

Time-dependent lipid profile inversely associates with mortality in hemodialysis patients – independent of inflammation/malnutrition

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Abstract. Ebert T, Qureshi AR, Lamina C, Fotheringham J, Froissart M, Eckardt K-U, Wheeler DC, Floege J, Kronenberg F, Stenvinkel P (Karolinska Institutet, Stockholm, Sweden; Medical University of Innsbruck, Innsbruck, Austria; Northern General Hospital, Sheffield; University of Sheffield, Sheffield, UK; Lausanne University Hospital, Lausanne, Switzerland; Charité-Universitätsmedizin Berlin, Berlin, Germany; University College London, London, UK; RWTH University of Aachen, Aachen, Germany). Time-dependent lipid profile inversely associates with mortality in hemodialysis patients – independent of inflammation/malnutrition. *J Intern Med* 2021; <https://doi.org/10.1111/joim.13291>

Background. Patients with end-stage kidney disease have an extremely high cardiovascular mortality rate, but there is a paradoxical relationship between lipid profile and survival in haemodialysis patients. To investigate whether inflammation/malnutrition confounds the associations between lipids and mortality, we studied a full lipid profile comprising of five clinically well-established lipid parameters and its associations with mortality in a large, multinational European cohort with a median follow-up >3 years.

Methods. The association between quartiles of total, high-density lipoprotein (HDL), non-HDL, low-density lipoprotein (LDL) cholesterol, as well as triglyceride, levels and the end-points of all-cause,

cardiovascular and non-cardiovascular mortality was assessed in a cohort of 5,382 incident, adult haemodialysis patients from >250 Fresenius Medical Care dialysis centres out of 14 participating countries using baseline and time-dependent Cox models. Analyses were fully adjusted and stratified for inflammation/malnutrition status and other patient-level variables.

Results. Time-dependent quartiles of total, HDL, non-HDL and LDL cholesterol were inversely associated with the hazard for all-cause, cardiovascular and non-cardiovascular mortality. Compared with the lowest quartile of the respective lipid parameter, hazard ratios of other quartiles were <0.86. Similar, albeit weaker, associations were found with baseline lipid profile and mortality. Neither time-dependent nor baseline associations between lipid profile and mortality were affected by inflammation/malnutrition, statin use or geography.

Conclusions. Baseline and time-dependent lipid profile are inversely associated with mortality in a large, multicentre cohort of incident haemodialysis patients. Inflammation/malnutrition is not a confounder nor effect modifier of the associations between lipid profile and mortality in European haemodialysis patients.

Keywords: albumin, cholesterol, chronic kidney disease, haemodialysis, inflammation, lipid profile, mortality.

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Introduction

Patients with chronic kidney disease (CKD) undergo premature vascular ageing [1], which leads to a substantially higher cardiovascular risk [1–4]. When patients have progressed to end-stage kidney disease (ESKD), cardiovascular mortality rates are extremely high, especially for patients receiving dialysis treatment [5]. Despite considerable efforts and progress in cardiometabolic research, the pathophysiological mechanisms of the high mortality in the uremic milieu have not been fully understood.

In the general population, total cholesterol [6] and triglycerides (TG) [7] predict increased vascular [6] and overall [7] mortality whilst lowering of low-density lipoprotein (LDL) cholesterol decreases mortality [8]. In contrast, in CKD cholesterol shows a paradoxical relationship with mortality, that is ‘reverse epidemiology’: Hypocholesterolaemia was an independent predictor of death in a Japanese study comprising of 1,167 haemodialysis (HD) patients [9]. Furthermore, we reported that high apoB/apoA-I ratio was associated with a paradoxical survival advantage in incident dialysis patients [10]. Potential explanations for this apparent reversed epidemiology include ability of lipoproteins to bind bacterial endotoxins and modulate inflammatory immune response [11], time differential of competing risks [12] and/or confounding by protein-energy wasting (PEW) and chronic inflammatory conditions [13].

Liu *et al.* [14] reported that systemic inflammation/malnutrition is a major confounder of the association between lipid profile and mortality after a 2.4-year follow-up in 832 US dialysis patients. Whereas the ‘cholesterol paradox’ was evident in 634 dialysis patients with inflammation/malnutrition, high cholesterol (like in the general population) predicted poor outcome in 189 dialysis patients without inflammation and/or malnutrition [14]. However, a number of limitations possibly influencing the results were evident, including (a) a limited sample size; (b) inclusion of only total cholesterol in the models; (c) mixed ethnic backgrounds and both HD and peritoneal dialysis (PD) treatment; and (d) a cross-sectional but not time-varying analysis. To address these issues and to investigate whether these effects of inflammation/malnutrition on the lipid–mortality associations could be confirmed in a large multinational European dialysis cohort, we analysed the

association between a full lipid profile comprising of five clinically well-established lipid parameters (i.e. total cholesterol, high-density lipoprotein [HDL] cholesterol, non-HDL cholesterol, LDL cholesterol and TG) and all-cause mortality, cardiovascular mortality and non-cardiovascular mortality in a cohort of 5,382 European, incident, adult HD patients from >250 Fresenius Medical Care (FMC) dialysis centres out of 14 participating countries. Using a validated clinical database [15], all patients were followed for a period of 3.2 years and all analyses were stratified based on the presence of inflammation/malnutrition with comprehensive adjustment for multiple variables.

