Blood Calcification Propensity, Cardiovascular Events, and Survival in Patients Receiving Hemodialysis in the EVOLVE Trial

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Abstract

Background and objectives Patients receiving hemodialysis are at risk of cardiovascular events. A novel blood test (T_{50} test) determines the individual calcification propensity of blood.

Design, setting, participants, & measurements T_{50} was determined in 2785 baseline serum samples of patients receiving hemodialysis enrolled in the Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events (EVOLVE) trial and the T_{50} results were related to patient outcomes.

Results Serum albumin, bicarbonate, HDL cholesterol, and creatinine were the main factors positively/directly and phosphate was the main factor negatively/inversely associated with T_{50}. The primary composite end point (all-cause mortality, myocardial infarction [MI], hospitalization for unstable angina, heart failure, or peripheral vascular event [PVE]) was reached in 1350 patients after a median follow-up time of 619 days. After adjustments for confounding, a lower T_{50} was independently associated with a higher risk of the primary composite end point as a continuous measure (hazard ratio [HR] per 1 SD lower T_{50}, 1.15; 95% confidence interval [95% CI], 1.08 to 1.22; $P<0.001$). Furthermore, lower T_{50} was associated with a higher risk in all-cause mortality (HR per 1 SD lower T_{50}, 1.10; 95% CI, 1.02 to 1.17; $P=0.001$), MI (HR per 1 SD lower T_{50}, 1.38; 95% CI, 1.19 to 1.60; $P<0.001$), and PVE (HR per 1 SD lower T_{50}, 1.22; 95% CI, 1.05 to 1.42; $P=0.01$). T_{50} improved risk prediction (integrated discrimination improvement and net reclassification improvement, $P<0.001$ and $P=0.001$) of the primary composite end point.

Conclusions Blood calcification propensity was independently associated with the primary composite end point, all-cause mortality, MI, and PVE in the EVOLVE study and improved risk prediction. Prospective trials should clarify whether T_{50}-guided therapies improve outcomes.

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Introduction

Patients receiving hemodialysis suffer from a dramatically increased cardiovascular (CV) morbidity and mortality compared with age-matched persons with normal or near normal kidney function (1,2). A large portion of this excess risk is attributable to CV causes, which can only partially be explained by the traditional CV risk factors BP, cholesterol, smoking, body mass index (BMI), and diabetes (3,4). Rather, so-called nontraditional CV risk factors (5,6) reflecting disturbances in bone and mineral metabolism appear to play an important role (7,8). Current therapeutic concepts are accordingly aimed at lowering elevated serum concentrations of phosphate and parathyroid hormone (PTH), which are associated with an increased mortality in patients receiving hemodialysis (9,10,11) and with dystrophic calcification within vascular walls and cardiac valves (12,13). Such ectopic calcifications are often present even at young age (14,15) and may progress rapidly (16). The number of sites with calcified vessels (17) and the degree of calcifications at specific sites have been associated with mortality in this patient population (18–20).

Recently, a novel functional *in vitro* test (T_{50} test) for the determination of calcification propensity in blood was developed (21). This test quantifies the calcification inhibition inherent in blood by challenging the patient's serum with supersaturated calcium and phosphate solutions. This leads to the instantaneous formation of primary calciprotein particles (CPP). The timing of the spontaneous transformation of these particles into secondary CPP depends on the individual composition of serum, and more specifically on the concentrations and interplay of well established calcification-inhibiting factors, including the proteins fetuin-A and albumin, and the small molecules calcium, phosphate, magnesium, pyrophosphate, and others (21,22). A shorter transformation time indicates a more rapid precipitation of calcium and phosphate in the presence of serum and lower T_{50} values have been associated with higher risk of worse outcome in cohort studies. Specifically, the T_{50} test

was recently shown to predict all-cause mortality and to outperform the predictive value of its individual components in patients with stages 3 and 4 CKD (22) and in kidney transplant recipients (23). Furthermore, the T_{50} value was closely associated with progressive stiffening of the aorta (22).

The Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events (EVOLVE) trial (24) is the largest (in size) and longest (in duration) prospective, randomized, placebo-controlled event-driven CV outcome trial performed in patients with secondary hyperparathyroidism receiving hemodialysis. This trial was conducted over 64 months and assessed the benefits and risks of cinacalcet (Mimpara/Sensipar) compared with placebo on all-cause mortality and major CV events (24). As the T_{50} test is a composite of established nontraditional CV risk factors related to bone and mineral metabolism, we hypothesized that the T_{50} result would be associated with the primary composite end point (all-cause mortality, myocardial infarction [MI], hospitalization for unstable angina, heart failure, or peripheral vascular event [PVE]), as well as its individual components.

