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The Association Between Chronic Hepatitis B, Chronic Hepatitis C, Sustained Liver Damage, and Features of Increased Cardiovascular Risk

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Original article

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It is thought that chronic liver disease affects a person's risk of cardiovascular disease (CVD) development. The aim of this study was to assess the effect of Chronic Hepatitis B (HBV) infection, Chronic Hepatitis C (HCV) infection, and liver damage on cardiovascular risk and selected vascular parameters contributing to CVD risk. This case-control study included a group of 114 patients composed of 34 patients with HBV, 35 patients with HCV, and 45 patients as the control group. Cardiovascular risk was assessed by analyzing classic risk factors, and the SCORE system. The following arterial properties were analyzed using applanation tonometry with SphygmoCor Vx technology: central systolic blood pressure (cSBP), central pulse pressure, augmentation pressure, augmentation index, and carotid-femoral pulse wave velocity (PWV). Asymmetric dimethyloarginine (ADMA) blood levels were analyzed using ELISA as a marker of vascular function. In a univariable analysis we found no significant differences between the hepatitis B, hepatitis C, and control groups in terms of PWV (respectively: median 7.2 [Q25-Q75 6.4-8.5], 7.3 [6.9-8.7], 7.8 [6.5-8.9]), cSBP (115 [109-126], 118 [107-123], 116 [107-129]), ADMA (0.52 [0.47-0.60], 0.53 [0.45-0.62], 0.58 [0.51-0.63]), SCORE (0 [0-1], 0 [0-2], 0 [0-2]). No significant differences in cardiovascular variables were observed between cirrhotic and non-cirrhotic patients. A multivariable analysis confirmed the above findings. (PWV, p=0.29; cSBP, p=0.26; ADMA, p=0.19). We concluded that chronic hepatitis B or C was not independently associated with an adverse cardiovascular risk profile nor with an unfavorable pattern of vascular parameters contributing to CVD risk in our study population, even in the case of liver cirrhosis. The same was true for blood ADMA levels.

Key words: ADMA, arterial stiffness, HBV, HCV, pulse wave velocity, PWV.

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It has been thought that liver damage, apart from the well-researched consequences such as impairment of detoxification, metabolism, synthesis, and storage, also affects the cardiovascular system. The exact mechanism of this, however, has remained unclear and many aspects of it are completely unexplored. Viral hepatitides are often a cause of liver impairment. Especially important among them are hepatitis B and C,

as these hepatitides can lead to a chronic inflammatory process, and in turn, in some patients, cirrhosis of the liver. Both infections are significantly problematic worldwide. Globally, approximately 400 million people suffer from chronic hepatitis B and 170-200 million, chronic hepatitis C (BADAWI *et al.* 2018, KARAKECILI *et al.* 2018). The majority of studies on the impact of liver disease on cardiovascular disease (CVD) development conducted thus far have focused on liver damage in the course of non-alcoholic, fatty liver disease (NAFLD) (BHALA *et al.* 2011; MUSSO *et al.* 2011; MORITA *et al.* 2015; VILLELA-NOGUEIRA *et al.* 2016). However, the correlation between NAFLD and CVD is not easy to interpret, the difficulty being the abundance of confounding factors usally related to both NAFLD and CVD. In a large percentage of patients, NAFLD is prompted by the same factors as CVD; therefore, it might be unclear whether isolated liver damage leads to CVD (FRANCQUE *et al.* 2016). In this context, liver damage caused by viral hepatitis might represent a better clinical model to assess the impact of liver dysfunction on CVD risk, and be less likely to be affected by confounders. However, the data available in literature in this regard are limited and inconclusive (TOMIYAMA *et al.* 2003; MORITANI *et al.* 2005; ADAM *et al.* 2008; PERTICONE *et al.* 2015).

The aim of the current study was to assess the relationship between HBV or HCV infection, and selected vascular parameters associated with CVD risk, while excluding other extrahepatic factors, which might influence the results. Additionally, we assessed the association between the presence of cirrhosis and cardiovascular variables.

