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NEPHROLOGY - ORIGINAL PAPER

Evaluation of cardiovascular events and progression to end-stage renal disease in patients with dyslipidemia and chronic kidney disease from the North-Eastern area of Romania

Cristiana-Elena Vlad[1,2] · Liliana Foia[2,4] · Mariana Pavel-Tanasa[2] · Vasilica Toma[2] · Laura Florea[1,2] · Luminita Voroneanu[2] · Mugurel Apetrii[2] · Gianina Dodi[2] · Adrian Covic[1,2,3]

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Abstract

Purpose The aim of this prospective cohort study was: to identify the association between different biomarkers [proprotein convertase subtilisin/kexin 9-PCSK9, lipoprotein(a)-Lp(a) and high-sensitivity C-reactive protein-hsCRP] and the cardiovascular events; to evaluate the relationship between the 3 biomarkers mentioned above and the renal outcomes that contributed to end-stage renal disease (ESRD).

Methods We studied 110 patients with chronic kidney disease (CKD) stages 2 to 4. The identification of the new cardiovascular events and the renal outcomes were performed by clinical and paraclinical explorations.

Results 350 patients were examined and 110 (31.4%) were included in this study. The mean age was 55.6 ± 10.9 years, with a higher number of men compared to women. The CKD patients with de novo cardiovascular events and new renal outcome during the study, had significantly increased values of total cholesterol (TC), low density cholesterol lipoprotein (LDL-C) at 6 and 12 months and higher levels of Lp(a), PCSK9, hsCRP and low ankle–brachial index (ABI) and ejection fraction (EF) values compared to patients without cardiovascular and renal events. In CKD patients, PCSK9 > 220 ng/mL was a predictor of cardiovascular events, while the EF < 50% was a predictor for renal outcomes. For CKD patients with PCSK9 > 220 ng/mL and hsCRP > 3 mg/L levels, the time-interval for the new cardiovascular and renal events occurrence were significantly decreased compared to patients displaying low values of these biomarkers.

Conclusion The results of this study show that PCSK9 > 220 ng/mL was predictor for cardiovascular events, while EF < 50% was predictor for CKD progression to ESRD. PCSK9 > 220 ng/mL and hsCRP > 3 mg/L were associated with the occurrence of renal and cardiovascular events earlier.

Keywords Dyslipidemia · Chronic kidney disease · Lipoprotein(a) · Proprotein convertase subtilisin/kexin 9 · High-sensitivity C-reactive protein · Cardiovascular events · Atherosclerotic cardiovascular disease · Renal outcome

Cristiana-Elena Vlad, Mariana Pavel-Tanasa and Laura Florea have contributed equally to this work.

✉ Liliana Foia
lilifoia@yahoo.co.uk

1 Department of Nephrology-Internal Medicine, "Dr. C. I. Parhon" Clinical Hospital Iasi, Iasi, Romania

2 "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania

3 The Academy of Romanian Scientists (AOSR), Bucharest, Romania

4 Departament of Biochemistry, "Grigore T. Popa" University of Medicine and Pharmacy, Universitatii Street, 700115 Iasi, Romania

Abbreviations

CKD	Chronic kidney disease
ASCVD	Atherosclerotic cardiovascular disease
CVD	Cardiovascular diseases
CHD	Coronary heart disease
PAD	Peripheral arterial disease
ABI	Ankle–brachial index
ECG	Electrocardiogram
LV	Left ventricular
EF	Ejection fraction
TC	Total cholesterol
LDL-C	Low density cholesterol lipoprotein
HDL-C	High density cholesterol lipoprotein
TG	Triglycerides
hsCRP	High-sensitivity C-reactive protein

PCSK9 Proprotein convertase subtilisin/kexin 9
Lp(a) Lipoprotein(a)

Background

Dyslipidemia in chronic kidney disease (CKD) is represented by increasing levels of total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides and lipoprotein(a)-Lp(a) levels and decreased high density lipoprotein cholesterol (HDL-C) values [1, 2]. Atherosclerosis is a complex multifactorial disorder, consisting of chronic inflammatory response which causes plaque formation in the intima and media of medium and large arteries [3]. Atherosclerotic complications of coronary heart disease (CHD), peripheral vascular (PAD) and cerebrovascular disease are common causes of morbidity and mortality in CKD [4]. Dyslipidemia, diabetes mellitus, and high blood pressure are significant contributors to the atherosclerotic plaques formation, especially in CKD patients [3, 5]. Others novel risk factors such as chronic inflammatory state (high-sensitivity C-reactive protein-hsCRP), abnormal mineral and bone metabolism, Lp(a) levels, some uremic toxins, are also significantly associated with cardiovascular morbidity and mortality in CKD patients [2, 4, 6, 7].

Lp(a), proprotein convertase subtilisin/kexin type 9 (PCSK9) and hsCRP are predictors of cardiovascular outcomes and potential biomarkers for cardiovascular mortality in both the general population and CKD patients [2, 8]. Lp(a) was first described as a dominantly inherited LDL-like particle synthesized in the liver and its structure differs from LDL by a high polymorph glycoprotein apo(a) [9, 10]. Lp(a) has atherogenic and thrombogenic activities, promotes the oxidation of LDL, and facilitates monocyte adhesion [2, 9]. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease of the subtilase family, secreted primarily by the liver and kidney via sterol regulatory element-binding protein 2 (SREBP-2) regulation [11, 12]. PCSK9 is a new biomarker for the lipid metabolism, a novel therapeutic target for hypercholesterolemia, for lowering cardiovascular risk (PCSK9 is observed in carotid atherosclerotic lesions) [8, 13, 14]. PCSK9 determines the degradation of low density lipoprotein receptor (LDLR), and inhibits receptor recycling in the hepatocyte membrane [8, 15]. In the kidney, PCSK9 modulates sodium absorption by degrading the epithelial sodium channel [8, 16]. High-sensitivity C-reactive protein is an acute-phase reactant included in the protein family pentraxin and it is synthesized by the liver in response to pro-inflammatory cytokines. hsCRP levels have been linked with the development of atherosclerosis in CKD patients [17], possibly via acceleration of LDL uptake by macrophages, endothelial dysfunction, and migration and proliferation of vascular smooth muscle cell [18].

This prospective cohort study included patients with CKD stages 2 to 4, with the following objectives: (a) to identify the association between different biomarkers [PCSK9, Lp(a) and hsCRP] and the cardiovascular events triggered by atherosclerosis; (b) to evaluate the relationship between the 3 biomarkers mentioned above and the renal outcomes that contributed to ESRD.

Methods

Inclusion and exclusion criteria for CKD patients

Study design

Observational, prospective, 1 year study (February 2019 to February 2020) in a referral nephrology center in the North-Eastern region of Romania, which includes eight counties and a population of over 3,980,000 inhabitants.

