ACTA ABP BIOCHIMICA POLONICA

Vol. 59, No 4/2012
661–667
on-line at: www.actabp.pl

Regular paper

Ischemia-modified albumin (IMA) is increased in patients with chronic hepatitis C infection and related to markers of oxidative stress and inflammation

Jolanta Zuwała-Jagiełło[1]✉, Maria Warwas[1] and Monika Pazgan-Simon[2]

[1]Department of Pharmaceutical Biochemistry; [2]Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency, Wrocław Medical University, Wrocław, Poland

Inflammation and oxidative stress have been reported in patients with chronic hepatitis C (CHC) infection, but their influence on ischemia-modified albumin (IMA) levels and diabetes prevalence remains unknown. Sixty-three CHC patients, 28 with diabetes, and 40 healthy controls were enrolled in the study. Circulating levels of oxidative stress markers [Nε-(carboxymethyl)lysine-advanced glycation end products (CML-AGEs) and advanced oxidation protein products-(AOPPs)], pro-inflammatory cytokines (interleukin-6, and tumor necrosis factor α), and high-sensitivity C-reactive protein (hsCRP) were assessed. Compared with the controls, the CHC patients with diabetes showed a significant increase in plasma concentrations of IMA, AOPPs, interleukin-6 and hsCRP ($P < 0.05$). The values of IMA and hsCRP were more elevated in patients with diabetes than without diabetes (both $P < 0.01$). The positive relationships were found between hsCRP and presence of diabetes, IMA (both $P < 0.01$) and AOPP levels ($P < 0.05$). CML-AGEs did not show any significant correlation with IMA, markers of inflammation and presence of diabetes. In conclusion, we have documented significant elevation in plasma levels of IMA and AOPPs in CHC patients. In addition, circulating IMA was associated with inflammation markers and diabetes prevalence. This observation suggests a relationship between IMA and inflammation in CHC patients with diabetes, which may represent one of the mechanisms involved in the accelerated atherosclerosis in this population.

Key words: Ischemia-modified albumin, oxidative stress, HCV infection, diabetes, inflammation

Received: 17July, 2012; revised: 07 November, 2012; accepted: 27 November, 2012; available on-line 06 December, 2012

INTRODUCTION

It is now widely recognized that chronic hepatitis C is a metabolic disease, strongly associated with type 2 diabetes mellitus. Type 2 diabetes has been observed to occur more frequently in association with HCV infection than with other chronic inflammatory liver diseases. Diabetes in patients with chronic hepatitis C (CHC) infection has a unique and complex pathogenesis which distinguishes this metabolic disorder from type 2 diabetes mellitus (Douglas & George, 2009). It has been observed that type 2 diabetes mellitus occurs in the early stages of liver disease (Petit *et al.*, 2001). The mechanism through which chronic hepatitis C infection is associated with insulin resistance may involve direct viral effects, pro-inflammatory cytokines and suppressors of cytokine signaling (Douglas & George, 2009).

Type 2 diabetes is associated with atherosclerosis and an increased risk of cardiovascular disease. The mechanism by which diabetes promotes atherosclerosis is complex and involves chronic inflammatory condition, oxidative stress, hypoxia and ischemia (Pu *et al.*, 2006). Among the inflammatory biomarkers, the C-reactive protein (CRP) and cytokines such as tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6), seem to be the most significant for cardiovascular risk detection, which relies on evidence suggesting atherosclerosis as a chronic inflammatory process (Corrado *et al.*, 2010). Oxidative stress may also contribute to the inflammatory state in atherosclerosis. A growing number of studies suggest that reactive oxygen species, produced during ischemia, can generate the highly reactive hydroxyl radical, resulting in site-specific modification to the N-terminus of the albumin moiety, especially at the N-Asp-Ala-His-Lys sequence, thereby producing ischemia-modified albumin (IMA) (reviewed in Sbarouni *et al.*, 2008). IMA was shown to be a better myocardial infarction marker than the C-reactive protein (Mastella *et al.*, 2009). However, IMA may increase in conditions of non-cardiac ischemia, such as liver cirrhosis and metabolic syndrome (Valle Gottlieb *et al.*, 2010; Chen *et al.*, 2011). In addition, there is some recent suggestion that the albumin molecule in the plasma of diabetic patients is modified in chronic hypoxia conditions induced mainly by hyperglycemia and oxidative stress (Piwowar *et al.*, 2008). To our knowledge no paper has been published to date on the plasma levels of the ischemia-modified albumin in the population of CHC patients with diabetes.

The aim of the present study was to investigate the circulating IMA levels in CHC patients with and without diabetes and their probable association with oxidative stress and inflammation markers.

