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Neopterin is associated with cardiovascular events and all-cause mortality in renal transplant patients

Pihlstrøm H, Mjøen G, März W, Dahle DO, Abedini S, Holme I, Fellström B, Jardine A, Pilz S, Holdaas H. Neopterin is associated with cardiovascular events and all-cause mortality in renal transplant patients.

Abstract: Background: Inflammatory markers show significant associations with cardiovascular events and all-cause mortality after kidney transplantation. Neopterin, reflecting interferon-γ-release, may better reflect the proinflammatory state of recipients than less specific markers.
Methods: Kidney transplant recipients in the Assessment of LEscol in Renal Transplant (ALERT) trial were examined and investigated for an association between serum neopterin and subsequent clinical events: graft loss, major cardiovascular events (MACE) and all-cause mortality.
Results: After adjustment for established and emerging risk factors neopterin expressed as neopterin-to-creatinine ratio was significantly associated with MACE ($p = 0.009$) and all-cause mortality ($p = 0.002$). Endpoints were more frequent with increasing quartiles of neopterin-to-creatinine ratio. The incidence rates of MACE and all-cause mortality were significantly increased in the upper quartiles compared with the first.
Conclusions: This long-term prospective analysis in stable kidney allograft recipients suggests that neopterin is associated with long-term risk of cardiovascular events and all-cause mortality, but not renal outcomes.

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Key words: inflammatory marker – kidney transplantation – long-term – neopterin – outcomes

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Cardiovascular (CV) events and premature deaths are significantly more frequent in kidney transplant recipients (KTR) compared with the general population, even when adjusting for the higher prevalence of traditional risk factors such as diabetes mellitus, hypercholesterolemia, and hypertension (1). Although long-term statin therapy reduces the incidence of major cardiovascular events (MACE) in this population, there is significant residual risk for both cardiac events and all-cause mortality (2). Several non-traditional risk factors, both modifiable and non-modifiable, have been proposed to contribute to this excessive risk (3). We have previously demonstrated in KTR with stable graft function that the inflammatory markers interleukin 6 (IL-6) and C-reactive protein (CRP) show significant associations with CV events and all-cause mortality (4). In this study, we explored the possibility that neopterin may be a more appropriate inflammatory marker for patients undergoing renal transplantation (5).

Neopterin (D-erythro-1-2-3-trihydroxypropylpterin) is produced from guanosine triphosphate (6) by activated human monocytes, monocyte-derived dendritic cells, and macrophages. Release and production of neopterin is stimulated mainly by interferon-γ (IFN-γ) released by activated Th1-lymphocytes during the cellular immune response (7). In contrast to IFN-γ, which quickly binds to target structures or is neutralized by soluble receptors, neopterin is biochemical inert, and its serum concentrations were closely linked to the activity of the cellular immune system (8). Neopterin is shown to be a marker of disease in a variety of conditions (9) and has previously been associated with CV events and mortality in non-transplant populations (10, 11). As a marker of cellular immune response activation depending on IFN-γ release, neopterin may better reflect the proinflammatory state of KTR than less specific markers of inflammation, but the predictive value of neopterin for clinical outcomes in stable KTR is unknown.

In the current analysis, long-term data from the randomized Assessment of LEscol in Renal Transplant (ALERT) trial (1) were examined to investigate the association between serum neopterin level and subsequent adverse clinical outcomes in a population of KTR.