Materials and Methods

Participants

The study was conducted in accordance with the Declaration of Helsinki (as revised in Brazil 2013). Participants had provided written informed consent to participate in the study. The study protocol was approved by ethics committees of the Jagiellonian University (1072.6120.21.2017).

This case-control study included 114 patients recruited at the Department of Infectious Diseases, Department of Gastroenterology and Hepatology, University Hospital in Krakow, Poland and in the Unit of Cardiology of San Luca Hospital, Istituto Auxologico Italiano, Milano, Italy, from June 2014 to June 2019. All study participants were Caucasian and were recruited from the outpatient services of the institutions taking part in the study. The study group included 34 patients with chronic hepatitis B, 35 patients with chronic hepatitis C, and 45 patients as a control group. Patients recruited into the control group were correspondent in age, sex and BMI. HBV and HCV infection had been excluded before participation and the exclusion criteria had been met. Patients from the HBV and HCV groups were divided into 2 subgroups: non-cirrhotic and cirrhotic. Patients from the control group were divided into 2 subgroups: with no liver disease and those with liver cirrhosis due to some cause other than infection or an autoimmune issue. A diagnosis of cirrhosis was established based on typical laboratory results, imaging tests and/or histopathology, as well as the clinical picture. Exclusion criteria were as follows: age less than 18 years, pregnancy, a history of CVD (myocardial infarction, angina pectoris, heart failure, stroke, transient ischaemic attacks, or claudication), uncontrolled hypertension (defined as a brachial systolic blood pressure (bSBP) $\geq$140 mmHg and a brachial diastolic blood pressure (bDBP) $\geq$ 90 mmHg under treatment), diabetes mellitus, a history of dyslipidemia, and a use of antiaggregants, anticoagulants, or any other CV drugs besides antihypertensive agents. Diagnosis of hepatitis B was based on the presence of HBV-DNA and for hepatitis C of HCV-RNA in the patients' blood.

Anthropometric and laboratory assessments

We measured height, body weight, and waist circumference. Body mass index (BMI) was calculated as weight/height^2. We assessed complete blood count with differential leukocyte count and the levels of serum alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptydase (GGTP), cholinesterase, bilirubin, glucose, albumin, ammonia, lipid profile, C reactive protein (CRP), prothrombin time (PT), and activated partial thromboplastin time (APTT). All tests were performed according to generally accepted standard procedures. In some cases, the data was incomplete, therefore the corresponding values of n were given.

Asymmetric dimethyl arginine (ADMA) analysis

Early-morning blood samples after fasting for at least 12 hours were drawn and collected into serum separator tubes, samples were allowed to clot for 30 minutes at room temperature and subsequently centrifuged for 15 minutes at 1000xg at room temperature. Blood samples were stored at -80^{o}C after centrifugation. The serum level of ADMA was determined by the ADMA Fast ELISA Kit (DLD, Germany), according to the manufacturer's instructions. All samples (standard, control solution 1&2, 66 patients and 29 controls) were analyzed in duplicate. The optical density was measured at 450 nm using a microplate reader (GloMax Multi+ Detection System, Promega, Madison, WI, US) within 15 minutes. A four parameters logistic (4-PL) curve-fit was used for quantitative analysis of the samples (Instinct Software, Promega, Madison, WI, US).

Cardiovascular assessments

We performed office blood pressure measurements twice on the non-dominant arm after 10 minutes of rest using a Omron M5-I device (Omron, Kyoto, Japan) and results were averaged, then bSBP, bDBP, pulse pressure (PP = SBP - DBP), and mean arterial pressure (MAP) were determined. MAP was calculated according to the formula: MAP = DBP + 1/3 PP. Central systolic blood pressure in the aorta (cSBP), central pulse pressure (cPP), augmentation pressure (AP), augmentation index (AIx), and pulse wave velocity (PWV) were measured using applanation tonometry with the SphygmoCor Vx system (AtCor Medical, Sydney, NSW, Australia). SCORE (Systematic Coronary Risk Evaluation) was used to estimate the risk of cardiovascular disease development among the studied groups (CONROY *et al.* 2003).