Research design and methods

Patients and study design

The design of the ARO research initiative has been described previously [16]. Briefly, the present study focuses on the AROii cohort, which enrolled 11,211 European, incident (maximum 90 days since initiation), adult patients on HD from >300 European FMC facilities across 15 participating countries enrolled in 2007–2009. All patients were followed prospectively [17] using a validated clinical database comprising of anonymized patient-level medical history, longitudinal laboratory, dialysis and medication data; as well as ICD-10-coded hospitalization and death data [15]. The study has been approved by the institutional review board of the Medical University of Innsbruck (EK-Nr. 1339/2020). Exclusion criteria for this analysis were a history of renal transplant, non-commencement of HD, peritoneal dialysis (PD) on admission, as well as missing baseline data on lipid profile. The main reason for excluding patients was missing lipid data. Differences between excluded and included patients are presented in Table S1. The remaining 5,382 patients (derived from 279 FMC facilities across 14 countries) were stratified into two groups according to the presence of inflammation/malnutrition at baseline, that is, if any of the two following cut-off points were achieved: C-reactive protein (CRP) ≥ 10 mg/l and/or serum albumin < 36 g/l in accordance with Lui *et al.* [14] and other studies [18,19].

For geographical comparisons, European FMC facilities were coded as ‘Western Europe’ (participating centres in France, Italy, Portugal, Spain and United Kingdom) vs. ‘Central/Eastern Europe’ (participating centres in Czech Republic, Hungary, Poland, Romania, Russia, Serbia, Slovak Republic,

Slovenia and Turkey) similar to [4]. Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. LDL cholesterol was calculated by the equation: Total cholesterol [mmol/l] – HDL cholesterol [mmol/l] + (K × TG [mmol/l]) where K = 0.46 if TG ≤ 4.5 mmol/l and K = 0.37 if TG > 4.5 mmol/l [20]. LDL cholesterol was not to be derived by this equation when TG were > 9 mmol/l [21].

Biochemical analysis

In all patients, blood samples were drawn routinely at the time of HD start and were analysed at the local FMC facilities. Measurements of CRP, serum albumin, haemoglobin (Hb), ferritin, total cholesterol, HDL cholesterol, TG, creatinine, calcium, phosphate and parathyroid hormone (PTH) levels were performed at regular but different intervals depending on the biomarker and were included in the statistical analyses. Body mass index (BMI) was calculated as post-dialysis body weight divided by squared height.

Follow-up and end-points

Follow-up investigation using the clinical database started after the patients' first dialysis session. Patients were censored if they received either kidney transplantation, were lost to follow-up (45 days without continuous EU-FMC dialysis treatment) or study end (31 October 2014). Median follow-up time in the entire cohort was 3.2 (interquartile range: 1.6–5.2) years. Cause of death was identified using the validated clinical database [15] according to the WHO International Classification of Diseases (10th Revision; ICD-10) coding scheme [16].

Statistical analysis

Continuous variables were expressed as median with interquartile range (25th to 75th percentile). Differences between the two groups according to inflammation/malnutrition status were determined using non-parametric Mann–Whitney U-test for continuous parameters or chi-squared test for categorical variables. Missing values of potential confounders were imputed using multiple imputation with 20 sets [22]. Covariates included in the imputation models were age at study entry, sex, family history of cardiovascular diseases, diabetes status, smoking status, follow-up data on all-cause, cardiovascular, as well as non-cardiovascular,

mortality and days to censoring or death. Baseline biochemical, clinical characteristics and lipid parameters were not imputed (Table 1).

Since cardiovascular events and mortality in incident HD patients are extremely high in the first months [23], the cohort was left-truncated for the first six months. For time-dependent Cox regression analyses, person-time was split into consecutive 90-day periods similar to [24] and clinical parameters that were updated more frequently were averaged for each of the periods. After stratification of the cohort into four subgroups for each lipid marker according to quartiles [25], Cox proportional hazard regression modelling was performed to estimate the hazard ratio (HR) and 95% confidence intervals (CI) for each parameter comparing with patients within the lowest quartile of the respective lipid parameter, that is quartile 1. The proportional hazard assumption was checked graphically by plotting Schoenfeld residuals and Loess smoothing [26]. Statistical adjustment for the models is given in the respective table legends. Interaction *p* values for inflammation/malnutrition status on the associations of each lipid particle with all-cause, cardiovascular and non-cardiovascular mortality were calculated. In all analyses, statistical significance was set at the level of *p* < 0.05. All statistical analyses were performed using statistical software SAS version 9.4 (SAS Campus Drive, Cary, NC, USA) and Stata 16.1 (Stata Corporation, College Station, TX, USA).

