

Elevation of *N*ε-(carboxymethyl)lysine-modified advanced glycation end products in chronic liver disease is an indicator of liver cirrhosis

Eray Yagmur^{a,*}, Frank Tacke^b, Claudia Weiss^c, Birgit Lahme^a, Michael P. Manns^b, Paul Kiefer^a, Christian Trautwein^{b,1}, Axel M. Gressner^a

^a Institute of Clinical Chemistry and Pathobiochemistry, University Hospital Aachen (UKA), Aachen University (RWTH), Pauwelsstraße 30, 52074 Aachen, Germany

^b Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Germany

^c Institute of Medical Statistics, RWTH Aachen University, Germany

Received 8 February 2005; received in revised form 29 July 2005; accepted 29 July 2005

Available online 29 November 2005

Abstract

Objectives: Progression of liver fibrosis to cirrhosis is a dire consequence of chronic liver diseases (CLD). *N*ε-(carboxymethyl)lysine (CML)-modified advanced glycation end products (AGEs) in patients with CLD could reflect the degree of severity of the disease.

Design and methods: In 110 patients with CLD and 124 healthy controls, CML serum levels and their diagnostic sensitivity and specificity were determined and compared to hyaluronan (HA).

Results: Serum levels of CML were significantly affected by the stage of liver cirrhosis and were closely associated with liver function capacity. CML correlated positively with HA ($r = 0.639$, $P < 0.0001$). In ROC analysis, the diagnostic sensitivity and specificity in distinguishing healthy controls from liver disease patients for CML (AUC 0.908; 95%-CI 0.863–0.942, cut-off 640 ng/mL, sensitivity 74.5% and specificity 97.6%) resembled HA (AUC 0.948; 95%-CI 0.907–0.974; cut-off 50 ng/mL, sensitivity 80.7% and specificity 97.9%). The combination of CML and HA shows an AUC of 0.932; 95%-CI 0.888–0.962; sensitivity 82.6%; and specificity 95.8%.

Conclusions: Our data suggest that serum levels of CML could provide a supplementary diagnostic marker for advanced stages of liver cirrhosis. However, the quality of interaction needs further investigation.

© 2005 The Canadian Society of Clinical Chemists. All rights reserved.

Keywords: Chronic liver disease; Liver cirrhosis; Serum marker; Advanced glycation end products; Carboxymethyl(lysine); Hyaluronan; Hyaluronic acid

Introduction

Liver cirrhosis is characterized by progressive fibrosis and simultaneous tissue breakdown. This fibrosis is the result of increased synthesis and deposition of extracellular matrix, which leads to organ dysfunction [1,2]. Fine needle biopsy is still the reference method for distinguishing between cirrhotic and non-cirrhotic stages with certainty [3]. Nevertheless, this invasive diagnostic procedure is associated with patient risk, especially as bleeding disorders are common in advanced liver diseases [4,5]. Therefore, serum markers that reflect the stage of the disease by non-invasive measures are highly desirable.

*N*ε-(carboxymethyl)lysine (CML) is one of the best characterized compounds of advanced glycation end products (AGEs) and can be detected in tissue and serum proteins by specific antisera [6,7]. Non-enzymatic glycation and glycooxidation of amino acids, proteins and components of DNA are accelerated by hyperglycemia, but also lipids; that is, during hyperlipidemia in atherosclerosis, this process can lead to chemical formation of AGEs and advanced lipoxidation end products (ALEs), respectively [8–10]. An increase in AGEs is seen in the blood of patients with diabetes mellitus or atherosclerosis. This accumulation is believed to play a causal role in vascular endothelial damage and in diabetic neuropathy, retinopathy and nephropathy [11–15].

We have previously shown that RAGE (receptor for advanced glycation end products), a multi-ligand receptor and the main receptor for CML-modified serum proteins, is expressed by hepatic stellate cells (HSC) and myofibroblasts,

* Corresponding author. Fax: +49 241 80 82512.

E-mail address: eyagmur@ukaachen.de (E. Yagmur).

¹ Present address: Department of Medicine III, RWTH-University Hospital Aachen, Germany.

which are the relevant cells for fibrogenesis of CLD [16]. CML adducts have the capacity to activate cells via RAGE-mediated signaling cascades, resulting in triggering a multitude of intracellular responses. RAGE–ligand interaction is a propagation factor in a range of chronic disorders, based on the accumulation of the ligands in diseased organs [17–19]. In addition, elevated serum CML may, itself, represent a risk factor for further perpetuation of the fibrotic process in the liver.

The objective of this study was to investigate CML serum concentrations in patients with CLD compared to HA, as a marker to assess liver function capacity and liver fibrosis.

