in a considerable proportion of patients with chronic hepatitis C.(6)In this study a total of 100 patients were studied for their liver biopsy staging by histopathology (metavir,staging), liver fibrosis was considered significant when it spread out the portal tract (stages 2,3,4,)whereas it was considered non significant when it was restricted to the portal tract (stage 0, or 1,) respectively. (9).and by using GGT, platelets count and cholesterol level and recording their age, and by using a, formula constructed by Forns and colleagues we tried to find a cutoff value to discriminate between significant and non significant fibrosis to avoid unnecessary liver biopsies for HCV patients recommended for interferon therapy. The area under the R O C curve was 83.3% sensitivity and 74.1% specificity for the validation group and of 0.751 accuracy. According to statistical analysis results, using the best cut off score (5.42),presence of significant fibrosis (F2,F3,F4) could be excluded by score below 5.42 with 73% sensitivity i.e. high negative predictive value for excluding significant fibrosis,where it has a 66% positive predictive value for diagnosis significant fibrosis i.e. score >5.42.

2. Patients and Methods

This study includes 101 patients with chronic hepatitis C admitted to the liver unit to undergo liver biopsy between January 2012 and October 2012, clinical,biochemical and hematological data were recorded from each patients at the time of liver biopsy. All patients gave oral informed consent to use these data for scientific purpose and study was approved by hospital ethical committee Patients divided to 70 patients (estimation group),and 30 patients (validation group).Diagnosis of chronic hepatitis C was established by an elevation of alanin aminotransferase ALT on two separate determinations (more than 40 i. u.),a positive third –generation –anti HCV test,and the presence of the HCV –RNA in serum determined by both qualitative (Amplicor HCV 2.0 ROCH Diagnostic, Branchburg NG,USA) and quantitative assay (COBAS Amplicor HCV Monitor 2.0;Roach DIAGNOSTICS, Branchburg, NG).

Exclusion criteria:

Patients with age greater than 65 years,regular alcohol intake,morbid obesity,infection with hepatitis B or HIV virus,Previous interferon treatment,liver transplantation, malignancy and clinical or ultrasonographic evidence of cirrhosis (6).

Histological staging;

Ultrasonogrphic –guided liver biopsy was performed according to a standardized protocol and after patient written informed consent..Specimens were fixed,paraffin –embedded, and stained with hematoxilin and eosin,A minimum of 6 portal tracts in the specimen were required for diagnosis.ALL liver biopsies samples were evaluated by the same histo-pathologist,who was blinded for the clinical data. Liver fibrosis was considered significant when it spread out of the portal tract (stages 2,3,or 4), whereas it was considered absent when restricted to the portal tract (stage 0 or 1,respectively).(9)

Age, sex, ALT, AST, GGT, cholesterol, platelets count, bilirubin, prothrombin time,albumin was recorded as percentage of the daily internal control.

2.1 Statistical analysis

NOTE. Quantitative variables are expressed as median (centile 25; centile 75); categorical variables are expressed as n;(%) there were no significant differences between the estimation and the validation groups in any of the variable.

Data of patients characteristics were statistically described in terms of mean and standard deviation (±SD), chi-square test of significance was used in order compare between two categorical variables. for comparing between two means, t-test of significance was done.When data were not normally distributed, nonparametric Mann-Whitney test was used, probability value, (p value) less than 0.05 was considered significant, the final study includes 100 patients. Predictors of fibrosis, in the estimation group,

all variables were considered as predictors of fibrosis, Five variables were identified as independent predictors of fibrosis.This markers were in a decreasing rank; Age (t =+0.523),Platelets count (t = -0.416), Cholesterol level (t = -0.363), and Formal(t =+0.363), GGT (t = +0.228). A predictive model was constructed by modeling the values of the independent variables and their coefficient of regression.The diagnostic value of the model was assessed by calculating the areas under the receiver operating characteristic (R O C) curves.An area under the curve (A U C) of 1.0 is characteristic of an ideal test,wheals 0.5 indicates a test of no diagnostic value The diagnostic accuracy was calculated sensitivity,specificity, positive and negative predictive values, considering significant fibrosis as the disease.

The diagnostic value of the Forns model in the estimation group by R O C curve, showing an A U C of 0.86.

