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Circulating Advanced Oxidation Protein Products, N ϵ -(Carboxymethyl) Lysine and Pro-Inflammatory Cytokines in Patients with Liver Cirrhosis: Correlations with Clinical Parameters

Jolanta Zuwala-Jagiello¹,

Eugenia Murawska-Cialowicz² and Monika Pazgan-Simon³

¹Department of Pharmaceutical Biochemistry, Wroclaw Medical University,

²Department of Physiology and Biochemistry, University of Physical Education, Wroclaw,

³Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency,

Wroclaw Medical University,

Poland

1. Introduction

Patients with chronic liver disease are characterized by hepatic inflammation and the destruction of hepatocytes. Viral antigen-specific cytotoxic T lymphocytes, polyclonal cytokines, immune modulators, and oxidized biomolecules have been shown to induce damage and destruction of hepatocytes in these patients (Tsutsui *et al.*, 2003). The contribution of oxidative stress *per se* to the chronic inflammatory state has been suggested, and consistent evidence has been afforded that both monocyte/macrophage activation and a defect in antioxidant systems occur early in the course of chronic liver failure and gradually increase with its progression to end-stage liver disease (Kirkham, 2007; Videla, 2009). Oxidative stress lead to formation of glycoxidation products, including advanced glycation endproducts (AGEs - among them N ϵ -(carboxymethyl)lysine (CML) is best known), and advanced oxidation protein products (AOPPs). AOPPs can be formed *in vitro* by exposure of serum albumin to hypochlorous acid. *In vivo*, plasma AOPPs are mainly carried by albumin and their concentrations are closely correlated with the levels of dityrosine. Within the heterogeneous group of AGEs, N ϵ -(carboxymethyl)lysine has been identified as a major AGEs *in vivo* (Reddy *et al.*, 1995). Plasma concentrations of AGEs (closely correlating with AOPPs levels) increase with progression of chronic diseases (Witko-Sarsat *et al.*, 1996; 1998), therefore CML has been considered as liver disease-related biomarker for oxidative stress (Sebeková *et al.*, 2002; Yagmur *et al.*, 2006).

The receptor for advanced glycation endproducts (RAGE) is a signal transduction receptor that binds both AGEs and AOPPs. RAGE is expressed by various cell types, including

monocytes/macrophages, endothelial cells, smooth muscle cells and renal cells (Miyata *et al.*, 1994). Advanced glycation endproducts have been found to act as pro-inflammatory factors (Sparvero *et al.*, 2009). Nevertheless, AOPPs are believed to be more closely related to inflammation (Alderman *et al.*, 2002; Baskol *et al.*, 2006; Fialova *et al.*, 2006; Witko-Sarsat *et al.*, 2003; Yazici *et al.*, 2004) than AGEs, whose receptor for advanced glycation endproducts participates in AOPPs-mediated signal transduction (Kalousova *et al.*, 2003; 2005). These interactions enhance reactive oxygen species formation, with activation of nuclear factor NF- κ B and release of pro-inflammatory cytokines (Bierhaus *et al.*, 2006; Hyogo & Yamagishi, 2008; Saito & Ishii, 2004). Moreover, the monocyte/macrophage RAGE can be up-regulated by tumor necrosis factor- α (TNF- α) (Miyata *et al.*, 1994). Peripheral blood monocytes showed activity and elevated expression of TNF- α which correlated with liver disease severity (Hanck *et al.*, 2000). The concentrations of advanced oxidation protein products are high in liver cirrhosis of various etiologies (Zuwala-Jagiello *et al.*, 2009) and can reflect hemodynamic alterations in the liver (Guo *et al.*, 2008). This is accompanied by the activation of monocytes and increased expression of TNF- α (Giron-González *et al.*, 2004). High serum levels of TNF- α and interleukin-6 (IL-6) have been found in cirrhotic patients with ascites in the absence of demonstrable infection (Tilg *et al.*, 1992; Zeni *et al.*, 1993).

The accumulation of AGEs has been linked to vascular lesions in diabetes, chronic renal insufficiency, and atherosclerosis. Activation of NF- κ B, mediated by RAGE, promotes expression of the cytokines, as well as pro-inflammatory adhesion molecules (Basta *et al.*, 2002; Bierhaus *et al.*, 1998; Esposito *et al.*, 1989), what may enhance interaction of cirrhotic vasculature with circulating monocytes (Cybulsky *et al.*, 1991; Li *et al.*, 1993). Recently, it has also been shown that AOPPs activates vascular endothelial cells *via* RAGE-mediated signals (Guo *et al.*, 2008).

Endothelial activation plays an active role in the modifications of circulatory status of cirrhotic patients (Genesca *et al.*, 1999). The circulatory changes are more evident in advanced stages of liver cirrhosis, such as those represented by the presence of ascites or hepatorenal syndrome (Porcel *et al.*, 2002). We have demonstrated that elevated levels of AOPPs modified-albumin (AOPPs-albumin) are related to the severity of liver cirrhosis (Zuwala-Jagiello *et al.*, 2009; 2011). The role of AOPPs-albumin in liver cirrhosis and portal hypertension has not yet been studied. The effects of pro-inflammatory cytokines on the vessels and on liver function would influence the liver cirrhosis, with higher plasma levels of AOPPs indicating a poor prognosis. In the present study, plasma levels of AOPPs-albumin, as well as of N ϵ (carboxymethyl)lysine modified-albumin (CML-albumin) and pro-inflammatory cytokines, such as TNF- α and IL-6, have been analyzed in cirrhotic patients and were found to be correlated with clinical parameters of liver dysfunction.

2. Patients and methods

2.1 Patients

This study was performed on 129 patients with chronic liver disease admitted to the Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency for evaluation. The experimental group consisted of 68 men and 61 women with age of 18–74 years (median age was 66). The control group contained 40 healthy subjects (23 men and 17 women) with age of 19–56 (median age was 55). Blood samples were collected in the Department of

Physiology and Biochemistry, University of Physical Education in Wrocław. Clinical and biochemical characteristics of the study group are reported in detail in Table 1.

	Healthy controls	All patients	Non-cirrhotic patients	Cirrhotic patients
(n)	40	129	41	88
Male:Female ratio	23:17	68:61	15:26	53:35
Age (years)	55 (19-56)	66 (18-74)	56 (18-69)	55 (21-74)
Etiology (n)				
Virus hepatitis		62	18	44
Alcohol		54	21	33
Biliary		13	2	11
Albumin (g/L)	45 (36-57)	34 (16-45)	37* (29-49)	30* (16-45)
ALT (U/L)	24 (20-28)	40 (16-79)	28 (24-33)	47** (16-79)
AST (U/L)	27 (23-30)	70 (19-150)	41 (19-64)	79** (19-150)
Bilirubin (mg/dL)	0.7 (0.6-0.9)	0.98 (1.0-3.6)	0.92 (0.90-0.95)	1.6* (1.0-3.6)
γGT (U/L)	26 (25.3-27.8)	70 (41-106)	48 (41-56)	92** (78-106)
AP (U/L)	90 (50-130)	125 (100-163)	105 (100-147)	152** (141-163)
Serum creatinine (mg/dL)	0.8 (0.7-1.0)	1.4 (0.7-2.4)	0.96 (0.9-1.2)	1.39* (0.7-2.4)
Serum sodium (mEq/L)	140 (138-141)	137 (129-142)	136 (129-138)	130** (129-142)

Table 1. Clinical and biochemical characteristics of the study subjects. Statistical significance: * $P < 0.05$; ** $P < 0.01$ vs. healthy controls. AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; γGT, γ-glutamyltransferase; INR, normalised international ratio; MELD, model of end-stage liver disease.

The diagnosis of liver cirrhosis was based on clinical, laboratory and ultrasonographic findings or histological criteria. Alcohol-related liver cirrhosis was diagnosed in 33 of patients, primary biliary cirrhosis in 11, cirrhosis caused by hepatitis C virus in 30, whereas cirrhosis caused by hepatitis B virus in 14 patients. The Child-Pugh score was used to assess the severity of liver disease. Three biochemical variables [serum albumin, bilirubin, and prothrombin time (international normalized ratio, INR)] in addition to the two clinical characteristics (presence or absence of ascites and clinical signs of encephalopathy) were determined. Patients were scored as follows: 5–6 as class (group) A, 7–9 as class (group) B and 10–15 as class (group) C. The patients with cirrhosis were divided into compensated (Child-Pugh class A) and decompensated (Child-Pugh classes B and C) groups. At the time of the study no Child-Pugh A patients showed clinical features of decompensated liver cirrhosis (ascites or hepatic encephalopathy). At enrollment, esophageal varices were detected by endoscopy in 88% of patients, ascites and hepatic encephalopathy grade were present by physical examination in 53 (60%) and 23 (26%) patients, respectively.