Patients and methods

Patients and control collective

The study population consisted of 110 patients (65 males/45 females, median age 46 years, range 18–70 years) with CLD who were evaluated as inpatients for potential liver transplantation [20]. They underwent a highly standardized routine procedure with an extensive laboratory medical analysis and diagnostics. The Child–Pugh score was used as a prognostic score for the stage of the liver cirrhosis [21]. The control group consisted of 124 healthy blood donors with normal aminotransferases, normal blood counts and negative markers for virus hepatitis and HIV (82 males/42 females, median age 32.5 years, range 19–65 years).

Measurement of CML serum concentrations

CML-modified proteins in the patient and control groups were determined using a competitive enzyme-linked immunosorbent assay (ELISA) (Roche, Penzberg, Germany). Streptavidin-coated microtiter plates were coated with biotinylated BSA-AGE at room temperature for 1 h. Proteolytic pretreatment of the serum sample with proteinase K (final concentration 1 mg/mL) (Roche, Mannheim, Germany) for 3 h at 37°C followed by addition of phenylmethylsulfonyl fluoride (PMSF) (final concentration 1 mmol/L) and incubation for 30 min (Roche, Mannheim, Germany) at 37°C resulted in an optimal exposure of the CML epitopes. A 50 µL aliquot of standard, positive control (CML-modified human serum albumin prepared as previously described [22]) and serum samples in assay buffer and 50 µL of horseradish-peroxidase-labeled monoclonal CML antibody (Roche, Penzberg, Germany; research only, not commercially available) with a dilution of 1:1500 were added. The plate was incubated for 1 h at room temperature and washed. Then, 100 µL of 2,2'-amino-di(3-ethylbenzthiazoline sulfonate) substrate (ABTS) (Roche, Mannheim, Germany) was added and incubated for 30 min. Absorption was measured at 405 nm. Samples were analyzed in duplicate.

In human serum, the assay was linear over a dilution range from 1:10 to 1:40. The recovery of CML from serum was 101% and 94%, respectively. The coefficient of variation for intra-assay variability was 2.7% and 13.4% for inter-assay variability [22]. The standard calibration material for the standard calibration curve is 6-(*N*-carboxymethylamino)caproate (CMC),

which represents the singular epitope for the monoclonal anti-CML antibody. The CMC content of the samples was quantified using a standard calibration curve (range 0–140 ng/mL).

Measuring HA in serum

The serum concentration of HA was determined with a commercial quantitative kit (ELISA), which is based on the specific reaction of HA-binding protein (HABP) with HA (Corgenix HA Test Kit, Cat. no. 029-001, Corgenix Inc., Westminster, CO, USA). The assay uses microwells coated with a highly specific HABP from bovine cartilage to capture HA and an enzyme-conjugated version of HABP to detect and measure HA in serum samples. Reference solutions (prepared from rooster comb HA) were used to calculate the test results in ng/mL. Diluted samples (100 µL) and HA reference solutions (100 µL) as the standard were incubated in HABP-coated microwells for 60 min at room temperature and washed 4 times with washing buffer. Then, 100 µL HABP, conjugated with horseradish peroxidase, was added. The plate was incubated for 30 min at room temperature and washed 4 times. A chromogenic substrate 3,3',5,5'-tetra methyl benzidine and hydrogen peroxide (TMB/H₂O₂) (100 µL) was added and incubated for 30 min. HA concentrations were calculated by comparing the absorbance of the samples against a reference curve prepared from the reagent blank and five reference solutions (50–800 ng/mL). The standard calibration material for the standard calibration curve was the HA reference solution [23].

The assay was linear over a dilution range from 1:2 to 1:25. The recovery of HA from serum was 100.6% and 95.9%, respectively. The coefficient of variation for intra-assay variability was 4.2% and 6.3% for inter-assay variability.

Statistical analysis

Due to the skewed distributions of most parameters, values are given as median and range. Spearman rank correlations are given in order to quantify the degree of association between two variables. Comparisons between subgroups are illustrated using Box-and-Whiskers plots, which display a statistical summary of the median, quartiles, range and extreme values. Specifically, the whiskers extend from the minimum to the maximum value excluding outside and far out values, which are displayed as separate points. An outside value is defined as a value that is smaller than the lower quartile minus 1.5 times the interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range. A far out value is defined as a value that is smaller than the lower quartile minus 3 times the interquartile range, or larger than the upper quartile plus 3 times the interquartile range. Comparison of the parameters between two different groups was conducted with the Mann–Whitney *U* Test and with the Kruskal–Wallis analysis of variances (ANOVA) for multiple comparisons. ROC analysis was carried out for the diagnostic sensitivity and specificity of CML and HA in order to divide patients from healthy controls as well as between the different grades of cirrhosis (Child–Pugh score). Multivariate analysis was performed using the logistic regression model in

order to quantify the interaction between the two diagnostic parameters, CML and HA. Adjustment of the level of significance was not done as the results are to be understood as explorative. Statistical analysis was performed using MedCalc Version 6.01.001 (MedCalc software, Mariakere, Belgium) and SAS (The SAS System for Windows Release 8.02 TSL 02M0; SAS Institute, Inc., Cary, NC, USA).

