

Antibodies against N-homocysteinylation proteins and their determinants in patients on long-term hemodialysis

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KEY WORDS

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ABSTRACT

INTRODUCTION The factors that determine the level of antibodies against N-homocysteinylation (N-Hcy) proteins have not been established so far. The clinical significance of these antibodies and their effect on cardiovascular (CV) risk in patients with end-stage renal disease (ESRD) are still unknown.

OBJECTIVES The aim of this study was to identify the factors that determine the level of antibodies against N-Hcy albumin and N-Hcy hemoglobin in patients on long-term hemodialysis (HD).

PATIENTS AND METHODS The study involved 247 subjects on long-term HD (110 women, 137 men; age range, 23–89 years) and 60 controls matched for age, sex, and CV risk factors (serum creatinine level <140 $\mu\text{mol/l}$). Serum antibodies against N-Hcy albumin and N-Hcy hemoglobin were determined using an in-house enzyme-linked immunosorbent assay. Total homocysteine (tHcy), folate, and 8-isoprostaglandin F_{2a} (8-iso-PGF_{2a}) were also measured.

RESULTS Patients on HD had higher serum levels of anti-N-Hcy-albumin (absorbance at 490 nm: 0.56 [0.49–0.623] vs. 0.259 [0.198–0.338], $P < 0.0001$) and anti-N-Hcy-hemoglobin antibodies (0.659 [0.597–0.723] vs. 0.379 [0.289–0.442], $P < 0.0001$) as compared with controls. The level of both antibodies correlated with tHcy ($r = 0.56$, $P < 0.0001$ and $r = 0.67$, $P < 0.0001$, respectively), 8-iso-PGF_{2a} ($r = 0.48$, $P < 0.0001$ and $r = 0.63$, $P < 0.0001$, respectively), and folate ($r = -0.18$, $P = 0.0054$ and $r = -0.38$, $P < 0.0001$, respectively), but not with HD duration, the initial cause of ESRD, and CV comorbidity.

CONCLUSIONS In HD patients, tHcy is an independent predictor of antibodies against N-Hcy proteins. Folate and 8-iso-PGF_{2a} concentrations were not independently associated with the levels of both antibodies.

INTRODUCTION Cardiovascular (CV) diseases are the leading cause of morbidity and mortality in patients with chronic kidney disease (CKD).^{1–3} It has been estimated that 40% of patients with end-stage renal disease (ESRD) develop coronary artery disease (CAD) with 10 to 20-fold higher mortality compared with the general population.^{4,5}

Although the results of studies on the role of hyperhomocysteinemia in the development of CV diseases in patients with CKD remain controversial, there is growing evidence that elevated total homocysteine (tHcy) levels are one of the CV risk factors in this population.^{6–11} Hyperhomocysteinemia is observed in more than 80% of patients with ESRD.¹² The mechanisms by which

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homocysteine (Hcy) is involved in the pathogenesis of atherosclerosis are still unclear. One of the possible mechanisms is an autoimmune response to protein modification by one of Hcy derivatives, i.e., Hcy thiolactone (HCTL).^{13,14} Elevated levels of antibodies against N-homocysteinylated (N-Hcy) proteins have been observed in patients with stroke,¹⁵ CAD,¹⁶ and systemic lupus erythematosus.¹⁷ Undas et al.¹⁸ demonstrated elevated levels of these antibodies in 43 ESRD patients on long-term HD, but the role of these antibodies in the pathogenesis of CV diseases in this group of patients remains unclear. Neither the factors that determine the level of these antibodies, nor the clinical mechanisms by which the antibodies are involved in the pathogenesis of CV diseases in ESRD patients have been established so far.

The aim of the present study was to identify the factors that determine the level of antibodies against N-Hcy albumin and N-Hcy hemoglobin in ESRD patients on long-term HD.