Results

Baseline characteristics

Baseline characteristics of the entire cohort, as well as after stratification between presence of inflammation/malnutrition, are shown in Table 1. Median [interquartile range] age of the entire cohort was 67 [56–76] years, and 56.3% suffered from inflammation/malnutrition. Patients with inflammation/malnutrition were significantly older and had a higher prevalence of diabetes mellitus, cardiovascular diseases and lower statin usage compared with the non-inflammation/non-malnutrition group (all *p* < 0.05; Table 1). Furthermore, patients with inflammation/malnutrition had slightly lower levels of total cholesterol and HDL cholesterol compared to subjects without inflammation/malnutrition (all *p* < 0.05; Table 1) but did not differ in circulating levels of non-HDL cholesterol, LDL cholesterol, as well as TG (all *p* > 0.05; Table 1).

Table 1 Baseline characteristics of the entire study cohort and after stratification based on the presence of inflammation and/or malnutrition

	Entire cohort	Patients without inflammation/malnutrition	Patients with inflammation/malnutrition	<i>p</i> -value
<i>N</i>	5,382	2,352 (43.7%)	3,030 (56.3%)	
Age (years)	67 (56–76)	65 (52–74)	69 (58–77)	<0.001
Sex (male) (%)	3,234 (60.1)	1,388 (59.0)	1,846 (61.0)	0.140
Diabetes mellitus (%)	2,026 (37.6)	795 (33.8)	1,231 (40.6)	<0.001
CV disease (%)	3,171 (58.9)	1,259 (53.5)	1,912 (63.1)	<0.001
Statin users (%)	2,436 (45.3)	1,133 (48.2)	1,303 (43.0)	<0.001
Smokers (%)	3,599 (66.9)	1,545 (65.7)	2,054 (67.8)	0.100
Serum albumin (g/l)	38 (34–41)	40 (38–43)	35 (32–38)	<0.001
CRP (mg/l)	6.9 (2.5–17.9)	3.2 (1.3–5.5)	15.6 (7.5–31.9)	<0.001
BMI (kg/m ²)	25.8 (22.8–29.4)	25.7 (22.8–29.2)	25.8 (22.8–29.6)	0.160
SBP (mmHg)	138 (120–153)	139 (122–154)	136 (120–153)	0.021
DBP (mmHg)	70 (61–80)	71 (63–80)	70 (60–80)	<0.001
Hb (g/l)	106 (95–119)	112 (100–123)	102 (92–114)	<0.001
Total protein (g/l)	67 (62–71)	68 (64–72)	65 (60–70)	<0.001
Calcium (mmol/l)	2.2 (2.0–2.3)	2.2 (2.1–2.4)	2.1 (2.0–2.3)	<0.001
Phosphate (mmol/l)	1.5 (1.2–1.8)	1.5 (1.2–1.8)	1.4 (1.1–1.8)	<0.001
PTH (ng/l)	227.1 (123.1–398.0)	238.0 (131.0–408.4)	219.5 (116.4–386.4)	<0.001
eKt/V	1.3 (1.0–1.5)	1.3 (1.1–1.5)	1.3 (1.0–1.5)	0.014
All-cause mortality (%)	2,021 (37.6)	670 (28.5)	1,351 (44.6)	<0.001
CV mortality (%)	917 (17.0)	304 (12.9)	613 (20.2)	<0.001
Non-CV mortality (%)	1,104 (20.5)	366 (15.6)	738 (24.4)	<0.001
Total chol. (mg/dl)	163.3 (138.2–193.4)	166.2 (142.2–195.5)	161.3 (134.2–193.3)	<0.001
HDL chol. (mg/dl)	39.5 (32.0–49.1)	42.1 (34.1–51.1)	37.9 (30.0–47.1)	<0.001
Non-HDL chol. (mg/dl)	121.2 (96.7–150.2)	121.2 (97.8–149.3)	120.6 (96.1–151.2)	0.550
LDL chol. (mg/dl)	89.6 (67.6–114.4)	88.9 (68.0–114.5)	90.1 (67.2–114.3)	0.750
TG (mg/dl)	142.1 (102.1–205.2)	143.1 (103.0–205.2)	141.7 (101.1–204.2)	0.470

BMI, body mass index; BP, blood pressure; Chol, cholesterol; CRP, C-reactive protein; CV, cardiovascular; DBP, diastolic blood pressure; Hb, haemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; SBP, systolic blood pressure; TG, triglycerides.

Data are presented as median (IQR) for continuous measures and n (%) for categorical measures. *p* values for differences between patients with or without inflammation/malnutrition were assessed by non-parametric Mann–Whitney U-test for continuous parameters or chi-squared test for categorical variables, and significant *p* values (< 0.05) are depicted in bold.