Statistical analysis

All data are presented as means ± standard deviation (SD) or medians with lower (Q25) and upper (Q75) quartiles. A normal distribution of variables was verified using the Shapiro-Wilk test. Univariable comparisons between study groups were performed using the Mann-Whitney U-test and Kruskal-Wallis ANOVA, as appropriate. In order to determine the associations of peripheral and central haemodynamic variables (bSBP, MAP, cSBP, cPP, AP, AIx, PWV) and of ADMA with the clinical characteristics of the study participants, multivariable linear models were analyzed. In each model, one of the above variables was included as a dependent variable and the following as independent variables: hepatitis (HBV, HCV or none), presence of cirrhosis, age, sex, BMI, smoking, and total cholesterol. Analyses were performed using STATISTICA 13.1 software (StatSoft, Inc., Tulsa, OK, USA), and statistical significance was defined as $p<0.05$.

Results

Patient characteristics

The study group included 34 patients with chronic hepatitis B (mean age 43±12 years, 15 women and 19 men), 35 patients with chronic hepatitis C (mean age 47±13 years, 11 women and 24 men), and 45 patients as a control group (mean age 46±12, 20 women and 25 men).

Among the 34 participants with chronic hepatitis B, two patients were cirrhotic (liver fibrosis F4), and 32

Table 1

General characteristics of study participants according to the presence of hepatitis

Parameters	HBV		HCV		Control		p#	p##
	n	Median (Q25-Q75)	n	Median (Q25-Q75)	n	Median (Q25-Q75)		
Age (years)	34	41.5 (32-51)	35	47 (40-53)	45	44 (38-54)	0.23	0.002
BMI (kg/m^2)	34	25.6 (21.9-28.7)	35	25.8 (24.6-28.0)	45	24.7 (22.9-26.7)	0.49	0.19
waist cir. (cm)	33	92 (78-100)	35	94 (82-100)	35	88 (77-102)	0.40	0.09
Glycemia (mmol/l)	34	5.1 (4.6-5.3)	33	4.9 (4.7-5.8)	38	5.0 (4.7-5.3)	0.68	0.10
Bilirubine (µmol)	34	9.3 (6.6-15.3)	35	11.2 (8.7-19.4)	36	10.7 (6.6-18.4)	0.53	0.18
Albumin (g/l)	32	45.8 (43.7-47.5)	35	44.5 (40.8-47.0)	35	44.1 (40-46)	0.05	0.001
ALT (U/l)	34	26 (18-38)	35	38 (18-58)	37	24 (17-31)	0.06	0.11
AST (U/l)	34	25 (20-30)	33	30 (24-50)	34	20 (17-30)	0.002	0.01
ALP (U/l)	34	70 (57-86)	33	65 (53-84)	33	66 (52-93)	0.77	0.007
GGTP (U/l)	34	21 (15-41)	33	40 (23-55)	36	33 (18-82)	0.04	<0.001
ChE (Ux10^3/l)	34	7.67 (6.86-8.53)	33	6.95 (4.94-8.96)	29	6.87 (5.31-8.83)	0.26	0.003
PT (sec)	33	11.6 (11.3-12.1)	35	11.8 (11.2-13.3)	36	11.5 (10.7-12.3)	0.23	0.01
APTT (sec)	33	30.8 (28.5-32.1)	32	31 (28.2-34.8)	35	32 (28.6-34.1)	0.62	<0.001
Platelets (10^3/µl)	34	231 (194-265)	34	190 (106-225)	40	240 (158-269)	0.02	<0.001
Ammonia (µmol/l)	30	40.5 (31.4-49.8)	30	46.9 (37.6-68.4)	22	49.1 (38.5-67.1)	0.06	0.003
CRP (mg/l)	34	1.3 (0.5-2.1)	32	0.74 (0.30-1.63)	30	1.8 (1.0-6.4)	0.02	0.01

