Clinical impact of serum transforming growth factor- α mRNA as a predictive biomarker for the prognosis of fulminant hepatitis

Norimasa Miura · Hiroko Kabashima · Mika Shimizu · Reina Sato ·
Tomoe Tsukamoto · Tomomi Harada · Shunsaku Takahashi ·
Ryujin Endo · Nobuaki Nakayama · Yasuhiro Takikawa · Satoshi Mochida ·
Kazuyuki Suzuki · Junichi Hasegawa · Goshi Shiota

Received: 8 August 2007 / Accepted: 23 January 2008 / Published online: 9 April 2008
© Asian Pacific Association for the Study of the Liver 2008

Abstract

Purpose We previously reported that measuring serum telomerase reverse transcriptase (hTERT) mRNA with a quantitative, one-step, real-time RT-PCR was superior to conventional tumor markers for hepatocellular carcinoma and lung cancer. Here, we examined serum regeneration-related mRNA detection as a biomarker for fulminant hepatitis (FH).

Methods In 53 patients, including 17 patients with acute hepatitis (AH), seven with severe hepatitis (SH), four with late-onset hepatic failure (LOHF), and 25 with FH, we measured serum mRNA levels of hTERT, hepatocyte growth factor (HGF), hepatocyte growth factor receptor (c-met), epidermal growth factor receptor

(EGFR), and transforming growth factor- α (TGF- α). We examined the sensitivity and specificity of the technique in FH diagnosis as well as its clinical and prognostic significance compared with other clinical and prognostic tests.

Results Serum copy number of TGF- α mRNA in FH on admission was significantly smaller than in AH and SH. In FH, TGF- α mRNA level was 10^6 -fold higher in survivors than in patients who died or received liver transplants ($P = 0.034$), although these patients were not discriminated by other clinical parameters. The sensitivity/specificity for prognosis in FH was 74.3/65.5% for TGF- α mRNA. Of four prognostic scoring systems, only logit- λ was useful for prognosis assessment.

Conclusions TGF- α mRNA is an early predictor of FH outcome and a sensitive biomarker of lower regenerative liver capacity. This assay could help facilitate early therapy choice, such as liver transplantation.

N. Miura (✉) · H. Kabashima · M. Shimizu · R. Sato ·
T. Tsukamoto · T. Harada · S. Takahashi · J. Hasegawa
Division of Pharmacotherapeutics, Department of
Pathophysiological and Therapeutic Science, Faculty of
Medicine, Tottori University, Nishicho 86, Yonago,
Tottori 683-8503, Japan
e-mail: mnmiura@grape.med.tottori-u.ac.jp

R. Endo · Y. Takikawa · K. Suzuki
First Department of Internal Medicine, School of Medicine,
Iwate Medical School, Uchimaru 19-1, Morioka,
Iwate 020-8505, Japan

N. Nakayama · S. Mochida
Division of Gastroenterology and Hepatology, School of
Medicine, Saitama Medical University Hospital, 38 Morohongo,
Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan

G. Shiota
Division of Molecular and Genetic Medicine, Department of
Genetic Medicine and Regenerative Therapeutics, Tottori
University, Nishicho 86, Yonago, Tottori 683-8503, Japan

Keywords Fulminant hepatitis · Biomarker · TGF- α · mRNA · Diagnosis

Abbreviations

hTERT	Human telomerase reverse transcriptase protein
EGFR	Epidermal growth factor receptor
TGF- α	Transforming growth factor- α
HGF	Hepatocytes growth factor
FH	Fulminant hepatitis
FHF	Fulminant hepatitis failure
SAH	Severe acute hepatitis
AH	Acute hepatitis
LOFH	Late-onset failure of hepatitis
MELD	The Mayo End-Stage Liver Disease
KCC	King's College (Hospital) Criteria
JARFSG	Japanese Acute Liver Failure Study Group

Introduction

Fulminant hepatitis (FH) affects approximately 1,000 patients in Japan per year [1]. The survival rate of FH patients treated conservatively is approximately 30% [1], but liver transplantation can rescue approximately 70% of patients, even with subacute FH [2]. FH is a potentially reversible condition if diagnosed early, and early diagnosis in patients with acute hepatitis (AH) and severe acute hepatitis (SAH) can prevent the development of hepatic encephalopathy [3, 4]. Liver transplant should be considered early when common therapies, including artificial liver support, plasma exchange, and hemodiafiltration, are ineffective.

Regeneration after liver injury involves many cytokines and apoptotic factors, including hepatocyte mitogens isolated from primary cultures [5–7]. Transforming growth factor alpha (TGF- α) and hepatocyte growth factor (HGF) act as autocrine and paracrine hepatocyte mitogens, both in vitro and in vivo [8–13]. TGF- α binds to the epidermal growth factor receptor (EGFR) on the cell surface, and HGF stimulates hepatocyte proliferation via the c-Met receptor [14–20].

