ORIGINAL ARTICLE

Prognostic Value of Gc-Globulin in Chinese Patients with Acute-On-Chronic Hepatitis B Liver Failure

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ABSTRACT

Objective: To determine dynamic Gc-globulin level change in Acute-on-Chronic Hepatitis B Liver Failure (ACHBLF) patients, and evaluate the prognostic value of Gc-globulin.

Study Design: An analytical study.

Place and Duration of Study: The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China, from January 2010 to December 2012.

Methodology: A total of 54 consecutive Chinese ACHBLF patients and 30 healthy volunteers as controls were recruited from 2010 to 2012. The patients were divided into improved group and aggravated group. Gc-globulin levels were determined in both groups and mean values compared with significance at $p < 0.05$. Cut-off value was also determined.

Results: The Gc-globulin level was significantly decreased in ACHBLF patients ($p < 0.001$). Gc-globulin levels were significantly higher in improved patients than in aggravated patients, and a 215 mg/L cut-off value carried the best prognostic information. On longitudinal observations, Gc-globulin gradually elevated in improved groups. However, in aggravated groups, the Gc-globulin levels were always below normal levels and no significant change was observed before or after the treatment ($p > 0.05$).

Conclusion: Gc-globulin monitoring offers a rapid and accurate method to estimate treatment outcomes on admission and an effective temporal indicator of curative effects in ACHBLF patients at an optimal cut-off value of 215 mg/L.

Key Words: *Liver failure. Gc-globulin. Prognostic value. Acute on chronic hepatitis B.*

INTRODUCTION

Chronic Hepatitis B (CHB) infection causes significant morbidity and mortality in more than 400 million people worldwide of whom ~75% are Asian.[1,2] In western countries, drugs and/or alcohol are usually the major cause of CHB disease,[3] whereas in China, acute-to-chronic liver failure due to CHB is a leading cause of Hepatitis B-related deaths.[4,5]

Gc-globulin is widely recognized as a multifunctional protein that is a useful prognostic factor of fulminant hepatic failure, acetaminophen (paracetamol) overdose, multiple trauma, Multiple Organ Dysfunction Syndrome (MODS), and sepsis. This is in addition to its role as a carrier of vitamin D.[6,7] The Extracellular Actin Scavenger System (EASS), which is comprised of gelsolin and Gc-globulin, protects against potential deleterious effects of intravascular actin polymerization. Gc-globulin levels in peripheral blood are markedly decreased in inflammatory and necrotic diseases. Previous studies have shown that Gc-globulin monitoring can provide important prognostic information on the severity of liver damage in fulminant and acute liver failure.[8,9] However, none have demonstrated a relationship between serum Gc-globulin levels in Acute-on-Chronic Hepatitis B Liver Failure (ACHBLF) or in patients on admission and for clinical outcomes, or shown the correlation of Gc-globulin level changes during the course of curative therapy.

In order to clarify these relationships, longitudinal observations are necessary; hence, the present study was performed to characterize temporal Gc-globulin concentrations in consecutive ACHBLF patients upon admission to the hospital.

METHODOLOGY

All experimental protocols were approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University, China. Prior to the collection of blood samples, informed consent was obtained from each subject.

A total of consecutive Chinese ACHBLF patients and healthy volunteers as controls were recruited from 2010 to 2012 from the First Affiliated Hospital, College of Medicine, Zhejiang University, China. Based on the changes in the patients' clinical symptoms and laboratory markers, 54 patients were divided into two

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groups: an improved group (n = 28, including those in recovery or experiencing symptomatic relief) and a group who developed progressive liver failure (n = 26, including those who died, underwent liver transplantation).

Plasma was obtained from peripheral blood by centrifugation and stored at -80°C on hospital admission (day 1) and days 7, 14, 21, 28, and 40, during treatment, for Gc-globulin detection using an Enzyme-Linked Immunosorbent Assay (ELISA). The Gc-globulin concentration on day 1 was used as baseline level (pre-treatment) and the concentration on day 40 as the post-treatment level. In patients that died or received a liver transplant, the last acquired concentration of Gc-globulin was used as the post-treatment level.

All measurements of serum Gc-globulin and other indexes were made in duplicate and blinded to the patients' clinical or pre-clinical status. Gc-globulin analysis were performed using a commercially available ELISA kit (NovaTec Immundiagnostica GmbH, Dietzenbach, Germany).

