Original Article

The correlation of ADMA with proinflammatory, liver injury and cancer biomarkers in patients with liver dysfunction

Running head: ADMA in patients with liver dysfunction

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Abstract

Background & Aims: Asymmetric dimethylarginine (ADMA) is an enzyme involved in vascular tonus, blood pressure, and platelet activation. Serum ADMA levels are increased in liver diseases such as liver cirrhosis, hepatitis and acute liver failure. The aim of our study was the assessment of the correlation of ADMA with proinflammatory, liver injury and cancer biomarkers in patients with liver dysfunction of various etiology.

Materials and Methods: We analyzed the demographic and clinical data, including serum ADMA concentration and other biochemical markers such as albumin, platelets count, international normalized ratio, bilirubin and others in patients with hepatitis, compensated and decompensated liver cirrhosis and hepatocellular carcinoma. The one-way ANOVA, Student t-test, Mann-Whitney

U test, univariate and multivariate correlations were performed and p-value <0.05 was set as significant.

Results: In n=83 analyzed patients we have observed negative correlation of ADMA with albumin concentration (p=0.049). We have found negative correlation between ADMA and platelet count in n=31 patients with compensated liver cirrhosis (p=0.022). We have observed no significant correlations of ADMA and proinflammatory and cancer biomarkers in patients with hepatitis, compensated and decompensated liver cirrhosis and hepatocellular carcinoma.

Conclusions: ADMA can potentially be used as a subsidiary marker of disease progression in patients with liver dysfunction. Our research suggests that ADMA cannot be useful in detecting HCC.

Keywords: ADMA, biomarker, liver dysfunction, cirrhosis, hepatocellular carcinoma, hepatitis

Introduction

Asymmetric dimethylarginine (ADMA) is an inhibitor of nitric oxide synthase (NOS) since it is a competitor of L-arginine necessary to synthesize nitric oxide (NO) [1]. Elevated ADMA serum concentration leads to vasoconstriction, increases platelet aggregation, increases cell adhesion to the endothelium, and vascular muscle cell proliferation [2]. As a consequence, ADMA has also been reported as a risk marker of cardiovascular diseases and its concentration above the upper normal range was also identified as a risk factor of multiorgan dysfunction in critically-ill patients [3,4]. In addition to this, some researchers suggest the reduction of serum ADMA as one of the methods for lowering the risk of atherosclerosis and diabetes mellitus or as hypertension treatment [5].

Free ADMA, beside symmetric dimethylarginine (SDMA), is created in a process of proteolytic degradation of methylarginine. ADMA is mostly metabolized in the liver and the kidneys and then excreted by these organs. ADMA is degraded to citrulline and dimethylamine by dimethylarginine dimethylaminohydrolase (DDAH), an enzyme that is distributed mostly in the liver, kidneys and pancreas [1]. Thus, in chronic kidney disease (CKD) an elevation of serum ADMA concentration can occur and is considered an independent mortality risk factor in CKD patients [6]. Moreover, the serum accumulation of ADMA is observed in patients with viral hepatitis, non-alcoholic fatty liver disease (NAFLD) and acute liver failure (ALF) [4,7,8]. Higher plasma levels of ADMA are related to liver cirrhosis but not all metabolic pathways of NO and connections with ADMA in the cirrhotic liver are explained. On the one hand liver cirrhosis is associated with vasodilatation as an effect of higher activity of NO but the activity of endothelial NO synthase (eNOS) is decreased. Results of Lluch et al. study may suggest a different regulation of eNOS in the liver in the splanchnic vessels [9]. Another mechanism is probably due to impaired DDAH activity which can lead to the elevation of ADMA concentrations [10].

In patients with end-stage liver disease, CV risk is elevated with an increase of serum concentration of ADMA, NO, fasting glucose, HDL cholesterol, and absence of hepatocellular carcinoma (HCC) [11]. Elevated serum ADMA levels are also associated with the onset of multi-organ failure (MOF) [4].

The aim of this study was the assessment of the correlation of ADMA with proinflammatory, liver injury and cancer biomarkers in patients with liver dysfunction, including hepatitis, compensated and decompensated liver cirrhosis and hepatocellular carcinoma of various etiology in order to evaluate its potential use as a subsidiary marker in these liver diseases.