PATIENTS AND METHODS The study involved 247 patients (110 women) aged 23 to 89 years (median 61 years) on maintenance HD for the average time of 37 months, and 60 controls, matched for age, sex, and CV risk factors, and with serum creatinine levels <140 $\mu\text{mol/l}$. The subjects were recruited from 6 hemodialysis (HD) centers in Poland. The causes of ESRD were as follows: chronic glomerulonephritis in 57 patients (23%), chronic tubulointerstitial nephritis in 45 (18.2%), diabetic nephropathy in 45 (18.2%), polycystic kidney disease in 22 (8.9%), arterial hypertension in 13 (5.3%), amyloidosis in 5 (2%), bilateral nephrectomy due to renal cancer in 5 (2%), and renal tuberculosis in 2 (0.8%). In 53 patients (21.5%), the cause of ESRD was unknown.

Exclusion criteria were as follows: symptoms of acute infection, acute CV episode 3 months prior to the study, known cancer, and hypo- or hyperthyroidism. Maintenance HD was performed 3 times a week with single-use polysulfone membranes.

The study was approved by the Regional Medical Council Ethics Board in Kraków, Poland. All participants gave written informed consent.

Kt/V was calculated using the Daugirdas model,¹⁹ and glomerular filtration rate in controls using the abbreviated Modification of Diet in Renal Disease formula.²⁰

The data concerning CV comorbidity were obtained from medical records.

Blood samples from HD patients were collected in the middle of the week before a dialysis session. Total blood cell count, lipid profile, glucose, protein, uric acid, and high-sensitivity C-reactive protein (CRP) were assessed by standard automated techniques. Plasma tHcy was determined using high-performance liquid chromatography. Serum 8-isoprostaglandin $F_{2\alpha}$ (8-iso-PGF_{2 α}) levels were determined by an immunoenzymatic assay (Cayman Chemicals, Ann Arbor, MI, United States). Folic acid was measured using

the Immunolite analyzer (Diagnostic Products Corporation). Serum levels of antibodies against N-Hcy albumin and N-Hcy hemoglobin were measured by an enzyme-linked immunosorbent assay according to the protocol described in detail elsewhere.^{15,16}

The methylenetetrahydrofolate (MTHFR) C677T polymorphism was determined in peripheral white blood cells by the polymerase chain reaction (PCR) using the TaqMan SNP technique and ABI PRISM 7900HT Fast Real-Time PCR System (Applied Biosystems).

Statistical analysis Normally distributed data are shown as mean \pm standard derivation. Data not normally distributed are given as median and interquartile range (IQR). Intergroup differences were assessed by the Student t-test or univariate analysis of variance for normally distributed variables or otherwise by the Mann-Whitney and Kruskal-Wallis tests. The χ^2 test was used to assess intergroup differences between categorical variables. The Pearson's or Spearman's correlation coefficients were calculated to test the association between 2 variables with a normal or non-normal distribution, respectively. The univariate and multivariate linear regression analysis were used to determine the independent factors that influence the level of antibodies against N-Hcy proteins. The step-forward regression was used to assess the significance of variables that determine the level of antibodies. In the final model, all variables typed in step regression were considered (even if they correlated with each other), if their part in the regression did not affect the significance of other variables and the whole model.²¹ $P < 0.05$ was considered statistically significant. Data analysis was performed using the Stata v. 10.0 software (StataCorp, United States).

RESULTS Characteristics of the patient and control groups are presented in **TABLE 1**. Patients on HD had lower levels of hemoglobin, platelets, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and higher levels of triglycerides as compared with controls. The HD group had twice higher levels of tHcy (**FIGURE 1**), 2.2-fold higher levels of folate, 3-fold higher levels of CRP and 3.3-fold higher levels of 8-iso-PGF_{2 α} as compared with the control group.

In the HD group, levels of anti-N-Hcy-albumin and anti-N-Hcy-hemoglobin antibodies were significantly higher than in controls (**FIGURE 2**). There were no significant correlations between the levels of antibodies and demographic data. In the HD group, there was a strong correlation between tHcy and the level of antibodies against N-Hcy albumin ($r = 0.56$, $P < 0.0001$) (**FIGURE 3**) and N-Hcy hemoglobin ($r = 0.67$, $P < 0.0001$) (**FIGURE 4**). Similar correlations were observed in the control group ($r = 0.64$, $P < 0.0001$ and $r = 0.66$, $P < 0.0001$, respectively).