Human telomerase reverse transcriptase (hTERT) mRNA is detected in the serum of 40% of breast cancer patients, but not in normal controls or in two patients with benign breast disease [21]. Cell-free circulating hTERT mRNA in serum or plasma can be detected in significant numbers of patients with malignancies [22, 23] and may be a suitable diagnostic for lung cancer. We have reported a real-time, one-step RT-PCR method using RNA extracted from patient serum [24–26] that improved hepatocellular carcinoma (HCC) diagnosis compared with conventional tumor markers. There is no evidence for the specificity of a commercial antibody, thus suggesting that we face to the limitation of protein-based assay. The assay would be particularly useful for genes with low protein expression or secreted proteins, as well as for diagnosis of rapidly changing conditions such as inflammatory diseases.

Here, we found that TGF- α mRNA is a particularly good biomarker for predicting FH prognosis.

Materials and methods

Patients and sample collection

Fifty-three consecutive patients with acute liver failure (17 with AH, seven with SAH, 25 with FH, and four with late-onset failure of hepatitis (LOFH)) who were admitted to Tottori University hospital, Iwate Medical University Hospital, and Saitama Medical University Hospital,

between July 2003 and December 2005, were enrolled in this study (Table 1). The mean patient age was 57 years (range: 18–89). The diagnostic criteria of acute liver failure followed our previous reports [27–29]. AH was diagnosed if the onset of hepatitis was acute without a history of liver diseases. SAH was diagnosed if prothrombin time was less than 40% of normal individuals. FH was diagnosed if hepatic encephalopathy was more than grade II and prothrombin time was less than 40% within 8 weeks of onset. Acute or subacute FH were diagnosed if the onset of hepatic encephalopathy was within 10 days after the onset of illness or after 11 days, respectively. LOHF was diagnosed as encephalopathy more than 8 weeks and up to 24 weeks after the first symptom.

Clinicopathological findings such as age, etiology, diagnosis, prognosis, ascites, and encephalopathy were recorded. Twenty-two healthy individuals, including 11 females (from 22 to 72 years old: mean age 44 years), served as the controls. We quantified mRNA expression for hTERT, c-met, HGF, EGFR, and TGF- α expression in serum. Plasma exchange (PE) was performed in all the FH patients and some patients underwent liver transplantation. Informed consent was obtained from each patient and the study protocols followed the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the human research committee of Tottori University (approval No. 138, No. 138-1, 2001). The processing of blood and serum samples were performed as previously described [24]. Blood sampling was performed before plasma exchange and after the onset of hepatic encephalopathy.

RNA extraction and real-time quantitative RT-PCR

RNA from serum was extracted after DNase treatment as reported previously [24–26]. In brief, RNA from 200 μ l of serum was dissolved in 200 μ l of H₂O. Quantitative RT-PCR was performed by using 1 μ l of RNA extract and 2 μ l of SYBR Green I (Roche, Basel, Switzerland) in a one-step RT-PCR kit (Qiagen, Tokyo, Japan). RNA was extracted from HCC tissues by using the same volume of serum dried up to 20-fold concentration. RNAs from HCC tissues were extracted using TRIzol reagent according to manufacturer's instructions (Invitrogen Corp., Carlsbad, CA, USA). The RT-PCR condition was an initial incubation at 50°C for 30 min followed by a 12-min incubation at 95°C, then 50 cycles at 95°C (0 s), 55°C (10 s), and 72°C (15 s), and a 20-s melting at 40°C. The final concentration of primers was 1 μ M, and PCR was performed as reported previously for hTERT mRNA (the primers do not target some splice variants; Refs. [13, 30]): for EGFR-F, AACTGTGAGG TGGTCCTTGG, and EGFR-R, GTTGAGGGCAATGAG GACAT, (the amplified target corresponds to a tyrosine

Table 1 A profile of each patient with hepatitis

Clinical parameters		Number of patient					
		Total	AH	SAH	Acute FH	Subacute FH	LOFH
Age	Mean (range:18–89)	44	43	40	49	58	44
Gender	Male	27	8	3	3	11	2
	Female	26	9	4	2	9	2
Etiology	HAV	5	5	0	0	0	0
	HBV	14	6	2	3	3	0
	HEV	5	3	1	0	1	0
	Drug	4	2	1	0	1	0
Diagnosis	Unknown	25	1	3	2	15	4
	AH	17	17	–	–	–	–
	SAH	7	–	7	–	–	–
	FH	25	–	–	–	–	–
	Acute	5	–	–	5	–	–
	Subacute	20	–	–	–	20	–
	LOFH	4	–	–	–	–	4
Prognosis	Alive	30	17	7	3	3	0
	Death or transplantation	23	0	–	2	17	4
Ascites	–	38	16	7	2	11	2
	+	9	1	0	1	6	1
	++	5	0	0	1	3	1
	Unknown	1	0	0	1	0	0
Encephalopathy	–	24	17	7	0	0	0
	+	29	0	0	5	20	4