PATIENTS AND METHODS

Patients and study design. This study was performed in 63 patients with chronic hepatitis C (CHC) infection admitted to the Clinic of Infectious Diseases, Liver Diseases and Acquired Immune Deficiency for

✉e-mail: jolanta.zuwala-jagiello@am.wroc.pl

Abbreviations: AOPPs, advanced oxidation protein products; AGEs, advanced glycation end-products; CHC, chronic hepatitis C; CML, Nε-(carboxymethyl)lysine; CRP, C-reactive protein; hsCRP, high-sensitivity C-reactive protein; IMA, ischemia-modified albumin; IL-6, interleukin-6; RAGE, receptor for advanced glycation end-products; TNF-α, tumor necrosis factor α.

evaluation. The control group consisted of healthy blood donors with normal aminotransferases, normal blood counts and negative markers for viral hepatitis and HIV (11 males/29 females, mean age 46 ± 9 years). No statistically significant difference was indicated between the CHC patients and healthy controls in the male/female ratio. There was a significant difference in age ($P < 0.05$), but IMA level is not age-related (Sbarouni *et al.*, 2008). Clinical and biochemical characteristics of the study group are reported in detail in Table 1. In all subjects, age, body mass index, systolic blood pressure, diastolic blood pressure were obtained together with measurements of albumin, various liver enzymes (alanine aminotransferase-ALT; aspartate aminotransferase-AST and gamma-glutamyltranspeptidase), fasting plasma glucose, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol and triglyceride concentrations (Table 1). The levels of ALT and AST were significantly lower and the age was significantly higher in healthy controls as compared with CHC patients. There was no significant difference between patients with CHC and control subjects with respect to gender, systolic blood pressure, diastolic blood pressure, body mass index, serum albumin, serum total protein and lipid profile (Table 1). The exclusion criteria were as follows. 1) Liver cirrhosis. 2) Conditions other than diabetes and HCV infection that could influence serum glucose levels: irregular menstrual cycles in premenopausal women (both CHC and control groups); treatment with steroid or nonsteroidal anti-inflammatory drugs; alcohol consumption > 40 g/day; concomitant infection; chronic diseases other than diabetes. 3) Type 1 diabetes (history of diabetic ketoacidosis or age < 30 years with insulin requirement) and secondary diabetes due to chronic pancreatitis or pancreatic tumor. Other exclusion criteria were conditions that could influence either plasma IMA or oxidative marker levels, including microvascular diabetic complications, smoking and the use of antioxidant or multivitamin supplements.

Liver cirrhosis was ruled out by liver biopsy performed within 18 months before inclusion (compensated patients) or by typical clinical features such as signs of portal hypertension (splenomegaly, ascites, and esophageal varices), hematologic evidence of hypersplenism, or biochemical evidence of hepatocellular failure.

The diagnosis of chronic hepatitis C infection was based on persistently increased alanine aminotransferase values, anti-HCV and HCV-RNA positivity and liver histology features. The HCV inflammation was confirmed by measurement of HCV Ab and HCV RNA in the serum, using the enzyme immunoassay (EIA) and RT PCR – Cobas Amplicor Roche methods, respectively. For the anti–HCV positive patients with normal aminotransferase levels and no liver biopsy, we ensured that aminotransferase levels and liver function tests results were persistently normal.

Patients were divided in two groups according to their HCV antibody status and the presence of diabetes: anti–

Table 1. Clinical and biochemical parameters in patients with chronic hepatitis C (CHC) infection and healthy controls.

	Healthy controls	CHC patients	*P*-value
(n)	40	63	
Male:Female Ratio	11:29	33:30	NS
Age (years)	35 ± 9	51 ± 10*	< 0.05
Systolic blood pressure (mmHg)	131 ± 12	135 ± 19	NS
Diastolic blood pressure (mmHg)	80 ± 7	81 ± 10	NS
Duration of hepatitis C (years)	–	6.3 ± 2.9	–
HCV RNA (copies/mL)	–	3.14×10^5 (0.015×10^5–6.13×10^5)	–
Alanine aminotransferase (IU/L)	23 (20–28)	95 (33–190)**	< 0.01
Aspartate aminotransferase (IU/L)	28 (23–30)	78 (28–140)**	< 0.01
Gamma-glutamyltranspeptidase (IU/L)	26 (25–29)	62 (41–106)*	< 0.05
Body mass index (kg/m^2)	24 ± 2.6	25.7 ± 2.5	NS
Diabetes mellitus type 2 (%)	_	44%	_
Fasting plasma glucose (mmol/L)	5.0 ± 0.6	6.8 ± 2.3	NS
Total cholesterol (mg/dL)	140 ± 55	179 ± 43	NS
Low density lipoprotein (mg/dL)	93± 31	113 ± 25	NS
High density lipoprotein cholesterol (mg/dL)	40 ± 16	45 ± 10	NS
Triglycerides (mg/dL)	42 ± 10	46 ± 11	NS
Albumin (g/dL)	4.8 (3.6–5.7)	4.4 (3.4–4.8)	NS
Total protein (g/dL)	7.5 (6.8–8.1)	7.6 (6.8–8.2)	NS

Values are expressed as means ± S.D. or as medians (interquartile range) for skewed data. Statistical significance: *$P < 0.05$; **$P < 0.01$ *vs* healthy controls. NS, not significant ($P > 0.05$).

HCV-positive diabetic patients (n = 28) and anti-HCV-positive non-diabetic patients (n = 35). Diabetes was defined on the basis of a history of therapy with oral hypoglycemic agents or insulin at the time of inclusion. Based on the clinical information, all diabetic patients in this study were assumed to have type 2 diabetes mellitus. Patients with diabetes were investigated for diabetic complications using clinical examination findings and laboratory test results. No diabetes was defined as absence of a history of diabetes and fasting plasma glucose < 7 mmol/L. Glycated hemoglobin (HbA_{1c}) was used as a marker for glucose control; values <6.5% indicated well-controlled glucose and values >6.5% indicated poor glucose control.