Study population

350 patients with dyslipidemia were identified, and 110 patients met the following: *inclusion criteria* subjects with full mental capacity who signed the informed consent form; men and women aged over 18 years, biological identification of total cholesterol (TC) > 300 mg/dL, low density lipoprotein cholesterol (LDL-C) > 190 mg/dL without treatment or > 100 mg/dL following treatment with maximum doses of statins (40 mg rosuvastatin, 80 mg atorvastatin, in combination with ezetimibe), nephrotic and non-nephrotic proteinuria.

Exclusion criteria subjects lacking discernment or those who refused to sign the informed consent, patients under the age of 18, pregnant and breast-feeding women; patients with acute kidney injury (AKI) episodes; subjects with severe physical disabilities, dementia, neoplasms, familial hypercholesterolemia and others secondary hypercholesterolemia causes (uncontrolled diabetes, hypothyroidism, drug-induced dyslipidemia) [19].

Clinical and biological evaluation in CKD patients

The patients included in the study were coded with the letter C and the corresponding ID number. The medical history revealed that certain patients had received antihypertensive medication (those with BP > 140/90 mmHg), or oral antidiabetic medication (those diagnosed with type 2 diabetes), which was allowed throughout the study, according to the specialist doctors' prescription. The serum and urine analysis, the cardiovascular explorations were assessed at baseline, at 6 months and 12 months.

Table 1 Baseline characteristics of CKD patients

Characteristics	Patients with CKD and dyslipidemia			
	Total	G2–G3	G4	*P*
N	110	58	52	
Age—yo (mean ± SD)	55.6 ± 12.9	52.5 ± 13.2	59.2 ± 11.9	0.006*
Gender (male) *n* (%)	61 (55.5%)	36 (62.1%)	25 (48.1%)	0.14
Smoker *n* (%)	48 (43.6%)	25 (43.1%)	23 (44.2%)	0.91
High blood pressure *n* (%)	81 (73.6%)	37 (63.8%)	44 (84.6%)	0.01*
CHD history *n* (%)	28 (25.5%)	12 (20.6%)	16 (30.7%)	0.38
PAD history *n* (%)	17 (15.5%)	12 (20.7%)	5 (9.6%)	
Stroke history *n* (%)	9 (8.2%)	4 (6.9%)	5 (9.6%)	
CHD + PAD history *n* (%)	19 (17.3%)	8 (13.8%)	11 (21.2%)	
CHD + PAD + stroke history *n* (%)	7 (6.4%)	2 (3.4%)	5 (9.6%)	
Obesity *n* (%)	38 (34.5%)	17 (29.3%)	21 (40.4%)	0.23
Type 2 diabetes *n* (%)	36 (32.7%)	16 (27.6%)	20 (38.5%)	0.23
TC mg/dL (mean ± SD)	316.5 ± 38.7	311.6 ± 42.7	296.8 ± 28	0.04*
LDL-C mg/dL (mean ± SD)	241.9 ± 42.7	239.7 ± 43.3	221 ± 28.9	0.01*
HDL-C mg/dL (mean ± SD)	38.6 ± 7.5	40.9 ± 9.4	40.8 ± 9.1	0.95
TG mg/dL (median ± IQR)	197.2 ± 60.9	176 ± 101	223 ± 76	0.04*
Creatinine mg/dL (mean ± SD)	2.3 ± 0.8	1.6 ± 0.5	2.8 ± 0.5	0.001*
eGFR mL/min/1.73 m^2 (mean ± SD)	33.5 ± 20.3	47.9 ± 17.9	21.1 ± 4.6	0.001*
Uric acid mg/dL (mean ± SD)	7.1 ± 1.9	6.7 ± 1.9	7.2 ± 1.8	0.11
hsCRP mg/L (mean ± SD)	5.2 ± 2.1	5.2 ± 2.3	4.9 ± 2.1	0.52
Lp(a) mg/dL (mean ± SD)	19.1 ± 4.5	19.6 ± 4.3	17.2 ± 4.5	0.005*
PCSK9 ng/mL (mean ± SD)	322.8 ± 92.3	309.9 ± 95.9	287.9 ± 96.9	0.24
Proteinuria g/24 h (mean ± SD)	3.7 ± 3.3	3.8 ± 3.6	3.5 ± 2.9	0.57
Non-nephrotic proteinuria *n* (%)	58 (52.7%)	29 (26.4%)	29 (26.4%)	0.44
Nephrotic proteinuria *n* (%)	52 (47.3%)	30 (27.3%)	22 (20%)	
ECG changes *n* (%)	37 (33.6%)	13 (22.4%)	24 (46.2%)	0.008*
LV wall motion abnormalities *n* (%)	37 (33.6%)	13 (22.4%)	24 (46.2%)	0.008*
ABI (median ± IQR)	0.83 ± 0.08	0.85 ± 0.15	0.82 ± 0.01	0.13
Lipid-lowering agents				0.94
Statin *n* (%)	33 (30%)	17 (29.3%)	16 (30.8%)	
Statin + ezetimibe *n* (%)	16 (14.5%)	11 (19%)	5 (9.6%)	
Statin + omega 3 *n* (%)	17 (15.5%)	8 (13.8%)	9 (17.3%)	
Statin + fenofibrate + Omega 3 *n* (%)	20 (18.2%)	7 (12.1%)	13 (25%)	
RAS inhibitors				0.001*
Initiated treatment *n* (%)	28 (25.5%)	26 (23.6%)	2 (1.8%)	
Changes doses *n* (%)	35 (31.8%)	22 (20%)	13 (11.8%)	
Stopped treatment *n* (%)	47 (42.7%)	11 (10%)	36 (32.7%)	
EPO treatment	24 (21.8%)	3 (5.2%)	21 (40.4%)	0.001*
MBD treatment	30 (27.3%)	9 (15.5%)	21 (40.4%)	0.003*

CHD coronary heart disease, *PAD* peripheral arterial disease, *TC* total cholesterol, *LDL-C* low density cholesterol lipoprotein, *HDL-C* high density cholesterol lipoprotein, *TG* triglycerides, *hsCRP* high-sensitivity C-reactive protein, *ECG* electrocardiogram, *LV* left ventricular, *ABI* ankle–brachial index, *Lp(a)* lipoprotein(a) PCSK9-proprotein convertase subtilisin/kexin type 9, *RAS inhibitors* renin–angiotensin system, *EPO* erythropoietin, *MBD* mineral and bone disorder