Patients and methods

Study design

The study design and baseline data of the ALERT trial have been described previously (12). In brief, ALERT was a randomized, double-blind, placebo-controlled study of the effect of fluvastatin (40–80 mg/d vs. placebo) on cardiac and renal outcomes in 2102 male and female KTR aged 30–75 yr, included from June 1996 to October 1997. Patients had received a renal transplant more than six months previously, had a stable graft function and a total serum cholesterol between 4.0 and 9.0 mM (155–348 mg/dL). Exclusion criteria were familial hypercholesterolemia, recent acute rejection episodes, predicted life expectancy of less than one yr or ongoing statin therapy. Follow-up was 5–6 yr in the core study, after which trial participants were offered open-label fluvastatin 80 mg/d in a two-yr extension trial. Mean total follow-up time for the extension study was 6.7 yr. Prior to unblinding the ALERT study, neopterin was chosen as one of the pre-specified cardiovascular risk factors to be analyzed. Serum neopterin concentration was measured in 30% of patients (randomly chosen) by radioimmunoassay (IBL Diagnostics, Hamburg, Germany) in samples taken at the time of study entry (baseline), a mean of 5.4 yr after transplantation.

The study adhered to the International Conference on Harmonization guidelines for Good Clinical Practice and was conducted in accordance with the Declaration of Helsinki Principles. All participants provided written informed consent, and the ethics committee at each participating center approved the trial.

Outcome definitions

Renal endpoint was the time to graft loss (RGL), defined as return to dialysis or retransplantation. Cardiac endpoint was the occurrence of a MACE,

defined as cardiac death, non-fatal myocardial infarction verified by hospital records, or coronary revascularization procedure, including coronary artery bypass graft or percutaneous coronary intervention. Death by any cause was also chosen as study outcome. All endpoints were validated by an independent clinical end point committee blinded to study randomization.

Statistical analysis

Since treatment and placebo arms of the original study showed no significant heterogeneity in relation to demographics, known cardiovascular or renal risk factors or levels of inflammatory markers (12), the current analysis was based on the pooled patient population.

In comparing baseline characteristics between groups, independent samples *t*-test and Mann–Whitney *U*-test for continuous variables and chi-square test for associations between categorical variables were used. Spearman's rank correlation was used in checking for statistical associations between neopterin and creatinine, as well as the inflammatory markers IL-6 and CRP.

Univariable and multivariable Cox proportional hazard models were used to evaluate the influence of possible prognostic variables, including conventional cardiovascular risk factors, other inflammatory markers and factors associated with graft survival. These models were used to examine the association between neopterin and MACE, all-cause mortality and graft loss. Since high sensitivity CRP (hsCRP) and IL-6 are closely linked etiologically and considered to be mutual confounders, they were included as covariates in separate multivariable regression models, the other variables remaining the same. Covariates were examined using Schoenenfeld residuals and found to fulfill the assumptions of proportionality. Hazard ratios (HR) were estimated with 95% confidence intervals.

Neopterin estimates

As neopterin is chemically inert and its elimination is solely through the kidneys, compromised renal function leads to a rise in serum neopterin that is not caused by increased inflammatory activity (13). We found a high degree of correlation between neopterin and creatinine. Therefore, as done in the majority of previous studies on neopterin in populations with compromised renal function, neopterin levels were calculated relative to the serum concentration of creatinine, thus adjusting for kidney function. Values are expressed in μmol/mol of creatinine.

SPSS version 18 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses except for generation of Figs. 1 and 2 where we used Stata version 11 (StataCorp, College Station, TX, USA).

Results

Baseline characteristics

Table 1 lists baseline characteristics for the ALERT participants, comparing those with and without available baseline neopterin data. Demographics, risk factors, and neopterin levels were similar in the fluvastatin and placebo arms (not shown). Since there were no clinically important differences, neither between the two treatment arms nor between the overall study population and those for whom neopterin levels were available – subsequent analysis was performed on the pooled population with available neopterin measurements (4, 14).

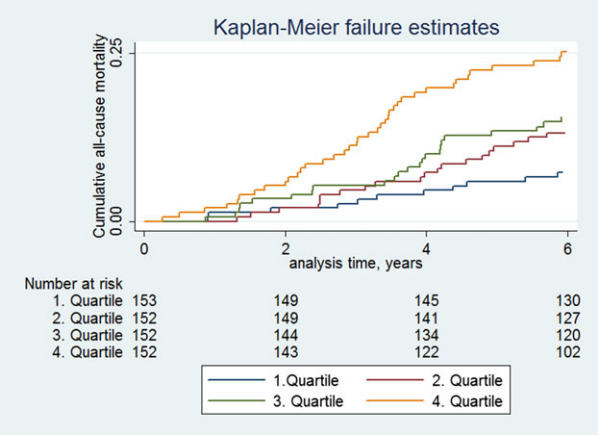

Fig. 1. Cumulative all-cause mortality according to quartiles of neopterin-to-creatinine ratio.