Results

CML serum concentrations in patients with CLD and healthy controls

We analyzed 110 patients (65 males/45 females, median age 46 years, range 18–70 years) with CLD. The distribution of the stages of liver cirrhosis as defined according to the Child–Pugh score, the underlying etiologies and measurements of CML, HA and albumin serum concentrations are presented in Table 1. The concentration of CML was measured in all serum samples with a median of 869 ng/mL (range 277–10762 ng/mL). CML serum concentrations were significantly higher in CLD patients than in healthy controls (controls median 424.5 ng/mL, range 45–924 ng/mL, $P < 0.0001$).

CML and liver cirrhosis

CML serum concentration was significantly higher ($P < 0.0001$) in patients with CLD and Child A to C ($n = 93$, median 907 ng/mL, range 418–10762 ng/mL) compared to patients with CLD without cirrhosis ($n = 17$, median 445 ng/mL, range 277–2786 ng/mL) ($P < 0.0001$, Fig. 1A). Although we could not find an association between the serum concentration of

CML and the etiology of the liver disease (Fig. 1B), CML serum levels were considerably higher in the group with biliary and autoimmune diseases than in the other groups ($n = 27$, median 1093 ng/mL, range 418–10,762 ng/mL). However, the finding was not related to a specific diagnosis within this group. Seventeen patients (15%) displayed a CML serum concentration of >2000 ng/mL. Seven of these patients had a biliary or autoimmune disease, while the others did not have major common striking features with respect to their degree of cirrhosis. The increase of CML concentration correlated positively with the degree of severity of liver cirrhosis illustrated by the Child–Pugh score ($r = 0.543$, $P < 0.0001$).

CML and hepatic function

The serum albumin concentration was determined in all patients ($n = 110$, median 34 g/L, range 16–49 g/L) and healthy control subjects ($n = 124$, median 45 g/L, range 36–57 g/L). In patients with CLD, CML correlated inversely with the serum albumin ($r = 0.413$, $P < 0.0001$). As expected, patients with CLD exhibited significantly reduced albumin concentrations ($P < 0.0001$).

HA serum concentration in patients with CLD and healthy controls

In patients with CLD, HA had a median value of 201 ng/mL (range 4–1195 ng/mL). HA was measured in 96 of the 124 healthy controls. These control persons had a median value of 7 ng/mL (range 0–103 ng/mL). CML and HA correlated positively ($r = 0.639$; $P < 0.0001$, Fig. 2B). Similar to CML, serum HA concentrations were significantly higher in Child A

Table 1
Patient characteristics

	Healthy controls	All patients	No cirrhosis	Stages of cirrhosis		
				Child A	Child B	Child C
(n)	124	110	17	35	44	14
Gender (male/female) (n)	82/42	65/45	10/7	18/17	30/14	7/7
Age (years)						
Median	32.5	46	46	41	48	40
Range	(19–65)	(18–70)	(18–65)	(18–64)	(20–70)	(26–69)
Etiology of liver disease (n)						
Virus hepatitis		32	1	9	17	5
Biliary or autoimmune		27	2	13	10	2
Alcohol or cryptogenic		30	1	12	10	7
Other etiology		21	13	1	7	0
Ne-(carboxymethyl)lysine (CML) (ng/mL)						
Median	425	869	445	689	1014	1244
Range	(45–924)	(277–10,762)	(277–2786)	(418–5457)	(429–10,762)	(769–2938)
Hyaluronan (ng/mL) [normal 0–100]						
Median	7	201	21	136	287	588
Range	(0–103)	(4–1195)	(4–414)	(6–1195)	(48–1184)	(83–1136)
Creatinine (μmol/l) [normal 44–106]						
Median	88	61	70	61	60	82
Range	(62–124)	(38–661)	(43–661)	(40–130)	(38–373)	(42–167)
Albumin (g/l) [normal 37–51]						
Median	45	34	40	39	30	26
Range	(36–57)	(16–49)	(29–49)	(28–45)	(20–40)	(16–32)