*$P < 0.05$

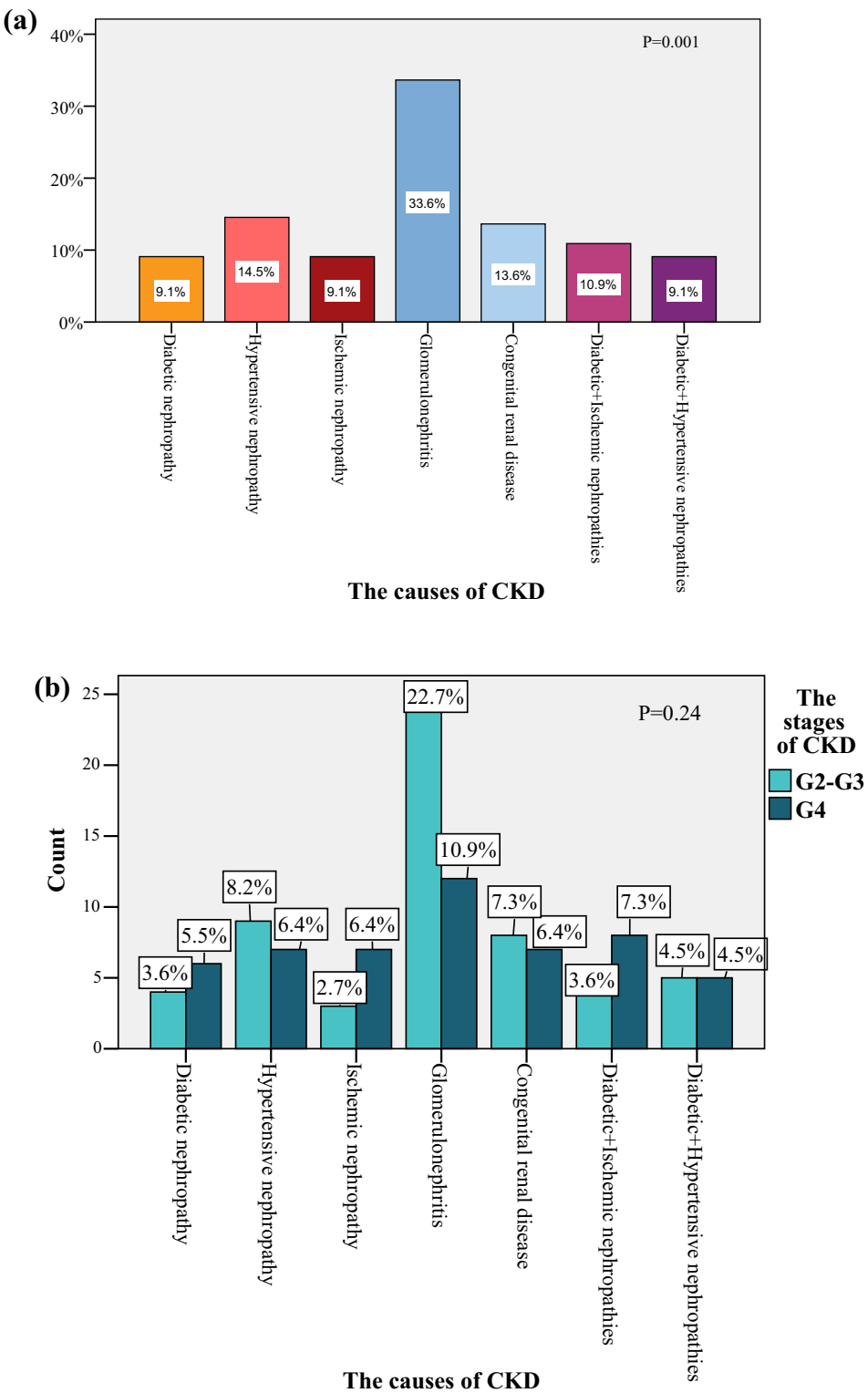

Fig. 1 The frequency of CKD causes **a** in all CKD population **b** by CKD stages

The lipid profile pointed out the total cholesterol (TC) mg/dL, low density cholesterol lipoprotein (LDL-C) mg/dL, high density cholesterol lipoprotein (HDL-C) mg/dL, triglycerides (TG) mg/dL measured by the spectrophotometric method; blood glucose (mg/dL), uric acid (UA) mg/dL measured by spectrophotometric method. Serum PCSK9, serum hsCRP and serum Lp(a) were assessed by

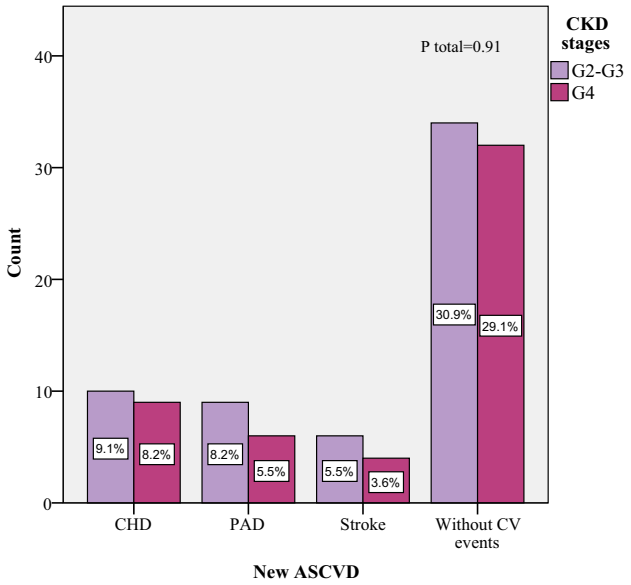

Fig. 2 The frequency of the new ASCVD by CKD stages

dual monoclonal antibody sandwich ELISA. The samples for the measurement of Lp(a) and PCSK9 were kept frozen at − 80 °C prior to the assessment. The estimated glomerular filtration rate (eGFR) was calculated by applying the Chronic Kidney Disease Epidemiology Collaboration equation and proteinuria was defined as ≥ 0.5 g protein/24-h urine [20]. CKD A and G categories were defined according to KDIGO as follows: for albuminuria (A1: < 30 mg/g; A2: 30–300 mg/g; A3: > 300 mg/g), for GFR (G2: eGFR 89–60 mL/min/1.73 m^2; G3a: eGFR 5–45 mL/min/1.73 m^2; G3b: eGFR 44–30 mL/min/1.73 m^2; G4: eGFR 29–15 mL/min/1.73 m^2) [21].

Further explorations for cardiovascular evaluation included:

- an electrocardiogram (ECG) for ischemic changes assessment;
- an ABI measurement with a sphygmomanometer and a portable ultrasonography device, which determines sounds that detect systolic blood pressure in the lower limbs; the reference ABI values were between 0.9 and 1.3;
- echocardiography (Siemens Acuson CV70 Cardiac Vascular Ultrasound Machine) highlighting left ventricular (LV) wall motion abnormalities and ejection fraction values, which are important predictors of left ventricular systolic dysfunction.

Evaluation of the new cardiovascular events and of renal outcomes

Atherosclerotic cardiovascular disease (ASCVD) was defined as one of the following diseases as identified in the medical records: coronary heart disease (CHD) with the following particularities: acute coronary syndrome, myocardial infarction (MI), stable angina, coronary revascularization, ischemic stroke, or transient ischemic attack and peripheral artery disease (PAD) [22].

