

INFLAMMATION PREDICTS ALL-CAUSE AND CARDIOVASCULAR MORTALITY IN HAEMODIALYSIS PATIENTS

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Abstract: Among non-traditional cardiovascular risk factors both malnutrition and inflammation appear to be strong predictors of mortality and morbidity in haemodialysis (HD) patients. Our study objective was to determine predictors of all-cause and cardiovascular mortality, considering the nutritional and immunologic parameters, in a cohort of HD patients treated in a single haemodialysis centre.

216 patients on HD were analyzed for *clinical, nutritional*-serum albumen and BMI, *immunologic*-serum CRP (C-reactive protein) and fibrinogen and *dialysis parameters* – ultrafiltration, length of dialysis in hours, HD dose (using spKt/V and eKt/V). Mortality was monitored prospectively over a two-year period.

Fifty-five of the 216 HD patients died during the follow-up period and the main cause of death was cardiovascular disease (CVD) – 33 patients out of 55 (60%), followed by infection/sepsis (13 pts, 24%). The patients who died were significantly older, had a significantly shorter duration of HD in hours, ultrafiltration was significantly less, HD doses were significantly lower, as were serum levels of albumin (36.06 ± 4.17 vs. 39.74 ± 3.31 ; $p = 0.000$) and Hg (93.14 ± 15.43 vs. 109.16 ± 12.08 ; $p = 0.000$), but they had significantly higher serum levels of CRP (40.26 ± 34.75 vs. 8.71 ± 7.68 , $p = 0.000$) and fibrinogen (5.28 ± 1.28 vs. 4.42 ± 0.97 , $p = 0.000$). Kaplan-Meier survival estimates showed that the group with the lowest levels of albumin (< 3.5 g/L), and with the greatest levels of CRP (> 20 mg/l) and fibrinogen (> 5 g/L) had the lowest

survival (log-rank test $p = 0.0008$, $p = 0.00000$, $p = 0.0000$). However, in the Cox proportional hazards model, a high CRP and low Hg level (chi-square = 96.467, $p = 0.0000$) were predictors of all-cause mortality, whereas serum level of albumin did not show to be predictive. When only cardiovascular mortality is entered into the Cox model, CRP and Hg levels are still more important in predicting mortality (chi-square = 70.055, $p = 0.0000$) and only if CRP is not taken into account in the multivariate analysis, serum albumin level remains, after Hg, the strongest predictor for both overall and cardiovascular mortality (chi-square = 76,564, $p = 0.0000$; chi-square 50.619 $p = 0.0000$).

It can be concluded that inflammation predicted all-cause and cardiovascular mortality in our study group, because high CRP, as a marker of inflammation and low haemoglobin, as a result of inflammation, remained powerful predictors of both overall and cardiovascular death.

Key words: cardiovascular mortality, haemodialysis, inflammation, acute phase reactants, CRP

Introduction

Although maintenance dialysis therapy for end-stage chronic renal failure has been used for almost 40 years, the mortality rate of dialysis patients remains unacceptably high. Cardiovascular disease is the major cause of morbidity and mortality in HD patients, but traditional risk factors alone cannot explain the unacceptably high prevalence and incidence of CVD in this population. Haemodialysis patients have disease-related non-traditional cardiovascular risk factors, and among these factors, both malnutrition and inflammation appear to be a strong predictor of mortality and morbidity in HD patients¹.

Many investigators have observed that both malnutrition and inflammation tend to occur concurrently and coexist in HD patients and many factors that engender one of these conditions also lead to the other. [2, 3] Inflammatory cytokines, such as interleukin-1 (IL1), IL-6 and tumor necrosis factor- α , increase the synthesis and release of positive acute-phase proteins such as CRP, serum amyloid A and fibrinogen, and decrease the synthesis and release of negative acute-phase proteins such as albumin and transferrin. But serum albumin, a negative acute-phase reactant, also is traditionally known as a nutritional marker because serum levels of albumin decrease with a decline in nutritional status [4]. The causes of inflammation in HD patients are multifactorial and, while it may reflect underlying CVD, an acute-phase reaction may also be a direct cause of vascular injury by several pathogenetic mechanisms. [5]

Our study objective was to determine predictors of all-cause and cardiovascular mortality, considering the nutritional and immunologic parameters, in a cohort of haemodialysis patients treated in a single haemodialysis centre.