Exclusion criteria were concurrent use of antioxidant drugs; co-existing diseases like diabetes mellitus, chronic kidney disease, cardiovascular disease, hepatocellular carcinoma; gastrointestinal bleeding, bacterial infection, and blood transfusion within previous two weeks.

Patients had not been receiving diuretic, antibiotic, vasoactive drug (nitrates, β -blockers), and lactulose or lactitol therapy during the eight days before inclusion in the study. After 2 h of bed rest, blood pressure was determined with an automatic digital sphygmomanometer and blood samples were collected in ice-cooled, ethylenediaminetetraacetic acid (EDTA)-containing tubes for the determination of plasma renin activity, antidiuretic hormone, and plasma AOPPs or N ϵ -(carboxymethyl)lysine, in tubes with no additive for routine biochemical study and aldosterone and cytokine concentrations. All samples were separated immediately by centrifugation at 4°C and stored at -80°C until further analysis.

The consent of the Bioethics Committee of the Wroclaw Medical University was obtained and all patients were informed about the character of analyses made. Studies were conducted in compliance with the ethical standards formulated in the Helsinki Declaration of 1975 (revised in 1983).

2.2 Determination of circulating AOPPs

In vivo plasma levels of AOPPs closely correlate with levels of dityrosine, a hallmark of oxidized proteins and with pentosidine, a marker of protein glycation closely related to oxidative stress. A new chromogen is found which caused increased absorbance at 340 nm and its spectrophotometric determination is proposed as a novel index of oxidative stress measuring the level of AOPPs (Witko-Sarsat *et al.*, 1996). Two-hundred microliters of plasma diluted 1:5 in 20 mM phosphate buffer pH 7.4 containing 0.9% sodium chloride (PBS), or chloramine-T standard solutions (0 to 100 μ mol/L), were placed in each well of a 96-well microtiter plate (Becton Dickinson Labware, Lincoln Park, NJ, USA), followed by 20 μ L of 10% acetic acid. Ten microliters of 1.16 M potassium iodide (Sigma-Aldrich Co. LLC, Canada) were then added, followed by 20 μ L of 10% acetic acid. The absorbance of the reaction mixture was immediately read at 340 nm in a microplate reader against a blank containing 200 μ L of PBS, 10 μ L of KI and 20 μ L of 10% acetic acid. The chloramine-T

absorbance at 340 nm was linear within the range of 0 to 100 $\mu\text{mol/L}$. The ratio of AOPPs concentration to albumin level (AOPPs-albumin) was expressed in micromoles of AOPPs per gram of albumin ($\mu\text{mol/g}$). The ratio of AOPPs to albumin content allows the evaluation of whether the proportion of oxidatively modified albumin is altered. Coefficient of variation (CV) served as an indicator of precision. Intra-day and inter-day CV values were $<10\%$.

2.3 Determination of circulating N ϵ -(carboxymethyl) lysine

Plasma N ϵ -(carboxymethyl)lysine (CML) levels were determined using a specific competitive ELISA kit [CircuLex CML/N ϵ -(carboxymethyl)lysine ELISA Kit (CycLex Co., Ltd, Nagano, Japan)]. Measurements were performed in duplicate and the results were averaged. The ratio of CML concentration to albumin level (CML-albumin) was expressed in micrograms of CML per gram of albumin ($\mu\text{g/g}$).

2.4 Laboratory determinations

Biochemical parameters were measured using routine laboratory methods. Serum high-sensitivity C-reactive protein (hs-CRP) level was determined with a high-sensitivity nephelometric method using the Beckman Image Immunochemistry system (Beckman Instruments, Fullerton, CA), which has a minimum level of detection of 0.2 mg/L. Serum levels of TNF- α and IL-6 were assayed with enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The minimum levels of detection were 1.6 pg/mL and < 0.70 pg/mL for TNF- α and IL-6, respectively. The intra- and interassay coefficients of variation for measurements of CRP, IL-6, and TNF- α were 2.7%, 4.3%, and 5.0%, respectively, and 3.0%, 5.5, and 6.9%, respectively.

Aldosterone (Aldoctk-2-P2714; Sorin Biomedica Diagnostics, Barcelona, Spain. Normal values, 35-150 pg/mL) and plasma renin activity (Clinical Assays, Baxter, Cambridge, Mass., USA. Normal values, 0.4-2.3 ng mL $^{-1}$ h $^{-1}$) were measured by specific radioimmunoassays. Antidiuretic hormone was also tested by a commercial radioimmunoassay (Buhlman Laboratories, Basel, Switzerland. Normal values, less than 1 pg/mL).

2.5 Measurement of the total antioxidant status of plasma

The plasma antioxidant capacity was measured using a commercially available total antioxidant status TAS kit (Randox Laboratories, Crumlin, UK). The TAS assay is based on the inhibition by antioxidants of the absorbance of the radical cation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) formed by the interaction of ABTS with ferrylmyoglobin radical species. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of the total antioxidant capacity of the plasma. The assay has excellent precision values, lower than 3%, and the results are expressed as mmol/L.

2.6 Model for End-stage Liver Disease (MELD) score

The model for end-stage liver disease (MELD) score was calculated from the following equation:

$9.57 \times \log_e (\text{creatinine mg/dL}) + 3.78 \times \log_e (\text{total bilirubin mg/dL}) + 11.2 \times \log_e (\text{international normalized ratio-INR}) + 6.43$ (constant for liver disease etiology).

The maximal creatinine concentration considered in the MELD score is 4.0 mg/dL (Huo *et al.*, 2006).

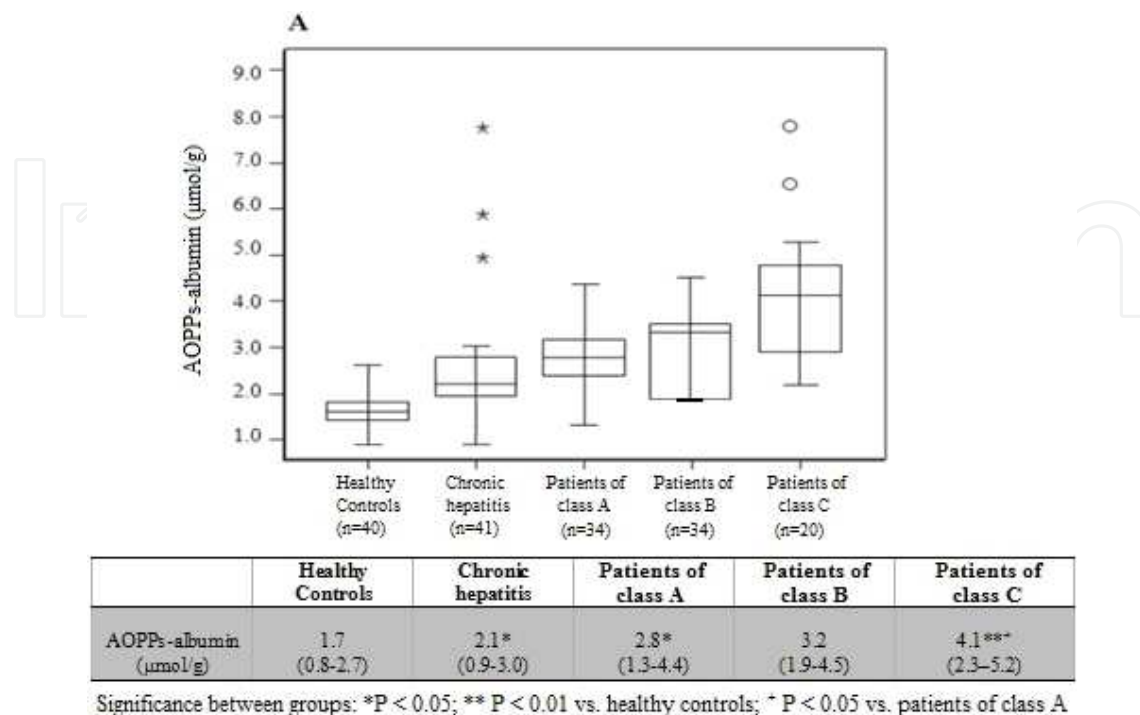
2.7 Statistical analysis

Results are expressed as median (25th percentile–75th percentile). Frequency data were compared using the χ^2 test or the Fischer's exact test when necessary. Because many of the variables analyzed did not have a normal distribution as determined by the Kolmogorov-Smirnov test, nonparametric tests were used for comparison of data. The Mann-Whitney U test and the Kruskal-Wallis test were used to analyze differences among two or more groups, respectively. Multivariate analysis by conditional logistical regression with a forward stepwise method was performed to find independent variables associated with the presence of ascites and low mean arterial pressure. Regression analysis to determine significant correlations among different parameters was performed using the Spearman correlation coefficient. Statistical significance was established at $P < 0.05$.