TABLE 1 Characteristics of patients on hemodialysis and controls

	Patients, n = 247	Controls, n = 60	P
age, yrs	61 (51–70)	58.5 (46.7–68)	NS
sex, male/female	137/110	29/31	NS
DM, n (%)	58 (23.5)	5 (8.3)	0.02
CAD, n (%)	48 (19.4)	23 (38.3)	0.003
stroke, n (%)	14 (5.6)	7 (11.6)	NS
hemoglobin, g/dl	11.2 (10.4–12.1)	13.8 (13.2–14.4)	<0.0001
platelets, × 10 ³ /μl	197.5 (163–229)	222.5 (182–252.5)	0.008
total cholesterol, mmol/l	4.46 (3.77–5.56)	5.02 (4.27–5.51)	0.05
HDL-C, mmol/l	1.06 (0.85–1.29)	1.25 (1.00–1.68)	<0.0001
LDL-C, mmol/l	2.38 (1.81–3.35)	3.12 (2.2–3.46)	0.01
triglycerides, mmol/l	1.83 (1.34–2.68)	1.16 (0.86–1.62)	<0.0001
glucose, mmol/l	5.58 (4.7–6.82)	5.58 (5.2–6.1)	NS
CRP, mg/l	5.55 (2.21–11.48)	1.76 (0.8–2.9)	<0.0001
tHcy, μmol/l	21.5 (17.3–27.7)	10.7 (8.93–13.2)	<0.0001
anti-N-Hcy-alb, A490	0.56 (0.49–0.623)	0.259 (0.198–0.338)	<0.0001
anti-N-Hcy-hb, A490	0.659 (0.597–0.723)	0.379 (0.289–0.442)	<0.0001
folate, ng/l	21.55 (14.6–27.8)	9.92 (7.74–15.1)	<0.0001
8-iso-PGF _{2α} , pg/ml	301 (260–380)	90 (36.4–136)	0.0012
C677T MTHFR CC, n (%)	55 (33.7)	34 (56.7)	0.003
C677T MTHFR CT, n (%)	65 (39.9)	21 (35)	NS
C677T MTHFR TT, n (%)	43 (26.4)	5 (8.3)	0.003
Kt/V ^a	1.34 (1.2–1.48)	–	
GFR ^b , ml/min	–	84.3 ± 16.7	
medications			
rHu-epo, n (%)	225 (91.1)	0	<0.0001
ferrum, n (%)	144 (58.3)	0	<0.0001
folic acid, n (%)	97 (39.3)	0	<0.0001
ACE inhibitors, n (%)	112 (45.3)	22 (36.6)	NS
statins, n (%)	61 (24.7)	29 (48.3)	0.0005
β-blockers, n (%)	87 (35.2)	21 (35)	NS
ASA, n (%)	52 (21.1)	30 (50)	<0.0001
oral anticoagulants, n (%)	15 (6.1)	0	<0.0001

a according to the Daugirdas formula

b according to the abbreviated Modification of Diet in Renal Disease formula

Normally distributed data are presented as mean ± standard deviation; not normally distributed data are presented as median and interquartile range

Abbreviations: ACE – angiotensin-converting enzyme, anti-N-Hcy-alb – antibodies against N-homocysteinylated albumin, anti-N-Hcy-hb – antibodies against N-homocysteinylated hemoglobin, ASA – acetylsalicylic acid, CAD – coronary artery disease, CRP – C-reactive protein, DM – diabetes mellitus, GFR – glomerular filtration rate, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, MTHFR – methylenetetrahydrofolate reductase, NS – nonsignificant, rHu-epo – human recombinant erythropoietin, tHcy – total homocysteine, 8-iso-PGF_{2α} – 8-isoprostaglandine F_{2α}

Genetic variants of MTHFR gene in the HD and control groups were represented as follows: CC – 33.7% (n = 55) vs. 56.7% (n = 34) (*P* = 0.003), CT – 39.9% (65) vs. 35% (n = 21) (*P* = 0.53) and TT – 26.4% (n = 43) vs. 8.3% (n = 5) (*P* = 0.003). Interestingly, in the HD group, the carriers of TT genotype were significantly overrepresented as compared with controls (TABLE 1). The levels of tHcy were significantly higher in patients with TT genotype of the MTHFR gene (median [IQR] 32.8 μmol/l [26.7–40.1]) than in patients with