kinase phosphorylation site of EGFR and the primers are designed in sequences without any mutations), for c-Met-F, AGTGAAGTGGATGCTTTGG, and c-Met-R, ATAAGGTGGGGCTCCTCTTG, for HGF-F, ATTTGGCCATGA-ATTTGACC, and HGF-R, CTCCAGGGCTGACATTTGAT, and for TGF- α -F, GGTGTTGGCTGTGGGTATTG, and TGF- α -R, GTTGCATGGAGAAGTG. The dynamic ranges of real-time PCR analysis for each mRNA was more than approximately 5–10 copies in this assay, and we could exclude false negative results using standardized hTERT mRNA generated using T7 RNA polymerase in pLIXN-hTERT cDNA kindly provided from Dr. H. Tahara (Hiroshima University, Japan), and the other four mRNAs were similarly generated using pCRII-TOPO-EGFR (Invitrogen Japan K.K, Tokyo, Japan) retrofitted from pME18SFL3-EGFR purchased as the FLJ cDNA clone commercially (TOYOBO, Tokyo, Japan). The PCR yielded products of 131, 114, 122, 128, and 118 bp for hTERT, EGFR, c-Met, HGF, and TGF- α , respectively (data not shown). All the primers were optimally designed (INTEC Web & Genome Informatics Corp., Tokyo, Japan). The RT-PCR assay was repeated twice and the quantification was confirmed with LineGene (TOYOBO, Tokyo, Japan) reproducibly. We confirmed that the amplicons were derived from the gene of interest by sequencing.

Assessment of the usefulness of prognostic scoring systems

For the assessment of prognosis for liver transplantation, four scoring systems, including the Mayo End-Stage Liver Disease (MELD) [31], King's College criteria (KCC) [32], logit-p from The Fulminant Hepatitis Study Group of Japan [33], and logit- λ , from the Japanese Acute Hepatic Failure Study Group (JAHFSG, [34]) were used; MELD = $9.57 \times \log_e(\text{Creatinine: mg/dl}) + 3.78 \times \log_e(\text{TB: mg/dl}) + 11.20 \times \log_e(\text{INR}) + 6.43$ [$\log_e(\text{INR}) = 1.585 - 0.01987 \times (\text{PT}\%)$]. KCC was classified by guidelines for the predictive scoring, in which evaluation 1 came up to the proposed criteria and evaluation 2 did not. Logit-p = $0.084 \times \text{age (year)} + 0.129 \times \text{serum total bilirubin (TB: mg/dl)} - 0.158 \times \text{PT}(\%) - 2.434$. Logit- $\lambda = -0.0649 \times (\text{PT}) + 0.0357 \times (\text{Age}) - 2.81 \times (\text{DB/TB}) + 0.703 \times \log_e(\text{TB}) + 1.04 \times (\text{O-C})$. (O-C: interval from initial symptoms to hepatic encephalopathy ≥ 11 days scored 1, ≤ 10 days scored 0.)

Statistical analysis

Clinical parameters were evaluated by a Friedman test, *t* test, multivariate analysis, or Kruskal–Wallis test. The

correlations among mRNA, conventional biomarkers for hepatitis, and clinical parameters for hepatitis were calculated by Pearson's relative test. To assess the accuracy of diagnostic tests, the matched data regarding age sets with and without FH, as well as the matched data between routine therapy, including plasma exchange (PE), hemo-absorption (HA), plasma absorption (PA), and continuous hemodiafiltration (CHDF), except for liver transplantation, and cases that led to death or liver transplantation were analyzed using receiver operator characteristic (ROC) curve analysis for each mRNA. A predictive cut-off value was evaluated as the nearest point from the right upper edge of the ROC curve analysis graph. Sensitivity was calculated as the mean of confidence interval (CI) of area under the curve (AUC) and specificity was calculated in the

output table of ROC (SPSS 13.0). The curves shown were obtained by processing raw data with SPSS 13.0.