Materials and Methods:

Blood samples were taken from patients admitted to our department with hepatitis, compensated and decompensated liver cirrhosis and hepatocellular carcinoma of various etiology. We analyzed demographic data and serum concentrations of ADMA (ELISA Cat. REA201/96 Enzyme Immunoassay for the Quantitative Determination of Endogenous ADMA in serum or plasma), vascular cell adhesion molecule 1 (VCAM-1) (BioSource International (VCAM-1 ELISA kit), alpha-fetoprotein (AFP), C-reactive protein (CRP), carcinoembryonic antigen (CEA), cancer antigen 19-9 (Ca 19-9), platelets count (PLT), international normalized ratio (INR), bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGTP), albumin, total cholesterol, low-density lipids (LDL), high-density lipids (HDL), triglycerides (TG). The two-sample Student t-test or Mann-Whitney U test were used to evaluate the difference in the mean values among quantitative variables. The one-way ANOVA test was performed to evaluate the difference in the mean values of analyzed biomarkers' levels. Correlations between quantitative variables were assessed using the Spearman correlation coefficient and multivariate linear regression. The p-value was set at 0.05. All statistical analyses were performed using Python 3.7 software and Statistica 13.1 program (StatSoft Poland, Kraków, Poland).

Results

The study included n=83 patients with n=37 female and n=46 male with a mean age of 56.3 range 29-87 years, compensated liver cirrhosis n=32, decompensated liver cirrhosis n=31, with the etiology of autoimmune hepatitis (AIH) n=4, ethanol cirrhosis (ETH) n=10, infection of hepatitis B virus (HBV) n=14, coinfection of hepatitis B and C virus (HBV/HCV) n=4, hepatitis C virus (HCV) n=28, primary biliary cirrhosis (PBC) n=3. HCC was confirmed in n=10 patients in n=2 with compensated and n=7 with decompensated liver cirrhosis. Among n=20 patients with hepatitis were: AIH n=3, ETH n=1, HBV n=6, HCV n=8, and PBC n=2. The baseline data of analyzed patients are presented below in Table 1.

All patients were divided into three groups and were analyzed according to various biomarkers. We have separately compared the data of patients diagnosed with hepatocellular carcinoma and with no HCC diagnosis. See Table 2.

We have performed post-hoc tests to evaluate the difference in mean VCAM-1, PLT, ALT, AST and albumin concentrations between the analyzed groups of patients. We observed a significant difference in mean concentration of VCAM-1 between patients with compensated and decompensated liver cirrhosis ($p=0.012$) and also in patients with hepatitis and compensated liver cirrhosis ($p=0.003$). We have also seen significant difference in PLT count between patients with compensated and decompensated liver cirrhosis ($p=0.001$) and among patients with hepatitis and decompensated liver cirrhosis ($p=0.001$). Significant difference in mean ALT concentrations was also observed among patients with hepatitis and decompensated liver cirrhosis ($p=0.004$) and among patients with compensated and decompensated liver cirrhosis ($p=0.031$). We have also seen significant difference in mean concentrations of AST between patients with hepatitis and decompensated liver cirrhosis ($p=0.020$) and significant difference among mean concentrations of albumin between patients with hepatitis and decompensated liver cirrhosis ($p<0.001$) and between patients with compensated and decompensated liver cirrhosis ($p=0.002$). Other mean differences did not achieve statistical significance.

We have further analyzed univariate and multivariate correlation of ADMA with other biomarkers. We have adjusted multivariate linear regression for age, gender and category of patients (with hepatitis, compensated and decompensated liver cirrhosis). We have found statistically significant multivariate correlation of ADMA and albumin concentrations. The results are presented in Table 3 and Figure 1.

We have also analyzed univariate and multivariate correlation of ADMA with other biomarkers among analyzed groups of patients independently. In multivariate linear regression for that analysis we have adjusted for age and gender. We have found statistically significant univariate correlation of ADMA and albumin concentrations in patients with hepatitis, however, the significance was not achieved after the adjustment for age and gender. We have also observed significant multivariate correlation of ADMA concentration and PLT count among patients with compensated liver cirrhosis. The results are presented in Table 4, Figure 2 and Figure 3.

Discussion

In our study we tried to define significance of serum ADMA levels and its correlation with various biomarkers in order to evaluate it as a potential subsidiary marker of liver diseases.