Associations of baseline lipid profile with mortality

After stratifying the cohort into quartiles of each baseline lipid particle, patients in quartile 2–4 were compared to the reference group with lowest lipid levels, respectively. Patients with total cholesterol levels in quartile 2–4 had a significantly lower all-cause and cardiovascular mortality compared to patients in quartile 1 (Table S2). Furthermore, patients with highest HDL cholesterol levels in

quartile 4 had a lower all-cause, cardiovascular and non-cardiovascular mortality compared to the reference group (Table S2). Moreover, patients in the second quartile of non-HDL cholesterol had a lower all-cause and cardiovascular mortality compared to the reference group (Table S2). In addition, patients with higher circulating LDL cholesterol had a lower all-cause mortality compared to patients in the lowest LDL cholesterol group (Table 2). Furthermore, patients in the

quartile 2 had a lower cardiovascular mortality, whereas patients in quartile 4 had lower non-cardiovascular mortality, compared with the reference group, that is quartile 1 (Table S2). In contrast, baseline TG were not associated with all-cause, cardiovascular and non-cardiovascular mortality in the entire cohort (Table S2). Importantly, effect estimates did not significantly differ between patients with and without inflammation and malnutrition (interaction p -values for inflammation/malnutrition status >0.05 , Table S2).

Associations of time-dependent lipid profile with mortality

After stratifying the cohort into quartiles of each time-dependent lipid particle, patients in quartile 2–4 were compared to the reference group with lowest lipid levels, respectively. Higher circulating total cholesterol was significantly and gradually associated with lower all-cause, cardiovascular and non-cardiovascular mortality compared with patients in quartile 1 (Table 2). HR [95% confidence interval] was lowest for patients in quartile 4 vs. 1: 0.633 [0.533;0.752] (all-cause mortality); 0.669 [0.523;0.857] (cardiovascular mortality); 0.603 [0.468;0.777] (non-cardiovascular mortality) (all $p < 0.05$; Table 2). Interaction effects of inflammation/malnutrition on the outcome were non-significant ($p > 0.05$; Table 2).

Patients with time-dependent HDL cholesterol levels in quartiles 2–4 had a lower all-cause, cardiovascular and non-cardiovascular mortality compared with the reference group (Table 2). Lowest HR was found in patients in quartile 3 (i.e. HDL cholesterol 39–48 mg/dl) vs. quartile 1: 0.705 [0.592;0.840] (all-cause mortality); 0.719 [0.556;0.930] (cardiovascular mortality); and 0.681 [0.529;0.877] (non-cardiovascular mortality) (all $p < 0.05$; Table 2). There was no effect of inflammation/malnutrition status on the outcome, respectively ($p > 0.05$; Table 2).

Patients in the 1st quartile of time-dependent non-HDL cholesterol had a higher all-cause, cardiovascular and non-cardiovascular mortality (Table 2). Thus, HR for all-cause and non-cardiovascular mortality gradually decreased in quartiles 2–4 compared with the reference group with lowest HR observed in quartile 4 for all-cause mortality (0.650 [0.542;0.779]) and non-cardiovascular mortality (0.597 [0.458;0.779]) (all $p < 0.05$; Table 2). HR for cardiovascular mortality was lowest in

quartile 2 (0.708 [0.560;0.894] ($p = 0.004$; Table 2). There was no interaction of inflammation/malnutrition on the outcome ($p > 0.05$; Table 2).

Patients with higher circulating LDL cholesterol had a lower all-cause, cardiovascular and non-cardiovascular mortality compared with patients in quartile 1 with lowest HR for all-cause (0.694 [0.581;0.829]) and non-cardiovascular mortality (0.661 [0.511;0.857]) in quartile 4 and for cardiovascular mortality (0.727 [0.571;0.924]) in quartile 2 (all $p < 0.05$; Table 2). Inflammation/malnutrition status was not a confounder of the observed associations ($p > 0.05$; Table 2).

Patients with time-dependent TG levels in the highest quartile had a lower all-cause (0.709 [0.591;0.851]) and non-cardiovascular (0.611 [0.462;0.808]) mortality compared with the reference group (all $p < 0.05$; Table 2) and inflammation/malnutrition status did not have an effect on the outcome ($p > 0.05$; Table 2).

As interaction p -values for inflammation/malnutrition status were non-significant for all lipid markers and all outcome analyses (Table 2), results, therefore, did not depend on the presence of inflammation/malnutrition.

Effect of potential confounders

To determine the effect of inflammation/malnutrition on the observed associations in more detail, all-cause (Table S3), cardiovascular (Table S4) and non-cardiovascular (Table S5) mortality were analysed separately in patients with or without inflammation/malnutrition, respectively (Fig. 1). In general, the associations between lipid profile and mortality were attenuated but comparable in patients with and without inflammation/malnutrition (Tables S3–S5, Fig. 1). Thus, patients without inflammation/malnutrition showed significant and reduced HR for all-cause mortality especially for total cholesterol and quartile 3 of non-HDL cholesterol (Table S3, Fig. 1). However, the direction of the adjusted HR estimates is comparable in terms of numbers and the direction is always similar to patients with inflammation/malnutrition (Table S3, Fig. 1). Importantly, we do not observe any inverse relationship of lipid particles in patients with inflammation/malnutrition compared with non-inflamed/non-malnourished patients (Table S3, Fig. 1) as reported by Liu

Table 2 Multivariate hazard regression analysis of groups of different time-dependent lipid parameters for all-cause mortality, cardiovascular mortality and non-cardiovascular mortality in the entire cohort (N = 5,382)