CC (16.4 μmol/l; [13.2–18.5], *P* < 0.0001) and CT variants (21.4 μmol/l; [19.2–24.6], *P* < 0.0001). The levels of antibodies against N-Hcy albumin were significantly higher in patients with TT genotype (0.640; [0.600–0.680]) than in patients with CC (0.480; [0.410–0.540], *P* < 0.0001) and CT genotypes (0.560; [0.500–0.600], *P* < 0.0001). Similarly, the levels of antibodies against N-Hcy hemoglobin were higher in the group with TT variant (0.770; [0.720–0.800]) as compared with

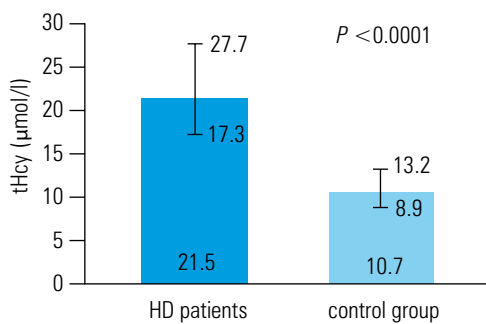


FIGURE 1 Total homocysteine (tHcy) in patients on hemodialysis (HD) and in controls (results shown as median, interquartile range)

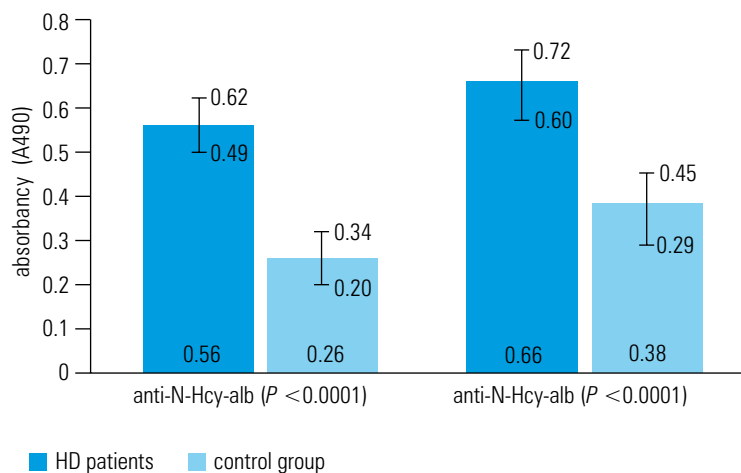


FIGURE 2 Antibodies against N-homocysteinylated albumin (anti-N-Hcy-alb) and hemoglobin (anti-N-Hcy-hb) in patients on hemodialysis (HD) and in controls (median, interquartile range)

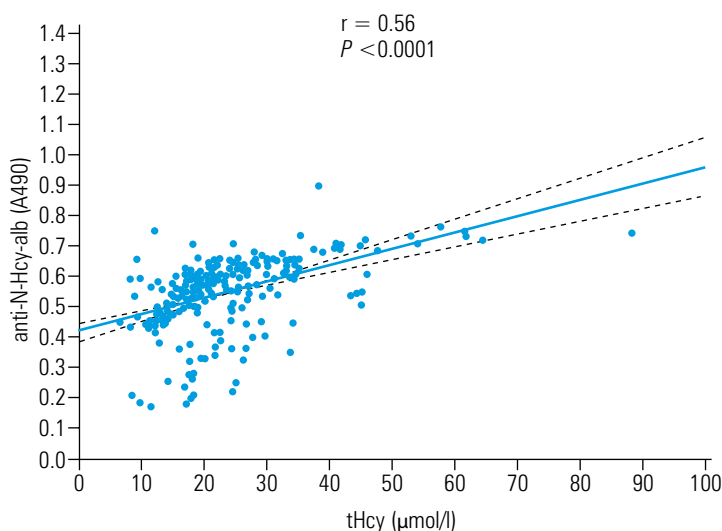


FIGURE 3 Correlation between antibodies against N-homocysteinylated albumin (anti-N-Hcy-alb) and total homocysteine (tHcy) in patients on hemodialysis