In the analyzed group of patients we have observed that compensated liver cirrhosis was associated with elevated concentration of serum ADMA, which was not observed in patients with hepatitis,

or decompensated liver cirrhosis. Available data states that ADMA levels are increased in patients with liver cirrhosis, alcoholic hepatitis and ALF and are higher in patients with decompensated than in compensated liver cirrhosis [4,12]. According to a paper by Karakecili et al. serum ADMA levels are significantly higher in patients with chronic active hepatitis B compared to inactive HBV carriers and a control group [7]. Similarly, Lluch et al. reports that in patients with chronic hepatitis C without signs of acute inflammatory activity ADMA serum concentrations remain unchanged compared to a control group [13]. Also, Mookrejee et al. state that patients with alcoholic hepatitis superimposed on cirrhosis were also suggested to have higher ADMA values compared to patients without inflammation [14]. Due to small study population we did not perform the analysis of ADMA concentration in patients with various hepatitis etiology.

A platelet count and albumin concentration, among other basic serum laboratory tests, is used to identify disease progression in patients with chronic liver diseases [15]. According to a study by Surana et al. platelets performed significantly better in identifying cirrhosis compared to other examined biomarkers such as AST, ALT, albumin etc. [16]. In our research we have observed a negative correlation between the platelet counts and serum ADMA levels in patients with compensated liver cirrhosis, which suggests that ADMA could potentially be used as an additional biomarker in this disease. Albumin is synthetized exclusively by the liver, thus serum albumin levels are reduced in acute and chronic liver disease [17]. It is a factor related to steatohepatitis, fibrosis and cirrhosis [18]. Available data also suggests that albumin levels are lower in patients with acute and higher in chronic viral hepatitis of different etiology in comparison to healthy individuals [19]. In our study population we have seen negative correlation of ADMA and albumin concentration. Since both lower albumin levels and higher ADMA levels are associated with the progression of liver diseases, serum ADMA concentrations could possibly serve as a marker of liver disease progression [4,15].

ADMA is significantly elevated in the plasma of cancer patients. Current literature mentions its elevated level in prostate, colon, stomach and other cancers [20]. However, in HCC ADMA levels are decreased due to induced expression of DDAH which catabolizes ADMA [21]. Plasma concentrations of biomarkers AFP, Ca 19-9 and CEA are significantly more elevated in patients with primary hepatic cancer compared to patients with liver cirrhosis and healthy control groups [22]. Our research seems to be consistent with current knowledge. In our study the group of patients with HCC had lower ADMA concentration in comparison to the individuals with no HCC diagnosis but that difference was not statistically significant. However, plasma levels of AFP and Ca 19-9 were significantly higher in patients with HCC. In our research we did not observe a correlation between mentioned cancer biomarkers and ADMA.

In our study we were not able to find statistically significant differences between other analyzed parameters and/or their correlation with serum ADMA levels. According to Pirisi et al. VCAM-1 concentrations are elevated in liver diseases and patients with acute hepatitis or cirrhosis have

higher VCAM-1 levels than those with mild chronic liver disease and VCAM-1 concentrations appear to reflect the severity of the liver disease but do not correlate with its etiology [23]. Another analyzed marker was INR, which is the earliest and most accurate marker of liver failure – in liver diseases hepatic synthesis is dysfunctional which results in increased INR. Bilirubin is a nonspecific marker of liver dysfunction and is not a sensitive marker of liver injury [24]. Bilirubin, ASP, ALT and ALP can be useful in defining the source of liver damage – for example elevated ALT and ASP in disproportion to ALP, and bilirubin can suggest HCC [25]. In patients with cirrhosis serum triglyceride, total, LDL and HDL cholesterol levels are notably decreased and correlate with the severity of the cirrhosis [26]. Furthermore, a study by Manka et al. suggests that HDL levels are significantly higher in patients with ALF caused by acetaminophen intoxication, compared to other analyzed etiologies [27]. CRP values can indicate the probability of future decompensation in liver cirrhosis patients or rehospitalization [28].

Many researches concerning liver diseases and ADMA levels that are currently available involved a limited number of participants. For example, a study by Czepiel et al. included 114 patients, a study by Karakecili et al. – 90 patients, a study by Mookerjee et al. – only 10 patients and a study by Lluch et al. – only 46 patients [7,13,29,30]. All this suggest that the importance of ADMA is yet to be learned and researchers should be encouraged to involve larger groups of patients in their studies on ADMA in liver diseases.