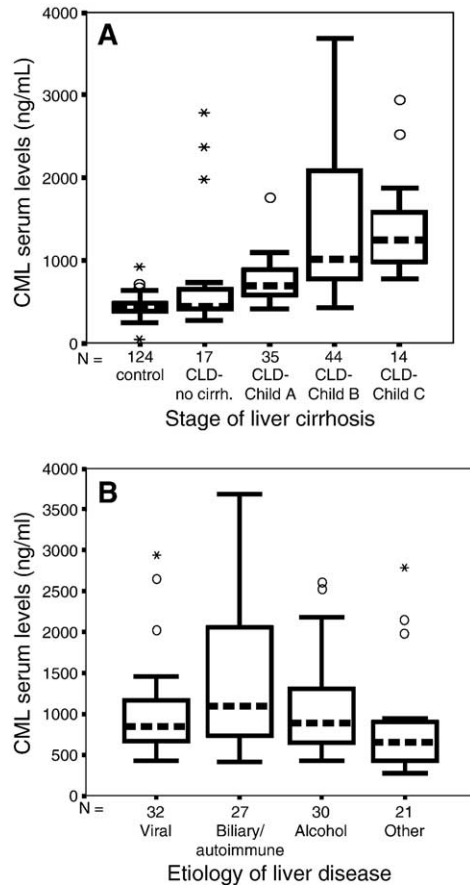


Fig. 1. (A) Association with Child's stage of liver cirrhosis. CML increases with degree of liver cirrhosis according to the Child–Pugh score (CLD: chronic liver disease). (B) Association with etiology of liver disease. Biliary or autoimmune etiology shows higher CML levels compared with other etiologies of liver disease.

to C cirrhosis patients than in patients without cirrhosis ($P < 0.0001$, Fig. 2A). However, the differences in HA between Child B and C cirrhotic patients were more pronounced than that observed for CML.

Suitability of CML as a diagnostic parameter

In order to examine the suitability of CML as a diagnostic parameter for liver disease, we performed ROC analyses, which we have depicted graphically in Fig. 3. This led to a favorable cut-off value of 640 ng/mL for CML, distinguishing liver disease patients from healthy controls with a sensitivity of 74.5% and a specificity of 97.6% (Fig. 3A). The area under the ROC curve (AUC) for CML was 0.908 (95%-CI 0.863–0.942). The optimal cut-off for HA was 50 ng/mL, with a sensitivity of 80.7% and specificity of 97.9% (Fig. 3A). The AUC for HA was 0.948 (95%-CI 0.907–0.974). Comparison of these two diagnostic tests led to a significant difference between the AUC of 0.043 favoring HA ($P = 0.048$, Fig. 3A). AUCs calculated for the diagnostic tests, CML and HA, dividing between the severity of liver cirrhosis (Child–Pugh score \geq A) and no cirrhosis (healthy controls and CLD-no cirrhosis group) all showed significant differences from the diagonal (lower confidence limits did not

show values below 0.833) with AUC values between 0.899 and 0.989. This analysis showed comparable AUC results for CML (0.938; 95%-CI 0.899–0.965) and HA (0.971; 95%-CI 0.937–0.989) (Fig. 3B). The combination of CML and HA showed an AUC 0.932; 95%-CI 0.888–0.962; sensitivity 82.6%; and specificity 95.8% and for Child–Pugh score \geq A an AUC 0.953; 95%-CI 0.915–0.978; sensitivity 82.6%; and specificity 95.8%, respectively (Figs. 3A and B).

To investigate whether the two diagnostic parameters are independent from each other, logistic regression was performed. This leads to the result that both factors, revealing significant effects in the univariate analyses ($P < 0.00005$ for both), showed significant interaction ($P = 0.0270$) in the combined model (HA: $P = 0.1472$, CML: $P = 0.6495$); therefore, a non-additive interaction can be assumed.

Discussion

In this study, we show that CML serum concentrations are significantly elevated in patients with chronic liver diseases compared to healthy controls. In liver disease patients, CML is