HBV – hepatitis B virus; HCV – hepatitis C virus; Q25, Q75 – lower, upper quartile; BMI – body mass index; ALT – alanine transaminase; AST – aspartate transaminase; ALP – alkaline phosphatase; GGTP – gamma-glutamyl transpeptydase; ChE – cholinesterase; PT – prothrombin time; APTT – activated partial thromboplastin time; CRP – C-reactive protein; p# – refers to the comparison of the HBV, HCV and control group; Kruskal-Wallis test; p## – refers to the comparison of patients with vs. without cirrhosis; Mann-Whitney U-test (for details see Suppl. 1. in Supplementary materials).

were non-cirrhotic. In 18 of the 32 non-cirrhotic patients, either core needle biopsy or dynamic elastography results were available to assess the extent of liver fibrosis. Six patients were in the F0 liver fibrosis stage, three in F1, six in F2, and three in F3. Average viraemia in the HBV group was 453 068 IU/ml.

Out of the 35 patients with chronic hepatitis C, 16 were cirrhotic (liver fibrosis F4) and 19 non-cirrhotic. Out of those 19, 18 had a core needle biopsy or dynamic elastography performed. One patient placed in F0, two in F1, twelve in F2, and three in F3. Average viraemia in the HCV group was 1.3×10^6 IU/ml.

Among the 45 patients from the control group, 34 had no liver disease, and 11 patients were cirrhotic.

Results of the parameters in corresponding groups

Tables 1 and 2 represent the results in the HBV and HCV groups and the control group. Data in Supplementary Materials (Suppl. 1, and Suppl. 2.) present data accounting for the cirrhotic and non-cirrhotic subgroups. There was a clear, statistically significant difference between the cirrhotic and non-cirrhotic group in terms of age and in several biochemical variables emblematic of liver damage (Table 1). Table 2 illustrates the parameters relevant to CVD risk and ADMA. The only evident, statistically significant difference was the total cholesterol level between the HBV and HCV groups, driven by the more numerous subgroup of cirrhotic patients in the HCV group. The rest of the parameters did not differ significantly. A statistically significant difference in SCORE between the cirrhotic and non-cirrhotic groups disappeared when SCORE was adjusted for age (p=0.79).

Chronic hepatitis B and C and their impact on arterial properties, cardiovascular risk, and ADMA, including multiple factor analysis

The next step was to compare the parameters relevant to CVD risk and ADMA between the studied groups, including multivariable analysis, as to avoid the influence of confounders (including negative ones) on the results yielded by univariable analysis (Table 3). Neither HBV, nor HCV infection, nor cirrhosis worsened the parameters in question. PWV was even lower in cirrhotic patients than the non-cirrhotic ones. The directions of the relations derived from the regression coefficients for the statistically significant parameters aligned as expected.

Table 2

Variables characterizing cardiovascular risk profile of study participants according to the presence of hepatitis