We understand that due to a limited number of patients involved in the research many possible correlations between different biomarker values could not be discovered. The study however points that such correlations may exist and therefore it is possible that ADMA can be found useful in thorough analyzing condition of patients with liver diseases. We strongly believe that this subject requires further and broader research in order to evaluate the clinical significance of assessing serum ADMA levels.

Conclusions

ADMA can potentially be used as a subsidiary marker of disease progression in patients with underlying liver diseases. Furthermore, our research suggests that ADMA cannot be useful in detecting HCC.

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Conflict of interest – the authors declare no conflict of interest.

Ethical approval – the study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board.

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Tables

Table 1. Baseline data of all analyzed patients.

	Mean	IQR	Median	SD	Minimum	Maximum
Age	56.3	15.0	57.0	13.4	29.0	87.0
ADMA *Normal range: 0.4–0.75 µmol/L*	0.8	0.3	0.5	3.4	0.0	31.0
VCAM-1 *Normal range:20.5- 2318.9 ng/ml*	199.1	151.7	193.5	89.1	21.9	335.6
AFP *Normal range: 2-12 ng/ml*	332.3	90.8	6.9	1741.6	2.1	12500.0
CRP *Normal range:<10.0 mg/l*	17.2	24.2	7.8	21.4	0.0	77.4
CEA *Normal range:<5.0 ng/ml*	5.5	1.1	2.3	13.4	0.1	87.9
Ca 19.9 *Normal range: <33.0 U/ml*	20.6	13.2	10.7	42.0	2.9	339.4
PLT *Normal range: 139-387 G/L*	136.9	120.5	127.0	85.4	12.0	539.0
INR *Normal range: 0.77-1.43*	1.2	0.2	1.2	0.3	0.8	2.5
Bilirubin *Normal range: 3-22 µmol/L*	59.7	39.2	32.5	84.5	8.1	518.7
ALT *Normal range: 4-50 U/L*	149.9	62.5	60.0	335.0	14.0	2276.0
AST *Normal range: 10-59 U/L*	147.3	106.5	74.0	247.5	20.0	1540.0
ALP *Normal range: 38-126 U/L*	176.6	165.5	135.5	123.6	53.0	618.0
GGTP *Normal range: 15-73 µmol/L*	204.9	125.5	97.0	354.5	12.0	2716.0
Albumin *Normal range: 35-55 g/L*	34.9	13.5	35.0	8.7	16.2	56.9
Total cholesterol *Normal range:<5.0 mmol/L*	4.9	0.4	4.8	1.4	2.2	10.3
LDL *Normal range:<2.5 mmol/L*	3.0	0.0	3.0	0.8	0.9	6.1
HDL *Normal range:>1.0 mmol/L*	1.4	0.0	1.4	0.5	0.5	3.5
TG *Normal range:<1.7 mmol/L*	1.5	0.4	1.4	0.6	0.7	3.4

Table 2. Mean results of analyzed biomarkers in patients with hepatitis, compensated and decompensated liver cirrhosis.