Material and methods

A total of 216 incidental and prevalent patients on HD (129 men and 87 women) were followed up for a period of 24 months (between January 2003 and December 2004). An inclusion criterion was patients undergoing HD for at least 3 months. In 211 patients, dialysis was performed three times per week, in 5 patients twice per week. End-stage renal failure was due to pyelonephritis and interstitial nephritis in 40 patients, diabetes mellitus in 38, nephroangiosclerosis in 37, glomerulonephritis in 34, polycystic kidney disease in 16 and other or unknown causes in 51 patients. Polysulfone dialyser and bicarbonate-buffered dialysate were used in all patients.

We analyzed four groups of parameters: *clinical parameters* (gender, age, months on haemodialysis, smoking, blood pressure, haemoglobin and blood urea nitrogen), *nutritional parameters*: serum albumen and BMI (body mass index, kg/m²), *immunologic parameters*: serum CRP (C-reactive protein) and fibrinogen, and *dialysis parameters*: ultrafiltration, length of dialysis in hours, HD dose using single-pool spKt/V and equilibrated eKt/V.

Serum albumin, CRP and haemoglobin (Hg) were measured monthly, and serum fibrinogen every third month. The serum concentration of CRP was measured by a nephelometry and the normal range for CRP was less than 6 mg/L. Single-pool spKt/V was calculated monthly, using a second-generation Daugirdas 2 formula and eKt/V was also calculated monthly, using the Daugirdas-Schneditz formula:

$$\text{spKt/V} = -\ln(R - 0.008 * t) + (4 - 3.5 R) * \text{UF/W}$$

where R = postdialysis/predialysis blood urea nitrogen, t = dialysis hours, UF = predialysis-postdialysis weight change, and W = postdialysis weight.

Statistical analysis: Data from haemodialysis patients who died and from those alive after 24 months of follow-up were compared using a t-test when normally distributed and a non-parametric Mann-Whitney rank-sum test when non-normally distributed. The risk of death among patients with varying subgroups of serum albumin, fibrinogen and CRP levels were compared using the Kaplan-Meier survival function analysis. Multivariate analysis was performed using the Cox proportional hazards model to determine which factors were most closely associated with the risk of death.

Results

The mean age of the patients was 56.56 ± 12.93 years, ranging from 21 to 86 years (men 56.08 ± 12.54 years, women 57.27 ± 13.46 years) and the

duration of haemodialysis treatment was 88.91 ± 71.89 months, ranging from 4 to 308 months (men 84.73 ± 71.18 months, women 95.11 ± 72.48 months). Fifty-five of the 216 HD patients died (31 men – 56.4%, 24 women – 43.6%) during the follow-up period. The main causes of death were CVD-myocardial infarction, congestive heart failure, sudden death and stroke – 33 patients out of 55 (60%), followed by sepsis (13 patients, 24%), neoplasma (4 patients, 7%) or other unknown causes (5 patients, 9%). CVD was the cause of death in twenty (64.5%) of the 31 men and 13 (54.1%) of the women who died.

Among clinical and dialytic data, the patients who died were significantly older, had a significantly shorter duration of HD treatment in hours, ultrafiltration was significantly less and the HD dose (spKt/V and eKt/V) was significantly lower than in those alive at 24 months. However, duration on HD in years, predialysis blood pressure and BMI did not differ between the two groups. During the follow-up period patients who died had lower serum levels of albumin and Hg, but they had significantly higher serum levels of CRP and fibrinogen. (Tables 1 and 2)

Table 1 – Табела 1

Parameters with significant differences between the patients who died vs. those alive during follow-up period

Сигнификантно значајни разлики во параметриите на починатите vs. живите пациенти во период од 24 месечно следење

Parameters	Died (No = 55)	Survived (No=161)	p
HD treatment (hours)	3.81 ± 0.33	4.04 ± 0.20	0.000
UF (l)	2.75 ± 0.73	3.31 ± 0.88	0.000
spKt/V	1.12 ± 0.28	1.21 ± 0.19	0.011
eKt/V	0.98 ± 0.25	1.06 ± 0.17	0.004

In this subset of 216 patients, there remains a negative linear correlation between CRP and serum albumin levels ($R = -0.365$, $p = 0.0000$), Hg ($R = -0.444$, $p = 0.0000$), spKt/V ($R = -0.211$, $p = 0.0021$), eKt/V ($R = -0.210$, $p = 0.0022$), and a positive linear correlation with age ($R = 0.221$, $p = 0.0013$) and fibrinogen ($R = 0.378$, $p = 0.0000$).