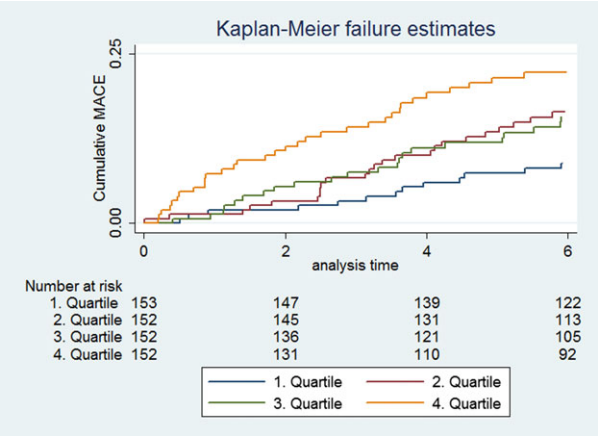

Fig. 2. Cumulative major cardiovascular events (MACE) according to quartiles of neopterin-to-creatinine ratio.

The correlation coefficient between neopterin and creatinine was 0.61. For purposes of survival analysis, we categorized patients into quartiles according to their neopterin-to-creatinine ratios at baseline. Levels ranged from 33 to 325 μmol/mol. In Table 2, demographic data and background risk factors are presented for each quartile of neopterin/creatinine. There was a tendency towards higher proportions of patients with hypertension, chronic heart disease, panel reactive antibodies, delayed graft function (DGF), longer time on dialysis prior to transplantation and treatment for CMV-infection/reactivation in the highest or the two highest quartiles. IL-6 and hsCRP increased progressively across the quartiles, as did age.

Outcomes

The proportions of patients reaching the renal, cardiac and mortality endpoints in each quartile for neopterin-to-creatinine ratio are listed in Table 3. Log-rank tests were used to check the significance of differences between each of the three upper quartiles compared with the lowest.

Table 1. Demographic and baseline data for patients with or without measurement of neopterin

Variables	Available data (n)	Neopterin measured (n = 629)	Neopterin not measured (n = 1473)
Age at baseline, yr	2102	49.9 (10.9)	49.6 (10.9)
Male gender	2102	420 (66.8)	967 (65.6)
Current smoker	2100	133 (21.1)	256 (17.4)
Body mass index, kg/m^2	2051	25.5 (4.3)	25.9 (4.6)
Diabetes mellitus	2101	133 (21.1)	263 (17.9)
Hypertension	2102	483 (76.8)	1092 (74.1)
Systolic blood pressure, mmHg	2094	142.6 (19.0)	144.5 (18.8)
Diastolic blood pressure, mmHg	2093	85.5 (9.3)	85.7 (10.4)
Coronary heart disease	2101	58 (9.2)	143 (9.7)
Serum creatinine, μM	2028	146.2 (51.6)	145.0 (53.6)
Proteinuria, g/24 h	1981	0.40 (0.76)	0.45 (1.11)
HDL cholesterol, mM	2017	1.33 (0.46)	1.34 (0.45)
LDL cholesterol, mM	2001	4.19 (1.01)	4.12 (1.02)
Triglycerides, mM	2028	2.26 (1.24)	2.19 (1.42)
hsCRP, mg/L	1910	3.70 (6.82)	3.85 (6.66)
IL-6, pg/mL	1751	3.05 (1.92)	2.85 (1.84)
Time since last transplant, yr	2101	5.4 (3.5)	5.0 (3.4)
Time on dialysis, yr	2092	2.2 (3.6)	2.3 (3.4)
Cold ischemia time, hours	1520	17.9 (7.3)	20.5 (7.8)
Panel reactive antibodies	1845	117 (20.4)	210 (16.5)
Delayed graft function	2063	93 (15.0)	272 (18.8)
Treatment for cytomegalovirus	2030	86 (14.1)	200 (14.1)

Continuous variables are shown as mean (SD); categorical variables as n (%).
HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high sensitivity CRP; IL-6, interleukin-6.