3. Results

3.1 AOPPs-albumin plasma concentrations in patients with chronic liver disease and healthy controls

We analyzed 129 patients (68 males/61 females, median age 66 years, range 18–74 years) with chronic liver disease. The distribution of the stages of liver cirrhosis as defined according to the Child-Pugh score, and measurements of AOPPs-albumin and CML-albumin plasma concentrations are presented in Fig.1. The concentration of AOPPs-albumin



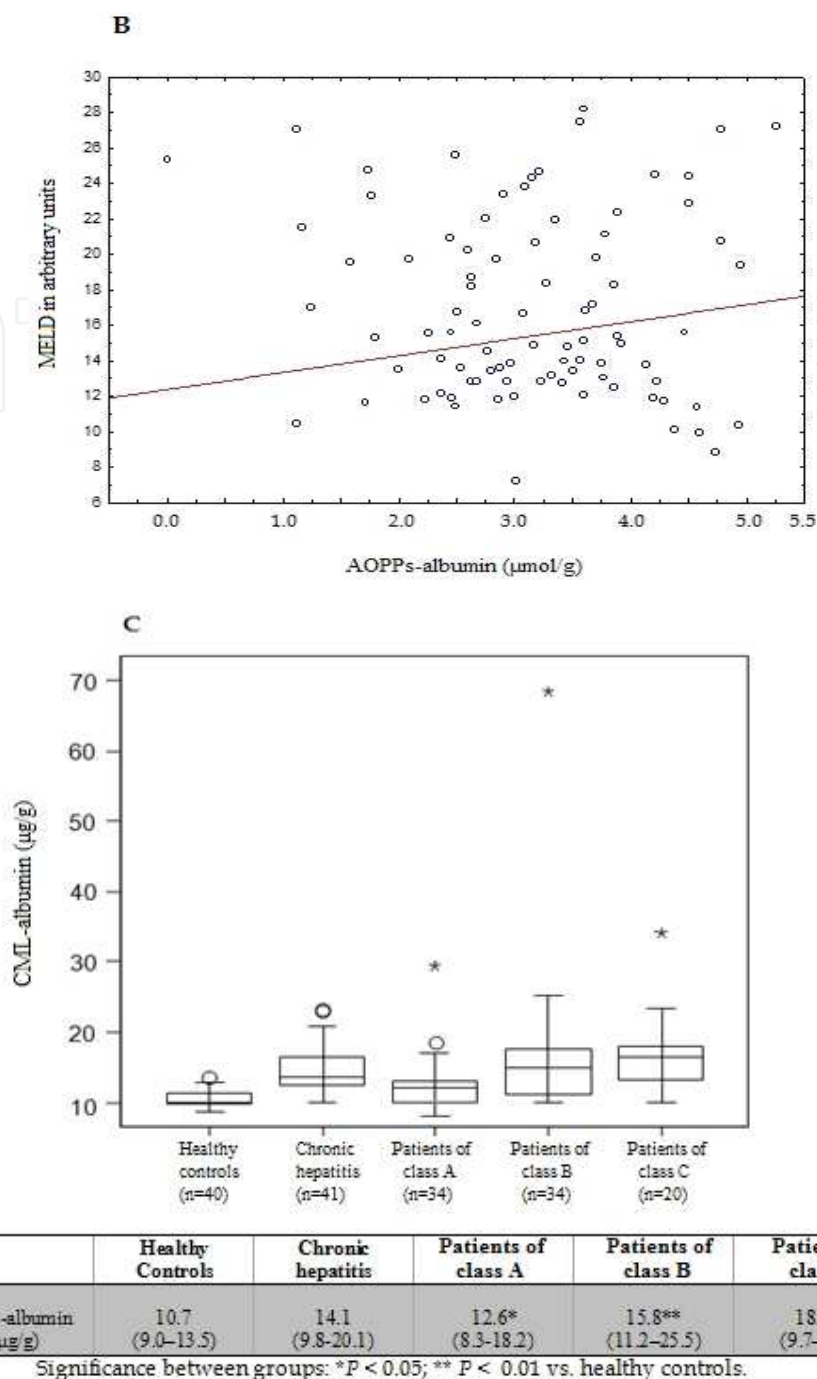


Fig. 1. (A) AOPPs-albumin serum concentrations in 129 patients with chronic liver disease, according to Child's stage of cirrhosis, and in a control group of 40 healthy blood donors. *P* values are given in the table. Comparisons between subgroups are illustrated with box plot graphics, where the dotted line indicates the median per group, the box represents 50% of the values, and horizontal lines show minimum and maximum values of the calculated non-outlier values; asterisks and open circles indicate outlier values. (B) AOPPs-albumin serum concentrations in patients with cirrhosis are correlated with the MELD (model of end-stage liver disease) score ($r = 0.43$, $P < 0.01$, Spearman rank correlation test). (C) CML-albumin serum concentrations increase with the stage of liver cirrhosis in patients with chronic liver disease. *P* values are given in the table.

in healthy subjects was 1.7 $\mu\text{mol/g}$ (range 0.8-2.7 $\mu\text{mol/g}$, $P < 0.05$). In patients with chronic liver disease, AOPPs-albumin plasma concentrations were 1.3-fold higher. In healthy controls, the plasma AOPPs or CML were similar to those in control groups in other studies (Sebeková *et al.*, 2002; Kalousová *et al.*, 2003).

3.2 AOPPs-albumin and liver cirrhosis

AOPPs-albumin plasma concentration was significantly higher in patients with liver cirrhosis ($n = 88$, median 2.4 $\mu\text{mol/g}$, range 1.3-5.6 $\mu\text{mol/g}$) compared to patients with chronic liver disease without cirrhosis ($n = 41$, median 2.1 $\mu\text{mol/g}$, range 0.9-3.0 $\mu\text{mol/g}$) ($P < 0.05$, Fig.1A). Patients with Child-Pugh class C exhibited significantly higher plasma concentrations of AOPPs-albumin than patients with Child-Pugh class A and controls ($P < 0.05$, $P < 0.01$, respectively) (Fig.1A). There was no significant difference in AOPPs concentrations between control subjects and Child-Pugh B cirrhotic patients.

Differences in plasma AOPPs-albumin or CML-albumin were not significant in patients with liver cirrhosis of various etiologies (Table 2). Only in the group with primary biliary cirrhosis AOPPs-albumin were decreased ($n = 11$, median 1.3 $\mu\text{mol/g}$, range 0.80-2.2 $\mu\text{mol/g}$), though it should be consider with caution since small number of subjects included in this group.

	AOPPs-albumin ($\mu\text{mol/g}$)	CML-albumin ($\mu\text{g/g}$)	TAS (mmol/L)
Healthy controls ($n=40$)	1.7 (0.80-2.7)	10.7 (9.0-13.5)	1.31 (1.12-1.5)
Viral hepatitis-related cirrhosis ($n=44$)	3.09* (1.5-5.2)	13.3* (11.6-18.1)	0.65* (0.48-0.75)
Alcohol-related cirrhosis ($n=33$)	2.9* (1.6-4.3)	16.3* (13.3-25.5)	0.71* (0.60-0.73)
Primary biliary cirrhosis ($n=11$)	1.3 (0.80-2.2)	11.8 (8.3-12.5)	0.98 (0.76-0.83)

Biliary etiology shows lower AOPPs-albumin levels compared with other etiologies of liver disease. Significance levels between groups: * $P < 0.05$ vs. healthy controls.

AOPPs, advanced oxidation protein products; CML, N ϵ -(carboxymethyl)lysine; TAS, total antioxidant status.

Table 2. Plasma AOPPs-albumin, CML-albumin and TAS in liver cirrhosis patients of various etiologies.

The MELD scores were determined in the 88 patients with liver cirrhosis (Table 3). These were higher in the Child-Pugh C cirrhotic patients than in the Child-Pugh A cirrhotic patients ($p < 0.01$). Significant correlations between AOPPs levels and MELD scores ($r = 0.43$, $P < 0.01$; Fig. 1B) were observed among the cirrhotic patients belonging to all three Child-Pugh classes.