As shown in Fig 1., Kaplan-Meier survival estimates of patients from varying CRP subgroups (< 6; 6–10; 10–20; > 20 mg/l) differed among the four groups (log-rank test, $p = 0.00000$). The group with the greatest CRP (> 20 mg/l) had the lowest survival curve ($n = 46$, 35 died, 76.1 %), compared with the

survival in the other three subgroups: CRP < 6 (n = 76, 1 died, 1.3 %), CRP between 6–10 (n = 46, 7 died, 15.2 %), CRP between 10–20 (n = 44, 8 died, 18.2 %).

Table 2 – Табела 2

Parameters with significant differences between the patients who died vs. those alive during follow-up period

Сигнификантно значајни разлики во параметријите на починалиите vs. живите пациенти во период од 24 месечно следење

Parameters	Died (No = 55)	Survived (No=161)	p
Age	60.78 ± 11.10	55.12 ± 13.26	0.005
Hg (g/l)	93.14 ± 15.43	109.16 ± 12.08	0.000
Albumin (g/l)	36.06 ± 4.17	39.74 ± 3.31	0.000
Fibrinogen (g/l)	5.28 ± 1.28	4.42 ± 0.97	0.000
CRP (mg/l)	40.26 ± 34.75	8.71 ± 7.68	0.000

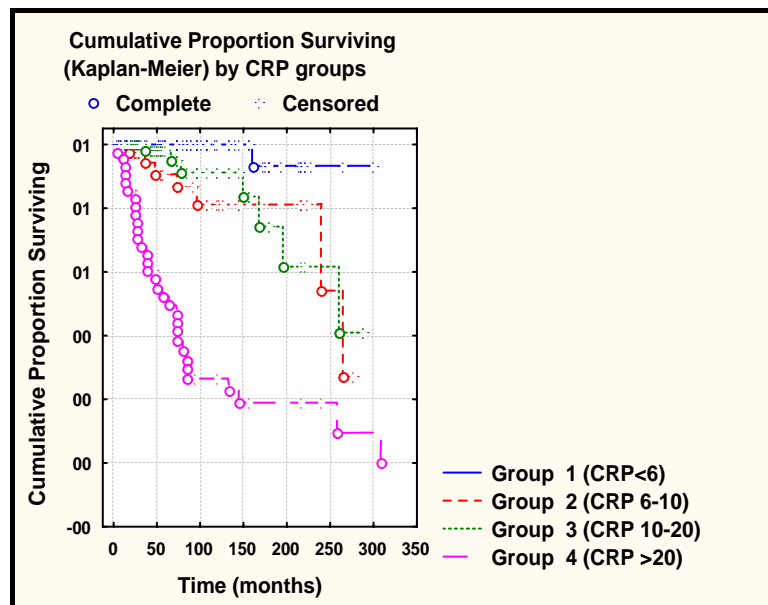


Figure 1 – Kaplan-Meier survival curves for patients with subgroups of CRP levels

Слика 1 – Kaplan-Meier криви на преживување кај пациентите со подгрупи на ЦРП

As shown in Fig 2, the Kaplan-Meier survival curves among HD patients with varying serum albumin levels (< 35, 35 to 40, and > 40 g/L) were statistically significantly different ($p = 0.0008$). Those with serum albumin levels in the lowest subgroup (< 35 g/L) had the lowest survival ($n = 32$, 21 died, 65.6%), compared with the survival of those with serum albumin which was between 35 to 40 g/L, ($n = 118$, 26 died, 22 %) and serum albumin > 40 g/L ($n = 64$, 7 died, 10.9 %).

