Coll. Antropol. **25** (2001) 1: 341–348
UDC 616.36-004:613.81
Original scientific paper

Hepatocyte Growth Factor Levels in Croatian Healthy and Alcoholic Liver Cirrhosis Patients

N. Antoljak[1], E. Topić[1], M. Duvnjak[2], N. Vrkić[1] and I. Žuntar[1]

[1] Clinical Institute of Chemistry, »Sestre milosrdnice« University Hospital, Zagreb, Croatia
[2] Departments of Gastroenterology and Hepatology, »Sestre milosrdnice« University Hospital, Zagreb, Croatia

A B S T R A C T

Hepatocyte growth factor (HGF) is a most potent hepatocyte mitogen, and plays a mayor role in liver regeneration during injury. The aim of this study was to evaluate HGF values in Croatian healthy and alcoholic liver cirrhosis patients (AC). The HGF and standard laboratory tests of liver damage were measured in 33 AC patients, and 41 healthy subjects. HGF was measured by using an ELISA method. The HGF levels were higher in cirrhotic patients than in healthy subjects (median value is 0.78 vs. 0.19 ng/ml, $p < 0.001$). Japanese study showed similar values of HGF for healthy subjects and AC subjects. The HGF values in patients depend on grade of illness. There was found significant correlation between HGF and almost all standard liver damage tests. The ROC analysis showed that measuring of HGF had convincingly best accuracy than other parameters, and seems to be useful in classifying grade of illness.

Introduction

Alcohol consumption causes not only psycho-social problems but also many organ diseases among which liver cirrhosis is most usual[1,2]. One of the latest significant developments has been the purification and characterization of hepatocyte growth factor, a multifunctional cytokine greatly released from Ito, but also from many other cell types, such as Kupffer, endothelial and lung fibroblasts[3]. HGF has morphogenic, mitogenic and angiogenic activities, and one of its fundamental role is effect on liver growth and development[3,4]. HGF is a species non-specific heterodimer with molecular weight of 105 kD and consists of two polypeptide chains, with 38 kD, and with 68 kD, linked together by disulfide bonds[5]. In humans HGF gene is localized on chromosome 7 and composed of 18 exons and 17 introns, spans

Received for publication March 9, 1998.

approximately 70 kb[3]. Receptor for HGF (Met-protein), has the configuration of a type II tyrosine kinase receptor, and consists of an chain (50 kDa) which remains entirely extracellular, and a large polypeptide chain (145 kDa)[6–8]. It is expressed in normal epithelium of almost every tissue and a variety of tumor cell lines. The gene coding receptor is the c-met protooncogen and is also localized on chromosome 7[7]. The regeneration process in the liver causes activation of HGF gene resulted by releasing inactive precursor pro-HGF which is bio-activated by proteolytic digestion[8]. During the liver regeneration increased HGF levels were found in sera of liver disease patients correlating with the severity of liver damage and histological activity index score, so could be a good marker of degree of liver damage[9,10]. The aim of this study was to evaluate HGF values in healthy and alcoholic liver cirrhosis patients in north Croatia as well as to investigate clinical significance of HGF measurement in patients with various degrees of illness.

Materials and Methods

Subjects

Thirty-three patients with clinical and laboratory evidence of alcoholic cirrhosis and 41 sex and age matched healthy subjects were included in the study. Inclusion criteria for patients were: > 80 g of alcohol ingested daily during a period of at least five years, absence of clinical evidence of other diseases or therapy with steroids or cytostatics, and negative hepatitis B and C virus serology. The degree of liver injury was determined according to Child-Turcot classification and patients divided into three groups (Child A, N = 10, and Child B, N = 9, Child C, N = 14). Venous blood-samples for HGF, total bilirubin, AST, ALT, GT, AP, albumin determination and other analyses were measured at the same morning. Serum for determination of HGF levels was stored at –20 °C.

Methods

Total bilirubin, AST, ALT, GT, AP and albumin levels were measured by proposed or reference IFCC method on 30 °C on Synchron CX4, Beckman and Spectrum, Abbott. Serum immunoglobulin levels were measured by electroimmunodiffusion, whereas prothrombin index by the one-stage method (Quick).