The rate of death from all causes increased in higher neopterin/creatinine quartiles, and the differences were statistically significant between all three upper quartiles and the first quartile. The number of events more than trebled from the first to the fourth quartile (9.5–33.6%). For MACE, the incidence rate was more than twice as high (11.1–25.0%) in the fourth neopterin/creatinine quartile compared with the first one, and the difference was statistically significant. For renal graft loss the incidence rates was slightly higher in the fourth quartile, but no statistical significance was reached.

Figs. 1 and 2 presents the Kaplan–Meier failure estimates plots for all-cause mortality and MACE showing time to event, or end of follow-up, by neopterin/creatinine quartile.

Multiple risk factor analysis

Table 4 shows risk factor evaluation using univariable and multivariable models of Cox regression analyses. For all study outcomes we present HR with 95% confidence intervals (95% CI) and their respective p-Values. In the univariable model, baseline neopterin expressed as neopterin-to-creatinine ratio, as well as potential risk factors and baseline demographics, is examined separately against the study outcomes. The multivariable model is adjusted for the following baseline covariates: age, gender, smoking habit, diagnosis of coronary heart disease, LDL-cholesterol, systolic blood pressure, diabetes mellitus, serum creatinine, and proteinuria. IL-6 and hsCRP were included in separate models, as they are part of the same etiological inflammatory pathway and thus have a high colinearity. Results are shown only for the analysis including hsCRP, as the hazard ratio for neopterin was virtually identical in the two models.

In the univariable model, neopterin was highly significantly associated with all endpoints in our study. After adjustment for other established and potentially important risk factors, we found neopterin (in μmol/mol creatinine) to have a significant positive association with all-cause of death (HR 1.06 per 10 units, p = 0.002, 95% CI 1.02–1.09) and MACE (HR 1.06 per 10 units, p = 0.009, 95% CI 1.01–1.10) but no independent predictive value for graft loss. HR and p-Values for neopterin remained the same for all endpoints when randomization group was included in the multivariate model (not shown). Though reaching significance for MACE in the univariable analyses, HsCRP was not independently associated with any of the

Table 2. Demographic and baseline data according to quartiles of neopterin-to-creatinine ratio