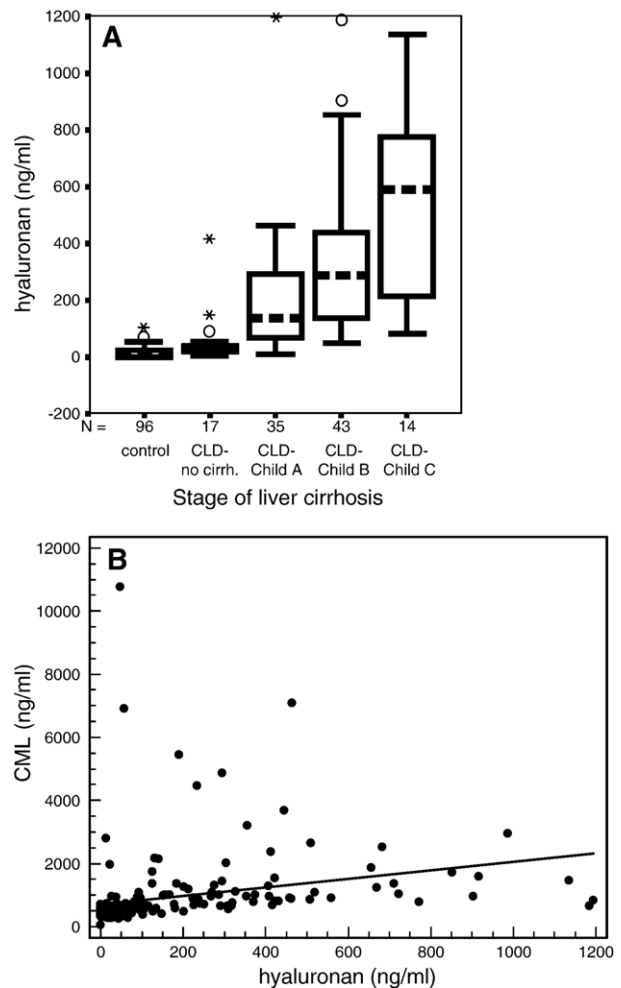


Fig. 2. (A) Association of hyaluronan (HA) with Child's stage of liver cirrhosis. (CLD: chronic liver disease). (B) CML and HA correlate with each other ($r = 0.639$; $P < 0.0001$, Spearman rank correlation analysis).