	Hepatitis (n=20)	Compensated liver cirrhosis (n=32)	Decompensated liver cirrhosis (n=31)	*P*	HCC (n=10)	No HCC (n=73)	*P*
Age mean (range) years	52.0 (31.0-87.0)	57.0 (35.0-86.0)	58.0 (29.0-81.0)	0.254	65.2 (51.0-82.0)	55.0 (29.0-87.0)	**0.020**
ADMA mean (range) *Normal range: 0.4–0.75 µmol/L*	0.5 (0.0–0.8)	1.4 (0.1–31.0)	0.5 (0.0–1.1)	0.445	0.5 (0.0-1.2)	0.9 (0.0-31.0)	0.278
VCAM-1 mean (range) *Normal range:20.5-2318.9 ng/ml*	166.7 (30.2–317.3)	181.4 (21.9–314.8)	238.1 (116.7–335.6)	**0.006**	226.2 (67.6-318.7)	195.4 (21.9-335.7)	0.183
AFP mean (range) *Normal range: 2-12 ng/ml*	43.5 (3.0–450.0)	365.6 (2.1-10000.0)	484.3 (2.7–12500.0)	0.676	2558.3 (92.1-12500.0)	27.4 (2.1-121.9)	**<0.001**
CRP mean (range) *Normal range:<10.0 mg/l*	11.4 (0.1–63.9)	16.5 (0.1–71.8)	21.7 (0.1–77.4)	0.240	21.1 (0.1-70.5)	16.7 (0.1-77.4)	0.431
CEA mean (range) *Normal range:<5.0 ng/ml*	3.0 (1.7–12.4)	5.5 (0.1–87.9)	7.3 (0.3–65.1)	0.531	6.7 (0.1-33.5)	5.4 (0.3-87.9)	0.373
Ca 19.9 mean (range) *Normal range: <33.0 U/ml*	10.3 (2.9–26.2)	23.8 (2.9–339.4)	24.1 (2.9–155.0)	0.452	36.2 (3.6-155.0)	18.5 (2.9-339.4)	**0.017**
PLT mean (range) *Normal range: 139-387 G/L*	173.6 (22.0–290.0)	153.7 (36.0–539.0)	95.8 (12.0–332.0)	**0.002**	115.5 (36.0-239.0)	139.8 (12.0-539.0)	0.240
INR mean (range) *Normal range: 0.77-1.43*	1.2 (0.9–2.2)	1.2 (0.8–2.5)	1.3 (1.0–2.1)	0.328	1.2 (1.0-1.6)	1.3 (0.9-2.5)	0.281
Bilirubin mean (range) *Normal range: 3-22 µmol/L*	75.8 (8.1–354.7)	46.5 (12.3–244.8)	63.2 (8.6–518.7)	0.465	39.1 (10.1-67.0)	62.6 (8.1-518.7)	0.494
ALT mean (range) *Normal range: 4-50 U/L*	382.1 (21.0–2276.0)	99.7 (14.0–724.0)	52.0 (16.0–93.0)	**0.001**	78.7 (33.0-141.0)	159.7 (14.0-2276.0)	0.138
AST mean (range) *Normal range: 10-59 U/L*	286.3 (20.0–1540.0)	116.5 (25.0–741.0)	89.5 (20.0–372.0)	**0.013**	122.3 (33.0-270.0)	150.8 (20.0-1540.0)	0.081
ALP mean (range)	132.5	185.7 (53.0–618.0)	195.9 (61.0–459.0)	0.177	164.4 (71.0-432.0)	178.3 (53.0-618.0)	0.469

Normal range: 38-126 U/L	(55.0–358.0)						
GGTP mean (range) *Normal range: 15-73 µmol/L*	283.2 (12.0–2716.0)	234.2 (23.0-1378.0)	124.1 (14.0–543.0)	0.248	111.7 (14.0-317.0)	217.7 (12.0-2716.0)	0.302
Albumin mean (range) *Normal range: 35 to 55 g/L*	39.6 (17.9–52.3)	36.6 (21.6–47.0)	30.2 (16.2–56.9)	**<0.001**	30.5 (16.2-39.8)	35.5 (17.9-56.9)	0.637
Total cholesterol mean (range) *Normal range:<5.0 mmol/L*	5.2 (3.3–8.0)	5.0 (2.1–10.3)	4.6 (2.2–7.1)	0.229	4.6 (3.4-5.8)	4.9 (2.2-10.3)	0.300
LDL mean (range) *Normal range:<2.5 mmol/L*	3.2 (2.2–6.1)	3.0 (1.0–4.7)	3.0 (0.9–5.1)	0.529	2.8 (1.0-3.3)	3.1 (0.9-6.1)	0.395
HDL mean (range) *Normal range:>1.0 mmol/L*	1.4 (0.5–2.3)	1.6 (0.5–3.5)	1.4 (0.7–2.7)	0.166	1.5 (1.4-2.1)	1.4 (0.5-3.5)	0.159
TG mean (range) *Normal range:<1.7 mmol/L*	1.5 (0.8–3.4)	1.4 (0.8–3.2)	1.6 (0.7–3.3)	0.639	1.3 (1.0-1.4)	1.6 (0.7-3.4)	0.220

Table 3. Correlation of ADMA with other biomarkers.