All laboratory measurements were performed on fasting blood samples. After 2 hours 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 IMA, AOPPs (advanced oxidation protein products), Nε-(carboxymethyl)lysine, and fasting glucose or HbA_{1c}, while tubes with no additive were used for routine biochemical study and assessment of cytokine concentrations. All samples were separated immediately by centrifugation at 4°C and stored at –80°C for 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).

Biochemical assays. The albumin cobalt binding test was analyzed according to the method defined by Bar-Or *et al.* (2000). In this method 100 μL serum was added to 25 μL of 0.1% (w/v) cobalt chloridine water solution (Sigma; $CoCl_2 \times 6H_2O$). It was mixed gently and waited for 10 minutes for sufficient cobalt-albumin binding. Then 25 μL dithiothreitol (DTT) (Sigma 1.5 mg/mL H_2O) was added as a colorizing agent. After waiting for two minutes 150 μL 0.9% NaCl was added to stop the cobalt binding process of albumin. Afterwards, the absorbance was measured in a spectrophotometer at 492 nm (Micro-plate reader model Stat Fax 2100). A sample without DTT was used as a blank. The results were reported as absorbance units (ABSU). The intra- and inter-assay coefficients of variation were lower than 3.6%.

Determination of AOPPs was based on spectrophotometric detection according to Witko-Sarsat et al. (1996). Coefficient of variation (CV) served as an indicator of precision. Intra-day and inter-day CV values were < 12%. Plasma Nε-(carboxymethyl)lysine (CML) levels were determined using a specific competitive ELISA kit [CircuLex CML/Nε-(carboxymethyl)lysine ELISA Kit (CycLex Co., Ltd, Nagano, Japan)]. 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 detection levels were 1.6 pg/ml and < 0.70 pg/ml for TNF-α and IL-6, respectively. Serum high-sensitivity C-reactive protein (hsCRP) level was determined by a high-sensitivity nephelometric method using the Beckman Image Immunochemistry system (Beckman Instruments, Fullerton, CA), which has a minimum detection level of 0.2 mg/L. HbA_{1c} levels were measured using ion-exchange high-performance liquid chromatography (Bio-Rad Variant™ II Turbo HbA_{1c} Kit). All other biochemical parameters were measured with routine laboratory methods.

Statistical analysis. The adjustment to normality was verified with the Kolmogorov–Smirnov test with Lilliefors correction. Skewed data were log transformed for analysis. All results are expressed as mean ± S.D. or median and interquartile range (25–75th percentile) as appropriate. The Mann–Whitney U test and the Kruskal–Wallis test were used to analyze differences among two or more groups, respectively. Correlations were determined using multiple regression analysis. A P value < 0.05 was considered the minimum level of statistical significance.

RESULTS

IMA and oxidative stress markers (AOPPs, CML-AGEs) plasma concentrations in patients with chronic hepatitis C infection and healthy controls

We analyzed 63 patients (33 males/30 females, mean age 51 ± 10 years) with chronic hepatitis C (CHC) infection. The plasma levels of IMA, AOPPs and CML-AGEs in the group of patients with CHC and subjects belonging to the control group are presented in Figure 1. All examined parameters were also checked for correlations with selected biochemical markers of liver function (albumin, prothrombin ratio, bilirubin concentration) and injury (aminotransferases). Plasma levels of IMA were significantly higher in all CHC patients than in healthy controls (n = 40, 0.28 ± 0.21 ABSU; $P < 0.05$). Plasma IMA concentrations were higher in CHC patients with diabetes than in patients without diabetes, and this difference was statistically significant (0.56 ± 0.16 ABSU vs. 0.34 ± 0.14 ABSU; $P < 0.01$) (Fig. 1). However, IMA levels in non-diabetic CHC patients were similar to those in the healthy controls.

The mean level of CML-AGEs in CHC patients was 2.74 ng/mL and no statistically significant difference was noted, compared with the control group ($P > 0.05$) (Fig. 1). Circulating CML-AGE levels were not significantly different between diabetic and non-diabetic CHC patients (2.84 ± 0.15 ng/mL *vs* 2.69 ± 0.20 ng/mL, $P > 0.05$) (Fig. 1). In contrast, in diabetic CHC patients the mean plasma concentrations of AOPPs were significantly higher than those measured in healthy controls or non-diabetic CHC patients ($P < 0.05$, $P < 0.01$; respectively) (Fig. 1). No significant difference was found between CHC patients and healthy controls in terms of total protein and albumin (Table 1); neither parameter correlated with IMA (data not shown). In the study group, no significant correlations were observed between CML-AGEs, AOPPs, IMA and biochemical markers of liver function and injury (not reported in detail).