	Healthy controls	Non-cirrhotic patients	Patients of class A	Patients of class B	Patients of class C
(n)	40	41	34	34	20
Age (years)	55 (19-56)	56 (18-69)	51 (21-74)	58 (24-71)	56 (29-69)
Albumin (g/L)	45 (36-57)	40 (29-49)	34* (28-45)	30* (20-40)	25* (16-32)
Bilirubin (mg/dL)	0.7 (0.6-0.9)	0.92 (0.90-0.95)	1.01 (1.02-1.03)	1.56* (1.0-2.0)	2.15* (1.1-3.6)
AOPPs-albumin (μmol/g)	1.7 (0.8-2.7)	2.1* (0.9-3.0)	2.8* (1.3-4.4)	3.2 (1.9-4.5)	4.1*** (2.3-5.2)
CML-albumin (μg/g)	10.7 (9.0-13.5)	14.1 (9.8-20.1)	12.6* (8.3-18.2)	15.8** (11.2-25.5)	18.3** (9.7-23.8)
Uric acid (mmol/L)	0.35 (0.25-0.39)	0.29 (0.21-0.33)	0.31 (0.26-0.34)	0.22 (0.16-0.24)	0.18* (0.19-0.31)
Vitamin C (μmol/L)	54.3 (38.3-70.3)	45.2 (31.9-58.6)	47.1 (28.3-64.0)	33.6 (20.2-45.7)	36.9 (29.2-54.2)
TAS (mmol/L)	1.3 (1.1-1.5)	1.1 (0.9-1.3)	0.9 (0.6-1.0)	0.63 (0.5-0.76)	0.53*** (0.5-0.8)
INR	0.8-1.1	-	0.9 (0.8-1.09)	1.2 (1.1-1.3)	2.3 (1.6-2.9)
MELD score	6-8	-	7.8 (5.2-10.3)	15.9* (8.7-23.1)	24.1** + (14.4-28.4)

Table 3. Plasma concentrations of AOPPs-albumin, CML-albumin and antioxidant parameters in patients with chronic liver disease without cirrhosis and in patients with cirrhosis. Significance between groups: * $P < 0.05$; ** $P < 0.01$ vs. healthy controls; + $P < 0.05$; ++ $P < 0.01$ vs. patients of class A. AOPPs, advanced oxidation protein products; CML, Nε-(carboxymethyl)lysine; TAS, total antioxidant status; MELD, model for end-stage liver disease score.

3.3 CML-albumin plasma concentrations in patients with CLD and healthy controls

In patients with chronic liver disease, CML-albumin had a median value of 14.1 $\mu\text{g/g}$ (range 9.8-20.1 $\mu\text{g/g}$). Plasma CML-albumin concentrations were higher in Child-Pugh A to C cirrhotic patients ($n = 88$, median 15.7 $\mu\text{g/g}$, range 8.3-25.5 $\mu\text{g/g}$) than in patients without cirrhosis, but this difference was not statistically significant (Fig. 1C). The levels of plasma CML-albumin in all liver cirrhotic patients were higher than those of the controls and this difference was statistically significant (Fig. 1C). Plasma CML-albumin in patients with Child-Pugh class C cirrhosis was only slightly elevated compared with those in Child-Pugh class A cirrhosis ($P = 0.17$) (Fig. 1C). There was no statistically significant correlation between CML-albumin levels and the Child-Pugh score in cirrhotic patients.

3.4 Antioxidant parameters and liver cirrhosis

As it is seen in Table 3, while all individual parameters of the antioxidant status tend to decrease, only the decrease of uric acid was statistically significant. There was a markedly decreased total antioxidant status (TAS) in patients with Child-Pugh class C cirrhosis compared to those with Child-Pugh class A cirrhosis or controls ($P < 0.05$, $P < 0.01$, respectively). Although differences between cirrhosis and chronic liver disease ($n = 41$, median 1.1, mmol/L, range 0.9-1.3 mmol/L) were not statistically significant, weak but significant correlation was observed between TAS and plasma AOPPs-albumin ($r = -0.31$, $P < 0.05$). We failed to find, however, any relation between circulating CML-albumin levels and TAS ($r = -0.22$, $P = 0.059$).

3.5 Markers of oxidative stress and hepatic function

The serum albumin concentration was determined in all patients ($n = 129$, median 34 g/L, range 16-45 g/L) and healthy control subjects ($n = 40$, median 45 g/L, range 36-57 g/L). Level of albumin, the main substrate in both AOPPs and CML formation (Kalousová *et al.*; 2005), was significantly depleted both in chronic liver disease ($n = 41$, median 37 g/L, range 29-49 g/L) and in cirrhosis (median 30 g/L, range 16-45 g/L) (Table 1). Plasma CML-albumin and foremost AOPPs-albumin showed significant associations with biochemical indices of liver function (albumin, prothrombin time, bilirubin concentration) but not with markers of liver injury - aminotransferases (Table 4). As expected, in patients with cirrhosis, AOPPs-albumin weakly but significantly correlated with the serum albumin ($r = -0.38$, $P < 0.05$).

3.6 AOPPs-albumin, CML-albumin and chronic inflammatory state in cirrhotic patients

We assessed the levels of several inflammatory markers and their association with the levels of AOPPs-albumin and CML-albumin. Serum high-sensitivity C-reactive protein (hs-CRP) levels and white blood cells (WBC) counts were significantly elevated in cirrhotic patients (Table 5). Serum TNF- α levels were higher in the Child-Pugh class C cirrhosis patients than in the Child-Pugh class A cirrhosis patients ($P < 0.05$) (Table 5). Moreover, TNF- α concentrations were weakly but significantly correlated with Child-Pugh score in cirrhotic group ($r = 0.31$, $P < 0.05$). The levels of serum IL-6 in cirrhotic patients were higher than those of the control group and this difference was statistically significant ($P < 0.05$) (Table 5). The levels of serum IL-6 in patients with Child-Pugh class C cirrhosis were higher than those in Child-Pugh class A cirrhosis, but this difference was not statistically significant.

	AOPPs-albumin ($\mu\text{mol/g}$)	CML-albumin ($\mu\text{g/g}$)
Albumin (g/L)	$r = -0.38, P < 0.05$	$r = -0.26, P = 0.07$
ALT (U/L)	$r = 0.10, P = 0.54$	$r = -0.14, P = 0.31$
AST (U/L)	$r = -0.20, P = 0.11$	$r = -0.15, P = 0.26$
Bilirubin (mg/dL)	$r = 0.23, P = 0.07$	$r = 0.24, P = 0.06$
Prothrombin ratio (%)	$r = -0.25, P < 0.05$	$r = -0.43, P < 0.001$
MELD score	$r = 0.43, P < 0.01$	$r = 0.23, P = 0.07$

Table 4. Correlations between plasma AOPPs-albumin and CML-albumin and selected biochemical indices of liver function and injury. ALT, alanine aminotransferase; AST, aspartate aminotransferase; MELD, model for end-stage liver disease score.

	Healthy controls (n=40)	Overall (n=88)	Patients of class A (n=34)	Patients of class B (n=34)	Patients of class C (n=20)
AOPPs-albumin ($\mu\text{mol/g}$)	1.7 (0.8-2.7)	2.4* (1.3-5.6)	2.8* (1.3-4.4)	3.2 (1.9-4.5)	4.1** (2.3-5.2)
CML-albumin ($\mu\text{g/g}$)	10.7 (9.0-13.5)	15.7* (8.3-25.5)	12.6* (8.3-18.2)	15.8** (11.2-25.5)	18.3** (9.7-23.8)
Albumin (g/L)	45 (36-57)	31** (16-35.4)	34* (28-45)	30* (20-40)	25* (16-32)
TNF- α (pg/mL)	32.9 (31.0-35.2)	41.5* (37.6-64.0)	36.9* (37.7-45.6)	42.0* (37.6-47.2)	51.7** (48.7-58.3)
IL-6 (pg/mL)	5.9 (5.4-6.8)	13.3** (6.4-39.9)	8.8* (6.4-34.6)	12.3* (6.8-33.9)	18.9* (9.0-39.9)
hsCRP (mg/L)	1.5 (0.63-2.0)	5.3** (4.9-11.0)	4.8* (5.5-7.0)	5.2** (4.9-7.7)	6.3** (6.8-10.1)

	Healthy controls (n=40)	Overall (n=88)	Patients of class A (n=34)	Patients of class B (n=34)	Patients of class C (n=20)
WBC ($\times 10^9/L$)	4.0 (4.2-5.0)	5.0 (1.1-8.6)	4.7 (1.1-8.6)	5.2* (3.0-7.8)	5.4*** (2.7-8.2)
MELD score	6-8	13.5* (5.2-28.4)	7.8 (5.2-10.3)	15.9* (8.7-23.1)	24.1**+ (14.4-28.4)

Table 5. Plasma concentrations of AOPPs-albumin, CML-albumin and inflammatory markers in healthy controls and in patients with liver cirrhosis. Significance levels between groups: * $P < 0.05$; ** $P < 0.01$ vs. healthy controls; + $P < 0.05$, ++ $P < 0.01$ vs. patients of class A. AOPPs, advanced oxidation protein products; CML, N ϵ -(carboxymethyl)lysine; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; WBC, white blood cells; MELD, model for end-stage liver disease score.

The association study revealed only a tendency toward an extremely weak but significant correlation between AOPPs-albumin and WBC in all cirrhotic patients ($r = 0.23$, $P < 0.05$). In turn, a weak but significant correlation between AOPPs-albumin levels and hs-CRP was observed among the cirrhotic patients belonging to all three Child-Pugh classes ($r = 0.33$, $P < 0.05$). There was a significant correlation between the IL-6 and the AOPPs-albumin level ($r = 0.42$, $P < 0.05$) and MELD score ($r = 0.38$, $P < 0.05$) in cirrhotic patients. As it was expected, a significant correlation between AOPPs-albumin levels and TNF- α ($r = 0.48$, $P < 0.05$) was observed in Child-Pugh class A cirrhosis patients. In the multivariate analysis the relationship between plasma AOPPs-albumin, TNF- α and Child-Pugh score was independent of age, sex and liver cirrhosis etiology (data not shown).