	Neopterin/creatinine quartile μmol/mol			
Variable	1 (n = 153) 33–68	2 (n = 152) 68–87	3 (n = 152) 87–109	4 (n = 152) 109–325
Age at baseline, yr	46.2 (10.0)	49.2 (10.6)	51.4 (10.6)	53.1 (11.0)
Male gender	111 (72.5)	106 (69.7)	94 (61.8)	97 (63.8)
Current smoker	40 (26.1)	29 (19.1)	34 (22.4)	27 (17.8)
Body mass index, kg/m^2	24.9 (4.0)	25.8 (4.1)	25.8 (4.9)	25.6 (4.3)
Diabetes mellitus	28 (18.3)	39 (25.7)	26 (17.1)	39 (25.7)
Hypertension	115 (75.2)	106 (69.7)	121 (79.6)	128 (84.2)
Systolic blood pressure, mmHg	141.2 (18.6)	141.7 (18.5)	144.1 (19.6)	143.5 (19.5)
Diastolic blood pressure, mmHg	85.5 (9.4)	85.2 (9.2)	85.5 (9.0)	85.7 (9.8)
Coronary heart disease	8 (5.2)	11 (7.2)	10 (6.6)	26 (17.1)
Serum creatinine, μM	145.9 (52.4)	144.1 (48.5)	145.2 (19.6)	149.7 (56.4)
Proteinuria, g/24 h	0.44 (0.99)	0.34 (0.48)	0.34 (0.66)	0.49 (0.81)
HDL cholesterol, mM	1.39 (0.42)	1.33 (0.46)	1.35 (0.50)	1.22 (0.46)
LDL cholesterol, mM	4.30 (0.95)	4.20 (1.09)	4.14 (0.97)	4.10 (1.04)
Triglycerides, mM	2.09 (1.01)	2.23 (1.48)	2.27 (1.27)	2.46 (1.16)
hsCRP, mg/L	2.15 (5.26)	3.61 (7.66)	4.71 (7.40)	4.54 (6.81)
IL-6, pg/mL	2.50 (1.43)	2.74 (1.78)	3.25 (2.20)	3.85 (2.15)
Time since last transplant, yr	5.7 (3.6)	5.7 (3.1)	5.3 (3.2)	4.8 (3.9)
Time on dialysis, yr	1.8 (2.9)	1.9 (2.6)	2.5 (4.2)	2.8 (4.2)
Cold ischemia time, hours	17.8 (8.0)	17.6 (6.4)	18.4 (6.1)	17.9 (8.5)
Panel reactive antibodies	26 (19.1)	28 (20.4)	26 (18.3)	34 (24.3)
Delayed graft function	17 (11.4)	15 (9.9)	31 (20.5)	29 (19.5)
Treatment for cytomegalovirus	18 (12.2)	17 (11.6)	19 (12.8)	31 (20.9)
Neopterin, nM	8.26 (3.03)	11.14 (3.80)	14.21 (4.93)	23.35 (13.02)
Randomized to fluvastatin	83 (54.2)	73 (48.0)	75 (49.3)	81 (53.3)

Continuous variables are shown as mean (SD); categorical variables as n (%).
HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high sensitivity CRP; IL-6, interleukin-6.

Table 3. Event occurrence in stable renal transplant patients according to neopterin/creatinine quartiles

	Neopterin/creatinine quartile μmol/mol			
Endpoint	1 (n = 153) 33–68	2 (n = 152) 68–87	3 (n = 152) 87–109	4 (n = 152) 109–325
Graft loss, n (%)	31 (20.3)	25 (16.4)	29 (19.1)	35 (23.0)
p-Value	–	0.426	0.987	0.238
MACE, n (%)	17 (11.1)	25 (16.4)	25 (16.4)	38 (25.0)
p-Value	–	0.159	0.105	0.001
All-cause mortality, n (%)	13 (9.5)	25 (16.4)	33 (21.7)	51 (33.6)
p-Value	–	0.043	0.001	<0.001

Data expressed as number of patients with the event in each quartile (%). MACE, major adverse cardiac event.

study endpoints in the multivariable analyses, nor was IL-6. Using Spearman's rank correlation, we found the correlation coefficient between neopterin and IL-6 to be 0.26, $p < 0.001$, while for neopterin and CRP it was 0.14, $p < 0.001$. Among the remaining risk factors entered into the model, diabetes mellitus was strongly associated with all outcomes, as was current smoking for all outcomes but MACE. As might have been expected, coronary heart disease was predictive of future MACE and all-cause mortality, while serum creatinine and level of proteinuria was significantly associated with the renal endpoint. Also, LDL-cholesterol was significantly associated with MACE.

Neopterin was also included in a multivariate model with osteoprotegrin, asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) to assess its independency of other markers related to inflammation and endothelial function. No relevant change in HR was seen, and the association with MACE and all-cause death remained highly significant (data not shown).