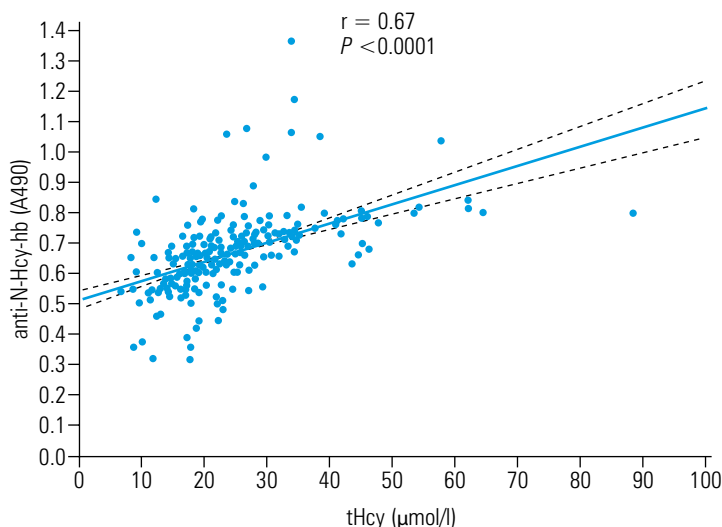


FIGURE 4 Correlation between antibodies against N-homocysteinylated hemoglobin (anti-N-Hcy-hb) and total homocysteine (tHcy) in patients on hemodialysis

CC (0.560; [0.530–0.650], $P < 0.0001$) and CT variants (0.650; [0.600–0.700], $P < 0.0001$).

In the HD group, there were correlations between the level of anti-N-Hcy-albumin antibodies and 8-iso-PGF_{2α} ($r = 0.48$, $P < 0.0001$) (FIGURE 5), folate ($r = -0.18$, $P = 0.0054$) and parathyroid hormone ($r = 0.17$, $P = 0.0083$). The level of anti-N-Hcy-hemoglobin antibodies in the HD group correlated with 8-iso-PGF_{2α} ($r = 0.63$, $P < 0.0001$) (FIGURE 6) and folate ($r = -0.38$, $P < 0.0001$). No significant correlations between the level of both antibodies and the remaining laboratory tests were found.

In the HD group, the level of anti-N-Hcy-albumin antibodies was significantly lower in those patients who reported regular supplementation with folic acid ($n = 97$) as compared with those who did not take this supplement (median [IQR], 0.530 [0.408–0.615] vs. 0.559 [0.515–0.625], $P = 0.002$). No other correlations between the level of antibodies and the medications were detected.

In the HD group, there was a weak, negative correlation between the level of anti-N-Hcy-albumin antibodies and the dialysis adequacy measured as Kt/V coefficient ($r = -0.18$, $P = 0.0303$). No such correlation was observed for anti-N-Hcy-hemoglobin antibodies. There was no significant association between the level of the 2 antibodies and the duration of HD maintenance therapy (data not shown).

The level of anti-N-Hcy-albumin and anti-N-Hcy-hemoglobin antibodies did not differ between patients with a history of CAD ($n = 48$) and patients without this comorbidity (0.55 [0.495–0.599] vs. 0.56 [0.451–0.618], $P = 0.93$ and 0.656 [0.602–0.73] vs. 0.655 [0.573–0.717], $P = 0.47$, respectively). There were no significant differences in the levels of anti-N-Hcy-albumin and anti-N-Hcy-hemoglobin antibodies between patients with a history of stroke ($n = 15$) as compared with those without cerebrovascular events (0.559 [0.446–0.609] vs. 0.558 [0.471–0.614], $P = 0.83$