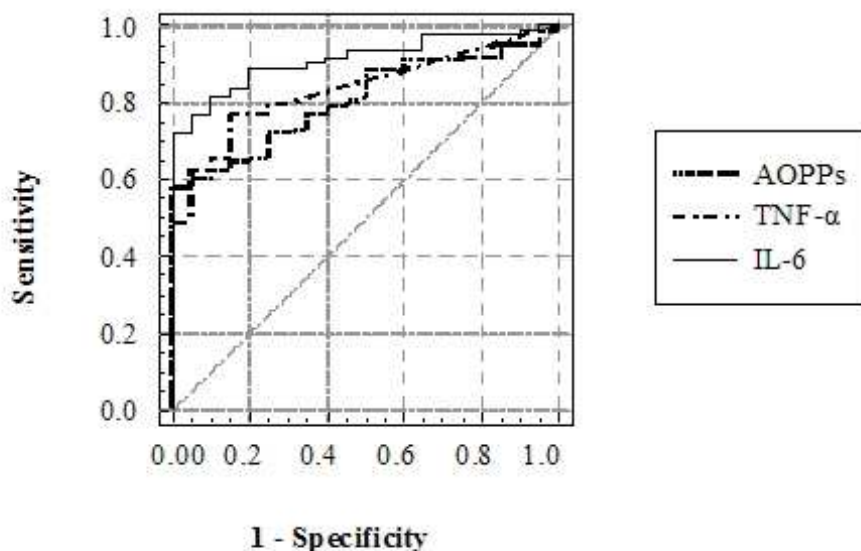
There was no statistically significant correlation between CML-albumin level and hs-CRP or cytokines levels in all liver cirrhotic patients (data not shown).

The ROC curve analyses are shown in Fig. 2 (sensitivity versus 1-specificity). The cut-off values of plasma AOPPs-albumin, TNF- α and IL-6 to separate cirrhotic patients from healthy controls were 3.71 $\mu\text{mol/g}$, 37.2 pg/mL , and 8.95 pg/mL , respectively.

3.7 Hemodynamic characteristics of the patients with liver cirrhosis

Hemodynamic characteristics of patients are shown in Tables 1 and 6. Cirrhotic patients had significantly lower values of mean arterial pressure (MAP) when compared with controls (Table 6). Parameters related to hemodynamic disturbances such as decreased mean arterial pressure and increased plasma renin activity and aldosterone levels deteriorated with increasing of Child-Pugh score. However, similar values of antidiuretic hormone were detected in all patients grouped according to the Child-Pugh classification.

Among clinical parameters of liver dysfunction ascites revealed significant association with plasma AOPPs-albumin, TNF- α and IL-6 (Table 6). By contrast, patients presented similar AOPPs-albumin levels when classified according to the presence or absence of encephalopathy grade I (data not shown). Ascitic patients had a more intense alteration of hemodynamic parameters (plasma renin activity, aldosterone), along with higher levels of



	AUC	Standard error	95% confidence interval	Sensitivity (%)	Specificity (%)	Optimal cut-off
AOPPs-albumin	0.802	0.018	0.766-0.838	57.6	100	3.71 $\mu\text{mol/g}$
TNF- α	0.902	0.015	0.872-0.932	71.7	100	37.2 pg/mL
IL-6	0.832	0.012	0.808-0.856	76.1	85	8.95 pg/mL

Fig. 2. Receiver operating characteristic (ROC) curve and optimal cut-off levels of advanced oxidation protein products (AOPPs), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) for distinguishing cirrhotic patients from healthy controls; AUC, area under the curve.

AOPPs-albumin, TNF- α , and IL-6, compared with patients without ascites. Cirrhotic patients who had ascites showed higher AOPPs-albumin levels ($n = 53$, median $3.6 \mu\text{mol/g}$, range $1.5\text{-}5.3 \mu\text{mol/g}$) than patients without ascites ($n = 35$, median $2.2 \mu\text{mol/g}$, range $1.0\text{-}3.4 \mu\text{mol/g}$) ($P < 0.05$, Table 6). AOPPs-albumin levels were not different between cirrhotic patients without ascites and controls, while patients with ascites had higher AOPPs-albumin levels than controls (median $1.7 \mu\text{mol/g}$, range $0.8\text{-}2.7 \mu\text{mol/g}$) ($P < 0.01$). High IL-6 levels showed an independent association with the presence of ascites.

To differentiate cirrhotic patients with a more intense hemodynamic alteration (vasodilatation), we divided patients according to those with low mean arterial pressure ($\text{MAP} \leq 83 \text{ mm Hg}$) and high mean arterial pressure ($\text{MAP} > 83 \text{ mm Hg}$) (Table 7). Only IL-6 levels were significantly higher in patients with more severe vasodilatation; this association was independent of other factors.

Plasma levels of AOPPs-albumin very weakly but significantly correlated with MAP ($r = -0.25$, $P < 0.01$; Fig.3). Furthermore, IL-6 levels had a significant correlation with several

parameters, including plasma renin activity ($r = 0.39$, $P < 0.05$) and MAP ($r = -0.38$, $P < 0.01$), in addition to albumin ($r = -0.51$, $P < 0.001$). Neither TNF- α levels nor the CML-albumin levels correlated significantly with hemodynamic parameters.

	Overall (n=88)	Patients of class A (n=34)	Patients of class B (n=34)	Patients of class C (n=20)	Cirrhosis without ascites (n=35)	Cirrhosis with ascites (n=53)
Mean arterial pressure- MAP (mmHg)	83 (76-93)	89 (85-93)	83 (77-91)	76* (73-81)	88 (85-93)	77+ (73-89)
Plasma renin activity (ng mL ⁻¹ h ⁻¹)	0.6 (0.1-7.6)	0.37 (0.1-1.2)	1.9* (0.95-4.8)	6.0** (1.2-7.6)	0.48 (0.13-1.6)	1.9** (0.51-6.2)
Aldosterone (ng/dL)	15.0 (5.0-71.0)	12.0 (5.0-19.0)	20.6 (14.0-/51.0)	30.0* (21.0-71.0)	13.2 (5.5-20.9)	33.0+++ (13.7- 52.2)
Antidiuretic hormone (pg/mL)	4.9 (2.5-6.5)	3.8 (2.5-5.7)	4.5 (3.7-6.2)	4.8 (3.8-6.5)	4.6 (3.0-6.8)	4.5 (3.6-6.4)
AOPPs- albumin (μ mol/g)	2.4 (1.3-5.2)	2.8 (1.3-4.4)	3.2 (1.9-4.5)	4.1* (2.3-5.2)	2.2 (1.0-3.4)	3.6+ (1.5-5.3)
TNF- α (pg/mL)	41.5 (37.6-64.0)	36.9 (37.7-45.6)	42.0 (37.6-47.2)	51.7* (48.7-58.3)	37.5 (35.4-46.5)	57.5+ (52.1-69.7)
IL-6 (pg/mL)	13.3 (6.4-39.9)	8.8 (6.4-34.6)	12.3 (6.8-33.9)	18.9 (9.0-39.9)	6.4 (5.6-7.3)	10.8** (8.5-12.4)

Significance between groups: * $P < 0.05$; ** $P < 0.001$ vs. patients of class A. + $P < 0.05$; ++ $P < 0.01$, +++ $P < 0.001$ vs. liver cirrhosis without ascites. AOPPs, advanced oxidation protein products; TNF, tumor necrosis factor; IL, interleukin.

Table 6. Blood pressure, plasma renin activity and plasma concentrations of aldosterone, antidiuretic hormone and AOPPs-albumin according to the Child-Pugh class or presence of ascites.

	Mean arterial pressure (MAP) ≤ 83 mm Hg (n=51)	Mean arterial pressure (MAP) > 83 mm Hg (n=37)
Plasma renin activity (ng mL ⁻¹ h ⁻¹)	1.7 (0.46-5.5)	1.4 (0.37-4.4)
Aldosterone (ng/dL)	25.6 (10.6-40.5)	13.7 (5.7-21.6)
Antidiuretic hormone (pg/mL)	4.7 (3.7-6.7)	4.2 (3.3-5.9)
AOPPs-albumin (μmol/g)	3.3 (1.4-4.8)	3.01 (1.2-4.5)
IL-6 (pg/mL)	11.0 ⁺ (9.5-12.8)	6.3 (5.4-7.0)
TNF-α (pg/mL)	47.9 (43.4-58.0)	49.8 (45.1-60.3)

Table 7. Comparison between cirrhotic patients classified according to the finding of low (≤ 83 mm Hg) and high (>83 mm Hg) mean arterial pressure (MAP). Significance between groups: ⁺P < 0.01 by multivariate analysis.