Clinical and biochemical characteristics of patients with chronic hepatitis C infection with respect to the presence of diabetes

The biochemical and clinical characteristics of all the investigated groups are shown in Table 2. Distribution of sex was similar among groups. However, there was a significant difference in age among the groups; subjects in the CHC group with and without diabetes were older than subjects in the control group ($P < 0.05$). Fasting plasma glucose levels were higher in CHC patients with diabetes than in CHC patients without diabetes, and aminotransferase levels were higher in CHC patients with and without diabetes than in healthy controls (Table 2). The median hsCRP levels were higher in CHC patients

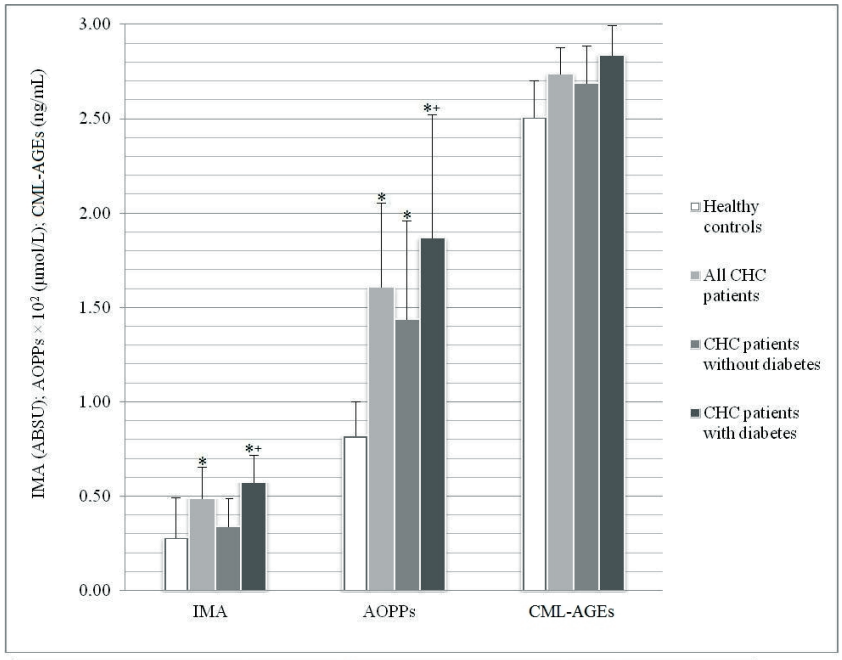
with diabetes than in healthy controls (3.84 mg/L *vs* 1.05 mg/L, respectively; $P<0.05$).

Significant difference was observed in serum levels of hsCRP between CHC patients with and without diabetes ($P<0.01$). IL-6 plasma concentrations were increased in the non-diabetic and diabetic CHC group, with higher concentrations in the latter (Table 2). Serum TNF-α concentrations were lower in CHC patients without diabetes than in diabetic CHC patients but neither group differed from the healthy controls (Table 2).

Using plasma IMA level of 0.70 ABSU as a cut-off value, the patients with CHC were divided into two groups (high or low levels of IMA). Differences in clinical parameters between patients with high (>0.70 ABSU) and lower (≤0.70 ABSU) IMA are outlined in Table 3. In CHC patients, high IMA levels were related to the presence of diabetes ($P<0.01$) and high levels of the inflammatory markers ($P<0.05$) but these correlations disappeared after excluding CHC patients with diabetes (Table 3).

Correlations between IMA, oxidative stress markers, inflammation, age and diabetes prevalence in patients with chronic hepatitis C infection

Correlations between IMA, AOPPs, inflammation, and diabetes prevalence are shown in Table 4. The inflammation markers and AOPP levels were associated with age. The positive relationships were found between hsCRP and diabetes as well as AOPP ($P<0.01$, $P<0.05$; respectively) and IMA levels ($P<0.01$). IMA and AOPP levels were related to diabetes prevalence ($P<0.05$, $P<0.001$; respectively). Fasting plasma glucose correlated with IMA concentrations and inflammation markers, and IMA was correlated with IL-6 levels (Table 4). Both IL-6 and TNF-α are known to induce the production of CRP from hepatocytes. Since only IL-6 concentrations were increased in CHC patients both with and without diabetes, it was expected that only this parameter would correlate with concentrations of hsCRP (Table 4). This was indeed the case as shown for TNF-α *vs* hsCRP ($P>0.05$) and IL-6 *vs* hsCRP ($P<0.001$). No significant correlations were found between TNF-α and other parameters (Table 4). CML-AGEs did not show significant correlation with IMA, AOPPs, inflammatory markers and the presence of diabetes (data not shown).



	Healthy controls	All CHC patients	CHC patients without diabetes	CHC patients with diabetes
(*n*)	40	63	35	28
IMA (ABSU)	0.28±0.21	0.48±0.18*	0.34±0.14	0.56±0.16*+
AOPPs × 10^2 (µmol/L)	0.82±0.18	1.61±0.45*	1.44±0.52*	1.86±0.66*+
CML-AGEs (ng/mL)	2.51±0.19	2.74±0.14	2.69±0.20	2.84±0.15

Figure 1. IMA, AOPPs and CML-AGEs concentrations in 63 patients with chronic hepatitis C (CHC) infection, with regard to the presence of diabetes, and in a control group of 40 healthy blood donors.
P values are given in the table. Data reported as means ±S.D. Significance levels between groups: *$P<0.05$ *vs* healthy controls; +$P<0.01$ *vs* CHC patients without diabetes. AOPPs, advanced oxidation protein products; CML-AGEs, Nε-(carboxymethyl)lysine-advanced glycation end products; IMA, ischemia-modified albumin.