Quartile	N	All-cause mortality				Cardiovascular mortality				Non-cardiovascular mortality			
		HR	p	95% Conf. Interval	Pinteraction	HR	p	95% Conf. Interval	Pinteraction	HR	p	95% Conf. Interval	Pinteraction
		1.000				1.000				1.000			
Total chol.													
Q1 (<137 mg/dl)	1345	1.000				1.000				1.000			
Q2 (138–162 mg/dl)	1345	0.709	<0.001	0.612	0.822	0.680	0.001	0.545	0.849	0.754	0.007	0.613	0.926
Q3 (163–192 mg/dl)	1338	0.705	<0.001	0.604	0.822	0.718	0.004	0.572	0.900	0.692	0.001	0.554	0.865
Q4 (>193 mg/dl)	1354	0.633	<0.001	0.533	0.752	0.669	0.001	0.523	0.857	0.603	<0.001	0.468	0.777
				0.682						0.868			0.130
HDL chol.													
Q1 (<31 mg/dl)	1108	1.000				1.000				1.000			
Q2 (32–38 mg/dl)	1165	0.818	0.015	0.696	0.961	0.812	0.088	0.639	1.031	0.853	0.174	0.677	1.073
Q3 (39–48 mg/dl)	1097	0.705	<0.001	0.592	0.840	0.719	0.012	0.556	0.930	0.681	0.003	0.529	0.877
Q4 (>49 mg/dl)	1176	0.827	0.033	0.695	0.985	0.846	0.196	0.656	1.090	0.790	0.067	0.614	1.016
				0.754						0.544			0.104
Non-HDL chol.													
Q1 (<95 mg/dl)	1136	1.000				1.000				1.000			
Q2 (196–120 mg/dl)	1133	0.721	<0.001	0.618	0.842	0.708	0.004	0.560	0.894	0.703	0.002	0.564	0.875
Q3 (121–149 mg/dl)	1123	0.701	<0.001	0.596	0.826	0.754	0.020	0.594	0.956	0.670	0.001	0.530	0.847
Q4 (>150 mg/dl)	1154	0.650	<0.001	0.542	0.779	0.715	0.012	0.551	0.929	0.597	<0.001	0.458	0.779
				0.618						0.051			0.845
LDL chol.													
Q1 (<670.6 mg/dl)	1120	1.000				1.000				1.000			
Q2 (670.6–890.7 mg/dl)	1121	0.756	0.001	0.645	0.887	0.727	0.009	0.571	0.924	0.766	0.020	0.613	0.959
Q3 (890.7–1140.6 mg/dl)	1121	0.755	0.001	0.641	0.890	0.763	0.029	0.599	0.972	0.732	0.009	0.579	0.926
Q4 (>1140.6 mg/dl)	1120	0.694	<0.001	0.581	0.829	0.744	0.024	0.576	0.962	0.661	0.002	0.511	0.857
				0.932						0.682			0.929
Triglycerides													
Q1 (<101 mg/dl)	1296	1.000				1.000				1.000			
Q2 (102–141 mg/dl)	1300	1.021	0.780	0.884	1.178	0.972	0.795	0.782	1.207	1.114	0.295	0.911	1.362
Q3 (142–204 mg/dl)	1302	0.883	0.126	0.740	1.035	0.916	0.464	0.724	1.159	0.884	0.290	0.704	1.111
Q4 (>205 mg/dl)	1305	0.709	<0.001	0.591	0.851	0.861	0.252	0.666	1.112	0.611	0.001	0.462	0.808
				0.538						0.126			0.994

Multivariate hazard regression analysis adjusted for age, sex, presence of diabetes mellitus, presence of cardiovascular diseases, presence of cancer, dialysis vintage, smoking status, equilibrated Kt/V (eKt/V), presence of inflammation/malnutrition, calcium, phosphate, parathyroid hormone, haemoglobin, statin treatment, region and hospitalization. Conf. Interval, confidence interval; HR, hazard ratio; all other abbreviations are indicated in Table 1. Groups were defined according to quartiles (Q) and Q1 was used as the reference group for all lipid parameters. HR, 95% CI and p values for comparisons against Q1, as well as for interaction effects of inflammation/malnutrition on the outcome, are given, and significant p values (< 0.05) are depicted in bold.