Renal outcomes were represented by the doubling of serum creatinine, the renal replacement therapy (RRT) and ≥ 30% decline in eGFR from baseline measures [20, 23].

Statistical analysis

The data of the CKD patients were introduced into a database and processed through the statistical functions of the SPSS version 20.0 system. One-sample Kolmogorov–Smirnov for normal distribution tests were performed, with the data being calculated as: mean and standard deviation (SD) for normal distribution variables, percent for categorical variables by using a frequency test, median and interquartile range (IQR) for continuous variables with asymmetrical distribution. Bivariate correlation analysis was performed between the scale variables, using the Spearman correlation coefficient. Specific association coefficients [Cramer's, Phi, contingency coefficient, Chi-square (χ^2)] were used to evaluate the associations between nominal variables. Comparative analyses between the pathological history, clinical and paraclinical history according to CKD stages were performed for the values that did not meet the criteria of normal homogeneity. The nonparametric tests were performed: Mann–Whitney *U* sample, Wilcoxon signed-rank, Kruskal–Wallis *H* test, Friedman test. Survival free of ASCVD and renal outcomes, during follow-up, were estimated using the Kaplan–Meier method. The duration of follow-up was calculated from the date of inclusion in the study to the date of the cardiovascular events and renal events, respectively. Multiple logistic regression analysis was applied to identify the independent factors for cardiovascular events and for renal outcomes. The *P* value < 0.05 was considered statistically significant.

Results

Baseline data of CKD patients in the North-Eastern part of Romania

The study group included 110 patients (31.4% of all patients examined), with a mean age of 55.6 ± 10.9 years, all subjects being Caucasian, with a higher number of men compared to women (55.5% versus 44.5%). The laboratory results were: TC 316.5 ± 38.7 mg/dL, LDL-C 241.9 ± 42.7 mg/dL, HDL-C 38.6 ± 7.5 mg/dL, TG 197.2 ± 60.9 mg/dL (for all patients), creatinine 2.3 ± 0.8 mg/dL, eGFR 33.5 ± 20.3 mL/min/1.73 m^2, nephrotic range-proteinuria 3.7 ± 3.3 g/24 h. Also, 72.9% of the patients had ASCVD history (Table 1).

Table 2 The characteristics of CKD patients according to cardiovascular events

Characteristics	Patients with CKD and dyslipidemia		*P*
	ASCVD	Without ASCVD	
N (%)	44 (40%)	66 (60%)	
TC baseline mg/dL (mean ± SD)	318.3 ± 42.9	296.6 ± 31.9	0.001*
TC 6 mo mg/dL (mean ± SD)	283.8 ± 37.7	266.4 ± 31.1	0.01*
TC 12 mo mg/dL (mean ± SD)	250.1 ± 38.9	236.7 ± 28.9	0.06
LDL-C baseline mg/dL (mean ± SD)	247.1 ± 45.1	220.3 ± 29.2	0.001*
LDL-C 6 mo mg/dL (mean ± SD)	203.2 ± 42.6	185.9 ± 32.5	0.02*
LDL-C 12 mo mg/dL (mean ± SD)	168.7 ± 44.7	153.7 ± 28.9	0.06
HDL-C baseline mg/dL (mean ± SD)	37.9 ± 9.4	47.8 ± 8.6	0.001*
HDL-C 6 mo mg/dL (mean ± SD)	44.2 ± 8.5	48.5 ± 8.7	0.003*
HDL-C 12 mo mg/dL (mean ± SD)	50.2 ± 7.8	55.3 ± 6.7	0.001*
TG mg/dL baseline (median ± IQR)	188 ± 50.7	160.5 ± 60.5	0.08
TG mg/dL 6 mo (median ± IQR)	173 ± 54.8	158.5 ± 58.3	0.07
TG mg/dL 12 mo (median ± IQR)	146.5 ± 58.2	131 ± 52.9	0.06
eGFR baseline mL/min/1.73 m^2 (mean ± SD)	34.3 ± 18.3	36.2 ± 19.2	0.58
eGFR 6 mo mL/min/1.73 m^2 (mean ± SD)	32.5 ± 24.8	34.4 ± 19.8	0.23
eGFR 12 mo mL/min/1.73 m^2 (mean ± SD)	31.4 ± 27.5	34.5 ± 24.3	0.32
EF baseline % (mean ± SD)	49.2 ± 12.8	52.7 ± 9.3	0.24
EF 6 mo % (mean ± SD)	41.6 ± 11.9	51.1 ± 8.4	0.001*
EF 12 mo % (mean ± SD)	38.6 ± 11.4	50 ± 8.3	0.001*
ABI baseline (median ± IQR)	0.83 ± 0.08	0.89 ± 0.08	0.001*
ABI 6 mo (median ± IQR)	0.81 ± 0.08	0.9 ± 0.05	0.001*
ABI 12 mo (median ± IQR)	0.86 ± 0.05	0.93 ± 0.05	0.01*
hsCRP mg/L (mean ± SD)	6.7 ± 1.5	3.9 ± 1.8	0.001*
Lp(a) mg/dL (mean ± SD)	19.5 ± 4.1	17.8 ± 4.7	0.03*
PCSK9 ng/mL (mean ± SD)	380.8 ± 67.8	245.3 ± 71.7	0.001*
RAS inhibitors			0.02*
Initiated treatment *n* (%)	8 (7.3%)	20 (18.2%)	
Changes doses *n* (%)	10 (9.1%)	25 (22.7%)	
Stopped treatment *n* (%)	26 (23.6%)	21 (19.1%)	

ASCVD atherosclerotic cardiovascular disease, *TC* total cholesterol, *LDL-C* low density cholesterol lipoprotein, *HDL-C* high density cholesterol lipoprotein, *TG* triglycerides, *EF* ejection fraction, *ABI* ankle–brachial index, *hsCRP* high-sensitivity C-reactive protein, *Lp(a)* lipoprotein(a), *PCSK9* proprotein convertase subtilisin/kexin 9, *RAS inhibitors* renin–angiotensin system inhibitors

*$P < 0.05$

CKD patients had non-nephrotic proteinuria in 52.7% of cases and nephrotic proteinuria in 47.3% of cases, without significant difference between the CKD stages (Table 1).

Table 1 presents the demographic and clinical data of the patients with CKD stages G2–G4, as well as the main associated cardiovascular risk factors. The main etiology of CKD in patients included in our study was glomerulonephritis (33.6%), followed by hypertensive nephropathy (14.5%), autosomal dominant polycystic disease (ADPKD) in 13.6% of individuals, diabetic and ischemic nephropathies (10.9%); while de mixed nephropathies had similar frequencies—9.1% (Fig. 1a). No significant differences were observed between the CKD causes depending on the CKD stages ($P = 0.24$) (Fig. 1b). The CKD stage G4 patients were older, had higher blood pressure and more ischemic changes (abnormalities on the ECG and kinetics of the left ventricle) than CKD stages G2–G3 patients. Biologically, it was identified that CKD stages G2–G3 patients had significantly higher values of TC, LDL-C, Lp(a) compared to CKD stage G4 patients, while CKD stage G4 patients had significantly higher TG values (Table 1).