The concentration of HGF was measured by ELISA »sandwich« enzyme immunoassay (Otsuka Pharmaceuticals Co, Tokyo, Japan). Briefly, samples and standards dispensed into a 96 wells microtiter plate coated with a monoclonal antibody against human HGF were incubated for 1 h, and washed three times. After washing anti-h-HGF rabbit serum (h-HGF antibody) is added and after incubation washed away. After the addition of anti-rabbit IgG (HRP conjugate anti-rabbit IgG marked by enzyme) the plate was incubated for 1 h, then washed three times. An aliquot (0.1 ml) of 0.25% o-phenylenediamine (OPD) was added as a substrate and the plate allowed to stand for 10 min. After the reaction was stopped by the addition of 0.1 ml of 1 N sulphuric acid, the absorbency was measured at 492 nm by an automated plate reader with a reference wavelength of 690 nm. The detection limit of this assay is 0.1 ng/ml and normal range is less than 0.34 ng/ml[10].

Statistical analysis

Kruskal-Wallis one way analysis of variance on ranks was used to compare the means obtained in four groups. Mann-Whitney test was used for testing differences between whole patient group and healthy subjects. The linear and polynomial regression analysis was used to test correlation between parameters. To test the diagnostic accuracy of each liver func-

tion test and the HGF levels the receiver operator curve analysis (ROC) was done. All statistic analyses were made by the computer program Excel Version 7.0 and SigmaStat. Jandel. Version 2.0.

Results

The medians and range of HGF values in healthy subjects and groups of cirrhotic patients are presented in Figure 1. The HGF levels were significantly increased in the whole group of patients when compared with healthy subjects. The median of whole cirrhotic group was 0.78ng/ml vs. 0.19 ng/ml in healthy subjects ($p < 0.001$, Mann-Whitney test).

Statistically significant differences were also found comparing the median values of HGF in patient s subgroups and healthy subjects: HGF median value was 0.44 ng/ml in Child A, 0.68 ng/ml in Child B and 1.02 ng/ml in Child C vs. 0.19 ng/ml in healthy volunteers, ($p < 0.001$, Kruskal-Wallis test).

There was found statistically significant difference between subgroup s at self, too ($p < 0.001$, Kruskal-Wallis test). The values of other biochemical markers of liver damage in patient group as a whole, and in healthy subjects are presented in Table 1. Albumin were significantly lower

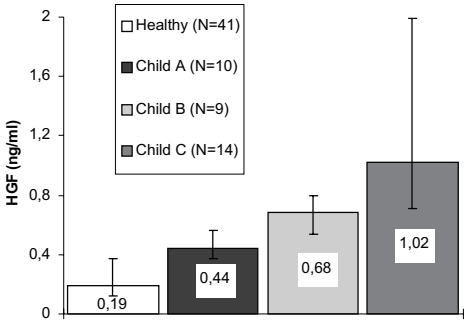

Fig. 1. HGF in healthy subjects and subgroups of patients presented as median and range. Statistically significant difference was found between each subgroup and healthy subjects and between subgroups, too ($p < 0.001$, Kruskal -Wallis test).

in the patients ($p < 0.001$), suggesting a decrease in the liver capacity to synthesize proteins on the one hand, and proving the loss of albumin in the ascitic fluid of all patient groups. The median values of AP, AST, ALT and GT showed significantly higher values in the patients compared to healthy subjects ($p < 0.001$). The values of other biochemical markers of liver damage in patient subgroups and healthy subjects are presented in Table 2. There was found a significant difference between the mean values of the same

TABLE 1
BIOCHEMICAL MARKERS (MEDIAN VALUES) OF LIVER DAMAGE IN ALCOHOLIC CIRRHOSIS PATIENTS AND HEALTHY SUBJECTS

Test	Healthy subjects (N = 41)	Patients (N = 33)
HGF (ng/ml)	0.19	0.78*
Albumin (g/l)	40.4	26.4*
Immunoglobulins (g/l)	19.5	33.4*
Bilirubin (mol/l)	17.0	80.6*
AP (IU/l)	48	81*
AST (IU/l)	11	47*
ALT (IU/l)	13	37*
GT (IU/l)	9	52*

* $p < 0.001$, Mann-Whitney test, patients vs. healthy subjects.