Results

Correlation of mRNAs with clinical parameters

A baseline analysis (A, B) and a strong linear relationship (C) were demonstrated between copy number and PCR cycles using RNA controls for concentration ($r^2 0.99$ for each mRNA, Fig. 1). The copy number of each EGFR mRNA was lower in FH and LOHF patients than in AH and SAH patients ($P < 0.01$), as was mRNA for TGF- α , the ligand of EGFR ($P < 0.01$ and $P < 0.01$, Fig. 2). These

Fig. 1 In each quantitative assay, a baseline analysis (a, b) and a strong linear relation (c) were demonstrated between copy number and PCR cycles using RNA controls for concentration ($r \geq 0.99$ for TGF- α mRNA). The right part of Fig. 1 corresponds to the representative results of RT-PCR as 17.7 ng/ μ l of tRNA template from 200 μ l of serum obtained from a patient with AH. The examined genes were TGF- α mRNA and hTERT mRNA. The upper, middle, and bottom parts show the actual start of reaction, melting curves, and the gel electrophoresis of each RT-PCR product

Fig. 2 Mean $\pm$ SE of quantified mRNA in the serum of patient with liver disease. The dotted line represents the mean copy number corresponding to the quantified mRNA level in the serum of healthy individuals. Longitudinal axis was depicted as the copy number in EGFR mRNA and otherwise as the logarithmic copy number. TGF- α mRNA in serum was detected as a stepwise down-regulated quantification

data suggest that TGF- α EGFR signaling was impaired in FH. c-Met mRNA was lower in FH than in AH and SAH ($P < 0.01$ and $P < 0.01$); however, it was higher in LOHF, but not significantly. HGF mRNA was lower in FH than in AH ($P < 0.01$), similar to the levels in SAH, and significantly higher in LOHF than AH, SAH, or FH ($P < 0.01$, each). hTERT mRNA was significantly lower in FH than in AH and SAH ($P < 0.01$ and $P < 0.01$). It tended to be higher in LOHF, but not significantly.

EGFR mRNA levels correlated with prothrombin time (PT) and TGF- α mRNA ($P < 0.026$ and $P < 0.032$). TGF- α mRNA significantly correlated with AST and α -feto-protein (AFP) ($P < 0.019$ and $P < 0.033$). hTERT mRNA levels significantly associated with platelet counts ($P = 0.001$) and HGF mRNA with ammonia levels ($P < 0.001$).

EGFR mRNA significantly correlated with the inflammatory exacerbation from AH to FH ($P = 0.033$ by

Friedman test). Comparison of acute with subacute FH on admission showed significant correlation in total bilirubin (TB) ($P = 0.034$) and TGF- α mRNA ($P = 0.049$). Surprisingly, TGF- α mRNA solely demonstrated a significant correlation with the prognosis of FH patients retrospectively by Kruskal–Wallis test ($P = 0.034$). Although other clinical parameters, such as PT, TB, direct bilirubin (DB)/TB, and ammonia (NH₃), also showed significant correlation with the prognosis, they were generated at the peak of clinical condition such as just before death or transplantation, and not on admission. Peak TB, DB/TB, and creatinine significantly correlated with FH prediction ($P = 0.034$, $P = 0.024$, and $P = 0.023$, respectively), as did TB and TGF- α mRNA on admission ($P = 0.036$ and $P = 0.049$, respectively). mRNA copy number was lower in acute liver injury patients than in healthy controls ($P < 0.01$), indicating that RNase from damaged hepatocytes destroyed the RNA.

Evaluation of serum TGF- α mRNA quantification as a predictor of prognosis in patients with hepatitis

There were no differences between surviving and non-surviving FH patients in conventional markers, including PT, TB, DB/TB, AST, ALT, PLT, NH₃, AFP, and HGF, or in serum mRNA levels. However, the copy number of TGF- α mRNA in survivors was 10⁶-fold higher than in non-survivors (7.21 ± 4.82 vs. 1.34 ± 0.020 , $P = 0.034$, Table 2). Thus, TGF- α mRNA can predict the outcome of FH patients. Logit- λ was the only prediction formula (of four test) for evaluating indications of liver transplantation for FH in Japan (Table 3).

ROC curve analysis

ROC curve analysis of serum mRNA expression for prognosis of FH was examined using patient data on admission, or just before or after the onset of encephalopathy. TGF- α mRNA was the best biomarker, with CI and AUC of 0.470–0.919 and 0.694, respectively (Fig. 3a). The optimal cut-off values for TGF- α mRNA were 10^{1.7} logarithmic copies/0.2 ml. For a logarithmic predictive cut-off value of 1.7, positive predictive value (PPV)/negative predictive value (NPV) of TGF- α mRNA was 86.4/63.2% and sensitivity/specificity was 74.3/65.5%. ROC curve analysis also measured predictive formulas: KCC, TGF- α , MELD, and JARFSG, with sensitivity/specificity of JARFSG criteria being the best at 88.9/86.7% (Fig. 3b). The AUC and CI for JARFSG are 0.905 and 0.703–1.106, respectively.