Parameters	HBV		HCV		Control		p#	p##
	n	Median (Q25-Q75)	n	Median (Q25-Q75)	n	Median (Q25-Q75)		
bSBP (mmHg)	32	128 (120-138)	30	130 (122-137)	40	122 (115-136)	0.33	0.99
bDBP (mmHg)	32	81 (75-86)	30	81 (75-89)	40	80 (72-88)	0.76	0.53
MAP (mmHg)	32	97 (88-103)	30	95 (88-105)	40	92 (84-101)	0.16	0.62
HR (/min)	31	70 (64-74)	24	66 (58-88)	39	76 (65-83)	0.11	0.36
cSBP (mmHg)	31	115 (109-126)	30	118 (107-123)	40	116 (107-129)	0.90	0.99
cPP (mmHg)	31	32 (30-37)	30	36 (30-41)	40	32 (29-37)	0.78	0.09
AP (mmHg)	31	14 (8-17)	30	14 (10-16)	40	12 (8-17)	0.44	0.19
Aix (%)	31	40.5 (25.0-51.7)	30	39.4 (27.5-53.3)	40	37.3 (20.2-51.4)	0.79	0.10
PWV (m/s)	31	7.2 (6.4-8.5)	30	7.3 (6.9-8.7)	40	7.8 (6.5-8.9)	0.57	0.28
SCORE (%)	32	0 (0-1)	30	0 (0-2)	33	0 (0-2)	0.99	0.02
TChol (mmol/l)	34	4.4 (3.9-5.7)	34	4.1 (3.2-4.7)	38	4.6 (3.9-5.0)	0.04	0.19
LDL (mmol/l)	34	2.5 (1.8-3.5)	32	2.1 (1.5-2.7)	37	2.5 (1.8-2.9)	0.21	0.18
HDL (mmol/l)	34	1.6 (1.2-1.9)	32	1.4 (1-1.7)	38	1.3 (1.1-1.7)	0.16	0.87
TG (mmol/l)	34	1.1 (0.7-1.3)	32	0.9 (0.6-1.3)	36	1.1 (0.8-1.4)	0.20	0.45
ADMA (μmol/l)	33	0.52 (0.47-0.60)	30	0.53 (0.45-0.62)	29	0.58 (0.51-0.63)	0.20	0.45

HBV – hepatitis B virus; HCV – hepatitis C virus; Q25, Q75 – lower, upper quartile; bSBP – brachial systolic blood pressure; bDBP – brachial diastolic blood pressure; MAP – mean arterial pressure; HR – heart rate; cSBP – central systolic blood pressure in aorta; cPP – central pulse pressure; AP – augmentation pressure; Aix – augmentation index; PWV – pulse wave velocity; TChol – total cholesterol; LDL – low density lipoprotein cholesterol; HDL – high density lipoprotein cholesterol; TG – triglycerydes; ADMA – asymmetric dimethyl arginine; p# – refers to the comparison of the HBV, HCV and control group; Kruskal-Wallis test; p## – refers to the comparison of patients with vs. without cirrhosis; Mann-Whitney U-test (for details see Suppl. 2. in Supplementary materials).

Table 3

Summary of variables associated with peripheral and central haemodynamic parameters and with ADMA in multivariable models