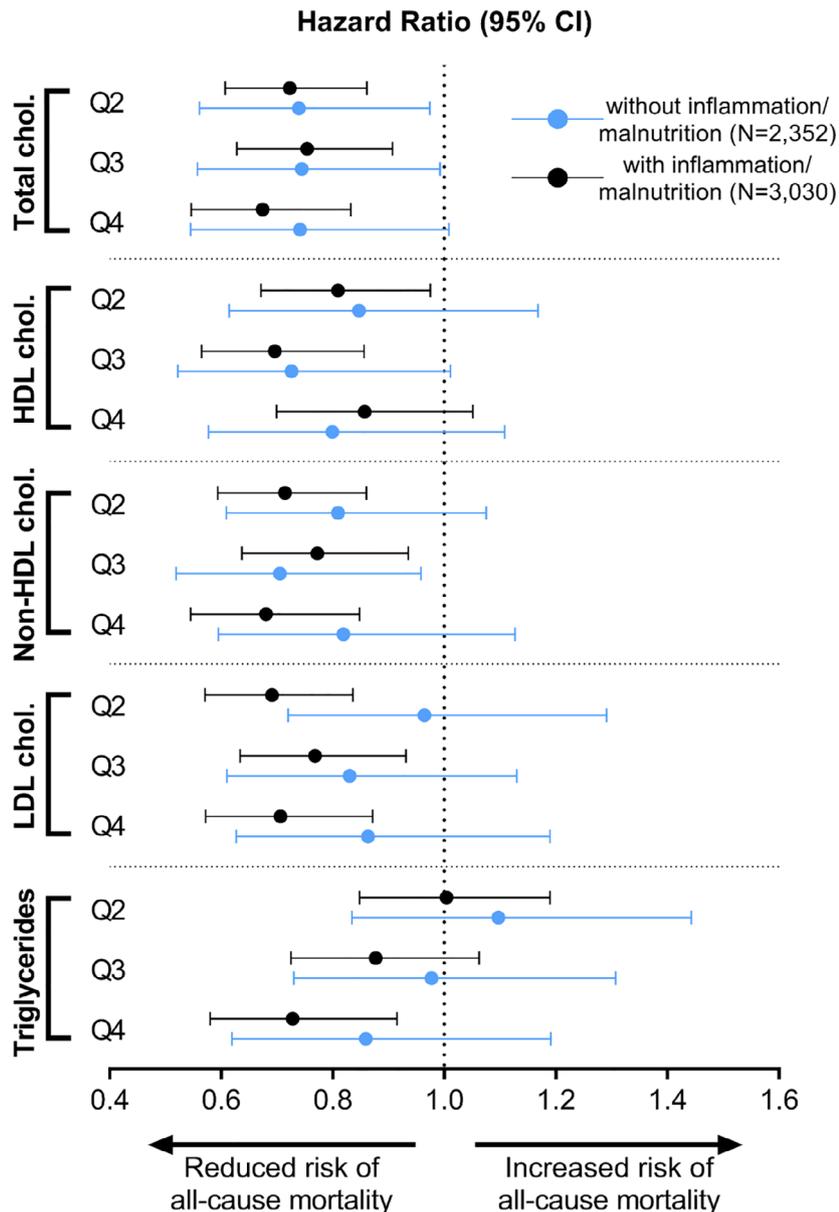


Fig. 1 Multivariate hazard regression analysis of quartiles of different time-dependent lipid parameters for all-cause mortality in patients with (black lines/circles) and without (blue lines/circles) inflammation/malnutrition. Multivariate hazard regression analysis was adjusted for age, sex, presence of diabetes mellitus, presence of cardiovascular diseases, presence of cancer, dialysis vintage, smoking status, equilibrated Kt/V, calcium, phosphate, parathyroid hormone, haemoglobin, statin treatment, region and hospitalization. Groups were defined according to quartiles (Q), and Q1 was used as the reference group for all lipid parameters (indicated as dotted line). Circles indicate adjusted hazard ratios (HR); lines indicate 95% confidence intervals. Chol, cholesterol; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

et al. [14]. Non-significant interaction p values for inflammation/malnutrition status on the observed associations (all $p > 0.05$, Table 2) confirmed

that effect estimates do not differ significantly between patients with and without inflammation/malnutrition.

Furthermore, we stratified the entire analyses by statin usage. In patients not on statin treatment (Table S6), associations in general were slightly weaker but comparable to the subgroup of patients receiving statin treatment (Table S7).

To investigate potential socio-economic differences, we have stratified the analysis for geographical area of the FMC dialysis facilities, that is Western (Table S8) vs. Central/Eastern (Table S9) Europe according to [4]. In general, the associations between lipid profile and outcome were comparable between patients treated in Western vs. Central/Eastern European countries (Tables S8 and S9).

To determine potential sex-specific associations, we have re-calculated the analysis for both sexes separately (Table S10). Here, results on all-cause mortality remain virtually the same, albeit some associations were weaker (cholesterol particles) or stronger (TG) in male compared with female subjects (Table S10). We do not observe significant interaction *p*-values for inflammation/malnutrition status in sex-specific analyses supporting our results in the entire cohort (Table S10).

Sensitivity analyses

We further re-analysed our cohort applying exactly the same time lag of four months, as well as lipid thresholds for baseline total cholesterol and non-HDL cholesterol previously used by Liu *et al.* [14] (Table S11). Using these cut-offs, baseline total cholesterol and non-HDL cholesterol were not associated with all-cause mortality in the entire cohort and in patients with or without inflammation/malnutrition, except for inflamed/malnourished patients with cholesterol and non-HDL cholesterol levels in group 2 (Table S11).

We have re-calculated the time-dependent analyses using a different CRP cut-off at 3 mg/l similar to the SHARP trial [27]. When using this definition of inflammation/malnutrition, that is CRP \geq 3 mg/l [27] and/or serum albumin $<$ 36 g/l, we confirm (Table S12) the results of our primary analysis applying a CRP cut-off at 10 mg/l based on Liu *et al.* [14] (Table 2). Thus, both HRs and *p*-values are comparable to the primary analyses (Table S12; Table 2). Furthermore, interaction *p*-values for inflammation/malnutrition status on all associations remained non-significant, except for HDL cholesterol and cardiovascular mortality (all

p $>$ 0.05, Table S12). As CRP assays differed at the local laboratories at each FMC facility, we did not use a lower CRP cut-off.