Table 3 Prognostic evaluation and comparison of published prediction formulas in case of FH patients on admission

Parameter	Alive ($N = 5$) mean $\pm$ SE	Death ($N = 20$) mean $\pm$ SE	K–W test P value
logit- p	-0.69 ± 0.74	0.36 ± 0.63	0.194 (NS)
MELD	24.60 ± 10.55	23.17 ± 1.82	0.913 (NS)
KCC	–	–	0.294 (NS)
logit- λ	2.23 ± 0.38	0.13 ± 0.27	0.001

Discussion

hTERT mRNA is the only significant expression change seen in malignancies that is detectable in blood and urine samples [21–26, 35]. hTERT mRNA derived from cancer cells does not seem to be detectable in serum due to its instability in serum containing RNase. However, since RNAs in serum are stable within 24 h after drawing blood [36–38], minimizing cell contamination may allow detection in serum [25, 26]. Early assessment of FH can help move toward a life-saving liver transplant more quickly. In this context, biomarkers for the assessment of FH prognosis are required.

TGF- α is a hepatocyte mitogen, and impaired liver regeneration may contribute to the poor prognosis in FH. Maximum serum TGF- α levels in patients with FH were significantly higher in survivors than in non-survivors [39]. TGF- α protects against Fas-mediated liver apoptosis in transgenic mice that constitutively express more Bcl-xL protein than wild-type mice [40], and TGF- α also protects against liver apoptosis induced by the Fas pathway. TGF- α concentration-dependently protects against c-Myc and

Table 2 Prognosis prediction of FH using diagnostic examination on admission

Parameter	Survivor ($N = 5$) mean $\pm$ SE	Non-survivor ($N = 20$) mean $\pm$ SE	K–W test P value
PT (%)	24.1 ± 7.14	22.32 ± 3.04	NS
TB (mg/ml)	10.8 ± 1.40	15.1 ± 1.94	NS
DB/TB	0.69 ± 0.03	0.59 ± 0.03	NS
AST (IU/L)	1338.0 ± 688.5	1526.4 ± 514.5	NS
ALT (IU/L)	2279.8 ± 910.8	1607.5 ± 557.2	NS
PLT ($\times 10^4$)	11.4 ± 2.25	12.8 ± 2.54	NS
NH ₃ (g/ml)	111.8 ± 30.0	139.5 ± 27.0	NS
AFP (ng/ml)	28.0 ± 23.1	20.6 ± 12.1	NS
HGF (ng/ml)	2.44 ± 0.96	2.72 ± 0.91	NS
HGF mRNA	1.09 ± 0.38	1.56 ± 0.65	NS
c-Met mRNA	3.19 ± 1.33	3.05 ± 0.84	NS
hTERT mRNA	1.74 ± 0.72	2.03 ± 0.47	NS
EGFR mRNA	1.51 ± 0.51	25.9 ± 16.4	NS
TGF- mRNA	7.21 ± 4.82	1.34 ± 0.20	0.034

P value was calculated by Kluskal–Wallis test. TGF-mRNA was treated logarithmically

Fig. 3 Receiver operator characteristic (ROC) curve analysis of (a) serum mRNA expression regarding the prognosis of FH and (b) evaluation of predictive formulas or the criteria for extensive use regarding the prognosis of FH. The curves shown were obtained by processing quantified raw data by the SPSS II software and the sensitivity/specificity values were calculated from the mean of CI of AUC and the calculated data. (a) The bold dotted line, dotted line,

solid line, fine dotted line, and bold solid line correspond to loghTERT, logc-Met, logHGF, EGFR mRNA, and logTGF- α mRNA, respectively. Sensitivity/specificity of logTGF- α mRNA is 74.3/65.5%. (b) The bold dotted line, dotted line, solid line, and bold solid line correspond to KCC, TGF- α , MELD, and JARFSG, respectively. Sensitivity/specificity of JARFSG criteria is 88.9/86.7%

TNF α -induced apoptosis, and overexpression of TGF- α might completely suppress apoptosis in vivo [41–44]. On the other hand, EGFR signaling controls the expression of Bcl-xL in keratinocytes, mammary epithelial cells, and hepatocytes in vitro [45]. TGF- α levels are increased with liver regeneration after necrosis in patients with AH, but such liver regeneration may be impaired depending on the extent of liver damage in patients with FH. TGF- α may contribute to liver regeneration and promote recovery from AH. Furthermore, adult human hepatocytes cultured from patients with FH still respond to EGF and TGF- α [46]. Thus, these molecules play crucial roles in the onset and progression of FH and LOFH [47–49].