SPSS version 21.0 statistic system was used to analyze the datum. Data was presented as median (minimum - maximum). For variables with non-normal distributions, non-parametric tests (Wilcoxon sign rank test) were used to determine differences between groups. Correlations between the indexes were calculated using the Spearman's rank correlation coefficient. Bivariate correlation analysis based the Spearman's rank test was used to draw matrix scatter plots to obtain the correlation between Gc-globulin and other liver function markers. A p-value < 0.05 was considered statistically significant.

RESULTS

There were 54 patients (median age = 47, range = 22 - 74 years) and 30 controls (median age = 46, range = 20 - 75 years). Serum albumin (ALB; r = -0.022, p = 0.874); alanine transaminase (ALT; r = 0.048, p = 0.730); aspartate aminotransferase (AST; r = -0.098, p = 0.482); total bilirubin (TB; r = 0.083, p = 0.549); TBA (r = 0.030, p = 0.832); and cholinesterase (ChE; r = 0.107, p = 0.443) was not significant.

The serum Gc-globulin levels on day 1 in all 54 ACHBLF patients (median = 282.95, range = 35.40 - 1227.87, mg/L; n = 54) was significantly lower than that in normal controls (median = 533.38, range = 310.41 - 667.07, mg/L, n = 30, Mann-Whitney U test, p < 0.001). Of the 54 ACHBLF patients, 28 were included in the improved group and 26 in the failure group. A comparison to normal controls showed that the Gc-globulin level of all 28 improved cases (median = 400.76, range = 94.33 - 1227.87, mg/L) was lower, the difference was significant (Mann-Whitney U test, p = 0.046). The Gc-globulin concentrations of the 26 failure cases (median = 143.93, range = 35.40 - 364.90, mg/L) were significantly lower than in the normal controls (Mann-Whitney U test, p < 0.001). Likewise, there was a significant difference between the improved and failing groups (Mann-Whitney U test, p < 0.001).

To assess the predictive ability of Gc-globulin, Receiver Operating Characteristic (ROC) curves and area under the ROC curves (AUC) were constructed. As shown in Figure 1, the ROC curve of Gc-globulin, used to evaluate the outcome prognoses, had an area of 0.907. After inspection of the ROC curve for Gc-globulin, a modified ROC curve (Figure 2) was drawn to determine the points with the greatest combined specificity and sensitivity for use as a cut-off value for prognostic validity. We found that a cut-off value of 215 mg/L provided the best discrimination between the improved and failure cases complicated by deterioration, transplantation, or death, and showed the highest Youden's index (0.66).

In the validation set, there was a statistically significant difference between improved and failing cases. We tested the 215-mg/L cut-off level against all 54 patients and found a Positive Predictive Value (PPV) of 87.0% (20/23), a Negative Predictive Value (NPV) of 80.6% (25/31), a sensitivity of 76.9% (20/26), a specificity of 89.3% (25/28), a positive Likelihood Ratio (LR+) of 7.179, and a negative Likelihood Ratio (LR-) of 0.258.

In all patients, the serum Gc-globulin levels from day 1 (pre-treatment) and 40 (post-treatment) were compared. In the improved group, the post-treatment Gc-globulin

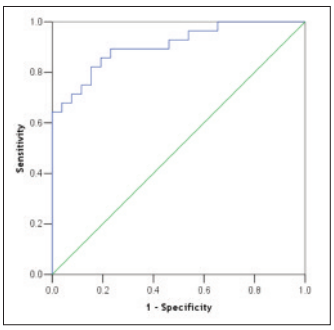

Figure 1: An ROC curve to predict treatment outcome

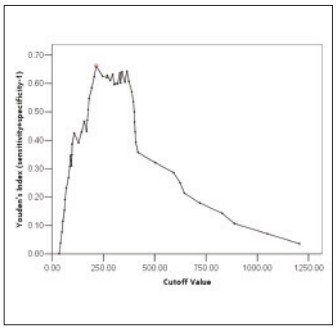

Figure 2: A modified ROC curve to determine the Gc-globulin cut-off value.

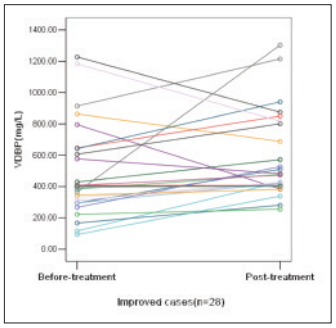

Figure 3A: Comparison of Gc-globulin levels in 28 improved ACHBLF patients before and after treatment.