DISCUSSION

In the present study we investigated the relationship between oxidative stress markers (AOPPs, CML-AGEs) and inflammation by comparing inflammatory markers (hsCRP, IL-6, TNF-α) in CHC patients showing high levels of IMA in the presence of high circulating AOPP concentrations. In the control group, the plasma IMA levels were similar to those in healthy controls in other studies (Valle Gottlieb *et al.*, 2010). The main findings were as follows: (1) Compared with the healthy controls, the CHC patients had higher concentrations of IMA, which were associated with increased markers of oxidative stress. (2) The plasma levels of IMA as well as those of AOPPs were higher in CHC patients with diabetes. (3) When the IMA levels were analyzed individually in CHC patients, there was a positive association between plasma IMA and the presence of diabetes, and between plasma IMA and CRP and IL-6 levels.

The amino terminal end (N-terminus) of the albumin molecule appears to be the primary binding site for transitional metals, such as cobalt, copper and nickel (Bar-Or *et al.*, 2001). When the aspartyl-alanyl-

Table 2. Clinical and biochemical characteristics of patients with chronic hepatitis C (CHC) infection with regard to the presence of diabetes.

	Healthy controls	CHC patients without diabetes	CHC patients with diabetes
(n)	40	35	28
Male:Female ratio	18:12	21:14	12:16
Age (years)	35 ± 9	48 ± 12	54 ± 9
Body mass index (kg/m^2)	24 ± 2.6	22 ± 2.3	23.3 ± 2.5
Fasting plasma glucose (mmol/L)	5.0 ± 0.6	5.6 ± 0.68	8.3 ± 1.0**$^+$
IMA (ABSU)	0.28 ± 0.21	0.34 ± 0.14	0.56 ± 0.16**$^+$
Alanine aminotransferase (IU/L)	23 (20–28)	89 (33–150)*	104 (37–190)**
Aspartate aminotransferase (IU/L)	28 (23–30)	76 (28–130)*	82 (31–140)*
Albumin (g/dL)	4.8 (3.6–5.7)	4.4 (4.2–4.8)	4.2 (3.4–4.8)
Platelet count (x 10^9/L)	200 (130–220)	214 (154–241)	217 (161–245)
White blood cells count (x 10^9/L)	4.7 (3.2–5.0)	4.9 (3.0–7.8)	6.0 (3.3–8.2)
hsCRP (mg/L)	1.05 (0.58–2.5)	1.88 (1.3–4.5)	3.84 (2.05–17.3)*$^+$
IL-6 (pg/mL)	5.8 (5.5–6.7)	7.3 (5.4–9.8)*	9.4 (7.4–12.1)*$^+$
TNF-α (pg/mL)	28.0 (25.5–35.0)	32.9 (31.0–35.2)	36.9 (31.7–45.6)

Values are expressed as means ± S.D. or as medians (interquartile range) for skewed data. Significance levels between groups: *$P < 0.05$; **$P < 0.01$ vs. healthy controls; $^+P < 0.01$ *vs* CHC patients without diabetes. hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IMA, ischemia-modified albumin; TNF-α, tumor necrosis factor α.

histidyl-lysine sequence in the N-terminus is damaged, it results in a reduced binding capacity for metals. The effect is related to albumin circulating through ischemic capillary beds such as those found in cardiovascular disease (Bar-Or *et al*., 2001; Bhagavan *et al*., 2003). Thus, IMA is considered as a cardiovascular disease-related biomarker that is sensitive to cardiac ischemia (Pantazopoulos *et al*., 2009). Recent studies have shown significantly higher IMA levels in metabolic syndrome, hyperlipidemia and type 2 diabetes mellitus (Piwowar *et al*., 2008; Duarte *et al*., 2009; Kaefer *et al*., 2010, Ma *et al*., 2011; 2012). These studies suggest that IMA formation may occur under chronic oxidative stress conditions and also at extra cardiac sites. However, we did not find a relationship between oxidative stress markers and IMA, because increased accumulation of the glycoxidation products is highly age-dependent, and it could require a larger number of patients to detect. Despite the attention paid to the validity of IMA and AOPPs as biomarkers for cardiovascular disease, the actual association between AOPPs and IMA in chronic hepatitis is unknown. An increased plasma IMA level as well as AOPP levels in CHC patients, particularly in the diabetic CHC group, were observed in the present study. Although we have not found any study in the literature that deals with plasma AOPP level in diabetic CHC patients, increased plasma AOPPs may reflect increased oxidative stress. Such supposition is supported by studies suggesting the importance of reactive oxygen species production in diabetes (Evans *et al*., 2003), which may lead to IMA formation. It is of importance that the presence of diabetes but not HCV infection was associated with increased circulating concentrations of AOPPs and IMA in patients with chronic hepatitis C infection. Based on the above results, it may be hypothesized that high concentrations of IMA might indicate chronic oxidative stress in chronic hepatitis C infection associated with metabolic complications.