TGF- α may be an indicator of FH prognosis. Actually, increased TGF- α level 14 days after portal vein embolization was the only independent factor related to the hypertrophy of the nonembolized liver, suggesting that TGF- α levels may be a useful indicator for scheduling subsequent extensive hepatectomies [50]. Even with HGF present, proliferation of hepatocytes is attenuated only if the effect of TGF- α is suppressed, indicating that TGF- α promotes hepatic regeneration. In our study, TGF- α significantly correlated with α -fetoprotein levels, which could confirm the involvement of TGF- α in liver regeneration in

FH patients [51]. TGF- α mRNA exclusively correlated with FH prognosis at the time of admission, with EGFR mRNA correlating with disease progression, indicating that they may be good biomarkers for FH or LOFH. mRNAs indicative of hepatocyte apoptosis may also contribute to accurate diagnosis of inflammatory progression or as a guideline for liver transplantation.

Although the KCC is used for emergency liver transplantation in drug-induced acute liver failure, strategies to improve sensitivity and facilitate earlier transplantation are required. MELD predicts the severity or prognosis of decompensated liver cirrhosis, with arterial blood lactate being added to the modified KCC [52]. Here, the most useful predictive formula for FH (including exacerbation from chronic liver diseases) on admission was an assessment proposed by JARFSG (the cut-off point is 1.9). However, these formulas only include clinical factors, and further refinement using molecular markers should be evaluated.

Here, we measured mRNAs in patients with hepatitis with a sensitive, established method. HGF mRNA was highly expressed in LOFH rather than in FH, whereas TGF- α mRNA was strongly down-regulated in both LOFH and FH, suggesting that interstitial liver tissue may be maintained in LOFH, with hepatocytes committing to

apoptosis. After hepatectomy, EGFR expression initially elevates within 5–6 h, with secondary increases in both HGF and TGF- α [53]. Our study shows that HGF and its receptor, c-met, were not directly correlated with proliferation. Serum HGF mRNA levels decreased in patients with FH, but serum HGF protein significantly increases, indicating that HGF mRNA does not accumulate in blood and HGF protein is greatly influenced by clearance. The proliferative capacity of the liver in FH is lost, and TGF- α may prompt hepatic proliferation under HGF expression. TGF- α protein levels are difficult to quantitate in serum [54], but measurement at the mRNA level may facilitate diagnosis in hepatitis.

In conclusion, we showed that TGF- α mRNA levels are associated with poor prognostic FH on admission. We propose a novel prediction formula incorporating serum TGF- α mRNA and logit- λ scores. Serial changes in TGF- α mRNAs or other mediators may also mark disease progressing during its course, and particularly patient survival. Further case-control studies and a large-scale study should be planned to evaluate this hypothesis.

Acknowledgments This study was financially supported by a Grant-in-Aid for scientific research from the Ministry of Education, Science.