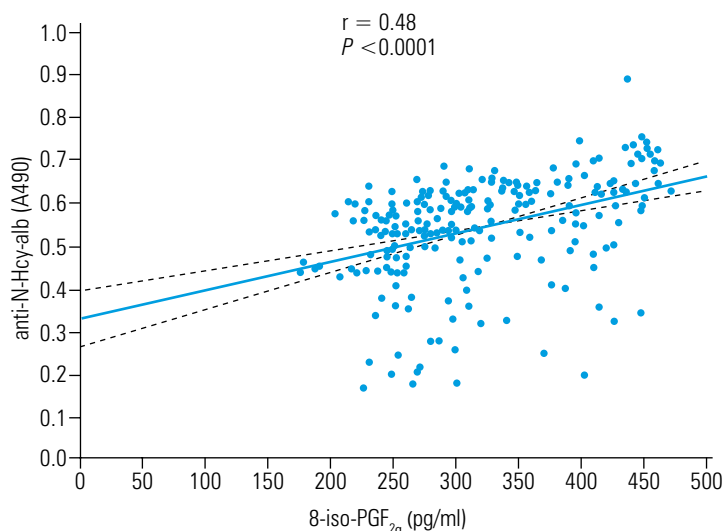


FIGURE 5 Correlation between antibodies against N-homocysteinylated albumin (anti-N-Hcy-alb) and 8-isoprostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$) in patients on hemodialysis

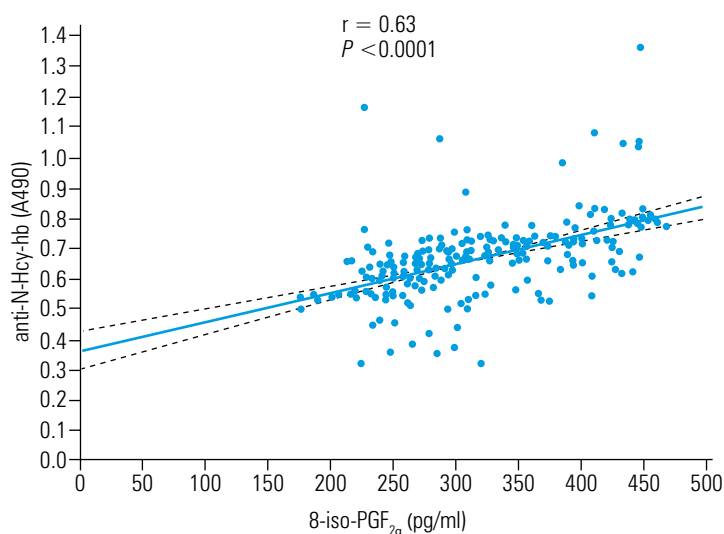


FIGURE 6 Correlation between antibodies against N-homocysteinylated hemoglobin (anti-N-Hcy-hb) and 8-isoprostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$) in patients on hemodialysis

TABLE 2 Multivariate linear regression analysis for independent predictors of anti-N-homocysteinylated albumin antibodies in patients on hemodialysis (n = 247)

	Coefficient (95% confidence interval)	Contribution to variance (%)	P
model		35	<0.0001
age, yrs	0.0003 (−0.0006–0.0012)	0.1	0.58
tHcy	0.006 (0.004–0.007)	23.8	<0.0001
folate	0.004 (0.003–0.006)	5.2	<0.0001
8-iso-PGF $_{2\alpha}$	0.0005 (0.0003–0.0007)	5.9	<0.0001

Abbreviations: see TABLE 1

and 0.649 [0.563–0.707] vs. 0.657 [0.594–0.722], $P = 0.79$, respectively).

The results of the multivariate linear regression incorporating the factors that determine the level of anti-N-Hcy-albumin antibodies are shown in TABLE 2. Because of a strong association between tHcy and folate and between tHcy

and 8-iso-PGF $_{2\alpha}$, the only independent predictor of anti-N-Hcy-albumin antibody level is tHcy. The predictors of anti-N-Hcy-hemoglobin antibody levels established by the multivariate linear regression are shown in TABLE 3. Because of the correlation between tHcy and 8-iso-PGF $_{2\alpha}$, the results of the multivariate analysis should be interpreted similarly to those concerning anti-N-Hcy-albumin antibodies.