Variable	Univariate r^2	Univariate p	Multivariate linear regression p
VCAM-1	0.017	0.246	0.070
AFP	0.001	0.842	0.341
CRP	0.007	0.461	0.251
CEA	0.001	0.856	0.343
Ca 19-9	0.001	0.807	0.280
PLT	0.011	0.342	0.088
INR	0.006	0.476	0.265
Albumin	0.023	0.171	**0.049** **r=0.364** **r^2=0.132**

Table 4. Correlation of ADMA with other biomarkers depending on the category of patients.

Category	Variable	Univariate correlation coefficient r^2	Univariate p	Multivariate linear regression p
Hepatitis	VCAM-1	0.013	0.634	0.501
	AFP	0.087	0.206	0.305

	CRP	0.104	0.165	0.441
	CEA	0.099	0.176	0.282
	Ca 19-9	0.034	0.437	0.398
	PLT	0.028	0.484	0.513
	INR	0.186	0.058	0.172
	Albumin	**0.247**	**0.026**	0.176
Compensated liver cirrhosis	VCAM-1	0.051	0.216	0.069
	AFP	0.000	0.908	0.219
	CRP	0.016	0.486	0.234
	CEA	0.001	0.873	0.233
	Ca 19-9	0.002	0.820	0.121
	PLT	0.036	0.299	**0.022** **r=0.536** **r^2=0.287**
	INR	0.020	0.439	0.192
	Albumin	0.105	0.070	0.060
Decompensated liver cirrhosis	VCAM-1	0.036	0.307	0.398
	AFP	0.058	0.192	0.252
	CRP	0.033	0.331	0.413
	CEA	0.000	0.999	0.514
	Ca 19-9	0.001	0.885	0.517
	PLT	0.039	0.289	0.391
	INR	0.000	0.987	0.479
	Albumin	0.029	0.358	0.397
Hepatocellular carcinoma	VCAM-1	0.118	0.331	0.057
	AFP	0.051	0.529	0.057
	CRP	0.188	0.211	0.075
	CEA	0.084	0.417	0.072
	Ca 19-9	0.148	0.272	0.079
	PLT	0.000	0.993	0.053
	INR	0.115	0.338	0.058
	Albumin	0.131	0.303	0.060

Figure legends

Fig. 1. Scatterplot of ADMA and albumin concentrations among all patients.

Fig. 2. Scatterplot of ADMA and albumin concentrations in patients with hepatitis.

Fig. 3. Scatterplot of ADMA concentration and PLT count in patients with compensated liver cirrhosis.

Figures

Fig. 1. Scatterplot of ADMA and albumin concentrations among all patients.