TABLE 2
BIOCHEMICAL MARKERS (MEDIAN VALUES) OF LIVER DAMAGE IN ALCOHOLIC CIRRHOSIS PATIENTS AND HEALTHY SUBJECTS

Test	Healthy subjects (N = 41)	Child A (n = 10)	Child B (N = 9)	Child C (N = 14)
Albumin (g/l)	40.4	30.1*	30.1*	23.3*
Immunoglobulins (g/l)	19.5	23.5*	32.7*	43.5*
Bilirubin (mol/l)	17.0	38.9*	43.0*	92.0*
AP (IU/l)	48	84*	77*	78*
AST (IU/l)	11	24*	50*	52*
ALT (IU/l)	13	41*	30*	38*
GT (IU/l)	9	95*	26*	50*

* $p < 0.001$, difference between each subgroup and healthy subjects, Kruskal-Wallis one-way analysis on ranks.

liver damage tests in the healthy subjects and either study subgroup, $p < 0.001$, Kruskal-Wallis one-way analysis on ranks.

Correlations between the tested parameters

A statistically significant ($p < 0.01$) positive linear correlation was found between HGF levels and AST, ALT, GT, bilirubin, AP, immunoglobulins and IgA of all tested subjects. These correlations and regression lines are shown in Figure 2.

A statistically significant nonlinear correlation with a regression line of the polynomial type was found between HGF levels and serum albumin ($r = –0.79$, $p < 0.01$) (Figure 2). Prothrombine index was measured only in patients and a statistically significant nonlinear correlation with a regression line of the polynomial type was found between HGF levels and prothrombine index in patient group ($r = –0.53$, $p < 0.01$). This correlation and regression line of polynomial type are shown in Figure 2.

ROC analysis

In order to evaluate diagnostic accuracy of performed tests the ROC analysis was done (Figure 3). The area (S) under ROC has been advocated as a measure of diagnostic value of each test. A curve with upward deviation toward the left corner denotes better test. ROC analysis also allows selection of the best cut-off point for each test, i.e. the point yielding the lowest proportion of false negative and false positive results. As shown in Figure 3, HGF is convincingly the best marker of liver lesion, and its diagnostic accuracy is near by 100%. The best cut-off HGF value is 0.37 ng/ml. Another excellent markers are bilirubin, serum albumin and immunoglobulins, whereas AST, ALT and GT have limited value in discrimination between liver cirrhosis patients and healthy subjects. On the contrary, AP is not of choice in testing liver cirrhosis because a low diagnostic accuracy.

Discussion

The main purpose of this study was to evaluate HGF values in healthy and alcoholic liver cirrhosis patients in north Croatia. The median value of HGF in healthy subjects was 0.19 ng/ml (range 0.12–0.38), what is similar to data given by Tsubouchi et al. (0.24 0.12 ng/ml)[10] and Hioki et al. (0.19 0.06 ng/ml)[11]. According to Hillan upper limit for healthy subjects is 0.5 ng/ml[12]. Mean values obtained in Jap-

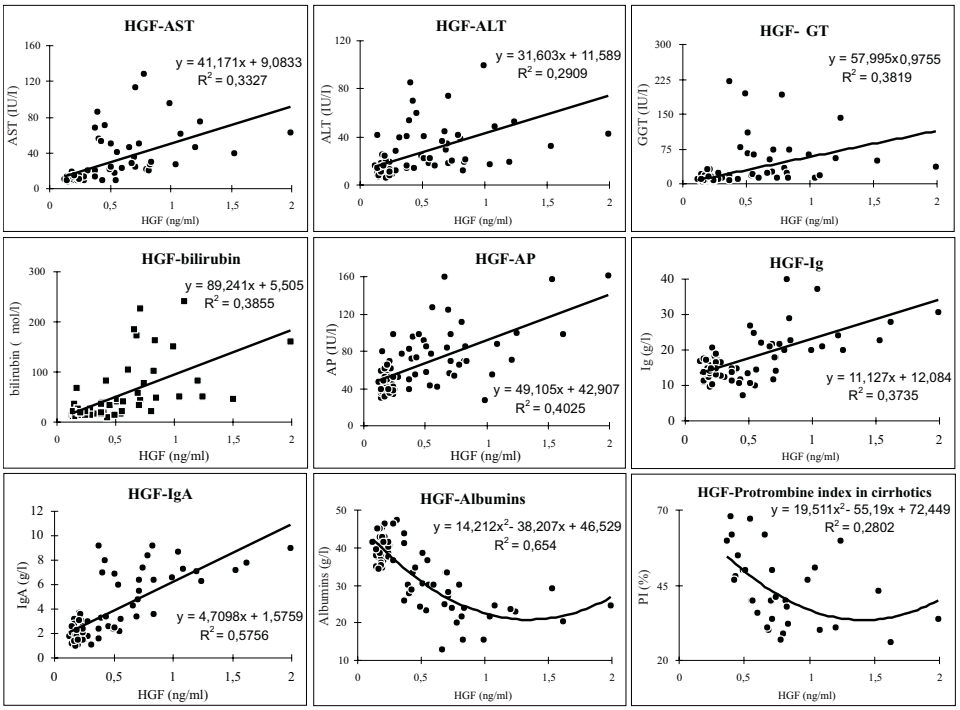

Fig. 2. The correlations between HGF levels and other liver damage tests in subjects presented at xy scatter diagrams with lines of regression and equations, $p < 0.01$.