DISCUSSION The current study provides additional evidence in a large real-life HD population, supporting the previous observations published by Undas et al.¹⁸ We demonstrated significantly higher levels of antibodies against N-Hcy proteins in 247 patients on long-term HD followed up for 1 to 3 years.

As expected, the level of antibodies against anti-N-Hcy proteins correlated strongly with tHcy. This correlation is reinforced by the results of the multivariate linear regression showing that tHcy is the only independent predictor of both antibodies in patients on HD.

This finding is, at least in part, contradictory to previous observations,^{14,22} namely that the level of antibodies against N-Hcy proteins is independent of tHcy levels. It might suggest that ESRD represents a particular chronic condition, in which the effects of other factors observed in CV diseases are eliminated, and the markedly higher tHcy levels relatively resistant to vitamin treatment overcome less potent modulators of autoimmune response to N-Hcy proteins.

An interesting finding of this study is a strong positive correlation between the level of antibodies against anti-N-Hcy proteins and the level of 8-isoprostanes, which have been demonstrated to be sensitive and reliable markers of oxidative stress.^{23,24} The level of 8-isoprostanes is associated with tHcy. However, this factor in the multivariate regression model explains the contribution to variance to a larger extent than could be expected from the correlation between 8-iso-PGF $_{2\alpha}$ and tHcy, not affecting the statistical significance of the whole model.

The link between oxidative stress and CV risk in patients with ESRD remains controversial. Observations made to date suggest that N-homocysteinylated proteins and the synthesis of antibodies against modified protein particles are not associated with oxidative stress, and the disturbances of the redox balance are the consequences of impaired function of proteins and cytochrome C caused by homocysteinylated.^{25,26} Our findings suggest that oxidative stress modifies homocysteinylated, probably due to protein oxidation, and amplifies the autoimmune response leading to synthesis of antibodies against N-Hcy proteins. The element that links the oxidative stress and protein homocysteinylated may be, at least in part, the activity of paraoxonase 1 (PON1). PON1, known earlier as thiolactonase, is an enzyme that catalyses the hydrolysis of HCTL. The activity of PON1 is reversely correlated with

TABLE 3 Multivariate linear regression analysis for independent predictors of anti-N-homocysteinylated hemoglobin antibodies in patients on hemodialysis (n = 247)

	Coefficient (95% confidence interval)	Contribution to variance, %	P
model		38.11	<0.0001
age, yrs	0.0002 (–0.0007–0.0011)	0.05	0.66
tHcy	0.004 (0.003–0.005)	29.01	<0.0001
8-iso-PGF _{2α}	0.0006 (0.0004–0.0008)	9.05	<0.0001

Abbreviations: see **TABLE 1**

the level of oxidative stress markers and the advancement of atherosclerotic lesions in patients with CAD.^{27,28} The activity of PON1 is decreased in CKD.²⁹ Further studies to validate this hypothesis are needed.

In our study, we have found a moderate adverse correlation between the level of folate and anti-N-Hcy proteins. This correlation is probably not linear, as shown by the results of multivariate regression. These findings may be in part explained by the results of folic acid supplementation on CV risk reduction in patients with ESRD that are independent of the tHcy level reduction. It was demonstrated that low-dose folic acid may improve endothelial function dependent on nitric acid synthase activity, and this effect is not dose-dependent.³⁰ Folic acid also stimulates intracellular antioxidative mechanisms.³¹

Contrary to the study by Undas et al.,¹⁸ we did not observe a higher level of antibodies against N-Hcy proteins in patients with diagnosed CAD and stroke. This discrepancy may be explained by the differences in the number of participants in the respective studies.

We have found a weak inverse correlation between the level of anti-N-Hcy-albumin antibodies and dialysis adequacy (Kt/V). This finding cannot be explained by the level of tHcy or by oxidative stress. It might be speculated that lower Kt/V, possibly caused by the accumulation of toxins, although it has not been determined so far, enhances the autoimmune response to homocysteinylated albumin, but not the proteins that have a long half-life, such as hemoglobin.