Variable		bSBP (mmHg) n=94	MAP (mmHg) n=94	cSBP (mmHg) n=94	cPP (mmHg) n=93	AP (mmHg) n=93	AIx (%) n=93	PWV (m/s) n=93	ADMA (μmol/l) n=93
	Model R^2	0.23	0.21	0.26	0.25	0.15	0.25	0.29	0.19
Hepatitis vs. controls:									
HBV	B (SE)	-0.37 (2.15)	0.09 (1.71)	-0.51 (2.01)	0.22 (1.29)	-0.3 (0.91)	-2.09 (2.99)	-0.28 (0.23)	-0.02 (0.02)
	p	0.87	0.96	0.80	0.86	0.74	0.49	0.24	0.40
HCV	B (SE)	1.29 (2.2)	1.45 (1.75)	0.38 (2.04)	-0.48 (1.31)	0.98 (0.93)	3.29 (3.03)	0.02 (0.24)	0.01 (0.02)
	p	0.56	0.41	0.85	0.72	0.29	0.28	0.92	0.58
Cirrhosis (yes/no)	B (SE)	-2.7 (1.99)	-2.77 (1.59)	-2.18 (1.85)	1.23 (1.19)	-0.98 (0.84)	-3.96 (2.75)	-0.48 (0.22)	0.002 (0.02)
	p	0.18	0.08	0.24	0.30	0.25	0.15	0.03	0.91
Age (years)	B (SE)	0.2 (0.13)	0.22 (0.1)	0.29 (0.12)	0.18 (0.08)	-0.13 (0.05)	-0.59 (0.18)	0.06 (0.01)	0.003 (0.001)
	p	0.13	0.03	0.02	0.02	0.02	0.001	<0.001	0.04
Sex (male/female)	B (SE)	1.97 (1.58)	0.55 (1.26)	0.6 (1.47)	-0.63 (0.95)	1.39 (0.67)	4.89 (2.18)	0.27 (0.17)	-0.03 (0.01)
	p	0.22	0.66	0.68	0.51	0.04	0.03	0.12	0.04
BMI (kg/m^2)	B (SE)	1.34 (0.43)	0.75 (0.34)	1.36 (0.4)	0.77 (0.26)	-0.04 (0.18)	-0.74 (0.6)	0.05 (0.05)	-0.01 (0.004)
	p	0.003	0.03	0.001	0.004	0.84	0.22	0.27	0.12
Smoking (yes/no)	B (SE)	-0.7 (1.69)	-1.75 (1.35)	-1.17 (1.57)	-0.19 (1.01)	0.35 (0.71)	2.22 (2.33)	0.04 (0.18)	0.01 (0.02)
	p	0.68	0.20	0.46	0.85	0.63	0.34	0.83	0.65
TChol (mmol/l)	B (SE)	1.11 (1.49)	1.54 (1.19)	1.29 (1.39)	-0.49 (0.89)	-0.06 (0.63)	-0.62 (2.06)	0.05 (0.16)	-0.02 (0.01)
	p	0.46	0.20	0.35	0.58	0.92	0.76	0.75	0.11

HBV – hepatitis B virus; HCV – hepatitis C virus; BMI – body mass index; TChol – total cholesterol; bSBP – brachial systolic blood pressure; MAP – mean arterial pressure; cSBP – central systolic blood pressure in aorta; cPP – central pulse pressure; AP – augmentation pressure; AIx – augmentation index; PWV – pulse wave velocity; ADMA – asymmetric dimethyl arginine.

Discussion

From a methodological viewpoint, the model of liver damage in the course of hepatitis B and hepatitis C is much better than in the course of NAFLD due to the fact that it lacks many other, interfering extrahepatic factors of CVD and metabolic disorders. Many patients suffering from chronic hepatitis B and C are people who, on the one hand exhibit extensive liver damage, but on the other, do not suffer from other risk factors of CVD such as diabetes or obesity. However, the data from hitherto performed studies in a population of HBV/HCV-infected patients is ambiguous, and furthermore, it is worth noting that the qualification of patients in the HCV group in some of these studies was based on the presence of anti-HCV antibodies. Moreover, exclusion criteria did not always include other cardiovascular diseases, antiaggregation and anticoagulation therapy, as well as dyslipidaemia.

There are several of mechanisms through which, theoretically, the liver could in fact influence the development of CVD; impact on arterial stiffness and endothelial dysfunction among them (Oni *et al.* 2013). Arterial stiffness is a product of complex interactions of external factors and structural aspects of the arterial wall (Gkaliagkousi & Douma 2009). It is directly proportional to morbidity and mortality in the course of CVD in patients with hypertension and those with diabetes as well as the general population (Laurent *et al.* 2001; Willum-Hansen *et al.* 2006; Mitchell *et al.* 2010; Cardoso *et al.* 2013). The research on arterial stiffness in chronic hepatitis B/C as contributing to CVD has hitherto been scarce.