References

1. Sato S, Suzuki K, Takikawa Y, Endo R, Omata M, Japanese National Study Group of Fulminant Hepatitis. Clinical epidemiology of fulminant hepatitis in Japan before the substantial introduction of liver transplantation: an analysis of 1309 cases in a 15-year national survey. *Hepatol Res* 2004;30:155–61.
2. Fujiwara K: Japanese national survey of FH in 2000. In: Omata M, editor. The 2001 annual report of research group of intractable hepatitis. Tokyo: The Ministry of Health and Welfare of Japan; 2002. p. 87–96.
3. Trey C, Lipworth L, Dabidson CS. Parameters influencing survival in the first 318 patients reported to the fulminant hepatic failure surveillance study. *Gastroenterology* 1970;73:306.
4. Horney JT, Galambos JT. The liver during and after fulminant hepatitis. *Gastroenterology* 1977;73:639–45.
5. Michalopoulos GK. Liver regeneration: molecular mechanisms of growth control. *FASEB J* 1990;4:176–87.
6. Zarnegar R, DeFrances MC, Michalopoulos GK. Hepatocyte growth factor; its role in hepatic growth, pathology. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, Shafritz DA, editors. Liver: biology and pathology. New York: Raven Press; 1994. p. 1047–57.
7. Fausto N. Hepatic regeneration. In: Zakim D, Boyer TD, editors. *Hepatology*. Philadelphia: WB Saunders; 1996. p. 32–57.
8. Jhappan C, Stahle C, Harkins RN, Fausto N, Smith GH, Merlino GT. TGF alpha overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. *Cell* 1990;61:1137–46.
9. Shiota G, Wang TC, Nakamura T, Schmidt EV. Hepatocyte growth factor in transgenic mice: effects on hepatocyte growth, liver regeneration and gene expression. *Hepatology* 1994;19:962–72.
10. Fujiwara K, Nagoshi S, Ohno A, Hirata K, Ohta Y, Mochida S, et al. Stimulation of liver growth by exogenous human hepatocyte growth factor in normal and partially hepatectomized rats. *Hepatology* 1993;18:1443–9.
11. Ishiki Y, Ohnishi H, Muto Y, Matsumoto K, Nakamura T. Direct evidence that hepatocyte growth factor is a hepatotrophic factor for liver regeneration and has a potent antihepatitis effect in vivo. *Hepatology* 1992;16:1227–35.
12. Webber EM, Godowski PJ, Fausto N. In vivo response of hepatocytes to growth factors requires an initial priming stimulus. *Hepatology* 1994;19:489–97.
13. Liu ML, Mars WM, Zarnegar R, Michalopoulos GK. Collagenase pretreatment and the mitogenic effects of hepatocyte growth factor and transforming growth factor-alpha in adult rat liver. *Hepatology* 1994;19:1521–7.
14. Todaro GJ, Fryling C, De Larco JE. Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc Natl Acad Sci USA* 1980;77:5258–62.
15. Massague J. Epidermal growth factor-like transforming growth factor. II. Interaction with epidermal growth factor receptors in human placenta membranes and A431 cells. *J Biol Chem* 1983;258:13614–20.
16. Derynck R. Transforming growth factor alpha. *Cell* 1988;54:593–5.
17. Johnson AC, Garfield SH, Merlino GT, Pastan I. Expression of epidermal growth factor receptor proto-oncogene mRNA in regenerating rat liver. *Biochem Biophys Res Commun* 1988;150:412–8.
18. Mead JE, Fausto N. Transforming growth factor alpha may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc Natl Acad Sci USA* 1989;86:1558–62.
19. Fisher DA, Lakshmanan J. Metabolism and effects of epidermal growth factor and related growth factors in mammals. *Endocr Rev* 1990;11:418–42.
20. Fausto N, Webber EM. Liver regeneration. In: Arias IM, Boyer JL, Fausto N, Jakoby WB, Schachter DA, Shafritz DA, editors. The liver: biology, pathology. New York: Raven Press; 1994. p. 1059–84.
21. Chen XQ, Bonnefoi H, Pelte MF, Lyautey J, Lederrey C, Movarekhi S, et al. Telomerase RNA as a detection marker in the serum of breast cancer patients. *Clin Cancer Res* 2000;6:3823–6.
22. Kopreski MS, Benko FA, Kwak LW, Gocke CD. Detection of tumor messenger RNA in the serum of patients with malignant melanoma. *Clin Cancer Res* 1999;5:1961–5.
23. Pelosi G, Schianchi E, Dell'Orto P, Veronesi G, Spaggiari L, Pasini F, et al. Detecting cell-free circulating hTERT mRNA in the plasma may identify a subset of nonsmall cell lung cancer patients. *Virchows Arch* 2005;29:1–9.
24. Miura N, Shiota G, Nakagawa T, Sano A, Marumoto A, Kishimoto Y, et al. Sensitive detection of hTERT mRNA in the serum of patients with hepatocellular carcinoma. *Oncology* 2003;64:430–4.
25. Miura N, Maeda Y, Kanbe T, Yazama H, Takeda Y, Sato R, et al. Serum human Telomerase reverse transcriptase messenger RNA as a novel tumor marker for hepatocellular carcinoma. *Clin Cancer Res* 2005;11:3205–9.
26. Miura N, Nakamura H, Sato R, Tsukamoto T, Harada T, Takahashi S, et al. Clinical usefulness of serum telomerase reverse transcriptase (hTERT) mRNA and epidermal growth factor receptor (EGFR) mRNA as a novel tumor marker for lung cancer. *Cancer Sci* 2006;97:1366–73.
27. Shiota G, Okano J, Kawasaki H, Kawamoto T, Nakamura T. Serum hepatocyte growth factor levels in liver diseases: clinical implications. *Hepatology* 1995;21:106–12.