The study has several limitations. The number of participants is relatively small, which might have affected the results. The analysis is based on a single measurement of laboratory parameters. Therefore, we cannot exclude that the production of the 2 antibodies might have changed during maintenance HD. Several potential factors that may affect the levels of antibodies, e.g., vitamin B₆, vitamin B₁₂, and the activity of antioxidative enzymes, have not been analyzed. The diagnosis of CV comorbidity was based on medical records, which might have underestimated the prevalence of CAD, excluding subjects with clinically silent disease. A number of limitations may have resulted from the model of statistical analysis. The linear regression is not the best method to assess the complex effect of several factors on biological

phenomena. Because of the study design and the small size of the study group, we were not able to perform more advanced analyses.

In conclusion, patients with ESRD on long-term HD have higher levels of antibodies against N-Hcy albumin and N-Hcy hemoglobin compared with the well-matched controls with the serum creatinine level of <140 μmol/l. The level of antibodies correlates with oxidative stress. tHcy is an independent predictor of the levels of anti-N-Hcy-albumin and anti-N-Hcy-hemoglobin antibodies. Folate and 8-iso-PGF_{2α} are not independently associated with the level of these antibodies in HD patients.

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Przeciwciała przeciwko N-homocysteinyłowanym białkom i czynniki warunkujące ich poziom u pacjentów leczonych przewlekłe hemodializami

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SŁOWA KLUCZOWE

autoprzeciwciała,
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białek, schyłkowa
niewydolność nerek

STRESZCZENIE

WPROWADZENIE Dotychczas nie ustalono, jakie czynniki warunkują miano przeciwciał przeciwko N-homocysteinyłowanym (N-Hcy) białkom. Znaczenie kliniczne tych przeciwciał w schyłkowej niewydolności nerek oraz ich związek z ryzykiem sercowo-naczyniowym pozostają nieznane.

CELE Celem niniejszej pracy było scharakteryzowanie czynników warunkujących miano przeciwciał przeciwko N-Hcy-albuminie i N-Hcy-hemoglobinie u chorych ze schyłkową niewydolnością nerek leczonych przewlekłe hemodializami (HD).

PACJENCI I METODY Do badania włączono 247 chorych leczonych przewlekłe HD (110 kobiet, 137 mężczyzn, zakres wieku 23–89 lat) oraz 60 osób z populacji ogólnej dobranych pod względem płci, wieku i czynników ryzyka sercowo-naczyniowego, z poziomem kreatyniny $< 140 \mu\text{mol/l}$. Oznaczono poziom przeciwciał przeciwko N-Hcy-albuminie i N-Hcy-hemoglobinie metodą immunoenzymatyczną. Ponadto oznaczono poziom homocysteiny całkowitej (*total homocysteine* – tHcy), kwasu foliowego i 8-izoprostaglandyny $F_{2\alpha}$ (8-izo-PGF_{2 α}).

WYNIKI U pacjentów leczonych HD stwierdzono wyższy poziom przeciwciał przeciwko N-Hcy-albuminie (absorbancja 490 nm: 0,56 [0,49–0,623] vs 0,259 [0,198–0,338], $P < 0,0001$) oraz przeciwko N-Hcy-hemoglobinie (0,659 [0,597–0,723] vs 0,379 [0,289–0,442], $P < 0,0001$), w porównaniu z grupą kontrolną. Stwierdzono korelację poziomu obu przeciwciał z tHcy (odpowiednio: $r = 0,56$, $P < 0,0001$ i $r = 0,67$, $P < 0,0001$), 8-izo-PGF_{2 α} (odpowiednio: $r = 0,48$, $P < 0,0001$ i $r = 0,63$, $P < 0,0001$) oraz kwasem foliowym (odpowiednio: $r = -0,18$, $p = 0,0054$ i $r = -0,38$, $P < 0,0001$). Nie stwierdzono zależności między poziomem przeciwciał a czasem trwania leczenia HD, przyczyną schyłkowej niewydolności nerek i współwystępowaniem chorób sercowo-naczyniowych.

WNIOSKI Stężenie tHcy jest niezależnym czynnikiem warunkującym poziom przeciwciał, a 8-izo-PGF_{2 α} ani kwas foliowy nie są niezależnie związane z tymi poziomami u chorych hemodializowanych.

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