The most interesting observation in the present study was the presence of direct correlation between diabetes prevalence and aging-associated inflammation. Furthermore, both the presence of diabetes as well as inflammation were associated with circulating AOPP and IMA levels. Although AOPPs can merely be markers of an underlying oxidative stress, it is possible that AOPPs may be directly involved in the pathogenesis of inflammation/atherosclerosis (Guo *et al*., 2008). Our findings extended the results of earlier studies (Zuwała-Jagiello *et al*., 2009; 2011) which suggested that AOPPs were more closely related to inflammation than CML-AGEs, whose receptor, RAGE (receptor for advanced glycation end-products), participates in AOPP-mediated signal transduction (Kalousová *et al*., 2005). Among systemic inflammatory biomarkers, CRP seems to be the most significant for cardiovascular risk detection, which relies on evidence suggesting atherosclerosis as an inflammatory process (Corrado *et al*., 2010). The elevation of plasma AOPP level resulted in excessive inflammatory response, as evidenced by increased levels of IL-6, which preceded the increase in CRP in the inflammatory cascade (Ceciliani *et al*., 2002). Even if there was no correlation between AOPPs and IL-6, we demonstrated weak association with inflammatory CRP, as detected by the hsCRP assay. Esposito *et al.* (2002) have noted the effect of hyperglycemia-induced oxidative stress on inflammation. Furthermore, inflammatory mediators such as IL-6 and TNF-α have been detected in significant amounts not only in patients with type 2 diabetes (Castoldi *et al*., 2007), but also in CHC patients (Gattoni *et al*., 2006). The simultaneous increment in IMA and inflammation, which we observed in CHC patients with diabetes, suggests that their local tissue production is linked. This hy-

Table 3. Comparison between all CHC patients (with and without diabetes) and non-diabetes CHC patients classified according to low (≤0.70 ABSU) or high (>0.70 ABSU) levels of ischemia-modified albumin (IMA).

	CHC patients (n=63)		CHC patients without diabetes (n=35)	
	IMA≤0.70 ABSU	IMA>0.70 ABSU	IMA≤0.70 ABSU	IMA>0.70 ABSU
n (%)	32 (51)	31 (49)	21 (61)	14 (39)
Age (years)	52±10.8	50±11.1	44±10	47±11.5
Systolic blood pressure (mmHg)	132±18	139±19	130±18.5	132±18
Diastolic blood pressure (mmHg)	79±9.0	83±10	78±10	79±9.0
Body mass index (kg/m^2)	25±2.4	25.8±2.5	21.2±2.0	22±1.9
Fasting plasma glucose (mmol/L)	5.8±0.7	7.2±1.0$^+$	4.4±0.65	5.2 ±0.7
HbA_{1c} (%)	6.3±0.94	7.6 ±1.13^{++}		
Diabetes n (%)	6 (11)	22 (35)$^{++}$	–	–
hsCRP (mg/L)	2.0 (1.3–3.1)	3.2 (2.4–17.3)$^+$	1.6 (1.3–3.6)	1.8 (1.45–4.5)
IL-6 (pg/mL)	7.0 (5.4–9.5)	10.2 (6.2–12.1)$^+$	7.4 (5.4–9.5)	7.6 (5.7–9.8)
Alanine aminotransferase (IU/L)	80 (33–160)	93 (35–190)	73 (35–150)	87 (33–135)
Aspartate aminotransferase (IU/L)	71 (28–120)	80 (28–140)	66 (30–125)	77 (28–130)
Total cholesterol (mg/dL)	144±48	155±51.8	152±60	147±58
Low density lipoprotein (mg/dL)	71±26	73±27	91±29	83±30
High density lipoprotein cholesterol (mg/dL)	34±15	33±17	34±15	39±13
Triglycerides (mg/dL)	46.5±10	48±12	46±10	44±10.5

Values are expressed as means ±S.D. or as medians (interquartile range) for skewed data. Significance between groups: $^+P<0.05$; $^{++}P<0.01$. hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6.

pothesis is supported by experimental data demonstrating that part or all of the pro-atherogenic action of CRP could be mediated *via* its local action in the vascular wall where the protein is deposited (Kobayashi *et al.*, 2003; Yu *et al.*, 2012). Other studies have demonstrated that albumin is converted to IMA in the circulation under conditions of oxidative stress produced in the environment of atheroma plaques (Sbarouni *et al.*, 2011). Since the C-reactive protein itself serves as a pro-inflammatory factor (Bisoendial *et al.*, 2010), the enhanced formation of CRP in diabetic CHC patients may be considered as a factor that contributes to the development of a vicious circle of oxidative inflammation and free radical-induced albumin modification. Accordingly, there are reasons to believe that this positive feedback loop could amplify the chronic inflammatory state caused by the hepatitis C virus, and thus contribute to atherosclerosis in CHC patients with diabetes, albeit the exact mechanism still needs to be elucidated.

Analyses of the associations between IMA and CRP have to deal with the presence of cardiovascular disease as a possible confounder. Therefore, in our study we have excluded diabetic CHC patients with overt cardiovascular disease. However, some of our subjects might

Table 4. Correlations between ischemia-modified albumin (IMA) and markers of inflammation, oxidative stress and diabetes prevalence in patients with chronic hepatitis C (CHC) infection (n=63).