Table 4. Hazard ratios for neopterin-to-creatinine ratio (per 10 units in μmol/mol) with covariates in relation to outcomes in 628 stable renal transplant patients by univariable and multivariable Cox regression analysis

Risk factors Univariable analysis	MACE 106/628		All-cause mortality 122/628		Graft loss (death-censored) 124/628	
	HR (95% CI)	p-Value	HR (95% CI)	p-Value	HR (95% CI)	p-Value
Age	1.03 (1.01–1.05)	0.002	1.07 (1.05–1.09)	<0.001	0.98 (0.96–0.99)	0.008
Male gender	1.51 (0.98–2.34)	0.064	1.05 (0.72–1.54)	0.792	1.42 (0.96–2.12)	0.083
Current smoking	1.03 (0.64–1.64)	0.917	1.55 (1.04–2.30)	0.030	2.02 (1.39–2.94)	<0.001
Coronary heart disease	4.00 (2.55–6.27)	<0.001	3.22 (2.09–4.99)	<0.001	1.00 (0.52–1.90)	0.994
LDL-cholesterol	1.42 (1.18–1.69)	<0.001	1.06 (0.88–1.26)	0.552	1.11 (0.92–1.32)	0.273
Systolic blood pressure	1.01 (1.00–1.02)	0.202	1.01 (1.01–1.02)	0.001	1.02 (1.01–1.02)	0.001
Diabetes mellitus	1.99 (1.32–2.99)	0.001	2.17 (1.49–3.16)	<0.001	1.64 (1.11–2.43)	0.013
Creatinine	1.01 (1.00–1.01)	0.002	1.01 (1.00–1.01)	<0.001	1.02 (1.02–1.02)	<0.001
Proteinuria	1.22 (1.02–1.45)	0.030	1.23 (1.04–1.45)	0.015	2.23 (1.96–2.54)	<0.001
hsCRP	1.03 (1.01–1.05)	0.004	1.02 (1.00–1.04)	0.062	1.01 (0.99–1.03)	0.331
Neopterin/creatinine	1.08 (1.04–1.12)	<0.001	1.11 (1.07–1.14)	<0.001	1.05 (1.01–1.09)	0.020
Risk factors Multivariable analysis	MACE 100/585		All-cause mortality 117/583		Graft loss (death-censored) 114/583	
	HR (95% CI)	p-Value	HR (95% CI)	p-Value	HR (95% CI)	p-Value
Age	1.02 (1.00–1.04)	0.057	1.07 (1.05–1.09)	<0.001	0.98 (0.96–1.00)	0.097
Male gender	1.54 (0.96–2.45)	0.073	0.90 (0.59–1.35)	0.596	0.92 (0.59–1.44)	0.723
Current smoking	1.00 (0.60–1.65)	0.990	1.75 (1.16–2.65)	0.008	1.71 (1.14–2.58)	0.010
Coronary heart disease	3.35 (2.06–5.46)	<0.001	2.02 (1.27–3.19)	0.003	1.04 (0.51–2.11)	0.921
LDL-cholesterol	1.47 (1.22–1.77)	<0.001	0.99 (0.83–1.19)	0.940	1.07 (0.89–1.28)	0.498
Systolic blood pressure	1.00 (0.99–1.01)	0.503	1.00 (0.99–1.01)	0.893	1.00 (0.99–1.01)	0.979
Diabetes mellitus	2.01 (1.31–3.10)	0.002	2.38 (1.60–3.55)	<0.001	1.82 (1.20–2.79)	0.005
Creatinine	1.00 (1.00–1.01)	0.056	1.01 (1.00–1.01)	<0.001	1.02 (1.01–1.02)	<0.001
Proteinuria	1.08 (0.87–1.34)	0.508	1.09 (0.87–1.35)	0.456	1.88 (1.60–2.21)	<0.001
hsCRP	1.02 (1.00–1.04)	0.127	1.01 (0.99–1.03)	0.343	1.01 (0.99–1.03)	0.526
Neopterin/creatinine	1.06 (1.01–1.10)	0.009	1.06 (1.02–1.09)	0.002	1.03 (1.00–1.08)	0.152

The multivariable analysis adjusts for relevant demographic covariates (age, gender), known renal/cardiovascular risk factors (smoking, coronary heart disease, LDL-cholesterol, systolic blood pressure, diabetes mellitus, creatinine, level of proteinuria), and the inflammation marker hsCRP.
CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ration; hsCRP, high sensitivity CRP; LDL, low-density lipoprotein; MACE, major adverse cardiac event.