28. Trey C, Davidson CS. The management of fulminant hepatic failure. In: Popper H, Schaffner F, editors. *Progress in liver diseases*, vol. III. Philadelphia: Grune, Stratton; 1970. p. 282–298.
29. Gimson AES, O'Grady J, Ede RJ, Portmann B, Williams R. Late onset hepatic failure :clinical, serological and histological features. *Hepatology* 1986;6:288–94.
30. Wege H, Chui MS, Le HT, Strom SC, Zern MA. In vitro expansion of human hepatocytes is restricted by telomere-dependent replicative aging. *Cell Transplant* 2003;12:897–906.
31. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001;33:464.
32. O'Grady JG, Alexander GJM, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology* 1989;97:439–45.
33. Takikawa Y, Endo R, Suzuki K, Fujiwara K, Omata M, The Fulminant Hepatitis Study Group of Japan. Prediction of hepatic encephalopathy development in patients with severe acute hepatitis. *Dig Dis Sci* 2006;51:359–64.
34. Sugihara J, Naito T, Ishiki Y, Murakami N, Naiki T, Koshino Y, et al. A multicenter study on the prognosis and indications of liver transplantation for fulminant hepatitis in Japan. Details of decision of the guideline for liver transplantation in Japanese acute hepatic failure study group. *Acta Hepatol Jap* 2001;42:543–57.
35. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994;266:2011–5.
36. Tatsuma T, Goto S, Kitano S, Lin YC, Lee CM, Chen CL. Telomerase activity in peripheral blood for diagnosis of hepatoma. *J Gastroenterol Hepatol* 2000;15:1064–70.
37. Ng EK, Tsui NB, Lam NY, Chiu RW, Yu SC, Wong SC, et al. Presence of filterable and non filterable mRNA in the plasma of cancer patients and healthy individuals. *Clin Chem* 2002;48:1212–7.
38. Tsui NB, Ng EK, Lo YM. Stability of endogeneous and added RNA in blood specimens, serum, and plasma. *Clin Chem* 2002;48:1647–53.
39. Tomiya T, Fujiwara K. Liver regeneration in fulminant hepatitis as evaluated by serum transforming growth factor alpha levels. *Hepatology* 1996;23:253–7.
40. Kanda D, Takagi H, Toyoda M, Horiguchi N, Nakajima H, Otsuka T, et al. Transforming growth factor alpha protects against Fas-mediated liver apoptosis in mice. *FEBS Lett* 2002;22:11–5.
41. Nass SJ, Li M, Amundadottir LT, Furth PA, Dickson RB. Role for Bcl-xL in the regulation of apoptosis by EGF and TGF beta 1 in c-myc overexpressing mammary epithelial cells. *Biochem Biophys Res Commun* 1996;3:248–56.
42. Amundadottir LT, Nass SJ, Berchem GJ, Johnson MD, Dickson RB. Cooperation of TGF alpha and c-Myc in mouse mammary tumorigenesis: coordinated stimulation of growth and suppression of apoptosis. *Oncogene* 1996;15:757–65.
43. Reinartz J, Bechtel MJ, Kramer MD. Tumor necrosis factor-alpha-induced apoptosis in a human keratinocyte cell line (Ha-CaT) is counteracted by transforming growth factor-alpha. *Exp Cell Res* 1996;1:334–40.
44. Christensen JG, Goldsworthy TL, Cattley RC. Dysregulation of apoptosis by c-myc in transgenic hepatocytes and effects of growth factors and nongenotoxic carcinogens. *Mol Carcinog* 1999;25:273–84.
45. Musallam L, Ethier C, Haddad PS, Bilodeau M. Role of EGF receptor tyrosine kinase activity in antiapoptotic effect of EGF on mouse hepatocytes. *Am J Physiol Gastrointest Liver Physiol* 2001;280:G1360–9.
46. Blanc P, Etienne H, Daujat M, Fabre I, Zindy F, Domergue J, et al. Mitotic responsiveness of cultured adult human hepatocytes to epidermal growth factor, transforming growth factor alpha, and human serum. *Gastroenterology* 1992;102:1340–50.
47. Shiota G, Okano J, Kawasaki H, Kawamoto T, Nakamura T. Serum hepatocyte growth factor levels in liver diseases: clinical implications. *Hepatology* 1995;21:106–12.
48. Tsubouchi H, Kawakami S, Hirono S, Miyazaki H, Kimoto M, Arima T, et al. Prediction of outcome in fulminant hepatic failure by serum human hepatocyte growth factor. *Lancet* 1992;1:307.
49. Tomiya T, Nagoshi S, Fujiwara K. Significance of serum human hepatocyte growth factor levels in patients with hepatic failure. *Hepatology* 1992;15:1–4.
50. Kusaka K, Imamura H, Tomiya T, Makuuchi M. Factors affecting liver regeneration after right portal vein embolization. *Hepato-gastroenterology* 2004;51:532–5.
51. Ostapowicz G, McCashland TM, Shakil O, the ALF Study Group. An increase in alpha-fetoprotein predicts survival in acute liver failure. *Hepatology* 1999;72:479–82.
52. Bernal W, Donaldson N, Wyncoll D, Wendon J. Blood lactate as an early predictor of outcome in paracetamol-induced acute liver failure: a cohort study. *Lancet* 2002;359:558–63.
53. Furuta K, Kakita A, Takahashi T, Tomiya T, Fujiwara K. Experimental study on liver regeneration after simultaneous partial hepatectomy and pancreatectomy. *Hepatology Res* 2000;17:223–36.
54. Efimova EA, Glanemann AK, Nussler G, Schumacher U, Settmacher S, Jonas N, et al. Changes in serum levels of growth factors in healthy individuals after living related liver donation. *Transplant Proc* 2005;37:1074–5.