The score system of the Forns formula is;

7.11- 3.131 (platelet count) + 0.781 (GGT) +3.467 (age) -.014 (cholesterol) =

The statistical analysis of this study determined a cutoff value of 5.42 to identify an absence of significant fibrosis (F0,F1) i.e.(no fibrosis or limited to portal tracts only) to be below 5.42., on the other hand the values more than 5.42 are confined to significant fibrosis i.e. (F2, F3,F4,) (fibrosis extending out of portal tracts,bridging fibrosis), Applying this cutoff value (5.42); a 19 cases were found to have non significant fibrosis,i.e. below 5.42; with percentage of (18.81%) of total 101 cases and of 70.4% predictive value i.e. (high –ve predictive value),and 8 cases of estimation group has significant fibrosis with percentage of 7.92% of total cases. Instead it is found that 62 cases with significant fibrosis,i.e..> 5.42 cutoff value in estimation group from 74 cases of validation group,with 83.8% predictive value i.e. (high +ve predictive value), and with percentage of 61.39% of total cases,and 12 cases with non significant fibrosis in estimation group with percentage of 11.88% of total cases i.e. the patients without significant fibrosis were correctly identified,,More importantly significant fibrosis i.e. >5.42 could be ascertained with high certainty,because from 74 patients in the validation group; it is found that 62 patients (61.39% of total cases) of them has significant fibrosis in estimation group,instead patients who is below the cut off value i.e. Less than 5.42, there is only 12 cases with 16.2% predictive value i.e(. low – ve predictive value), i.e. the cutoff value has a high +ve predictive value for the cases of significant fibrosis above 5.42 cutoff value.and near high –ve predictive value for cases below 5.42 as a cutoff value.

The main end point was identification of absence or presence of significant fibrosis with a combination of clinically relevant variables at the time of biopsy. Most of these variables had been previously identified as potential predictors of fibrosis or the absence thereof (10-12). Age, sex, fasting glucose, ALT, AST, GGT, bilirubin, cholesterol, albumin, leucocytes and platelet counts, and prothrombin time, measured as percentage of the daily internal control.

In the estimation group, univariant analysis identified predictors of fibrosis by using the Fisher exact test for categorical variables and the Students t test for quantitative variables.

Thereafter, all variables were included in a multivariate forward stepwise logistic regression analysis to determine the independent predictors of the absence or presence of significant fibrosis. Significant fibrosis was considered positive results and absence of significant fibrosis as negative result.

F0, and F1,Is considered negative (non – significant –fibrosis or fibrous septa localized to portal tracts),and F2,F3,F4,is considered positive for fibrosis (significant -or fibrous septa is extending outside portal tracts).

The predictive model constructed by Forns and colleagues was constructed by modeling the values of the independent variables and their coefficient of regression.

The diagnostic value of the model was assessed by calculating the areas under the receiver operating characteristic (R O C) curves. An area under the curve (AUC) of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value. The diagnostic accuracy was calculated by sensitivity, specificity, positive and negative predictive values, considering significant fibrosis as the disease., Statistical analysis was performed by (SPSS.2008.SPSS Statistical Software; SPSS corporation).

3. Results

The results are shown in the following Tables and Figures.

Table 1.1 baseline characteristics of the 101 patients with chronic hepatitis c at the time of liver biopsy: comparison between the estimation and the validation groups variable

Characteristics	Estimation group(27)	Validation group (74)	All patients,(101)
Age (y)	39 (33-51)	38 (32-51)	39 (33-51)
Male gender %	17 (64%)	19 (26%)	
AST (IU/L)	57 (41-84)	53 (41-74)	56 (41-84)
ALT (IU/L)	97 (66-149)	90 (64-135)	95 (66-149)
GGT(IU/L)	38 (24-68)	41 (22-68)	38 (24-68)
Bilirubin. (mg/dL)	0.8 (0.7-1)	0.9 (0.7-1)	0.8 (0.7-1)
Glucose. (mg/dL)	92 (86-100)	92 (85-98)	92 (86-100)
Choles. (mg/dL)	173 (150-195)	178 (150-195)	175 (150-195)
Albumin. (g/L)	44 (43-47)	45 (43-47)	45 (43-47)
Leucocytes. (109/L)	6.2 (5-7.5)	6.0 (5.2-7.5)	6.1 (5-7.5)
Platelets. (109/L)	185 (154-215)	188 (161-223)	187 (154-215)
Proth. time.(%)	100 (97-100)	100 (98-100)	100 (97-100)