anese AC subjects were 0.43 0.16 and in another study mean was 0.71 ng/ml[9,11]. In our study HGF median value in patient group was 0.78 ng/ml. Another aim was to investigate clinical significance of measuring HGF levels in alcoholic cirrhotic patients with various degree of liver failure. When we compared HGF levels according to modified Child classification of patients we found higher levels in Child C subgroup than in Child B or Child A, what suggests a correlation between the degree of liver injury and/or regeneration with HGF elevation. It could be a good early marker of hepatocyte regeneration during active phase of liver cirrhosis.

Histologic findings of liver biopsies are used for prognosis in patients with liver disease[13,14]. The degree of HGF elevation is also useful to differentiate fulminant and chronic types of hepatic failure[10]. Shiota also found positive correlation between HGF levels and histological activity index score (HAI), and especially with fibrosis, so HGF elevation may reflect liver necrosis and activity of liver regeneration[9]. In the study done by Hillan et al. authors found positive correlation between HGF levels and hepatocyte proliferation in liver biopsies of alcoholic hepatitis patients[12]. Positive correlation between HGF mRNA levels and indices of hepatocyte proliferation was found in acute and chronic liver disease patients[11]. In another study performed in patients with primary biliary cirrhosis HGF levels were higher than in healthy subjects, but levels did not correlate with indices of