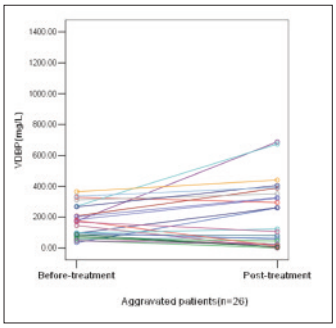

Figure 3B: Comparison of Gc-globulin levels in 26 aggravated ACHBLF patients before and after treatment.

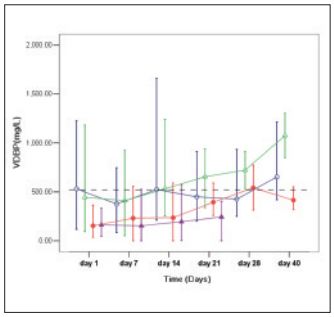

Figure 4: Gc-globulin concentrations in improved and aggravated cases (n = 54) treated with ALSS or medications after admission. The horizontal lines indicate the mean level in 30 healthy volunteers who served as the control group.

level was significantly greater compared to the pre-treatment level (median = 450.65, range = 255.51 - 1303.01, mg/L, vs. median = 400.76, range = 94.33 - 1227.87, mg/L), respectively; Wilcoxon sign rank test, p = 0.034, Figure 3A), but similar to normal controls = 533.38 (range, 310.41-667.07 mg/L; Wilcoxon sign rank test, p = 0.484). In the aggravated cases, the post-treatment Gc-globulin level (median = 114.93, range = 0.44 - 687.88 mg/L) was not significantly different from the pre-treatment level (median = 114.93, range = 0.44 -87.88, vs. 143.93, range = 35.40 - 364.90, mg/L); Wilcoxon sign rank test, (p = 0.328, Figure 3B), and significantly lower than the normal controls (533.38, range = 310.41-667.07, mg/L; Wilcoxon sign rank test, p < 0.001).

In order to compare the predictive effects of Gc-globulin in different treatment groups, the 54 ACHBLF patients were divided into four groups based on the disease outcome and treatment protocol: (1) improved cases that received ALSS treatment (n = 12); (2) improved cases that had not received ALSS treatment (n = 16); (3) failure cases that received ALSS treatment (n = 11); and (4) failure cases that had not received ALSS treatment (n=15). SPSS version 13.0 was used to produce high-low charts to illustrate the distribution of group data to chart the range of data within each group (the minimum, maximum, and average points for different groups temporally, Figure 4).

DISCUSSION

HBV infection continues to be a major public health concern in many Asian countries, including China, which reportedly has > 130 million chronic HBV carriers and roughly 30 million current chronic cases. Acute flare-ups in chronic hepatitis B are common and may be caused by a number of identifiable and potentially treatable factors.[10,11]

Gc-globulin is a multifunctional plasma protein that is primarily synthesized in the liver and has various important functions in addition to its role as a carrier of vitamin D. These include actin-scavenging in EASS, enhancement of complement factor 5 (C5)-mediated signaling,[12,13] and non-specific immune defense functions.[14,15] In addition, Gc-globulin plays an important role in trauma and inflammatory diseases, especially in the pathophysiology of liver injury in ACHBLF patients. Immune-mediated inflammatory responses result in necrosis of liver cells and subsequent release of actin into the systemic circulation after disruption of cell membranes. If actins are not efficiently eliminated, they participate in many protein-protein interactions, including self-association to form Filamentous actin (F-actin), which triggers Disseminated Intravascular Coagulation (DIC) that leads to a condition resembling MODS.[16] Various studies have demonstrated that serum Gc-globulin levels decreased shortly after liver failure, largely because of its increased consumption within the actin-scavenging system.[17] In addition to actin scavenging, Gc-globulin also plays a role in endotoxin transportation. Various experimental liver injuries and patients with liver diseases develop Intestinal Endotoxemia (IETM).[18] Severe IETM often leads to enhanced inflammation following serious hepatocellular necrosis, which may further develop to severe hepatitis or liver failure.[19] Gc-globulin counteracts the pathological consequences of endotoxin, protects hepatic cells from trauma, and improves the condition of the internal environment for the regeneration of hepatic cells.[7,13]

A decrease in Gc-globulin concentration was observed in patients in the aggravated group, in accordance with the hypothesis that hepatic necrosis, actin release, IETM, and subsequent stress on the EASS are characteristic in these patients. These observations were in agreement with the hypothesis that Gc-globulin could prevent DIC or MODS and rejuvenate liver cells. On the other hand, the correlation between Gc-globulin and other indexes of liver function (i.e., TB, ALT, AST, TBA, ChE, and ALB) was much weaker, suggesting that Gc-globulin was not a direct indicator of liver damage.