Table 1.2:predictive value of the model Obtained from the validation group:

ROC curve between fibrosis and formal					
Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
> 5.42	83.8%	71.4%	88.6	62.5	0.751

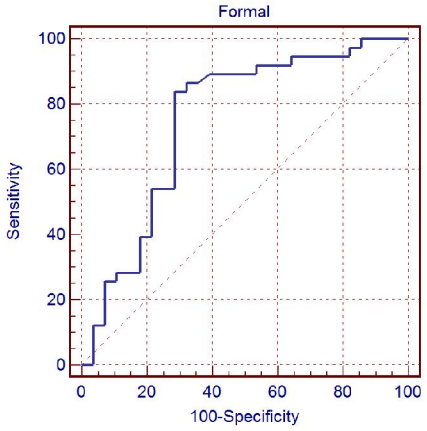

Figure 1

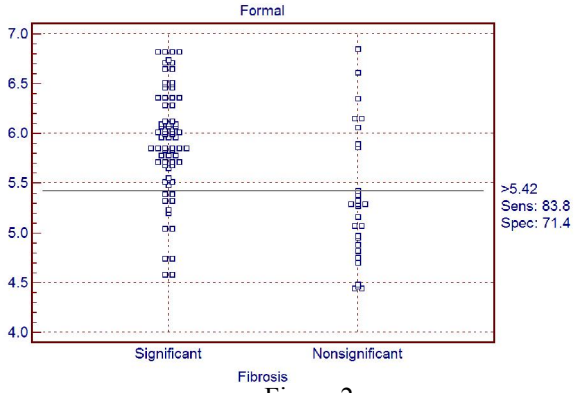

Figure 2

Table 2.1: correlation between fibrosis and other markers:

Fibrosis		
	R	*P*-value
Formal	0.363	<0.001*
Plat.	-0.416	<0.001*
Age	0.523	<0.001*
GGT	0.228	<0.001*
Cho.	-0.363	<0.001*

Table (2.2): comparison between significant fibrosis, non significant fibrosis.concerning all markers:

	Non significant			**Significant**			**T-test or Mann-whitny**	
	Mean	**±**	**SD**	**Mean**	**±**	**SD**	**test value**	***P*-value**
Formal	5.335	±	0.671	5.932	±	0.535	-4.686	0.000
Plat.	271.643	±	76.202	200.027	±	75.278	4.274	0.000
Age	33.071	±	9.067	42.173	±	7.792	-5.041	0.000
GGT	33.643	±	36.995	49.424	±	46.836	-2.273^{M}	0.023
Cho.	184.429	±	47.127	141.933	±	38.989	4.644	0.000

Table (3): multiple logistic regression between fibrosis and other variables

	B	S.E.	Wald	*P*-value	Odd ratio	95.0% C.I. for odd	
						Lower	Upper
Plat.	-0.008	0.004	4.422	0.035	0.992	0.984	0.999
Age	0.087	0.032	7.303	0.007*	1.091	1.024	1.163
GGT	-0.001	0.007	0.024	0.876	0.999	0.986	1.012
Cho.	-0.014	0.007	4.647	0.031*	0.986	0.973	0.999
Constant	1.986	2.022	0.965	0.326	7.285		

Table(4):true +ve and true -ve for cutoff value from formula:

FIB.		Insignificant f. < cutoff	Significant f. >cutoff	Total
Estimation group Validation group	N	19	8	27
	%	18.81%	7.92%	26.73%
	N	12	62	74
	%	11.88%	61.39%	73.27%
Total	N	31	70	101
	%	30.69%	69.31%	100.00%

4. Discussion

Liver biopsy is a valuable tool in assessing the natural history of chronic hepatitis C, particularly, when the data of onset of infection is known or when more than 1 biopsy is available (2-5). Because current antiviral therapy is expensive, has many side effects. Liver biopsy results influence treatment decisions. In fact the presence of mild disease in liver biopsy might permit differing treatments, particularly when viral load, and genotype are predictive of low response probability. On the contrary, the presence of advanced disease might recommended initiating antiviral therapy, even in patients with poor response profile. However, liver biopsy is an invasive procedure with associated morbidity that carries a significant cost. (2, 3,8,)For these reasons, several attempts have been made to find non invasive markers of disease activity and fibrosis. Most of these attempts have focused on the non invasive diagnosis of patients with advanced liver disease bridging fibrosis or cirrhosis. (9,12,16,19). Age and platelets count (16,19), ALT /AST ratio, and prothrombin index (12,16), as well as serum fibrosis markers such as hyaluronate, & 2 macroglobulin's (9,12) appear to identify correctly a significant proportion of HCV – infected patients with advanced hepatic fibrosis; however currently most patients with chronic hepatitis C are diagnosed at an early stage and have only mild lesions in the liver biopsy specimen.(6)