At baseline, 78.2% of CKD patients included in the study had received lipid-lowering therapies (about 1 year of treatment prior to their inclusion in the study), the most frequent being the treatment with statin as monotherapy—30%, followed by the triple associations between statin, fenofibrate and omega 3 (Table 1). In CKD patients, treatment with RAS inhibitors was discontinued in 42.7% of cases, doses

were changed in 31.8% of cases, followed by patients in whom these therapies were initiated (25.5%) (Table 1). In CKD patients with G2–G3 stages, RAS inhibitors were initiated in 23.6% of subjects, dose adjustment in 20% of them, while the patients with G4 stage had the most frequent stopped of RAS inhibitors therapy (32.7%) (Table 1).

Both ischemic changes on the ECG and LV wall abnormalities following echocardiography were observed in 37 CKD patients, being more common in patients with stage G4 compared to stages G2–G3 ($P=0.008$).

ASCVD in patients with CKD G2–G4 stages based on biomarkers

In this study, 40% ($n=44$ patients) of the CKD population had a new cardiovascular event, as follows: CHD in 17.3% of the enrolled patients ($n=19$), PAD in 13.6% ($n=15$ patients) and stroke in 9.1% of the enrolled patients ($n=10$). There were no significant differences between CKD stages (Fig. 2). The CKD patients with de novo cardiovascular events during the study, had significantly increased values of TC, LDL-C at 6 and 12 months and higher levels of biomarkers [e.g., Lp(a), PCSK9, hsCRP] and low ABI and EF values compared to patients without ASCVD (Table 2). In CKD patients with cardiovascular events, treatment with RAS inhibitors was found to be discontinued in 23.6% of cases, while subjects without CV events frequently had dose changes of RAS inhibitors in 22.7% of patients (Table 2).

Furthermore, in CKD patients, following the multiple logistic regression, only PCSK9 over 220 ng/mL ($P=0.001$) was a predictor of cardiovascular events (Table 3). Interestingly, lipid-lowering drugs did not act as a protective factor for cardiovascular disease in these patients (Table 3).

The time-interval for the occurrence of cardiovascular events was not significantly different between the CKD stages (Fig. 3a). For CKD patients with PCSK9 > 220 ng/mL and hsCRP > 3 mg/L levels, the time-interval for the new cardiovascular events occurrence was significantly decreased compared to patients displaying low values of these biomarkers ($P=0.001$) (Fig. 3b–d).

Chronic kidney disease progression to end-stage renal disease in CKD stages G2–G4 patients based on biomarkers

In this study, 9.1% ($n=10$ patients) of the CKD population displayed doubling of serum creatinine compared to baseline, in 17.3% of CKD patients ($n=19$ patients) initiation of dialysis was recommended, > 30% of GFR decline was observed in 14.5% ($n=16$ patients) and all 3 renal events were identified in 9.1% of patients enrolled ($n=10$ patients). In the patients included in the study, the renal events were significantly different between the stages, with the preponderance of doubling of creatinine in the G2–G3 stages, while in the G4 stage the other 2 renal events were significantly increased (RRT and > 30% GFR decline) (Fig. 4).

CKD patients with new renal outcome during the study had significantly increased values of TC, LDL-C at 6 and 12 months, and high PCSK9, hsCRP levels, but with low eGFR, ABI and EF values compared to patients without renal events (Table 4). In CKD patients with renal events, treatment with RAS inhibitors was stopped in 40.9% of cases, while subjects without renal events frequently had dose changes of RAS inhibitors in 26.4% of cases, followed by the initiation of treatment in 22.7% of patients (Table 4). After CKD stages classification, the hsCRP, Lp(a) and PCSK9 high values were not significantly different between the renal outcomes group versus patients without renal

Table 3 Independent factors for cardiovascular events in CKD patients

Variables	OR	95% CI for OR		*P*
		Lower	Upper	
PCSK9	1.041	1.016	1.066	0.001*
PCSK9 > 220 ng/mL	97.704	2.614	3651.961	0.013*
Lp(a)	1.224	0.995	1.506	0.056
Lp(a) > 10 mg/dL	0.029	0.001	2.990	0.134
hsCRP	1.297	0.591	2.846	0.517
hsCRP > 3 mg/L	1.635	0.046	57.492	0.787
LDL-C	0.994	0.973	1.014	0.549
EF	0.940	0.864	1.023	0.152
ABI	0.016	0.001	707.666	0.447
Lipid-lowering drugs				0.533
Statin	0.471	0.053	4.210	0.500
Statin + ezetimibe	3.543	0.299	41.965	0.316
Statin + omega 3	1.411	0.173	11.524	0.748
Statin + fenofibrate + omega 3	0.441	0.041	4.766	0.500

LDL-C low density cholesterol lipoprotein, *EF* ejection fraction, *ABI* ankle–brachial index, *hsCRP* high-sensitivity C-reactive protein, *Lp(a)* lipoprotein(a), *PCSK9* proprotein convertase subtilisin/kexin type 9, *OR* odds ratio