	IMA (ABSU)	AOPPs (μmol/L)	hsCRP (mg/L)	IL-6 (pg/mL)
Age	r=0.06, NS	r=0.32, $P<0.05$	r=0.53, $P<0.001$	r=0.51, $P<0.01$
Diabetes	$\chi^2=6.382$, $P<0.05$	$\chi^2=10.004$, $P<0.001$	$\chi^2=7.485$, $P<0.01$	$\chi^2=0.759$, NS
Fasting plasma glucose (mmol/L)	r=0.25, $P<0.05$	r=0.09, NS	r=0.19, $P<0.05$	r=0.18, $P<0.05$
IMA (ABSU)	–	r=0.05, NS	r=0.50, $P<0.01$	r=0.39, $P<0.05$
hsCRP (mg/L)	r=0.50, $P<0.01$	r=0.23, $P<0.05$	–	r=0.49, $P<0.001$
IL-6 (pg/mL)	r=0.39, $P<0.05$	r=0.41, NS	r=0.49, $P<0.001$	–
TNF-α (pg/mL)	r=0.15, NS	r=0.32, NS	r = 0.09, NS	r=0.01, NS

Results are shown as linear (r) regression coefficient or bivariate logistic (χ^2), and its statistical significance value. NS, not significant ($P>0.05$); hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IMA, ischemia-modified albumin.

have undiagnosed atherosclerosis, and we did not perform any surrogate measures of atherosclerosis, such as carotid intimamedia thickness. Our study is also limited by its cross-sectional design, and therefore the relationships between IMA and CRP cannot be considered as causal in the cascade of atherogenesis to end-point cardiovascular disease.

Some limitations deserve mentioning. First, our sample size is small and this might have resulted in weaker associations among some of the parameters. More extensive confirmatory studies should be performed to better estimate the association between IMA and oxidative stress, inflammation and the presence of diabetes in patients with CHC. A larger study would also have sufficient power to determine whether the association between IMA and inflammation in CHC patients with diabetes differs by sex. Secondly, the patients categorized as "CHC patients without diabetes" may have pre-diabetes (intermediate hyperglycemia), and many of them may develop diabetes in the future. Finally, we examined only three different inflammatory biomarkers. Studies in the general population have shown that other biomarkers, such as interleukin-18, may be associated with diabetes (Negi *et al*., 2012).

In conclusion, simultaneous monitoring of plasma AOPPs and IMA can be helpful for the cardiovascular disease control in CHC patients with diabetes, although the pathologic link between both biomarkers remains unclear. Therefore, in order to prevent cardiovascular diseases in chronic hepatitis C infection, it is crucial to monitor the subjects with biomarkers that are tailored for this population and that may turn to be more predictive of cardiovascular diseases in CHC patients with diabetes.

Acknowledgments

The project described was supported by a research grant from the Wrocław Medical University.

REFERENCES

Bar-Or D, Curtis G, Rao N, Bampos N, Lau E (2001) Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. *Eur J Biochem* **268**: 42–47.

Bar-Or D, Lau E, Winkler JV (2000) A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. *J Emerg Med* **19**: 311–315.

Bhagavan NV, Lai EM, Rios PA, Yang J, Ortega-Lopez AM, Shinoda H, Honda SA, Rios CN, Sugiyama CE, Ha CE (2003) Evaluation of human serum albumin cobalt binding assay for the assessment of myocardial ischemia and myocardial infarction. *Clin Chem* **49**: 581–585.

Bisoendial RJ, Boekholdt SM, Vergeer M, Stroes ES, Kastelein JJ (2010) C-reactive protein is a mediator of cardiovascular disease. *Eur Heart J* **31**: 2087–2091.

Castoldi G, Galimberti S, Riva C, Papagna R, Querci F, Casati M, Zerbini G, Caccianiga G, Ferrarese C, Baldoni M, Valsecchi MG, Stella A (2007) Association between serum values of C-reactive protein and cytokine production in whole blood of patients with type 2 diabetes. *Clin Sci (Lond)* **113**: 103–108.

Ceciliani F, Giordano A, Spagnolo V (2002) The systemic reaction during inflammation: the acute-phase proteins. *Protein Pept Lett* **9**: 211–223.

Corrado E, Rizzo M, Coppola G, Fattouch K, Novo G, Marturana I, Ferrara F, Novo S (2010) An update on the role of markers of inflammation in atherosclerosis. J Atheroscler Thromb **17**: 1–11.

Chen CY, Tsai WL, Lin PJ, Shiesh SC (2011) The value of serum ischemia-modified albumin for assessing liver function in patients with chronic liver disease. *Clin Chem Lab Med* **49**: 1817–1821.

Corrado E, Rizzo M, Coppola G, Fattouch K, Novo G, Marturana I, Ferrara F, Novo S (2010) An update on the role of markers of inflammation in atherosclerosis. J *Atheroscler Thromb* **17**: 1–11.

Douglas MW, George J (2009) Molecular mechanisms of insulin resistance in chronic hepatitis C. *World J Gastroenterol.* **15**: 4356–4364.

Duarte MM, Rocha JB, Moresco RN, Duarte T, Da Cruz IB, Loro VL, Schetinger MR (2009) Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. *Clin Biochem* **42**: 666–671.

Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D (2002) Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* **106**: 2067–2072.

Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2003) Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* **52**: 1–8.

Gattoni A, Parlato A, Vangieri B, Bresciani M, Derna R (2006) Interferon-gamma: biologic functions and HCV terapy (type I/II) (2 of 2 parts). *Clin Ter* **157**: 457–468.

Guo ZJ, Niu HX, Hou FF, Zhang L, Fu N, Nagai R, Lu X, Chen BH, Shan YX, Tian JW, Nagaraj RH, Xie D, Zhang X (2008) Advanced oxidation protein products activate vascular endothelial cells via a RAGE-mediated signaling pathway. *Antioxid Redox Signal* **10**: 1699–1712.

Kaefer M, Piva SJ, De Carvalho JA, Da Silva DB, Becker AM, Coelho AC, Duarte MM, Moresco RN (2010) Association between ischemia modified albumin, inflammation and hyperglycemia in type 2 diabetes mellitus. *Clin Biochem* **43**: 450–454.

Kalousová M, Zima T, Tesar V, Dusilová-Sulková S, Skrha J (2005) Advanced glycoxidation end products in chronic diseases-clinical chemistry and genetic background. *Mutat Res* **579**: 37–46.

Kobayashi S, Inoue N, Ohashi Y, Terashima M, Matsui K, Mori T, Fujita H, Awano K, Kobayashi K, Azumi H, Ejiri J, Hirata K, Kawashima S, Hayashi Y, Yokozaki H, Itoh H, Yokoyama M (2003) Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. *Arterioscler Thromb Vasc Biol* **23**: 1398–1404.

Ma SG, Wei CL, Hong B, Yu WN (2011) Ischemia-modified albumin in type 2 diabetic patients with and without peripheral arterial disease. *Clinics (Sao Paulo)* **66**: 1677–1680.

Ma SG, Jin Y, Hu W, Bai F, Xu W, Yu WN (2012) Evaluation of ischemia-modified albumin and C-reactive protein in type 2 diabetics with and without ketosis. *Biomark Insights* **7**: 19–26.

Mastella AK, Moresco RN, da Silva DB, Becker AM, Duarte MM, Giovelli LL, da Silva SH, Rossato L, Moretto MB, da Silva JE (2009) Evaluation of ischemia-modified albumin in myocardial infarction and prostatic diseases. *Biomed Pharmacother* **63**: 762–766.

Negi SI, Pankow JS, Fernstrom K, Hoogeveen RC, Zhu N, Couper D, Schmidt MI, Duncan BB, Ballantyne CM (2012) Racial differences in association of elevated interleukin-18 levels with type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes Care* **35**: 1513–1518.

Pantazopoulos I, Papadimitriou L, Dontas I, Demestiha T, Iakovidou N, Xanthos T (2009) Ischaemia modified albumin in the diagnosis of acute coronary syndromes. *Resuscitation* **80**: 306–310.

Petit JM, Bour JB, Galland-Jos C, Minello A, Verges B, Guiguet M, Brun JM, Hillon P (2001) Risk factors for diabetes mellitus and early insulin resistance in chronic hepatitis C. *J Hepatol* **35**: 279-283.

Piwowar A, Knapik-Kordecka M, Warwas M (2008) Ischemia-modified albumin level in type 2 diabetes mellitus — Preliminary report. *Dis Markers* **24**: 311–317.

Pu LJ, Lu L, Xu XW, Zhang RY, Zhang Q, Zhang JS, Hu J, Yang ZK, Ding FH, Chen QJ, Lou S, Shen J, Fang DH, Shen WF (2006) Value of serum glycated albumin and high-sensitivity C-reactive protein levels in the prediction of presence of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetol* **20**: 27.

Sbarouni E, Georgiadou P, Kremastinos DT, Voudris V (2008) Ischemia modified albumin: is this marker of ischemia ready for prime time use? *Hellenic J Cardiol* **49**: 260–266.

Sbarouni E, Georgiadou P, Voudris V (2011) Ischemia modified albumin changes — review and clinical implications. *Clin Chem Lab Med* **49**: 177–184.

Valle Gottlieb MG, da Cruz IB, Duarte MM, Moresco RN, Wiehe M, Schwanke H, Bodanese LC (2010) Associations among metabolic syndrome, ischemia, inflammatory, oxidatives, and lipids biomarkers. *J Clin Endocrinol Metab* **95**: 586–591.

Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, Jungers P, Descamps-Latscha B (1996) Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int* **49**: 1304–1313.

Yu Q, Li Y, Wang Y, Zhao S, Yang P, Chen Y, Fan J, Liu E (2012) C-reactive protein levels are associated with the progression of atherosclerotic lesions in rabbits. *Histol Histopathol* **27**: 529–535.

Zuwała-Jagiello J, Pazgan-Simon M, Simon K, Warwas M (2009) Elevated advanced oxidation protein products levels in patients with liver cirrhosis. *Acta Biochim Pol* **56**: 679–685.

Zuwała-Jagiello J, Pazgan-Simon M, Simon K, Warwas M (2011) Advanced oxidation protein products and inflammatory markers in liver cirrhosis: a comparison between alcohol-related and HCV-related cirrhosis. *Acta Biochim Pol* **58**: 59–65.