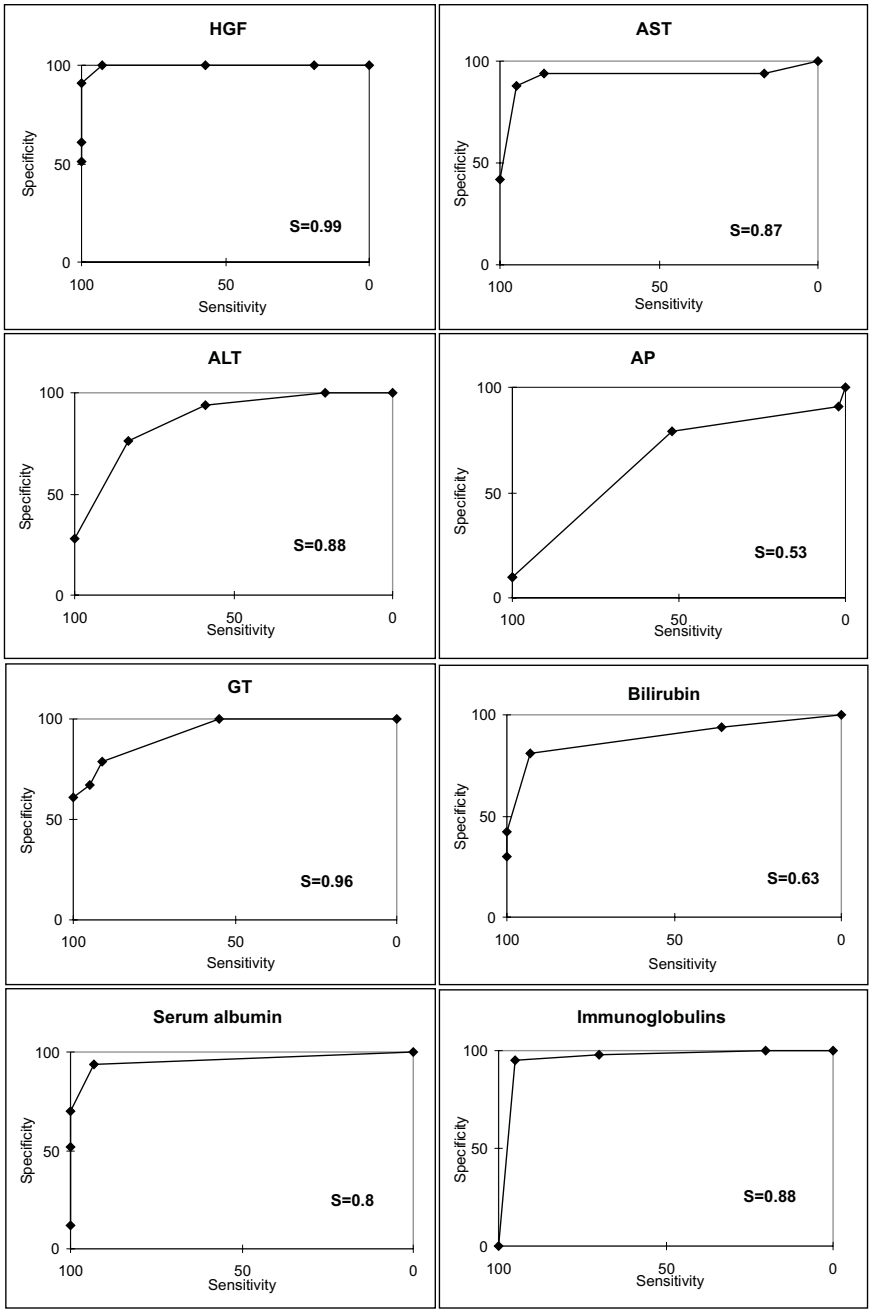

Fig. 3. The ROC analysis for performed tests shows the ability of each test to discriminate liver cirrhosis patients from healthy subjects. S = area under curve which represents ability to discriminate liver cirrhosis patient from healthy subjects.

hepatocyte proliferation[12]. This is caused, probably, by differences in ethiopathogenesis of that kind of cirrhosis. According to results of Appasamy elevation of HGF during liver regeneration is caused by decreased hepatic clearance on the one hand and by enhanced production, mainly in Ito cells, on another hand. In patients with hepato-renal dysfunction it could be a result of decreased renal filtration and elimination[8].

The results of our study also showed that HGF has the best diagnostic accuracy in discriminating healthy from liver cirrhosis patients, than any other standard liver function test, where near by 100% patients had true positive and 100% healthy subjects had true negative HGF levels (best cut-off is 0.37 ng/ml). Other standard liver damage tests like serum albumin, AST, ALT, GT, bilirubin and immunoglobulins, have also high efficacy. The role of elevated IgA is well known in liver cirrhosis patients[15]. On the contrary the AP value is not good marker of that kind of liver disease. Elevated AP in alcoholic liver cirrhosis patients could be a result of other changes such as concomitant biliary stasis in cirrhotic liver. Some authors represented a result of a long-term HGF monitoring in liver diseases. They found that persisting of elevated HGF is a bad prognostic sign and usually is recorded in patients who died within 6 months. Patients who recovered had a slight declination of HGF levels and that can be good prognostic sign[16,17]. We did not monitor HGF level changes during a such period, but six patients in group Child B or C died within 6 months after HGF measurement, while none of patients died in Child A, what also supports thesis that lower HGF levels are good prognostic sign in alcoholic liver cirrhosis. Some authors suggest measuring HGF levels as index of catabolism of patients awaiting liver transplantation[18]. The significance of this growth factor is in patients after hepato-biliary resection[19]. In animal models there was found that HGF prevents fulminant hepatic failure and inhibits massive hepatocyte apoptosis[20]. It is obvious that further investigations of HGF functions are required in liver disease patients. In conclusion, we confirmed that elevated HGF as a result of liver disease seems to be useful in grading alcoholic liver cirrhosis patients as well as in cases when there are some doubts about disease activity.