Discussion

We investigated the association of a full lipid profile comprising five clinically well-established lipid biomarkers on all-cause, cardiovascular and non-cardiovascular mortality in a large, multicentre cohort of incident HD patients with a median follow-up of 3.2 years using time-dependent analyses. Our main finding is the absence of a significant effect of inflammation/malnutrition on most of the associations between time-dependent lipid profile and mortality (Fig. 1). Furthermore, we show reduced all-cause, cardiovascular and non-cardiovascular mortality with increasing lipid levels.

A counter-intuitive association between cholesterol measurements and low mortality in dialysis patients has been demonstrated, and 12 out of 13 identified studies have reported a negative or non-significant association between total cholesterol or LDL cholesterol and mortality in dialysis patients [28]. These data are further supported by negative outcome trials of statins in patients on HD [29,30]. Possible explanations for the cholesterol paradox include reverse epidemiology, a unique phenotype and cardiovascular risk factor profile in ESKD [31], cholesterol-mediated endotoxin binding [10,32], an altered composition of cholesterol particles in the uremic milieu [33] and inflammation and/or malnutrition [14]. Liu *et al.* [14] reported an association between low cholesterol levels and all-cause mortality in a combined cohort of HD and PD patients only in the presence of inflammation/malnutrition. In patients without inflammation/malnutrition, the same association between higher cholesterol concentrations and outcomes was observed as in the general population. In contrast to these findings on baseline lipids, we show that the associations between time-dependent lipid profile and mortality outcomes are comparable in HD patients with or without inflammation/malnutrition. Furthermore, interaction *p*-values are non-significant for all lipid biomarkers and all outcomes. Importantly, when using baseline lipid values and the same criteria for inflammation/malnutrition as Liu *et al.* [14], we also cannot confirm an overall effect of inflammation/malnutrition on the association between lipid profile and mortality in our large European HD cohort. A number of

reasons potentially account for the difference between our data and the findings from Liu *et al* [14]: 1) the sample size of the two cohorts significantly differs and our cohort comprising 5,382 subjects is substantially larger than the cohort from Liu *et al.* [14] comprising 823 subjects. 2) Non-inflamed patients in our cohort are well-balanced ($N = 2,352$; 44% of the total population) with inflamed ones, whereas the non-inflamed group from Liu *et al.* [14] was smaller ($N = 189$; 23% of the total population). 3) The two cohorts differ in follow-up time (our cohort: 3.2 years vs 2.4 years) [14]. 4) Whereas ethnicity is heterogeneous in the US cohort, our data are based on an ethnically homogenous European cohort.

The SHARP study has also investigated the association between LDL cholesterol and cardiovascular diseases in CKD patients with and without inflammation as measured by CRP [27]. In this randomized controlled trial, a similar efficacy of lowering LDL cholesterol in non-dialysis and dialysis subgroups irrespective of CRP was reported [31], supporting our findings. Whereas the overall results of both cohorts are comparable, several differences between SHARP trial and our AROii cohort need to be pointed out: In contrast to the SHARP trial, we have investigated a pure HD cohort, whereas SHARP recruited patients with moderate-to-severe CKD with about 67% of all participants being not on dialysis [34]. Furthermore, in the subanalysis of the SHARP trial [27], a different CRP cut-off was used. Moreover, we have included measures of malnutrition/protein-energy wasting frequently observed in CKD and contributing to mortality [13]. Thus, our data confirm some of the results of the SHARP trial in our vulnerable HD patient group.

Inflammation/malnutrition modifies the association between lipid levels and a cardiovascular composite outcome including death in a cohort of African Americans with mild-moderate CKD [35]. Despite the smaller cohort, different ethnicity and less robust definition of inflammation/malnutrition [35]; these data further support the hypothesis that patients on dialysis show a unique phenotype and mortality risk and that they cannot be compared to mild-moderate CKD.

Besides total cholesterol and LDL cholesterol, our data on time-dependent non-HDL cholesterol associations are in accordance with results from a large US cohort of HD patients reporting a negative

association of baseline and time-dependent non-HDL cholesterol with all-cause and cardiovascular mortality [24]. Our time-dependent associations of higher HDL cholesterol levels with reduced all-cause, cardiovascular and non-cardiovascular mortality are confirmatory [36,37]. Recent data from the Monitoring Dialysis Outcomes (MONDO) database confirm the negative association of time-dependent HDL cholesterol with all-cause mortality [37]. Furthermore, baseline HDL cholesterol levels up to 50 mg/dl (corresponding to our quartile 3) have been related to better survival in HD also supporting our time-dependent analyses [36]. The negative association of time-dependent TG with mortality only at very high levels (>205 mg/dl) in our study supports a previous study showing negative baseline associations of TG with mortality at TG levels >200 mg/dl, which are lost after adjustment for inflammation/malnutrition [38]. Collectively, our data support the current guidelines recommending not to start lipid-lowering pharmaceutical treatment in patients on haemodialysis [39].