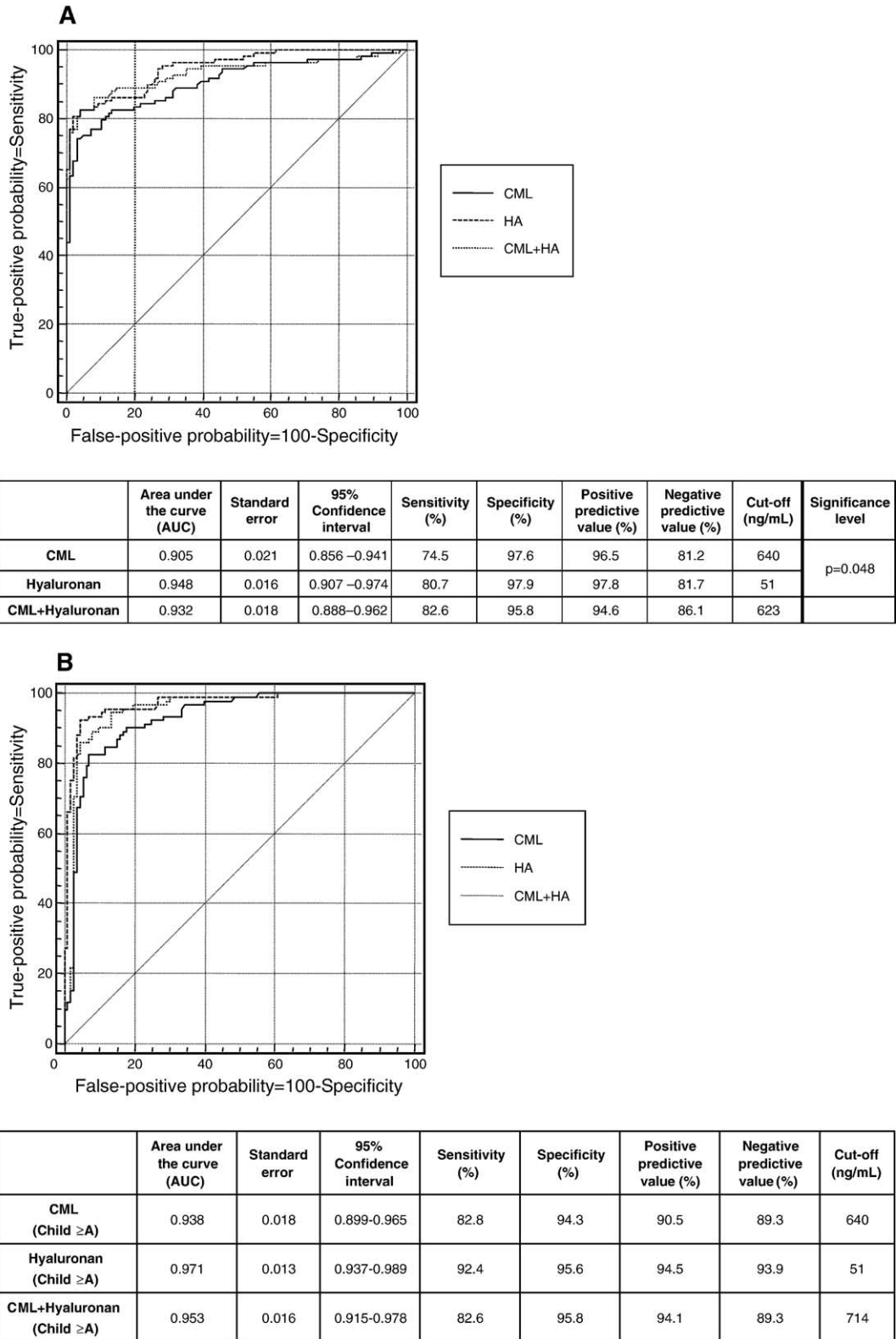


Fig. 3. (A) In receiver operating characteristics (ROC) analysis, the diagnostic sensitivity and specificity for CML and HA were calculated distinguishing between liver disease patients and healthy controls. CML and HA showed almost equal power. (B) Receiver operating characteristic curve (ROC) of CML and HA distinguishing Child A to C cirrhosis from non-cirrhotic patients or healthy controls. No difference was detected in the power for the two tests.

closely related with the disease severity as assessed by the Child–Pugh score or laboratory markers of liver function capacity (i.e. HA as an established biochemical marker for

progressive liver damage). CML and HA both displayed good diagnostic sensitivity and specificity in distinguishing healthy controls from patients and in distinguishing non-cirrhotic from

cirrhotic patients (Child \geq A). However, the comparison of these two diagnostic tests led to a significant difference between the AUCs, favoring HA. The correlation of CML with HA and its almost equal diagnostic sensitivity and specificity might be an indication that a reduced (sinusoidal) clearance of the AGE proteins in the human liver is an additional cause for the observed elevations in CML concentration.

These findings extended and confirmed the results of Sebekova et al. [24], which suggested that hepatic removal of AGEs is impaired in liver cirrhosis, thereby resulting in markedly elevated concentrations of AGEs [24]. Smedsrød et al. [25] were able to show that a great extent – approximately 90% – of the in vitro AGE-modified rat serum albumins are eliminated by sinusoidal endothelial cells of the liver in rats. In human liver, it is assumed that AGE-modified molecules are recognized, internalized by cell surface receptor-mediated endocytosis, degraded intracellularly and subsequently secreted as low molecular weight-AGEs (AGE-peptides, second generation AGEs) [26,27]. Similarly, the clearance of HA takes place mainly by receptor-mediated mechanisms by hepatic sinusoidal endothelial cells [28–30]. Serum HA corresponds to the function of the sinusoidal endothelial cells and/or the hemoperfusion of the liver and reflects the degree of liver damage as an objective marker [31–34]. Furthermore, HA was shown to be a valid prognostic indicator in liver cirrhosis [35].

In patients with CLD, clearance of the AGE proteins by sinusoidal endothelial cells may be affected. The capillarization of sinusoids during fibrosis and the diffusion barrier are associated with the severity of liver cirrhosis. Therefore, based on the formation of an incomplete subendothelial basal membrane, there is hindrance of substance exchange between hepatocytes and the sinusoidal blood stream, resulting in a decrease in clearance of circulating molecules. Additionally, constriction of the sinusoidal blood stream leads to the development of portal hypertension, a portocaval shunt and a reduced hemoperfusion [36]. Because intracellular degradation leads to even more reactive intermediates, the liver, especially in CLD patients, cannot prevent the accumulation of these substances effectively [37]. In contrast to HA, high CML levels may also reflect an increase of reactive intermediates and may be an early marker of liver damage. CML concentration in the circulation can increase because of severe diseases such as diabetes mellitus or uremia, which have high non-enzymatic activity rates. However, it is reasonable to assume that, analogous to HA, sinus endothelial cells in the liver play an important role in the elimination of CML-modified serum proteins. The CML clearance could be regulated just like the HA clearance [29] by a receptor mechanism in the hepatic sinusoidal endothelial cells as AGEs preferably bind to Kupffer and sinus endothelial cells in the liver [38]. Intra- as well extracellular disposal problems may occur due to the accumulation of AGEs. In the end, this may lead to an immune response, oxidative stress and the peroxidation of lipids. Disorders inflicted by AGEs are conceivable on very different functional levels in the organism or the organ.

In addition, we previously showed [16] that in rat liver RAGE mRNA is expressed in HSC and myofibroblasts and

the expression of RAGE protein is restricted to HSC and myofibroblasts — the key players in the pathogenesis of liver fibrosis/cirrhosis [2]. Being the most important ligand for RAGE on HSC and myofibroblasts, CML could trigger a multitude of intracellular signaling pathways, including the transcription factor NF κ B, the transcription factor AP-1 and the MAP-kinase pathway, which could lead to pathophysiologically relevant gene activation (i.e. extracellular matrix proteins) [18]. Therefore, CML may play a pathogenetically important role in the activation and matrix gene expression of liver fibrogenesis [39–41].