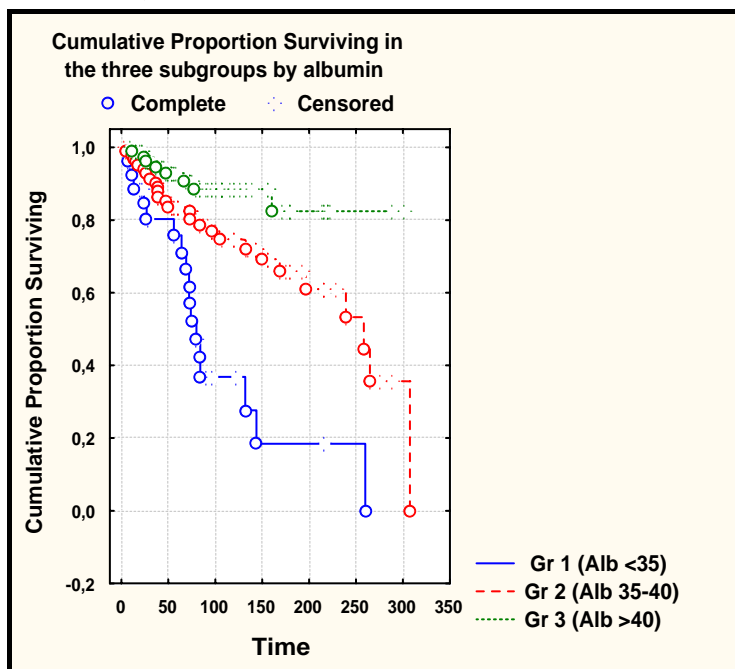


Figure 2 – Kaplan-Meier survival curves for patients with three subgroups of albumin levels

Слика 2 – Kaplan-Meier криви на преживување кај пациенти со три подгрупи на серумски албумин

Kaplan-Meier survival curves among the subgroups with varying fibrinogen levels (fibrinogen < 4, between 4 to 5, and > 5 g/L) also showed a statistically significant difference ($p = 0.0000$) respectively. (Fig. 3)

A high CRP level and low Hg level (chi-square = 96.467, $p = 0.0000$) were predictors of all-cause mortality in the Cox proportional hazards model, whereas serum level of albumin did not show to be predictive. But when CRP was excluded from the Cox model, a low serum albumin level did show to be a predictor of death, followed by a low Hg level (chi-square = 76,564, $p = 0.0000$). (Table 3)

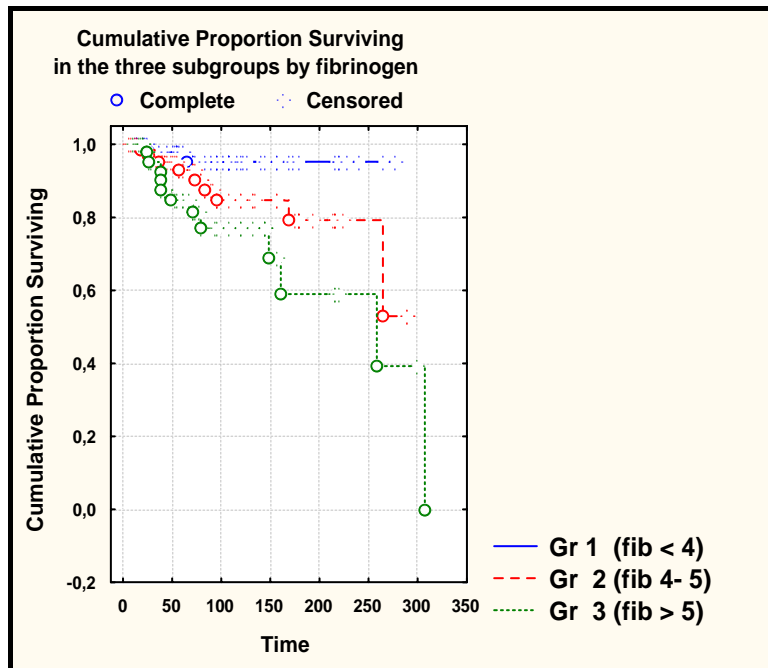


Figure 3 – Kaplan-Meier survival curves for patients with three subgroups of fibrinogen levels

Слика 3 – Kaplan-Meier криви на преживување кај пациенти со три подгрупи на серумски фибриноген

Table 3 – Табела 3

Parameters that predict all-cause mortality in the Cox Proportional Hazards Model