*$P < 0.05$

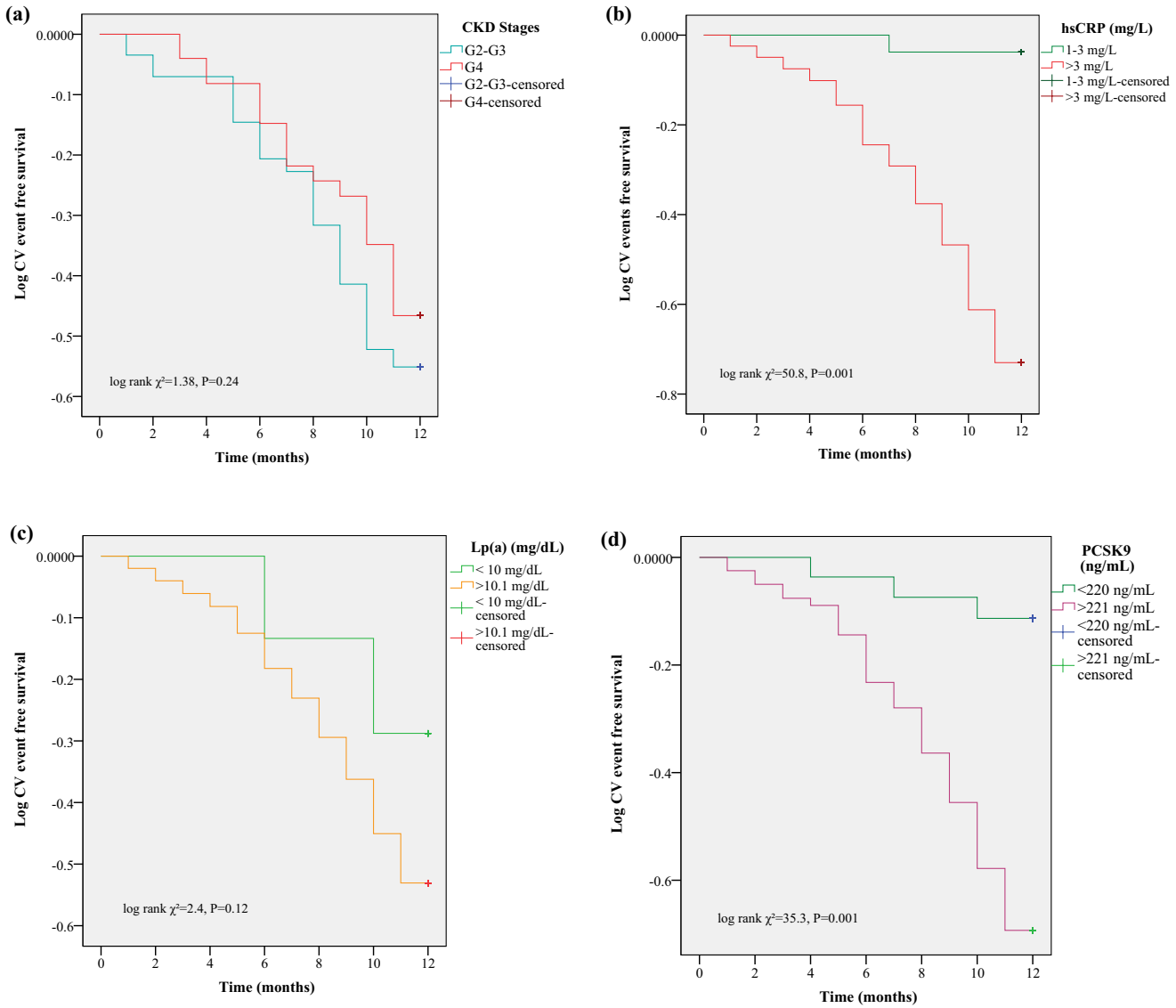

Fig. 3 Kaplan–Meier for ASCVD depending on **a** CKD stages and the biomarkers [**b** hsCRP, **c** Lp(a), **d** PCSK9] and time-interval for the occurrence of new CV events

outcomes. Also, in CKD patients, between stages G2–G3 and stage G4, there was no statistically significant difference between serum biomarkers values ($P > 0.05$).

Likewise, in CKD patients, following the multiple logistic regression, the ejection fraction under 50% ($P = 0.005$) was a predictor for renal outcomes (Table 5).

The time-interval for the renal events occurrence was not significantly different between the CKD stages (Fig. 5a). For CKD patients with PCSK9 > 220 ng/mL (8 months) and hsCRP > 3 mg/L (8 months), the time-interval for new renal events occurrence was significantly decreased compared to patients displaying low levels of these biomarkers ($P = 0.001$) (Fig. 5b–d).

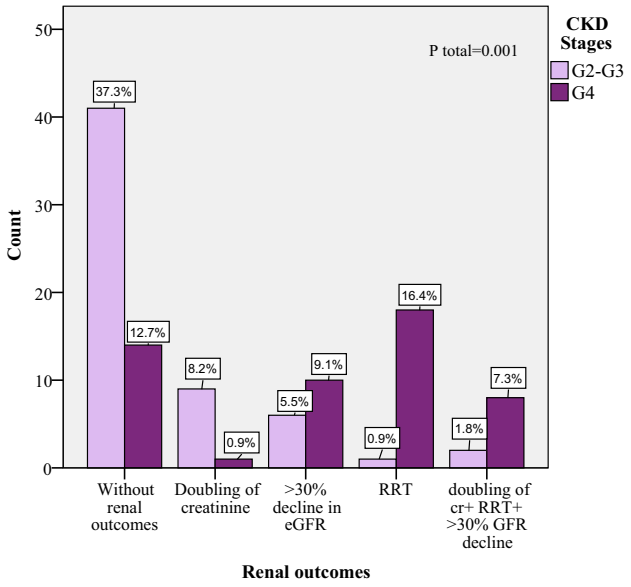

Fig. 4 The frequency of the renal outcomes by CKD stages

Discussion

This is the first epidemiological study in the NE region of Romania that included patients with CKD and dyslipidemia, in order to establish the link between specific biomarkers represented by PCSK9, Lp(a), hsCRP and cardiovascular events, respectively, renal events. This study represents an important step in identifying cases with dyslipidemia and CKD, to create a bridge between specialties (cardiology–nephrology–internal medicine).

The study evaluated 350 patients with dyslipidemia and CKD stages G2 to G4, from the North-Eastern part of Romania and included 110 patients who met the inclusion criteria. The patients with CKD stage G4 had an elevated blood pressure, ischemic changes revealed by changes on the ECG and kinetics of the left ventricle compared patients with CKD stages G2–G3. During the course of CKD progression, a rise in triglycerides (TG) was observed, whereas HDL-C decreased and LDL-C remained normal or decreased slightly [24], findings also confirmed by our study. In CKD patients with new cardiovascular events on the follow-up, the treatment with RAS inhibitors was found to be discontinued in 23.6% of cases, and RAS inhibitors doses were adjusted in 9.1% of patients. Furthermore, in CKD patients with new renal events on the follow-up, the treatment with RAS inhibitors was stopped in 40.9% of cases, and RAS inhibitors doses were adjusted in 5.5% of patients.

The quantification of serum Lp(a) levels was performed by the double sandwich ELISA method, as Longenecker et al. established that ELISA assay strongly predicted incident CVD [25]. Plasma levels of Lp(a) range from 0.1 to 200 mg/dL, and most Europeans have Lp(a) concentrations under 10 mg/dL, while only about 25% display concentrations above 30 mg/dL [2]. In our study, Lp(a) plasma concentrations was 19.1 ± 4.5 mg/dL, with high values in CKD stages G2–G3 compared to stage G4, an idea also supported by studies conducted by Kronenberg et al. and Kaysen et al. [10, 26]. Although Lp(a) is an independent risk factor for CHD and generalized atherosclerosis [2, 4, 10, 26], in our study Lp(a) was not associated with cardiovascular events, as an independent risk factor for atherothrombosis in CKD patients. In addition, our study did not identify high levels of Lp(a) as a risk factor for the development and/or progression of kidney disease. A possible explanation could be that in advanced CKD, the catabolism rate of Lp(a) does not correlate with its plasma concentrations, suggesting that Lp(a) levels are controlled by synthesis rather than by catabolism [2, 10].