We conclude that the concentration of CML-modified proteins, shown to be elevated in cirrhotic patients, may serve as a helpful supplementary marker in the assessment of the severity of the disease. Since CML was highest in biliary and autoimmune liver disease, the clearance of CML through the bile requires further studies. However, the clinical potential and prognostic impact of CML serum concentrations in patients with liver cirrhosis should be evaluated in future studies. In addition, the pathogenetic role of CML-modified AGEs and their receptor (RAGE) in liver cirrhosis needs to be clarified.

Acknowledgment

This work was supported by the German Research Foundation (DFG fellowship grant TA 434/1-1 to F.T.).

References

- [1] Reeves HL, Friedman SL. Activation of hepatic stellate cells—A key issue in liver fibrosis. *Front Biosci* 2002;7:808–26.
- [2] Gressner AM, Bachem MG. Molecular mechanisms of liver fibrogenesis—A homage to the role of activated fat-storing cells. *Digestion* 1995;56:335–46.
- [3] Desmet V, Fevery J. Liver biopsy. *Bailliere's Clin Gastroenterol* 1995;9:811–28.
- [4] Bedossa P, Dagere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003;38:1449–57.
- [5] McGill DB, Rakela J, Zinsmeister AR, Ott BJ. A 21-year experience with major hemorrhage after percutaneous liver biopsy. *Gastroenterology* 1990;99:1396–400.
- [6] Reddy S, Bichler J, Wells-Knecht KJ, Thorpe SR, Baynes JW. N ϵ (carboxymethyl)lysine is a dominant advanced glycation endproduct (AGE) antigen in tissue proteins. *Biochemistry* 1995;34:10872–8.
- [7] Ikeda K, Higashi T, Sano H, et al. N-epsilon-(carboxymethyl)lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. *Biochemistry* 1996;35:8075–83.
- [8] Niwa T, Katsuzaki T, Momoi T, et al. Modification of β 2m with advanced glycation end products as observed in dialysis-related amyloidosis by 3-DG accumulating in uremic serum. *Kidney Int* 1996;49:861–7.
- [9] Hayashi T, Namiki M. In: Fujinaki M, Namiki M, Kato H, editors. Amino-carbonyl reaction in food and biological systems. Amsterdam: Elsevier; 1986. p. 29–38.
- [10] Wells-Knecht KJ, Zyzak DV, Litchfield JE, Thorpe SR, Baynes JW. Mechanism of autoxidative glycosylation: identification of glyoxal and arabinose as intermediates in the autoxidative modification of proteins by glucosyl. *Biochemistry* 1995;34:3702–9.
- [11] Vasan S, Zhang X, Kapumiotu A, et al. An agent cleaving glucose-derived protein crosslinks in vitro and in vivo. *Nature* 1996;382:275–8.
- [12] Makino H, Shikata K, Hironaka K, et al. Ultrastructure of non-