It should be emphasized that most of the previous studies have related baseline or left-truncated (e.g. three months) lipid values with outcome. In the present study, we have used time-dependent, left-truncated (six months) lipid parameters to associate lipid profile with mortality independent of longitudinal changes in the lipid profile of each individual patient. This is of importance, as patients on HD show a very high mortality in the first months after dialysis initiation [23]. Thus, in contrast to previous studies, our results are not affected by dysregulated lipid and inflammatory status around time of dialysis initiation. Interestingly, when using baseline lipid markers, all effect estimates besides Q4 for TG show the same effect direction, although they are a bit weaker and, in some cases, not significant anymore (Table S2). This could be explained by a dilution of effects over the long observation period, which might not be predictive anymore after several years (median [interquartile range] follow-up time: 3.2 [1.6–5.2] years). It should also be noted that although significance was attenuated, our results remain virtually the same when statin users and non-statin users, as well as patients from Western and Central/Eastern Europe, were analysed separately (Tables S2–S9).

Limitations of the present study include inclusion of solely European FMC dialysis centres in an observational fashion, as well as the formula calculation of LDL cholesterol. Since the direct

measurement of LDL cholesterol heavily depends on the assay, this probably would have resulted in more misclassifications considering the large number of involved facilities [40]. In contrast to Liu *et al* [14], our study analysed a full lipid profile comprising of five clinically well-established lipid markers, the use of sophisticated time-dependent analyses with robust adjustment for multiple variables using a validated clinical database, as well as a high number of patients and well-defined mortality outcomes.

In conclusion, we report that time-dependent total, HDL, non-HDL, LDL cholesterol and, to a less extent, also TG, are inversely associated with all-cause, cardiovascular and non-cardiovascular mortality in incident European HD patients. In contrast to smaller studies from the United States, we find that inflammation/malnutrition is not a confounder of the associations between lipid parameters and mortality in incident HD patients.

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Conflict of interest

PS has served on scientific advisory boards of Baxter, Astra Zeneca, Vifor and REATA and has received honoraria from Astellas, Amgen, Fresenius and Pfizer. TE has served as a consultant for Sanofi-

Aventis, as well as on a scientific advisory board of Boehringer Ingelheim Pharma. FK serves in scientific advisory boards of Kaneka and Amgen. JF has received honoraria from Amgen, Bayer, Fresenius, Vifor. James F has received speaker honoraria from Fresenius medical care and conducts research funded by Vifor Pharma and Novartis. This specific research is funded by a National Institute for Health Research Clinician Scientist Award.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline characteristics of the entire study cohort and after stratification based on the excluded and included patients.

Table S2. Multivariate hazard regression analysis of groups of different baseline lipid parameters for all-cause mortality, cardiovascular mortality, and non-CV mortality in the entire cohort ($N = 5,382$).

Table S3. Multivariate hazard regression analysis of groups of different time-dependent lipid parameters for all-cause mortality stratified by inflammation/malnutrition status.

Table S4. Multivariate hazard regression analysis of groups of different time-dependent lipid parameters for cardiovascular mortality stratified by inflammation/malnutrition status.

Table S5. Multivariate hazard regression analysis of groups of different time-dependent lipid parameters for non-cardiovascular mortality stratified by inflammation/malnutrition status.

Table S6. Multivariate hazard regression analysis of quartiles of different time-dependent lipid parameters for all-cause mortality, cardiovascular (CV) mortality, and non-CV mortality in all patients not on statin treatment ($N = 2,946$).

Table S7. Multivariate hazard regression analysis of quartiles of different time-dependent lipid parameters for all-cause mortality, cardiovascular (CV) mortality, and non-CV mortality in all patients on statin treatment ($N = 2,436$).

Table S8. Multivariate hazard regression analysis of groups of different time-dependent lipid parameters for all-cause mortality, cardiovascular (CV) mortality, and non-CV mortality in patients being treated in Western European dialysis facilities.

Table S9. Multivariate hazard regression predictor of groups of different time-dependent lipid parameters for all-cause mortality, cardiovascular (CV) mortality, and non-CV mortality in patients being treated in Central/Eastern European dialysis facilities.

Table S10. Multivariate hazard regression analysis of groups of different time-dependent lipid parameters for all-cause mortality stratified by sex in the entire cohort ($N = 5,382$).

Table S11. Multivariate hazard regression analysis of groups of baseline total cholesterol and baseline non-HDL cholesterol for all-cause mortality in the entire cohort ($N = 5,382$), as well as stratified by inflammation/malnutrition status according to Liu *et al.* (13).

Table S12. Multivariate hazard regression analysis of groups of different time-dependent lipid parameters for all-cause mortality, cardiovascular mortality, and non-cardiovascular mortality in the entire cohort ($N = 5,382$) using a different definition of inflammation/malnutrition, that is CRP ≥ 3 mg/l [26] and/or serum albumin <36 g/l. ■