REFERENCES

1. BREITENFELD, D., B. LANG, V. THALLER, T. BREITENFELD, D. DE SYO, N. JAGETIĆ, Coll. Antropol., 22 (1998) 613. — 2. THALLER, V., D. BULJAN, D. BREITENFELD, S. MARUŠIĆ, T. BREITENFELD, D. DE SYO, Z. ZORIČIĆ, Coll. Antropol., 22 (1998) 603. — 3. MICHALOPOULOS, G. K., R. ZARNEGAR, Hepatology, 15 (1992) 149. — 4. RUHENSTROTH-BAUER, G., M. GOLDBERG, S. VOGL, Nature, 71 (1984) 404. — 5. ZARNEGAR, R., B. PETERSEN, M. C. DEFRANCES, G. MICHLOPOULOS, Genomics, 12 (1992) 147. — 6. BOTTARO, D. P., J. S. RUBIN, D. L. FALETTO, A. M. L. CHAN, T. E. KMECCIK, Science, 251 (1991) 802. — 7. VIGNA, E., L. NALDINI, L. TAMAGNONE, P. LONGATI, A. BARDELLI, F. MAINA, Cell. Mol. Biol., 40 (1994) 597. — 8. APPASAMY, R., M. TANABE, N. MURASE, Lab. Invest., 68 (1993) 270. — 9. SHIOTA, G., J. OKANO, H. KAWASAKI, T. KAWAMOTO, T. NAKAMURA, Hepatology, 21 (1995) 106. — 10. TSUBOUCHI, H., Y. NIITANI, S. HIRONO, H. NAKAYAMA, E. GOHDA, N. ARAKAKI, Hepatology, 13 (1991) 1. — 11. HIOKI, M. O., A. WATANABE, M MINEMURA, T. TSUCHIDA, J. Med., 24 (1993) 35. — 12. HILLAN, K. J., M. C. LOGAN, R. K. FERRIER, G. L. A. BIRD, G. L. BENETT, I. C. MCKAY, R. N. M. MACSWEEN, J. Hepatol., 24 (1996) 385. — 13. ČOLIĆ-CVRLJE, V., S. NAUMOVSKI-MIHALIĆ, M. PRSKALO, A. ČOLIĆ, B. CVJETIČANIN, B. ŠABARIĆ. Coll. Antropol., 24 (2000) 211. — 14. ČOLIĆ-CVRLJE, V., S. NAUMOVSKI-MIHALIĆ, M. PRSKALO, A. ČOLIĆ, B. CVJETIČANIN, B. ŠABARIĆ. Coll. Antropol., 24 (2000) 249. — 15. ULIJASZEK, S., Coll. Antropol., 21 (1997) 3. — 16. MASUHARA, M., M. YASUNAGA, K. TANIGAWA, F. TAMURA, S. YAMASHITA, I. SAKAIDA, K. OKITA, Hepatology, 24 (1996) 323. — 17. RUDI, J., R. WALDEHERR, R. RAEDSCH, W. STREMMEL,

Eur. J. Gastroenterol. Hepatol., 7 (1995) 1081. — 18. MIKI, C., A. D. MAYER, J. A. BUCKELS, K. IRIYAMA, H. SUZUKI, P. McMASTER, Gut, 44 (1999) 862. — 19. KURUMIYA, Y., Y. NIMURA, E. TAKEUCHI, K. NOZAWA, M. NAGINO, A. HAYATA, A. MAEDA, S. YOSHIDA, J. Hepatol., 30 (1999) 22. — 20. KOSAI, K., K. MATSUMOTO, H. FUNAKOSHI, T. NAKAMURA. Hepatology, 30 (1999) 151.

N. Antoljak

Clinical Institute of Chemistry, »Sestre milosrdnice« University Hospital, Vinogradska 29, 10000 Zagreb, Croatia

RAZINE HEPATOCITNOG ČINITELJA RASTA U BOLESNIKA S ALKOHOLNOM CIROZOM JETRE TE U ZDRAVIH ISPITANIKA IZ HRVATSKE

S A Ž E T A K

Hepatocitni činitelj rasta (engl. skraćenica HGF) je najjači mitogen za hepatocite s važnim učinkom za vrijeme regeneracije jetre. Cilj rada je bio određivanje vrijednosti HGF u bolesnika s alkoholnom cirozom jetre (AC) i zdravih ispitanika u sjevernoj Hrvatskoj. HGF i uobičajeni biljezi oštećenja jetre mjereni su u 33 bolesnika i 41 zdravog ispitanika. HGF je mjeren primjenom ELISA testa. Vrijednosti HGF su bile veće u bolesnika nego u zdravih ispitanika (medijan 0.78 u odnosu na 0.19 ng/ml, $p < 0.001$). U radu japanskih autora slične vrijednosti su dobivene za zdrave i bolesne ispitanike. Vrijednosti HGF-a u bolesnika su ovisne o težini bolesti. Dobivena je značajna korelacija između HGF i gotovo svih standardnih biljega. Prema rezultatima ROC analize HGF ima uvjerljivo najbolju kliničku valjanost u usporedbi s ostalim pokazateljima, stoga se čini pogodnim biljegom za određivanje stupnja bolesti.