- enzymatically glycosylated mesangial matrix in diabetic nephropathy. *Kidney Int* 1995;48:517–26.
- [13] Yamada K, Nakano H, Nakayama M, et al. Immunohisto-chemical study of human advanced glycolysation end products (AGE) in chronic renal failure. *Clin Nephrol* 1994;42:354–61.
- [14] Kume S, Takeya M, Mori T, et al. Immunohistological and ultrastructural detection of advanced glycation end products in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. *Am J Pathol* 1995;147:654–67.
- [15] Bucala R, Cerami A. Advanced glycolysation: chemistry, biology, and implications diabetes and aging. *Adv Pharmacol* 1992;23:1–34.
- [16] Fehrenbach H, Weiskirchen R, Kasper M, Gressner AM. Upregulated expression of the receptor for advanced glycation end products in cultured rat hepatic stellate cells during transdifferentiation to myofibroblasts. *Hepatology* 2001;34:943–52.
- [17] Bucciarelli LG, Wendt T, Rong L, et al. RAGE is a multiligand receptor of the immunoglobulin superfamily: implications for homeostasis and chronic disease. *Cell Mol Life Sci* 2002;59:1117–28.
- [18] Kislinger T, Fu C, Huber B, et al. *N*ε-(carboxymethyl)lysine adducts of proteins are ligands for receptor for advanced glycation end products that activate cell signalling pathways and modulate gene expression. *J Biol Chem* 1999;274:31740–9.
- [19] Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. *Biochim Biophys Acta* 2000;1498:99–111.
- [20] Tacke F, Brabant G, Kruck E, et al. Ghrelin in chronic liver disease. *J Hepatol* 2003;38:447–54.
- [21] Pugh RNH, Murray-Lyon M, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973;60:646–9.
- [22] Zhang X, Frischmann M, Kientsch-Engel R, et al. Two immunochemical assays advanced glycation and-products in serum from dialysis patients. *Clin Chem Lab Med* 2005;43:503–11.
- [23] Lindqvist M, Chichibu K, Delpech B, et al. Seven different assays of hyaluronan compared for clinical utility. *Clin Chem* 1992;38:127–32.
- [24] Sebekova K, Kupcova V, Schinzel R, Heidland A. Markedly elevated levels of plasma advanced glycation end products in patients with liver cirrhosis—Amelioration by liver transplantation. *J Hepatol* 2002;36:66–71.
- [25] Smedsrød B, Melkko J, Araki N, Sano H, Hourichi S. Advanced glycation end products are eliminated by scavenger-receptor-mediated endocytosis in hepatic sinusoidal Kupffer and endothelial cells. *Biochem J* 1997;322:567–73.
- [26] Vlassara H. Recent progress in advanced glycation end products and diabetic complications. *Diabetes* 1997;46:519–25.
- [27] Thornalley PJ. Cell activation by glycosylated proteins. AGE receptors, receptor recognition factors and functional classification of AGEs. *Mol Biol* 1998;44:1013–23.
- [28] Eriksson S, Fraser JRE, Pertoft H, Smedsrød B. Endothelial cells are a site of uptake and degradation of hyaluronic acid in the liver. *Exp Cell Res* 1983;144:223–8.
- [29] Smedsrød B, Pertoft H, Eriksson S, Fraser RE, Laurent TC. Studies in vitro on the uptake and degradation of sodium hyaluronate in rat liver endothelial cells. *Biochem J* 1984;222:617–26.
- [30] Fraser JRE, Alcorn D, Laurent TC, Robinson AD, Ryan GB. Uptake of circulating hyaluronic acid by rat liver endothelial cells: evidence for receptor recycling. *Biochem J* 1985;257:875–84.
- [31] McHutchison JG, Blatt LM, de Medina M, et al. Measurement of serum hyaluronate acid in patients with chronic hepatitis C and its relationship to liver histology. *J Gastroenterol Hepatol* 2000;15:945–51.
- [32] Nyberg A, Engstrom-Laurent A, Loof L. Serum hyaluronate in primary biliary cirrhosis: a biochemical marker for progressive liver damage. *Hepatology* 1988;8:142–6.
- [33] Ueno T, Inuzuka S, Sata M, et al. Serum hyaluronate predicts response to interferon alpha therapy in patients with chronic hepatitis C. *Hepatogastroenterology* 1995;42:522–7.
- [34] Bramley PN, Rathbone BJ, Forbes MA, Cooper EH, Losowsky MS. Serum hyaluronate as marker of hepatic derangement in acute liver damage. *J Hepatol* 1991;13:8–13.
- [35] Körner T, Kropf J, Kosche B, Kristahl H, Jaspersen D, Gressner AM. Improvement of prognostic power of the Child–Pugh classification of liver cirrhosis by hyaluronan. *J Hepatol* 2003;39:948–54.
- [36] Svistounov D, Smedsrød B. Hepatic clearance of advanced glycation end products (AGEs)—Myth or truth. *J Hepatol* 2004;41:1038–40.
- [37] Glomb MA, Monnier VM. Mechanisms of protein modification by Glyoxal and Glycolaldehyde, reactive intermediates of the Maillard reaction. *J Biol Chem* 1995;17:10017–26.
- [38] Youssef S, Soulis T, Cooper ME. Hepatic advanced glycation endproduct binding is increased in experimental diabetes. *Cell Mol Biol* 1998;44:1095–100.
- [39] Czaja MJ. Induction and regulation of hepatocyte apoptosis by oxidative stress. *Antioxid Redox Signal* 2002;4:759–67.
- [40] Lasnier E, Blanc MC, Housset C, Rey C, Roch Arveiller M, Vaubourdolle M. Cytotoxic response of sinusoidal endothelial cells to polymorphonuclear leukocytes and its potential implication in hypoxia-reoxygenation injury. *Liver* 2002;22:495–500.
- [41] Greenwel P, Dominguez-Rosales JA, Mavi G, Rivasestilla AM, Rojkind M. Hydrogen peroxide: a link between acetaldehyde-elicited alpha 1(I) collagen gene up-regulation and oxidative stress in mouse hepatic stellate cells. *Hepatology* 2000;31:109–16.