In patients with CKD, the available evidence for PCSK9 is insufficient, with few observational studies [8]. In a cross-sectional study, Elewa et al. identified that plasma PCSK9 was higher in CKD patients with lipid-lowering therapy, and the plasma PCSK9 levels did not vary between patients with different eGFR or albuminuria categories [27], the results being similar to those in our study. Elewa et al. pointed out the significant positive correlation between plasma PCSK9 levels and total cholesterol [27], a correlation that was also observed in our study, while Rogacev et al. revealed that plasma PCSK9 was poorly correlated with total cholesterol and triglycerides [15]. In addition, elevated PCSK9 levels were positively correlated with high LDL-C levels. In our study, in patients with CKD we found that elevated PCSK9 levels were significantly associated with cardiovascular events, especially in patients with CKD stages G2–G3, in contrast to Rogacev et al. who did not identify this association [15]. Although PCSK9 is a potential determinant of

Table 4 The characteristics of CKD patients according to renal outcomes

Characteristics	Patients with CKD and dyslipidemia		
	Renal outcomes	Without renal outcomes	*P*
N (%)	54 (49.1%)	56 (50.9%)	
TC baseline mg/dL (mean ± SD)	311.2 ± 36.2	299.6 ± 39.3	0.02*
TC 6 mo mg/dL (mean ± SD)	282.4 ± 34.7	264.6 ± 32.8	0.01*
TC 12 mo mg/dL (mean ± SD)	249.4 ± 35.9	235 ± 30.2	0.04*
LDL-C baseline mg/dL (mean ± SD)	236.1 ± 38.9	226.1 ± 37.9	0.1
LDL-C 6 mo mg/dL (mean ± SD)	202.5 ± 38.8	183.6 ± 34.3	0.02*
LDL-C 12 mo mg/dL (mean ± SD)	167.1 ± 38.6	152.6 ± 33.4	0.06
HDL-C baseline mg/dL (mean ± SD)	39.5 ± 9.1	42.1 ± 9.2	0.14
HDL-C 6 mo mg/dL (mean ± SD)	45.6 ± 8.3	47.9 ± 9.2	0.23
HDL-C 12 mo mg/dL (mean ± SD)	52.6 ± 6.8	53.8 ± 8.2	0.34
TG mg/dL baseline (median ± IQR)	189.5 ± 55.3	159.5 ± 57.9	0.09
TG mg/dL 6 mo (median ± IQR)	177 ± 57.1	156.5 ± 57.3	0.18
TG mg/dL 12 mo (median ± IQR)	146 ± 48.3	131.5 ± 62	0.11
eGFR baseline mL/min/1.73 m^2 (mean ± SD)	26.9 ± 14.9	43.6 ± 18.6	0.001*
eGFR 6 mo mL/min/1.73 m^2 (mean ± SD)	18.5 ± 10.6	48.2 ± 19.9	0.001*
eGFR 12 mo mL/min/1.73 m^2 (mean ± SD)	13.8 ± 8.4	52.1 ± 22.1	0.001*
EF baseline % (mean ± SD)	48.4 ± 11.8	54.1 ± 9.3	0.007*
EF 6 mo % (mean ± SD)	44.1 ± 11.8	50.4 ± 9.1	0.003*
EF 12 mo % (mean ± SD)	41.7 ± 12.2	49.1 ± 8.7	0.001*
ABI baseline (median ± IQR)	0.84 ± 0.08	0.89 ± 0.08	0.02*
ABI 6 mo (median ± IQR)	0.86 ± 0.07	0.9 ± 0.09	0.005*
ABI 12 mo (median ± IQR)	0.89 ± 0.05	0.92 ± 0.07	0.02*
hsCRP mg/L (mean ± SD)	5.8 ± 2.1	4.4 ± 2	0.001*
Lp(a) mg/dL (mean ± SD)	18.2 ± 4.9	18.7 ± 4.1	0.72
PCSK9 ng/mL (mean ± SD)	334.1 ± 97.1	266.2 ± 84.3	0.001*
RAS inhibitors			0.001*
Initiated treatment *n* (%)	3 (2.7%)	25 (22.7%)	
Changes doses *n* (%)	6 (5.5%)	29 (26.4%)	
Stopped treatment *n* (%)	45 (40.9%)	2 (1.8%)	

TC total cholesterol, *LDL-C* low density cholesterol lipoprotein, *HDL-C* high density cholesterol lipoprotein, *TG* triglycerides, *hsCRP* high-sensitivity C-reactive protein, *EF* ejection fraction, *ABI* ankle–brachial index, *Lp(a)* lipoprotein(a), *PCSK9* proprotein convertase subtilisin/kexin type 9, *RAS inhibitors* renin–angiotensin system

*$P < 0.05$

serum cholesterol, it cannot be considered a cardiovascular

Table 5 Independent factors for renal outcomes in CKD patients

Variables	OR	95% CI for OR		*P*
		Lower	Upper	
PCSK9	1.010	0.998	1.020	0.073
PCSK9 > 220 ng/mL	2.272	0.366	14.096	0.378
Lp(a)	0.975	0.838	1.135	0.748
Lp(a) > 10 mg/dL	1.733	0.172	17.487	0.641
hsCRP	1.308	0.813	2.105	0.268
hsCRP > 3 mg/L	1.352	0.169	10.834	0.776
LDL-C	1.002	0.987	1.018	0.786
EF	0.943	0.896	0.992	0.022*
ABI	41.767	0.032	55,150.295	0.309
Lipid-lowering drugs				0.473
Statin	1.802	0.476	6.815	0.386
Statin + ezetimibe	0.494	0.080	3.042	0.447
Statin + omega 3	1.798	0.401	8.053	0.443
Statin + fenofibrate + omega 3	2.293	0.475	11.063	0.301

LDL-C low density cholesterol lipoprotein, *EF* ejection fraction, *ABI* ankle–brachial index, *hsCRP* high-sensitivity C-reactive protein, *Lp(a)* lipoprotein(a), *PCSK9* proprotein convertase subtilisin/kexin type 9, *OR* odds ratio

*$P < 0.05$

risk factor in CKD patients [27]. Furthermore, serum PCSK9 levels did not predict cardiovascular events in any cohort (CARE FOR HOMe $P = 0.622$; LURIC $P = 0.729$) [15], while PCSK9 concentrations over 220 ng/mL represented a predictor of cardiovascular events in the CKD patients included in our study. Morena et al. observed that plasma PCSK9 concentrations do not vary between different stages of CKD [24], results similar to those obtained in our study. In CKD patients, elevated PCSK9 values were not associated with renal events [24], unlike our study where the PCSK9 > 220 ng/mL was associated with the occurrence of renal events earlier than the normal range of PCSK9 levels.

hsCRP is a nontraditional marker of cardiovascular risk and it correlates with various cardiovascular diseases [17]. Furthermore, these results are also found in our study, being identified by hsCRP > 3 mg/L which determined the occurrence of renal and cardiovascular events earlier than its normal values. The hsCRP levels under 1 mg/L, 1 to 3 mg/L and over 3 mg/L correspond to low, moderate and high-risk groups for future cardiovascular events [17]. In our study, hsCRP values were placed in the high-risk group (hsCRP = 5.2 ± 2.1 mg/L), without significant differences between CKD stages. In CKD patients, we identified that elevated hsCRP levels were significantly associated with cardiovascular events, especially in patients with CKD stages G2–G3. Fu et al. identified that the patients with hsCRP ≥ 2 mg/L were at a higher risk of CKD progression compared to those with hsCRP < 2 mg/L, hsCRP being a risk factor for kidney disease [28], a result also observed in our study. Besides, the ejection fraction under 50% was a predictor for progression toward ESRD, a conclusion also suggested by Stenvinkel et al. [29].