An ideal predictive tool should discriminate between beneficial and detrimental changes, be readily obtainable after acute exacerbation, be objective, and provide relevant information regarding patho-physiological processes of the disease state. In this study, the serum Gc-globulin level upon admission in improved cases was significantly higher than that in the aggravated cases. At the optimum admission cut-off point of 215 mg/L, the prognoses predicted by the Gc-globulin test were best. In previous studies by Schiodt *et al.*,[20] the admission cut-off level of 100 mg/L for patients with fulminant hepatic failure and 80 mg/L for patients with acute liver failure had the best predictive abilities. Primarily we suggest three explanations for the apparent discrepancy in reported cut-off values, including: (1) the fact that Gc-globulin production and consumption were more varied in patients with liver failure; (2) ethnic differences (Asian vs. European); and/or (3) different testing methods (ELISA vs. rocket immunoelectrophoresis).

In current clinical applications, a key decision is whether a patient should be considered for liver transplantation;

thus, Gc-globulin monitoring as a prognostic indicator in ACHBLF seems to be a feasible and effective marker over a long observational period. The PPV (87.0%) and NPV (80.6%) were most applicable for making clinical decisions. For example, when Gc-globulin was positive (≤ 215 mg/L), the likelihood of correctly predicting an aggravated case was 87.0%, whereas when Gc-globulin was negative (> 215 mg/L), the likelihood of correctly predicting improvement was 80.6%. Under ideal circumstances, both PPV and NPV would reach 100%, but this is essentially unattainable. Because the PPV and NPV are influenced by the choice of cut-off value, a high cut-off level will reduce PPV and increase NPV, so the choice of a cut-off level must be decided through clinical considerations. Once this value is determined, an accurate prediction of survival can be made and will prevent unnecessary transplantations and the consequent risks of surgical complications and death.[21]

During the course of treatment, we found that the Gc-globulin level in improved cases, regardless of the type of therapeutic regimen, was significantly greater than the admission level and similar to or greater than normal levels. In the failure cases, the Gc-globulin levels did not change and were always lower than the normal range. These results indicated that the initial decrease in Gc-globulin levels in the improved cases could be explained by increased consumption. Subsequently, the Gc-globulin level increased by compensatory synthesis, as previously reported in cases of muscle injury or trauma that stimulated Gc-globulin synthesis. In aggravated cases, actin and endotoxins were continuously released at high rates, which resulted in a prolonged load on the EASS or IETM. This was supported by the finding that Gc-globulin concentrations in aggravated and improved cases were inversely correlated. Therefore, the increase in Gc-globulin to supranormal levels reflected improvement of the hepatocytic inner environment, while persistently low Gc-globulin levels in aggravated cases were continuous due to the EASS or/and IETM. Since Gc-globulin is synthesized in the liver, which is one of the key organs in the acute phase response after injury, it is tempting to speculate that the increase in Gc-globulin level was a result of hepatic cytokine stimulation and release as part of the physiological responses to trauma. A possible interaction between IL-6 and Gc-globulin was demonstrated by Guha *et al*. via *in vitro* stimulation of hepatocytes,[22] in which IL-6 increased Gc-globulin mRNA concentrations and a subsequent increase in Gc-globulin secretion. These results indicated that the increase in Gc-globulin levels after the initial reduction was the result of IL-6-stimulated synthesis in the liver. The continuous decrease of Gc-globulin was correlated to the most severe signs of liver dysfunction, such as drug-induced liver failure, fibrogenic liver disease, or hepatocellular carcinoma.

CONCLUSION

The admission levels of serum Gc-globulin supplied information to predict treatment outcomes in both ALSS- and medication-treated cases. The best advantage of Gc-globulin as a prognostic factor was its ability to provide an estimate of the outcome as early as admission. More importantly, Gc-globulin monitoring can track the patient's progress and accurately reflect the patient's status in a timely manner during the course of treatment. Improved predictive outcome markers for ACHBLF will help to improve clinical decisions for patients.