MORITANI *et al.* (2005) assessed bSBP, ABI (ankle-brachial index), and PWV, showing no differences between HBV, HCV, and control groups. In TOMIYAMA *et al.*'s (2003) study, higher values of PWV in a HCV group were shown in comparison to a healthy group. No such relation was observed in the HBV group compared to the healthy group. In a subsequent study, there was no association of arterial stiffness in HCV patients with insulin resistance (ADAM *et al.* 2008). PERTICONE *et al. (*2015) showed higher PWV values in the HCV group compared to controls. WIJARNPREECHA *et al.* (2016) conducted a meta-analysis assessing coronary artery disease (CAD) in those infected with HBV. The authors concluded that HBV infection was not associated with an increased risk of coronary artery disease, however, the data obtained for this meta-analysis came mostly from Asia, therefore its results cannot be transposed to other populations with a different general cardiovascular risk. In the majority of studies included, the known risk factors of CAD had not been taken into consideration. BADAWI *et al.* (2018) analyzed data from the Canadian Health Measures Survey and the US-National Health and Nutrition Examination Survey, and suggested a potential relationship between HCV infection and clinical and subclinical cardiovascular disease assessed using the Framingham Risk Factors.

One of the most important results of our study is the lack of a difference in estimated cardiovascular risk using the SCORE system between the hepatitis B and C and control groups. This result supports the conclusion that patients with chronic hepatitis B or C are not associated with an increased cardiovascular risk. However, SCORE has a more prognostic, epidemiological value than just being useful in quantifying the real risk of CVD complications. The development of CVD complications is a continuum and might be better assessed by signs of early vascular damage. The 2018 guidelines of the European Society of Cardiology (ESC) and European Society of Hypertension (ESH) recommended the term HMOD (hypertension mediated organ damage) for those signs (WILIAMS *et al.* 2018). HMOD is very important for cardiovascular risk stratification, not only in patients with arterial hypertension. Increased arterial stiffness is one of the types of HMOD and aortic PWV is considered to be the gold standard for arterial stiffness evaluation (VAN BORTEL *et al.* 2012). The prognostic value of PWV for cardiovascular complications has been confirmed in different groups of patients (LAURENT *et al.* 2001; WILLUM-HANSEN *et al.* 2006; CARDOSO *et al.* 2013; SAFAR *et al.* 2018). The value of PWV in the effective prediction of cardiovascular events is comparable to (or stronger than) the Framingham risk score commonly used worldwide for this purpose (MITCHELL *et al.* 2010). Our results demonstrate that HBV, HCV, and liver damage, even having progressed to cirrhosis do not affect the indicators of arterial stiffness, closely associated with cardiovascular prognosis. The only observable correlation was the association between cirrhosis and PWV. Interestingly, the cirrhotic patients were characterized by lower PWV than the non-cirrhotic ones. As expected, age, sex, and BMI did impact CVD risk factors. No such effect was seen for smoking and blood cholesterol level. The absence of the effect of smoking could be explained by the fact that a large portion of the studied group did not smoke altogether. In addition, the population in question was relatively young, so the negative effects of smoking might not have been visible in the tested parameters. The lack of the effect of cholesterolemia was the result of subject selection. The patients in the HCV group were slightly older than the other groups, however, that difference was not statistically significant. The higher median age stemmed from the fact, that in that group, a larger percentage of patients suffered from liver cirrhosis, and those patients were older in all of the study groups (in the cirrhotic and non-cirrhotic subgroups, the median age was comparable in every main group). Patients with liver cirrhosis, despite being more advanced in age, could have been, to a degree, protected against atherosclerosis due to lower total cholesterol and LDL levels. Furthermore, the difference in age was relatively small considering the rather young age of the participants, so it was unlikely that such a small difference would translate to a discernible disparity in markers of atherosclerosis.