Предиктори на вкупниот морталитет при Cox Proportional Hazards Model

Including CRP				Excluding CRP			
Parameter	Beta	t-value	p	Parameter	Beta	t-value	P
Hg	-0.048	-4.433	0.000	Hg	-0.049	-4.573	0.000
CRP	0.022	5.656	0.000	Alb	-0.084	-1.980	0.047
Alb	-0.053	-1.185	0.235				
Fibrinogen	-0.003	-0.022	0.982	Fibrinogen	0.192	1.417	0.156

Chi-Square = 96.467, p = 0.0000

Chi-Square = 76,564 p = 0.0000

When only cardiovascular mortality is entered into the Cox model, a high CRP level is still more important in predicting mortality, and only if CRP is excluded from the Cox model does a low serum albumin level become predictive of mortality, followed by a low Hg level. (Table 4)

Table 4 – Табела 4

*Parameters that predict Cardiovascular mortality
in the Cox Proportional Hazards Model*
*Предиктори на кардиоваскуларниот морталиитет
при Cox Proportional Hazards Model*

Including CRP				Excluding CRP			
Parameter	Beta	t-value	p	Parameter	Beta	t-value	p
Hg	-0.036	-2.723	0.006	Hg	-0.036	-2.928	0.003
CRP	0.025	5.533	0.000	Alb	-0.132	-2.304	0.021
Alb	-0.084	-1.334	0.181				
Fibrinogen	0.144	0.855	0.393	Fibrinogen	0.252	1.518	0.128

Chi-Square = 70.055, p = 0.0000

Chi-Square = 50.619, p = 0.0000

Discussion

Although maintenance dialysis therapy for end-stage chronic renal failure has been used for almost 40 years, the mortality rate of dialysis patients remains unacceptably high. There is good evidence that cardiovascular disease is the leading cause of death in HD patients. The United States Renal Data System annual data (from prevalent patients in the years 1998–2000) shows that 75.47 (42.2%) of the 178.92 deaths per 1,000 patient years at risk have cardiovascular causes.[6] In accordance with other studies, CVD were the most common causes of death in this study. Our data show that among clinical and dialytic parameters, patients who died during the follow-up period were significantly older, had a shorter duration of HD treatment in hours, ultrafiltration was less and HD dose lower than in those alive, but in a multivariate analysis they were not predictive. CRP, albumin and fibrinogen, as acute-phase reactants, showed in the Kaplan-Meier curves that each of these factors was a significant predictors of mortality.

Hypoalbuminemia, a strong and reliable predictor of CVD and mortality in HD patients, is caused by both inflammation and malnutrition, and it is not clear which one of these two conditions has a greater influence on serum

albumin concentration. [7] Several studies have demonstrated that a low serum albumin concentration is strongly associated with both mortality [8, 9] and cardiac disease in HD patients. Moreover, in the HEMO study (1,411 HD patients), patients in the low albumin group had a significantly greater prevalence of CVD [10]. In our study, patients with serum albumin levels lower than 3.5 g/L had the lowest survival (21 died, 65.6%) compared with the survival of those with serum albumin levels above 40g/L (7 died, 10.9%), but in the multivariate Cox proportional hazards model albumin lost its significance as a risk factor for all-cause mortality.

Unlike other studies [3], where fibrinogen is one of the positive acute-phase proteins, in our study, in the multivariate Cox analysis it failed to be a predictor of mortality in dialysis patients.

Anemia is considered a "uraemia-specific" CVD risk factor, because the impact of anaemia on CVD and its association with poor outcomes is well described in dialysis patients. Numerous studies have reported an association between anaemia and inflammation in dialysis patients, reflected by a high serum concentration of CRP or such proinflammatory cytokines as IL-6 and TNF- α [4, 11]. In our study, in multivariate analysis using the Cox proportional hazards model, only haemoglobin, together with CRP, remained a predictor of both overall and cardiovascular death in dialysis patients, and also when CRP was excluded from the Cox model. This finding may explain why refractory anaemia is more common in HD patients who have malnutrition and inflammation.

Several studies have confirmed that inflammation, as reflected by elevated levels of CRP, is a significant independent predictor of mortality in dialysis patients [12, 13, 14]. Most studies with high applicability have shown that elevated CRP predicted *all-cause* and *cardiovascular* mortality in dialysis patients. [3, 12] In our study, only CRP, as representative of the acute-phase reactants, was a predictor of all-cause and cardiovascular mortality in the Cox proportional hazards model, whereas serum level of albumin and fibrinogen were not shown to be predictive. However, if CRP is not taken into account in the multivariate analysis, serum albumin remains, after low Hg, the strongest predictor for all-cause and cardiovascular mortality in dialysis patients. These results suggest that a high CRP and low Hg level, as two non-traditional cardiovascular risk factors, were predictors of all-cause and cardiovascular mortality in our study group.