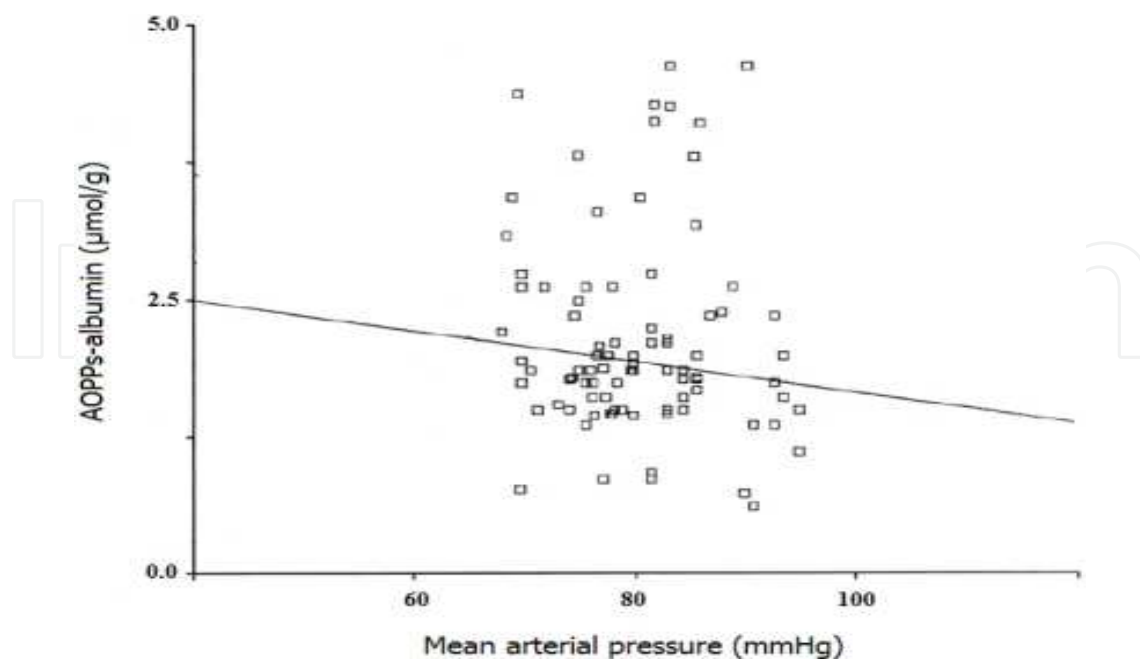


Fig. 3. AOPPs-albumin concentrations are very weakly but significantly correlated with the mean arterial pressure (MAP) in patients with chronic liver disease ($r = -0.25, P < 0.01$).

4. Discussion

Cirrhosis is characterized by inflammation of the liver, often caused by a rise in oxygen-derived free radicals within the liver. Under normal circumstances, the liver maintains a supply of internal antioxidants to neutralize the reactive species generated in response to viral infection and during metabolism of various endo- and exogenous compounds processed in the liver. However, when the liver antioxidants are low, or when the liver is undergone to continued oxidative insults (e.g., long-lasting alcohol abuse or infection with different hepatitis viruses), the damage from reactive species (Halliwell, 2007) may increase, resulting in inflammation and the formation of scar tissue (fibrosis) (Valko *et al.*, 2007). The progressive decrease of antioxidant reserves, the dysfunction of liver microcirculation through nitric oxide-mediated pathways, may determine the shift to liver cirrhosis. Advanced glycation and oxidation endproducts (AGEs and AOPPs, respectively) cause oxidative stress and trigger cytokine driven inflammatory reactions *in vitro*. (reviewed in Yan *et al.*, 2010). The net effects on markers of inflammation and hemodynamic changes in cirrhotic patients are unknown.

Advanced glycation endproducts are formed from the reaction of glucose and other reducing sugars with amino acid groups of proteins. This interaction generates a labile Schiff base followed by rearrangement to more stable Amadori-products, and subsequently these early glycation products may undergo further chemical rearrangements resulting in various irreversibly formed AGEs (Baynes & Thorpe, 1999). Three different mechanisms have been proposed by which AGEs lead to cirrhosis complications: 1) the binding of AGEs to the receptors for advanced glycation endproducts on different cell types including monocytes/macrophages, T lymphocytes, endothelial cells, smooth muscle cells, and activation of cell signaling pathways with subsequent modulation of gene expression, 2) intracellular AGEs formation leading to impaired cell function, and 3) the accumulation of AGEs in the extracellular matrix. CML-albumin levels (as prototype of the AGEs) were higher in cirrhosis groups than in the controls. In agreement with two previous reports (Zuwala-Jagiello *et al.*, 2009; 2011) these results indicate that reactive oxygen species are overproduced in patients with liver cirrhosis. CML may lead to progression of cirrhosis by interaction with receptors that induce production of reactive species followed by a release of inflammatory cytokines in different cell types (Bierhaus *et al.*, 1998; Raj *et al.*, 2000) such as IL-6, ultimately leading to the production of CRP by the liver. A correlation to high sensitive C-reactive protein (hs-CRP) could not be demonstrated, and the suggestion that high levels of CML may activate an inflammatory response was not demonstrated by serum markers (TNF- α and IL-6). It is likely that the blood load of CML resembles only a small fraction of body's AGEs content and that the serum levels reflect particular changes in the body's AGEs pool. Thus, circulating CML-albumin may not be an adequate parameter for demonstrating effects on inflammatory response in cirrhotic patients. Most likely the focus should be on intracellular AGEs (Thornalley *et al.*, 2003).

A role of oxidative stress in the pathogenesis of chronic liver disease has been proposed by several authors (Bandara *et al.*, 2005; Nagata *et al.*, 2007; Nakhjavani *et al.*, 2011; Serejo *et al.*, 2003; Zuwala-Jagiello *et al.*, 2007). Studies have shown increased plasma levels of markers of lipid peroxidation and reduced plasma antioxidant content. Protein oxidation products are

increasingly being used as markers instead of lipid peroxidation products in demonstrating oxidative stress (Dalle-Donne *et al.*, 2003). AOPPs measurements reflect the reactive species generation and the degree of protein oxidation (Witko-Sarsat *et al.*, 1996). It was reported that AOPPs generated by different oxidation patterns lead to the production of either hydrogen peroxide or nitric oxide (Servettaz *et al.*, 2007). Nitric oxide can interact with superoxide anion-radical forming reactive nitrogen species such as peroxynitrite. These reactive nitrogen species secondarily promote important reactions such as nitrosation, oxidation or nitration, leading to impaired cellular functions and enhanced inflammatory reactions (Friedman, 2008; Iwakiri & Groszmann, 2007). AOPPs are referred to as markers of oxidative stress as well as markers of neutrophil activation in chronic disease (Witko-Sarsat *et al.*, 2003). It has thus been shown that chlorinated oxidants of neutrophil origin may lead to oxidative stress, notably protein oxidation. Once formed, such AOPPs foci create a nidus for the amplification of oxidative stress. In addition to increased formation, decreased removal/detoxification of AOPPs may contribute to the stress. There is increasing evidence that the liver plays important roles in the elimination of AOPPs (Iwao *et al.*, 2006). In patients with chronic liver diseases, constriction of the sinusoidal blood stream leads to the development of portal hypertension with portocaval shunts (Svistounov & Smedsrød, 2004). The hindrance of substance exchange between hepatocytes and the sinusoidal blood stream could increase plasma level of AOPPs in these patients. Therefore, the liver, especially in cirrhotic patients, cannot prevent the accumulation of AOPPs effectively. Finally, our findings extended the results of Oettl *et al.* (2008) which suggested that albumin is oxidatively modified in patients with advanced liver disease depending on its severity. The present finding that AOPPs-albumin accumulation coexists with decreased TAS, while the plasma concentration of CML-modified albumin remains stable, supports the contention that AOPPs-albumin is more accurate marker of oxidative stress than glycooxidation products in cirrhotic patients. An increase in reactive species formation, manifested by increased hepatic and plasma levels of AOPPs (Gorka *et al.*, 2008; Sebeková *et al.*, 2002; Yagmur *et al.*, 2006; Zuwala-Jagiello *et al.*, 2006;) and as well as decreased antioxidant levels (Jain *et al.*, 2002; Zuwala-Jagiello *et al.*, 2009) have been reported in patients with liver cirrhosis. Finally, our previous study found that the patients with cirrhosis were exposed to oxidative stress and the level of AOPPs was significantly related to the severity of liver cirrhosis of various etiologies (Zuwala-Jagiello *et al.*, 2011).

The adverse effects of oxidative stress on the progression of cirrhosis may be categorized into effects on protein modifications and inflammatory response. Figure 4 presents a summary of the effects of AOPPs or AGEs and oxidative stress on markers of inflammation and hemodynamic changes in cirrhotic patients.