Discussion

In this analysis of a large cohort of KTR from the ALERT study, we have shown that the inflammatory marker neopterin is significantly associated with cardiovascular events and all cause mortality in KTR, even after adjustment for conventional and new risk factors.

In patients with pre-dialysis chronic kidney disease (CKD), serum neopterin is elevated and significantly correlated with markers of inflammation including hsCRP, IL-6, and IFN-γ. (15). We have shown previously that levels of IL-6 and hsCRP are associated with cardiovascular endpoints and all-cause mortality following kidney transplantation (4, 16). However, and of central importance, this study shows that in KTR the predictive power of neopterin was maintained after adjustment for hsCRP and IL-6. In the multivariable analyses including neopterin, CRP and IL-6 failed to reach significance as independent predictors of long-term outcomes. In addition, though statistically significant, the correlations between neopterin and these two inflammation markers were weak ones. Our epidemiological data on the predictive value of neopterin are in line with previous literature showing that neopterin rises quickly after macrophage activation (17), is an excellent marker for longer term activation of cellular immunity during the maintenance phase, and appear to remain relatively stable over time (18).

Inflammation is a key element of the development of atherosclerosis, with T-lymphocytes and monocyte-derived macrophages being detected in atherosclerotic lesions. In accordance with our results, studies have highlighted neopterin as a useful marker for long-term risk of all-cause and cardiovascular death in patients from diverse populations, including individuals undergoing coronary angiography (19), patients with stable

coronary artery disease (20), newly diagnosed diabetics (21), and dialysis-dependent CKD patients (22). Furthermore, it has recently been shown that elevated neopterin, but not CRP level, predicts left ventricular dysfunction in patients with chronic stable angina pectoris (23). A recent report from the Hordaland study demonstrated that in elderly patients, without pre-existing coronary heart disease, higher levels of neopterin are associated with an increased risk of subsequent coronary events (24). Chronic low-grade inflammation is one of the main conditions associated with increased cardiovascular morbidity and mortality in patients with CKD, especially those on dialysis (25). Thus, it is not surprising that persistent inflammation, endothelial dysfunction, and associated oxidative stress in KTR (26) is reflected in progression of atherosclerosis (27) and adversely affects cardiovascular outcomes (4). The significant association between neopterin and outcomes was maintained even after adjustment for markers of endothelial function (SDMA, ADMA).

Immunologic responses to allografts involve humoral and cellular components of both the adaptive and innate immune system, the T-cell playing a pivotal role in the initial recognition of anti-self (28). The stronger association that we found between neopterin and the clinical endpoints than for other inflammatory markers may possibly reflect the dominance of T-cell and macrophage activation in the ongoing inflammatory status of allograft recipients. Consistent with this, baseline neopterin values were substantially higher in our cohort compared with the general population (29) and patients with known CV disease (18). This is in harmony with earlier findings on clinically stable KTR (30, 31).

In one of the earliest publications to assess levels of neopterin in KTR (32), Margreiter et al. measured urinary neopterin daily during the early post-operative period and found that acute rejection and early viral infection were preceded by a rising or high level of urinary neopterin in 95% and 100% of cases, respectively. They later extended their data to include urinary neopterin measurements in 294 kidney allograft recipients (33). Subsequent studies by others have confirmed that elevation of serum or urinary neopterin precedes the rise in creatinine by up to several days in patients with acute early complications (34, 35), and routine daily post-operative neopterin measurements have been suggested for the early detection of immunologic complications in kidney allograft recipients (36).