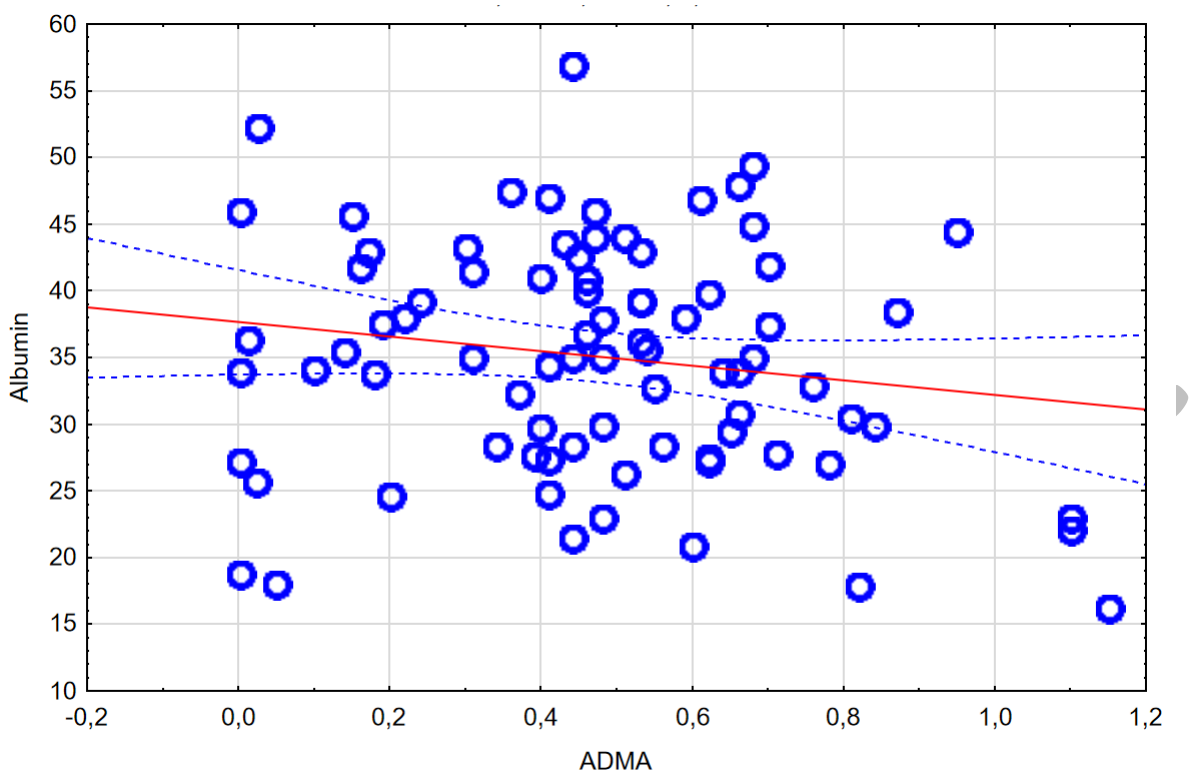

Fig. 2. Scatterplot of ADMA and albumin concentrations in patients with hepatitis.

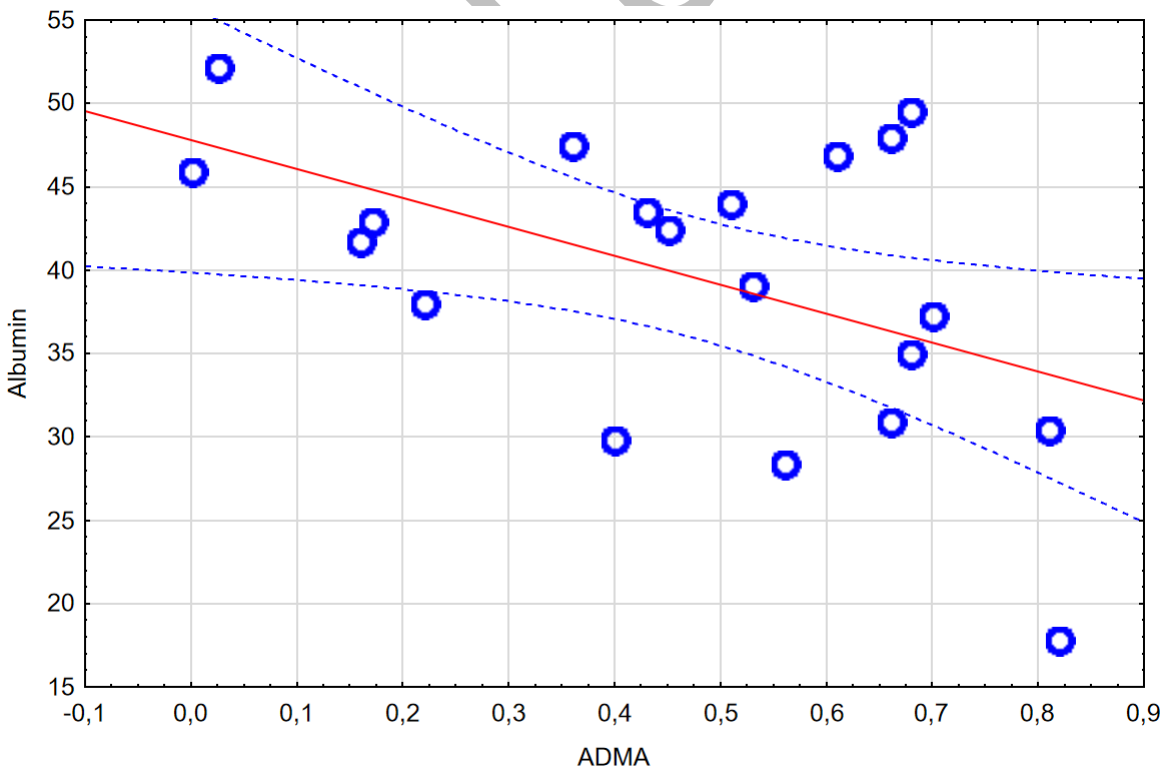

Fig. 3. Scatterplot of ADMA concentration and PLT count in patients with compensated liver cirrhosis.