Very recently, advanced glycation endproducts have been found to act as pro-inflammatory factors (Sparvero *et al.*, 2009). Nevertheless, AOPPs are believed to be more closely related to inflammation (Fialova *et al.*, 2006) than AGEs, whose RAGE participates in AOPPs-mediated signal transduction (Kalousová *et al.*, 2005; 2006). These interactions enhance reactive oxygen species formation, with activation of nuclear factor NF- κ B and release of pro-inflammatory cytokines (Bierhaus *et al.*, 2006; Hyogo & Yamagishi, 2008; Saito & Ishii, 2004) (Fig. 4). Moreover, the macrophage RAGE can be up-regulated by TNF- α (Miyata *et al.*,

1994). This is accompanied by the activation of macrophages and increased expression of TNF- α (Giron-González *et al.*, 2004). TNF- α production is also stimulated by macrophage sensing of intestinal microflora pathogen associated molecular patterns by toll-like receptors (Riordan *et al.*, 2003). It could therefore be consistent with observed increase levels of both AOPPs-albumin and TNF- α at an early stage of liver cirrhosis.

The hyperdynamic circulatory state associated with liver cirrhosis is characterized by vasodilatation and increased cardiac output; the arterial hypotension and relative hypovolemia caused by vasodilatation activate a number of vasoactive and neurohumoral systems (Wiest & Groszmann, 2002). TNF- α induces an endothelial activation, which can be detected by increased synthesis of nitric oxide (Spitzer, 1994). Endogenous nitric oxide, a powerful endothelium-derived vasodilator, has been implicated in hemodynamic changes present in cirrhotic patients (García-Tsao *et al.*, 1998). Treatment with both specific anti-TNF polyclonal antibodies and thalidomide, an inhibitor of TNF- α production, significantly prevent the development of the hyperdynamic circulation and reduces portal pressure (Gatta *et al.*, 2008). TNF- α levels in our patients were clearly different from control group and were much higher in ascitic patients. However, no differences existed between patients with high and low mean arterial pressure, and no significant correlations with hemodynamic values were found. These data suggest that, although TNF- α might be one of the inducers of nitric oxide generation in cirrhotic patients, other factors acting through different pathways probably exist.

AOPPs derived from *in vivo* sources stimulated endothelial cell generation of reactive oxygen species, in particular superoxide anion (Guo *et al.*, 2008), at least in part through NADPH-oxidase (Wautier *et al.*, 2001). However, the exact mechanisms and sources by which reactive oxygen species are generated in the vasculature are not yet known in detail. It has been observed in several experimental animal models, that the endothelium is one of the major sources for the generation of reactive oxygen species. In parallel with the vascular dysfunction the formation of superoxide anions became augmented, and removal of endothelium completely abolished the production of reactive oxygen species. (reviewed in Wright *et al.*, 2006). In another report, Rhee *et al.* (2003) demonstrated that growth factor-induced H₂O₂ production (e.g. PDGF, EGF) requires the activation of phosphoinositide 3-kinase. The essential role of phosphoinositide 3-kinase is likely to provide phosphatidylinositol (3, 4, 5)-trisphosphate that recruits and activates a guanine nucleotide exchange factor of Rac, which is required for the activation of NADPH-oxidase. Thus, the generation of reactive oxygen species is largely dependent on the activation of NADPH-oxidase that is present in endothelial cell. AOPPs stimulated endothelial cell activation of the following signaling mediators: NADPH oxidase and NF- κ B, the factors linked to increased expression of pro-inflammatory adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) (Kim *et al.*, 2001; Yan *et al.*, 2010) (Fig. 4). Endothelial activation plays an active role in the modifications of circulatory status of cirrhotic patients (Grangé & Amiot, 2004). Elevated levels of AOPPs-albumin were detected in the early stages of liver dysfunction: plasma concentrations were increased in patients in Child Pugh class A, with higher values found in those in class B or C. Plasma concentrations of AOPPs-albumin were very weakly correlated with hemodynamic alterations (mean arterial pressure).

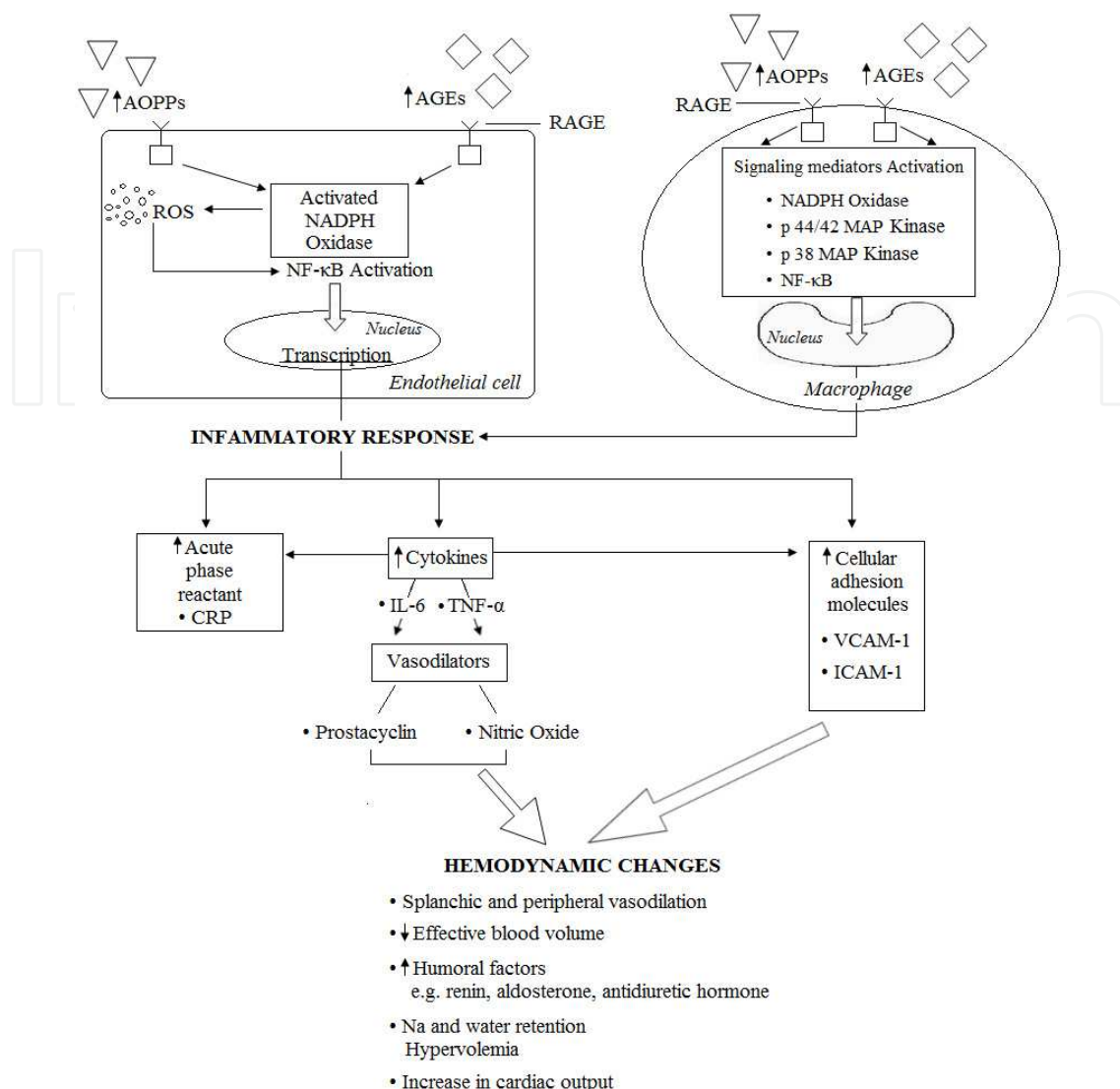


Fig. 4. Summary of the effects of AOPPs or AGEs and oxidative stress on markers of inflammation and hemodynamic changes in cirrhotic patients. AOPPs or AGEs bind with RAGE on the surface of endothelial cells lining blood vessels. AOPPs, AGEs ligands of RAGE sustain stimulation of RAGE. One consequence of RAGE signaling is the activation of NADPH oxidase and production of reactive oxygen species. Once formed, reactive oxygen species activate key transcription factor such as NF-κB, which results in the transcriptional activation of genes relevant for inflammation. Consequences include increased migration and activation of RAGE-expressing macrophages. This results in release of the pro-inflammatory cytokines. In this inflammatory environment, *via* interaction with RAGE on the surface of macrophages, AOPPs or AGEs magnify activation of NF-κB and other factors, thereby amplifying cellular stress and hepatic damage. In the aggregate, these processes may contribute to propagation of inflammation and vascular perturbation in liver cirrhosis. AGEs, advanced glycation endproducts; AOPPs, advanced oxidation protein products; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; MAP, mitogen activated protein; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); NF-κB, nuclear factor κB; RAGE, receptor for AGEs; ROS, reactive oxygen species; TNF-α, tumour necrosis factor α; VCAM-1, vascular cell adhesion molecule.