This study is aiming at discriminating patients with and without significant fibrosis with a non invasive method. The model identified around one /third of patients without fibrosis or fibrosis restricted to the portal tract (stage 0 and 1),with a high -ve predictive value. also patients with fibrosis stage 2 and stage 3 (bridging fibrosis),with a high +ve predictive value.

The model consisted of a combination of 4 variables identified by comparative analysis patients with and without significant fibrosis Age, platelets count, GGT and cholesterol level, In this study,A cutoff value(score) below 5.42 identified patients without significant fibrosis and above this level patients with significant fibrosis with 83.4% sensitivity and 71.4 specificity,

In fact, the accuracy of this model resembles other recently proposed models based on more sophisticated models.In study from Ember –Bismuth, the variables proposed are useful tools for research purpose and provide a high levels of certainty of the presence or absence of significant fibrosis,but its usefulness may be curtailed because some of the predictive markers (such as alpa2 macroglobulin's, haptoglobin, or apolipo protein A1) are not used in clinical practice in most centers.

In my serious, significant fibrosis (stages F 2,F3,F4) was present in about 62% percent of patients studied i.e. close to the spectrum of HCV patients at time of starting treatment by interferon. And more than one third has non significant fibrosis i.e., one third of non necessary liver biopsy can be avoided, therefore our model may be particularly useful and cost effective in community –based series of patients with HCV infection.

The value of age as a marker of fibrosis seems obvious as fibrosis progression is time dependant (6).Age at infection also has been shown to influence the outcome of hepatitis C and patients after the fourth decade have a higher risk of rapid disease progression (20).It is evident that duration of HCV would be a more precise indicator of fibrosis of fibrosis than age. However, in this study the mechanism of acquisition of HCV infection remains unknown in around half of the patients (6), and therefore, duration infection of HCV infection is

difficult to establish in many cases. The prognostic value of a low platelets count as a marker of sever fibrosis has been reported (16, 19).Thrombocytopenia in patients with advanced liver disease seems to be related to the development of portal hypertension and the decreased production of thrombopoietin (25).

The relationship between cholesterol levels and the liver fibrosis observed in our study is difficult to explain.The decrease in cholesterol levels seen in patients with advanced cirrhosis is caused by a reduction in cholesterol synthesis and, therefore, it is unlikely that the synthetic capacity of the hepatocytes can be altered before liver cirrhosis is established. However other mechanisms might influence the lipid profile in patients with a persistent infection.In a study by Fabris *et al.* (17)., patients with chronic HCV infection has lower cholesterol levels than patients infected with HBV. The lower cholesterol levels in HCV infected patients were not dependant on age,sex or liver function (13).

The value of GGT as a marker of liver fibrosis already has been described by others. (18) In this study its prognostic value was independent from the bilirubin and trasaminase levels. HCV frequently cause bile duct damage and steatosis (18),Bile duct lesion can partially explain increased GGT values and it appears that patients with bile duct lesions have significantly higher fibrosis scores.In a large study of Italian patients with chronic hepatitis C, Giannini *et al.* (18), showed that in patients with chronic HCV Bile duct damage was present in more than one third of patients with substantial fibrosis (stages 2-4)in comparison with less than 10%in patients with fibrosis stage 0-1. In this study,increased GGT was an independent predictor of bile duct damage.

5. Conclusion

Our results indicates,indicates that patients with chronic hepatitis C without significant fibrosis can be identified with a high accuracy with a combination of a few clinical,biochemical and hematological variables, More importantly,this model might render liver biopsy unnecessary in a significant proportion of the patients with chronic hepatitis C referred to hepatology clinics., Although these results need to be validated by other centers,we believe that this model might be widely used in clinical practice,especially in a community –based approach.

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