Endothelial dysfunction is an early and, therefore, crucial element leading to development of CVD. It occurs even before the formation of atherosclerotic plaque and fatty streaks (VANHOUTTE 2009). As a person's liver deteriorates, the endothelium of hepatic vessels deteriorates with it. This is a heavily pronounced process in cirrhosis (IWAKIRI *et al.* 2014). One of the possible mechanisms of the effect of liver disease on endothelial dysfunction is the accumulation of ADMA, an endogenic antagonist of NO (nitric oxide). An increase in ADMA is an established factor for CVD progression. The liver plays a crucial role in breaking down ADMA, this process begins to falter as liver damage progresses and might be the reason behind an increase in ADMA (DEANFIELD *et al.* 2007; KASUMOV *et al.* 2011; FERRIGNO *et al.* 2015). ADMA, by blocking the effect of NO, inhibits the endothelium-dependent vasodilation, as shown in both human and animal trials (BÖGER *et al.* 1998; BÖGER *et al.* 2000). ADMA is considered to be an important marker of endothelial dysfunction and correlates to risk factors of CVD (HE *et al.* 2013). It has been demonstrated that serum ADMA levels grow in non-infectious liver disease (NIJVELDT *et al.* 2003; LLUCH *et al.*2004; MOOKERJEE *et al.* 2007a). Moreover, those levels correspond to the severity of damage (MOOKERJEE *et al.* 2007b). MOOKERJEE *et al.* (2007a) have shown that acute liver failure is characterized by an increase in ADMA, which correlates

with the intensity of inflammation. During acute liver failure, ADMA levels grow and, subsequently, fall significantly in the first few days following a liver transplant, which points to the liver as key in ADMA metabolism (SIROEN *et al.* 2004). So far, there has been only one study assessing the levels of ADMA in HBV patients, showing elevated serum ADMA in patients with chronic hepatitis B (KARAKECILI *et al.* 2018). Several studies focusing on ADMA in HCV have yielded inconsistent results. LLUCH *et al.*'s (2009) small group study did not show differences in ADMA as well as NO in groups with hepatitis C, hepatitis C after a successful PegIFN+RBV treatment, and healthy people. However, only patients who retained full liver function qualified for the study. VIZZUTTI *et al.* (2007) have demonstrated HVPG (hepatic venous pressure gradient) to be directly proportional to ADMA levels and inversely proportional to NO levels in the serum of patients with hepatitis C, which could suggest an essential role of ADMA in portal hypertension in the course of this disease. Our study has shown neither a difference in ADMA stemming from HBV/HCV infection itself nor any effect of progressive liver damage on ADMA level.

The limitations of our study were as follows: a relatively low number of patients with cirrhosis and chronic hepatitis B met our strict inclusion/exclusion criteria. Another limitation, perhaps inherent to this type of study, was that even when we matched patients from the HBV, HCV, and control groups according to age and sex, the patients with cirrhosis were older than those without. Apart from the functional parameters, another important part of atherosclerosis assessment are the morphological parameters (e.g. the presence of atheromatous plaque in the carotid arteries or intima-media complex thickness), which, due to organizational limitations, have not been included in this study. However, it does seem that because of the relatively low age of the participants, the functional parameters have more quantifiable value in this population (incidence of clinically detectable lesions in people aged 40-50 is low). Another possible factor which could impact the studied parameters would be the duration of infection, however, considering the course of hepatitis, rarely are we able to confidently identify the point of transmission of HBV and HCV.

Conclusions

Chronic hepatitis B or C in our study population was not independently associated with an adverse cardiovascular risk profile nor with an unfavorable pattern of vascular parameters contributing to CVD risk, even in the case of liver cirrhosis. The same applied to blood ADMA levels.

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Research concept and design: J.C., M.R., G.Bl.; Collection and/or assembly of data: J.C., M.R., G.Bl., D.C., E.P.; Data analysis and interpretation: J.C., M.R., G.Bl., E.P.; Writing the article: J.C., M.R., G.Bl., E.P., M.M.; Critical revision of the article: J.C., M.R., G.Bl., G.P., G.Bs., D.C., E.P., P.W., M.M., A.G.; Final approval of article: J.C., M.R., G.Bl., G.P., G.Bs., D.C., E.P., P.W., M.M., A.G.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary Material to this article can be found online at: http://www.isez.pan.krakow.pl/en/folia-biologica.html

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