In the trial (33) conducted by Reibnegger and colleagues, a high neopterin level in the early post-transplant period was associated with a higher risk of graft failure in the long term. In a smaller cohort of patients, Grebe et al. (37) observed that elevated neopterin levels following transplantation were associated with inferior graft and patient survival, while a recent prospective study in 216 KTR showed an association between higher levels of neopterin and acute rejection in the first year of follow-up (38). Our population was recruited five yr after transplantation and was clinically stable with a reasonably good renal function. Elevated neopterin was not significantly associated with renal graft loss after adjusting for level of proteinuria. While Grebe et al. (37) were primarily interested in early post-transplant macrophage activation associated with elevated neopterin, our study suggests that in clinically stable allograft recipients, serum neopterin is not independently associated with renal graft failure and does not add significantly to the prognostic information given by the degree of proteinuria. Weimer et al. (39, 40) reported significant associations between neopterin concentration at one yr post-transplant and the development of chronic rejection and chronic allograft dysfunction within two yr, but proteinuria was not included in their multivariable analysis, possibly explaining this discrepancy.

A small study on children with primary nephrotic syndrome (41) showed a positive correlation between serum neopterin levels and proteinuria in patients with active disease. A link between neopterin levels and the progression rate in proteinuric diabetic nephropathy has been demonstrated (42), and one group (43) observed marked differences in serum and urine neopterin levels among diabetic patients with and without microalbuminuria. We are not proposing that enhanced macrophage activity is not an important factor in the development of a dysfunctional renal allograft, but the potential clinical value of neopterin with respect to graft function may be limited as long as proteinuria is considered a well established risk factor for poorer long-term renal outcomes in kidney allograft recipients (44, 45).

Reference ranges for neopterin are higher in the healthy elderly population (>75 yr). Several studies report rising neopterin levels from the age of 60–70 (46) or even earlier (47), probably reflecting the involvement of cellular immunity in the aging process, as well as the presence of low-grade inflammatory processes such as atherosclerosis, neurodegeneration, or unrecognized evolving disease (autoimmunity or malignancy) (29). However, we did not find significant differences in baseline neopterin between different age groups, perhaps because kidney transplants are not performed for

the oldest CKD patients (recipients in our cohort were aged 23.6–74.8 yr), and because comorbidity, the immunological consequences of long-standing uremia and the anti-allograft immune response may overshadow the component of neopterin production related to aging.

The strengths of this analysis are the prospective controlled design, the long follow-up time, the large patient cohort and the independent adjudication of all clinical endpoints. Potential limitations of our study, however, merit consideration. Although the results show a strong association between neopterin and clinical outcomes, the data do not prove a causal relationship. Residual confounding cannot be ruled out, though we have carefully adjusted for a wide range of covariates in our statistical models. Although neopterin may, at present, not be a clinically useful single parameter for risk prediction in KTR, it is conceivable that neopterin could be valuable for multi-marker risk prediction or for the evaluation of the clinical efficacy of established treatments in this patient group.

As in most large prospective trials, serum samples were obtained at inclusion and not followed consecutively. The study population, a cohort selected for entry into a clinical trial, is not necessarily fully representative of the general renal transplant population, although the qualitative relationships between neopterin and the specified clinical outcomes are likely to apply, at least for Caucasians. Mean neopterin values were not different for the two randomization groups, and there was no significant skewing in the proportion of patients receiving fluvastatin in each quartile of neopterin-to-creatinine ratio. Entering treatment group as a covariate in the multiple regression analysis did not change the hazard ratio for neopterin.

In conclusion, in clinically stable renal transplant recipients there appears to be an independent association between serum neopterin concentration and long-term clinical outcomes of MACE and all-cause mortality.

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Authors' contributions

Hege Pihlstrøm: Data collection, data analysis/interpretation, statistics, drafting the article. Hallvard Holdaas: Concept/design, study protocol, data collection, drafting and critical revision of the article. Bengt Fellström and Alan Jardine: Concept/design, study protocol, data collection, critical revision of the article. Geir Mjøen, Ingar Holme, Sadollah Abedini, and Dag Olav Dahle: Data analysis/interpretation, statistics, critical revision of the article. Winfried März and Stephan Pilz: Data analysis/interpretation, drafting and critical revision of the article.

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