Study strengths and limitations

The contradictory results of the studies conducted worldwide on CKD patients in pre-dialysis stages motivated us to conduct a new study in the NE region of Romania, in order to identify the impact of these biomarkers on cardiovascular events and on the progression to ESRD.

Nonetheless, this study had some limitations. The methodology of the study was observational, yet with a short follow-up period. Secondly, the number of patients included in the study was small (because for most eligible patients there were no values for EF, ABI or for lipid profile prior to their inclusion in the study). Thirdly, the current study enrolled subjects from the North-Eastern area of Romania, and this group of patients do not significantly reflect the entire Romanian population with ASCVD and CKD.

Conclusion

PCSK9 > 220 ng/mL was predictor for cardiovascular events, while EF < 50% was predictor for CKD progression to ESRD. PCSK9 > 220 ng/mL and hsCRP > 3 mg/L were associated with the occurrence of renal and cardiovascular events earlier.

Serum PCSK9 and hsCRP are potential biomarkers to identify the cardiovascular events and progression to ESRD in patients with CKD and dyslipidemia, who could benefit from PCSK9 monoclonal antibody therapies. Thus, the monitoring of the renal function, of the lipid profile, of PCSK9 and hsCRP may are useful for establishing a linkage between them and cardiovascular and renal events.

Author contributions CEV, MPT analyzed and interpreted the patients' data regarding the CKD criteria. CEV, AC, LF, LF, LV, MA, GD performed the clinical and imagistic examination. CEV, MPT, LF, AC were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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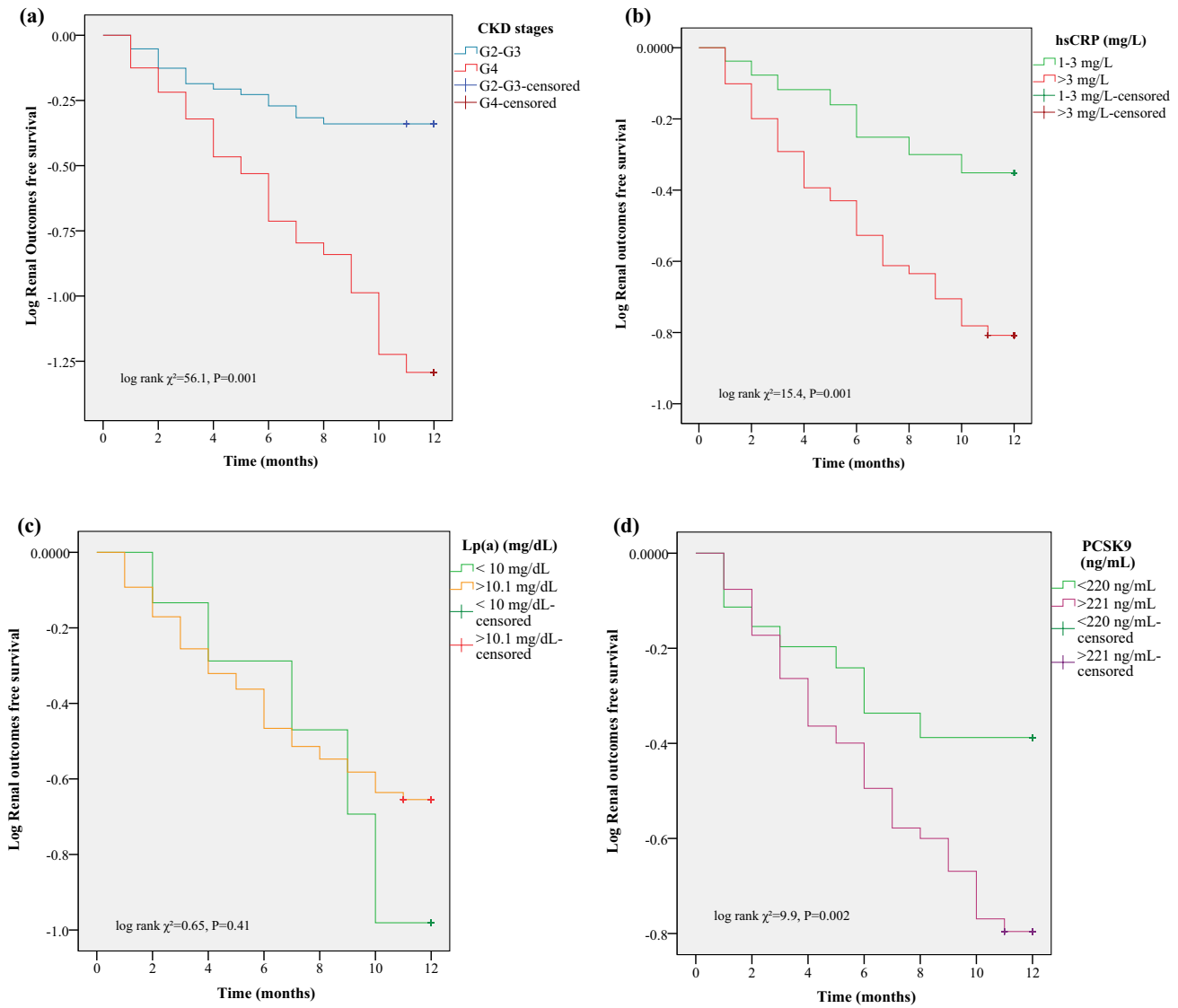

Fig. 5 Kaplan–Meier for renal outcomes depending on **a** CKD stages and the biomarkers [**b** hsCRP, **c** Lp(a), **d** PCSK9] and time-interval for the occurrence of new renal events

Availability of data and material The data and material of this study are available from the author (L.F.) on reasonable request.

Consent for publication All authors consent to the publication of the data.

Declarations

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical approval After the approval of the Informed Consent by the Ethics Commission of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, of "Dr. C. I Parhon" Clinical Hospital, the consent was signed by all the patients prior to their enrollment in the study. The research could not involve any physical or mental discomfort for the patients included in the study, nor any physical or mental risks, or obligation to participate in the study. The confidentiality of the personal and medical data of the subjects enrolled in the study was preserved.

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