These results suggested that this association of AOPPs with hemodynamic disturbances is dependent of the severity of cirrhosis. Additionally, AOPPs levels could be elevated as a result of insufficient renal elimination. However, the precise mechanism by which AOPPs is cleared from plasma is currently unknown. In addition, we found no correlation between AOPPs-albumin and serum creatinine levels. In any case, the values of AOPPs-albumin in patients with a low Child-Pugh score and absence of ascites suggests that AOPPs might have a role in the late stages of cirrhosis by aggravating the already initiated vasodilatation. Indeed, the presence of ascites, one of the major complications of cirrhosis and closely related to the hemodynamic disturbances of cirrhotic patients, was found in patients with higher levels of AOPPs. Finally, the extremely weak correlation between AOPPs-albumin levels and mean arterial pressure may suggest an indirect contribution of AOPPs to arterial vasodilatation through other mediators.

Structure and function of albumin are impaired in advanced liver disease by different mechanisms: plasma levels are decreased due to reduced synthesis and albumin is oxidatively modified. In this context, AOPPs-albumin may show altered binding capacities for several substances. Decreased bilirubin binding was reported for *in vitro* oxidized albumin (Oetl & Stauber, 2007). As bilirubin is preferentially bound by the fully reduced form of albumin, impaired binding of bilirubin and other ligands (e.g. nitric oxide) is likely to occur in liver cirrhosis. Theoretically, increased circulating AOPPs-albumin may indirectly lead to elevation of nitric oxide which can, in turn, contribute to oxidative stress in cells (La Villa & Gentilini, 2008). Finally, nitric oxide plays a major key role in the development of hyperdynamic circulation and portal hypertension in cirrhosis (Iwakiri & Groszmann, 2007). Infusion of albumin (Garcovich *et al.*, 2009) as well as albumin dialysis has been shown to improve the circulatory dysfunction as evidenced by an increase in mean blood pressure and systemic vascular resistance (Mitzner *et al.*, 2001). This improvement in systemic hemodynamics might be due to a reduction in vasodilation following removal of nitric oxide which results in deactivation of the neurohormonal systems and a decrease in plasma levels of renin, aldosterone, norepinephrine and vasopressin (Chen *et al.*, 2009). However, other findings of the present study imply that AOPPs are not only the factors responsible for the hemodynamic changes observed in cirrhotic patients. The AOPPs-albumin levels did not correlate significantly with the parameters that accompany important hemodynamic alterations in cirrhotic patients, such as plasma renin activity and aldosterone. Finally, when cirrhotic patients in our study were divided according to high and low mean arterial pressure, we found similar AOPPs-albumin levels in both groups.

Portal hypertension and cirrhosis can increase gut permeability to endotoxin and impair reticuloendothelial function of the liver that may result in increased serum endotoxin concentrations (Cariello *et al.*, 2010). This may be a stimulus for the production of pro-inflammatory cytokines, resulting in the increased production of acute phase proteins such as C-reactive protein. In turn, CRP is capable of stimulating IL-6 and TNF- α production by monocytes (Ballou & Lozanski, 1992) and reactive oxygen species formation (Wang *et al.*, 2003). Advanced oxidation protein products, as pro-inflammatory factors, accumulated in cirrhotic patients (Zuwala-Jagiello *et al.*, 2006, 2009, 2011) and played an important role in the occurrence and progression of complications such as dysfunction of endothelial cells (Witko-Sarsat *et al.*, 1998). AOPPs correlate well with certain cytokines (Kalousová *et al.*, 2005) as well as with some markers of inflammation, including fibrinogen, orosomucoid. Even if the correlation between AOPPs-albumin and hs-CRP were poor, other studies

demonstrated fair associations with other markers of oxidative stress, such as lipid peroxidation products and F2-isoprostanes. Furthermore, results from a recent study demonstrate that glycosylated and oxidized proteins indirectly up-regulate CRP expression in hepatocytes by stimulating monocytes to produce IL-6 (Li *et al.*, 2007). It seems, based on our study, even though there was functional loss of hepatocytes in patients with hepatic cirrhosis, the serum CRP level was still maintained in high level and dependent of AOPPs-albumin level with its significant correlation. The remaining viable hepatocytes may still contribute to this result. IL-6 is the main stimulant for hepatic production of CRP but also has other important roles leading to increased endothelial cell adhesiveness by up-regulating ICAM-1, and VCAM-1 and releasing inflammatory mediators, including IL-6 itself (Szmitko *et al.*, 2003). Finally, the significant correlation between the levels of AOPPs and IL-6 supports the existence of a link between AOPPs and hemodynamic changes present in cirrhotic patients (Fig. 4).

Portal hypertension is characterized by intrahepatic vascular resistance causing an increase of portal vein pressure, and leads to the development of ascite (Møller *et al.*, 2008). IL-6 levels in our cirrhotic patients were different from those in controls, increased with the severity of liver disease, were independently associated to the presence of ascites. IL-6 increased in serum of patients with ascites compared to compensated (Child-Pugh class A) patients without ascites, and similar results were obtained in a study where serum IL-6 was also analyzed (Zhang *et al.*, 2002). In a simplistic view, portal hypertension leads to the formation of portosystemic collateral veins in liver cirrhosis and the resulting shunting (Cichoz-Lach *et al.*, 2008) also contributes to impaired hepatic uptake of IL-6. It has been convincingly shown that hepatic uptake of IL-6 is significantly impaired in patients with liver cirrhosis, and this may at least in part explain elevated serum levels in these patients (Soresi *et al.*, 2006). In our study, IL-6 levels increase significantly in association with the severity of liver cirrhosis according to the MELD score. Moreover, IL-6 levels in all cirrhotic patients were independently associated to the presence of low mean arterial pressure, and showed significant correlations with parameters related to hemodynamic abnormalities. The mechanism by which IL-6 could cause vasodilatation is unknown. However, the effect appears to be independent of nitric oxide, possibly due to an important role of prostacyclin synthesis (Dagher & Moore, 2001). It is then possible that IL-6 would produce vasodilatation by inducing prostacyclin synthesis; the effect of IL-6 would be potentiated by AOPPs stimulation (Li *et al.*, 2007).

Further, investigators have tried to find more noninvasive biomarkers for cirrhotic patients for years (Schuppan & Afdhal, 2008). We observed good abilities of plasma AOPPs-albumin, TNF- α and IL-6 levels to distinguish cirrhotic patients from healthy controls, with good sensitivities and specificities by ROC analysis. Additionally, these parameters also were found to be elevated in concordance with the severity of cirrhosis. Thereby, it is possible that plasma levels of AOPPs-albumin, TNF- α and IL-6 levels could be evaluated as candidate biomarkers for initial and long-term assessment of liver cirrhosis.

5. Conclusion

In conclusion, there are differences between advanced oxidation protein products modified-albumin (AOPPs-albumin), which act as a pure oxidative stress marker, and N ϵ -(carboxymethyl)lysine modified-albumin (CML-albumin; as prototype of the advanced

glycation endproducts-AGEs), which behave as both oxidative and carbonyl stress markers. AOPPs-albumin shows a closer relationship to inflammation than CML-albumin. Because of the relationship of AOPPs-albumin with formation of inflammation, their relationship with certain inflammatory markers and their changes during progression of chronic liver disease, AOPPs may provide a marker of chronic long-lasting liver damage. Oxidative stress not only contributes to the derangement of hemodynamic chronic liver failure, but also gradually increases with its progression to end-stage liver disease.

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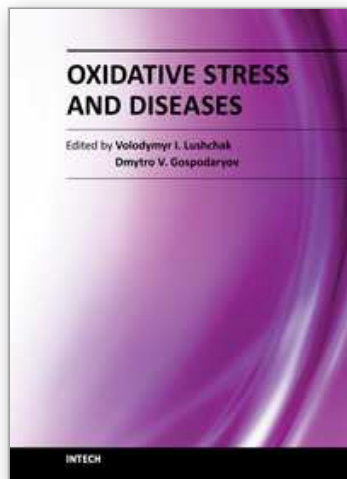
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The development of hypothesis of oxidative stress in the 1980s stimulated the interest of biological and biomedical sciences that extends to this day. The contributions in this book provide the reader with the knowledge accumulated to date on the involvement of reactive oxygen species in different pathologies in humans and animals. The chapters are organized into sections based on specific groups of pathologies such as cardiovascular diseases, diabetes, cancer, neuronal, hormonal, and systemic ones. A special section highlights potential of antioxidants to protect organisms against deleterious effects of reactive species. This book should appeal to many researchers, who should find its information useful for advancing their fields.

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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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