Conclusion

It can be concluded that inflammation, but not malnutrition, predicted all-cause and cardiovascular mortality in our study group, because a high CRP,

as a marker of inflammation and low haemoglobin, as result of inflammation, remained a more powerful predictor of both overall and cardiovascular death, than a low serum albumin level.

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Резиме

ИНФЛАМАЦИЈАТА КАКО ПРЕДИКТОР НА ВКУПНИОТ И КАРДИОВАСКУЛАРНИОТ МОРТАЛИТЕТ КАЈ ПАЦИЕНТИ НА ХЕМОДИЈАЛИЗА

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Во групата на не-традиционалните кардиоваскуларни ризик фактори, малнутрицијата и инфламацијата се појавуваат како значајни предиктори на морталитетот и морбидитетот кај пациентите на хемодијализа (ХД). Целта на студијата беше да се детерминираат нутритивно/имунолошките параметри како предиктори на вкупниот и кардиоваскуларниот морталитет кај пациенти на ХД.

Кај 216 пациенти на ХД беа анализирани *клинички, нутритивни* – серумски албумин и БМИ, *имунолошки* – серумски ЦРП (Ц – реактивен протеин) и фибриноген и *дијализни параметри* – ултрафилтрација, времетраење на ХД во часови, ХД доза (калкулирана преку $spKt/V$ и eKt/V). Морталитетот беше мониториран проспективно во текот на две години.

Од педесет и пет пациенти починати во текот на следењето, кардиоваскуларните болести беа главна причина за смртта на 33 пациенти (60%), по што следеа сепсите кај 13 пациенти (24%). Починатите пациенти беа сигнификантно постари, со пократко часовно траење на ХД, со помала ултрафилтрација и пониска ХД доза. Во текот на следењето починатите пациенти имаа сигнификантно пониски вредности на серумски албумин (36.06 ± 4.17 vs. 39.74 ± 3.31 ; $p = 0.000$) и хемоглобин (Хб) (93.14 ± 15.43 vs. 109.16 ± 12.08 ; $p = 0.000$), но сигнификантно повисоки вредности на серумски ЦРП (40.26 ± 34.75 vs. 8.71 ± 7.68 , $p = 0.000$) и фибриноген (5.28 ± 1.28 vs. 4.42 ± 0.97 , $p = 0.000$). Kaplan-Meier-овата крива на преживување покажа дека групата со најниска вредност за албумин (< 35 g/L), највисока вредност за ЦРП (> 20

mg/l) и фибриноген ($> 5\text{g/L}$) имаа најниско преживување (log-rank test $p = 0.0008$, $p = 0.00000$, $p = 0.0000$). Но, при Соx-овиот модел само високите вредности на ЦРП и ниските вредности на Хб (chi-square = 96.467, $p = 0.0000$) беа предиктори на вкупниот морталитет, додека серумскиот албумен не покажа статистичка значајност. При вклучување само на кардиоваскуларниот морталитет во Соx-овиот модел, повторно само високите вредности на ЦРП и ниските вредности на Хб (chi-square = 70.055, $p = 0.0000$) беа предиктори на вкупниот морталитет. При изземање на ЦРП од моделот, серумскиот албумен се појави како предиктор и на вкупниот и на кардиоваскуларниот морталитет, следејќи го повторно Хб (chi-square = 76,564, $p = 0.0000$; 50.619 $p = 0.0000$).

Би можеле да заклучиме дека инфламацијата е главната причина на вкупниот и кардиоваскуларниот морталитет кај пациентите на ХД, бидејќи единствено високите вредности на ЦРП, како маркер на инфламацијата, и ниските вредности на хемоглобинот, која е консеквенца на инфламацијата, се предиктори на вкупниот и кардиоваскуларниот морталитет.

Клучни зборови: кардиоваскуларен морталитет, хемодијализа, инфламација, акутни фазни реактанти, ЦРП

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