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REFERENCES

1. Lin KW, Kirchner JT. Hepatitis B. *Am Fam Physician* 2004; **69**:75-82.
2. Yuen MF, Yuan HJ, Wong DK, Yuen JC, Wong WM, Chan AO, *et al.* Prognostic determinants for chronic hepatitis B in Asians: therapeutic implications. *Gut* 2005; **54**:1610-4.
3. Seto WK, Lai CL, Yuen MF. Acute-on-chronic liver failure in chronic hepatitis B. *J Gastroenterol Hepatol* 2012; **27**:662-9.
4. Garg H, Sarin SK, Kumar M, Garg V, Sharma BC, Kumar A. Tenofovir improves the outcome in patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure. *Hepatology* 2011; **53**:774-80.
5. Wong VW, Wong GL, Yiu KK, Chim AM, Chu SH, Chan HY, *et al.* Entecavir treatment in patients with severe acute exacerbation of chronic hepatitis B. *J Hepatol* 2011; **54**: 236-42.
6. Lukaszewicz-Zajac M, Mroczko B, Kulakowska A, Szmitkowski M. The significance of Gc-globulin in clinical practice. *Postepy Hig Med Dosw* 2008; **62**:625-31.
7. Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. *Clin Chim Acta* 2006; **372**: 33-42.
8. Schiødt FV, Bangert K, Shakil AO, McCashland T, Murray N, Hay JE, *et al.* Predictive value of actin-free Gc-globulin in acute liver failure. *Liver Transpl* 2007; **13**:1324-9.
9. Schiodt FV, Bondesen S, Petersen I, Dalhoff K, Ott P, Tygstrup N. Admission levels of serum Gc-globulin: predictive value in fulminant hepatic failure. *Hepatology* 1996; **23**:713-8.
10. Massetto B, Menzaghi B, Giambelli C, Antinori S, Milazzo L. The good and evil of flare: flares in hepatitis B virus chronic hepatitis. *Eur J Gastroenterol Hepatol* 2007; **19**:821-3.
11. Perrillo RP. Acute flares in chronic hepatitis B: the natural and unnatural history of an immunologically mediated liver disease. *Gastroenterology* 2001; **120**:1009-22.
12. Antoniades CG1, Berry PA, Bruce M, Cross TJ, Portal AJ, Hussain MJ, *et al.* Actin-free Gc-globulin: a rapidly assessed

biomarker of organ dysfunction in acute liver failure and cirrhosis. *Liver Transpl* 2007; **13**:1254-61.

13. Meier U, Gressner O, Lammert F, Gressner AM. Gc-globulin: roles in response to injury. *Clin Chem* 2006; **52**:1247-53.
14. Antoniades CG, Berry PA, Bruce M, Cross TJ, Portal AJ, Hussain MJ, *et al.* Actin-free Gc-globulin: a rapidly assessed biomarker of organ dysfunction in acute liver failure and cirrhosis. *Liver Transpl* 2007; **13**:1254-61.
15. Hart GR, Furniss JL, Laurie D, Durham SK. Measurement of vitamin D status: background, clinical use, and methodologies. *Clin Lab* 2006; **52**:335-43.
16. Schiodt FV, Ott P, Bondesen S, Tygstrup N. Reduced serum Gc-globulin concentrations in patients with fulminant hepatic failure: association with multiple organ failure. *Crit Care Med* 1997; **25**:1366-70.
17. Jalan R. Gc-globulin to predict outcome in acute liver failure: a panacea? *Liver Transpl* 2005; **11**:1169-71.
18. Nolan JP. The role of intestinal endotoxin in liver injury: a long and evolving history. *Hepatology* 2010; **52**:1829-35.
19. Han DW. Intestinal endotoxemia as a pathogenetic mechanism in liver failure. *World J Gastroenterol* 2002; **8**:961-5.
20. Schiodt FV, Rossaro L, Stravitz RT, Shakil AO, Chung RT, Lee WM, *et al.* Gc-globulin and prognosis in acute liver failure. *Liver Transpl* 2005; **11**:1223-7.
21. Jalan R. Gc-globulin to predict outcome in acute liver failure: a panacea? *Liver Transpl* 2005; **11**:1169-71.
22. Guha C, Osawa M, Werner PA, Galbraith RM, Paddock GV. Regulation of human Gc (vitamin D-binding) protein levels: hormonal and cytokine control of gene expression *in vitro*. *Hepatology* 1995; **21**:1675-81.