Materials and Methods

Study Population and Design

The EVOLVE trial was an interventional trial which assessed the benefits and risks of cinacalcet (Mimpara/Sensipar) compared with placebo. The dose of cinacalcet was titrated according to PTH and calcium levels from 30 mg/d to a maximum dose of 180 mg/d. A tabulated overview of the baseline treatments relevant to mineral metabolism is given at the end of Supplemental Table 1. Blood was drawn before the first dialysis session of the week. Additional serum samples were collected in nonrandomly preselected 394 out of 467 sites from patients who had agreed to contribute additional serum samples for additional studies beyond the original EVOLVE study. Details on the patient characteristics at baseline (25), the study design (26), and the primary trial results (24) have been reported previously. The EVOLVE trial was sponsored by Amgen Inc. and was led by an academic executive committee. Ethics committee approval was obtained from all sites and all patients gave informed consent. The trial was registered under ClinicalTrials.gov number, NCT00345839.

Determination of Serum Calcification Propensity (T_{50})

For this *post hoc* analysis, serum calcification propensity was measured in a blinded manner at a single site (21) in 2785 patients with stored baseline (*i.e.*, week 0) serum samples (stored at −70°C throughout without thawing). In short, serum samples were challenged with highly concentrated calcium and phosphate solutions to induce the formation of CPP. Pipetting was performed with a high precision manual pipetting device using a 96-channel pipetting head (Liquidator; Mettler Toledo, Greifensee, Switzerland). The ripening and spontaneous transformation from primary to secondary CPP was then monitored in a time-resolved manner using a standard nephelometer (Nephelostar; BMG Labtech, Ortenberg, Germany). The results of these measurements were used to calculate the one-half maximal transition time (T_{50}). Upon analysis of the T_{50} values with reference to storage duration, no significant association between storage duration and T_{50} values was found: Period 1, September of 2006 to March of 2007: T_{50} median (10th percentile, 90th percentile): 215 (110, 326); Period 2, March of 2007 to August of 2007: 207 (106, 327); Period 3, August of 2007 to January of 2008: 214 (111, 334). The intra-assay coefficients of variation of standards precipitating at 130, 170, and 400 minutes were 3.3%, 3.2%, and 6.0%, respectively. The inter-assay coefficients of variation of standards precipitating at 120, 260, and 390 minutes were 7.8%, 5.1%, and 5.9%, respectively.

Clinical Study End Points

The EVOLVE primary composite end point was the time to death or the first nonfatal CV event (myocardial infarction, hospitalization for unstable angina, heart failure, or a PVE). PVE was defined as lower limb amputation for peripheral vascular disease, revascularization procedure for peripheral vascular disease, or hospitalization for ischemic rest pain with documented gangrene/tissue necrosis. Secondary end points included the time to the individual components of the primary composite end point, death from CV causes, and a tertiary CV composite end point (CV mortality, MI, heart failure, and hospitalization for unstable angina). All end points were adjudicated by an independent clinical events classification group.

Statistical Analyses

All randomized patients with baseline serum T_{50} were included in these analyses. To assess the associations of baseline variables and T_{50}, we performed a general multivariable linear regression analysis using a backward elimination procedure at significance level of 0.10. Demographics, patient characteristics, prior CV history, and laboratory measures at baseline were assessed as factors potentially associated with baseline T_{50}.

To examine the association of T_{50} and clinical events, hazard ratios (HRs) (per 1 SD decrease) and 95% confidence intervals (95% CIs) were calculated using Cox proportional hazards regression models. We performed multivariable analysis which adjusted for baseline covariates using a backward selection procedure at a significance level of 0.10. Potential baseline covariates assessed include patient characteristics, demographics, concomitant medication use, CV disease history, and laboratory measures (Supplemental Table 2).

Harrell C statistic was calculated to assess the capacity of the estimated risk score of the fitted survival model. Improvement in risk prediction was assessed using integrated discrimination improvement (IDI) and net reclassification improvement (NRI) methods. Three-year risk IDI and NRI were calculated accounting for time-to-event data using SAS macro and PROC PHREG (27).

Kaplan–Meier event-free survival times were computed and compared T_{50} groups using a two-sided log-rank test, stratified by country and diabetes status. Baseline quintile cutpoints were used to define the T_{50} groups within each randomized group.

As previously reported (24), the primary analysis of the EVOLVE trial did not reach statistical significance (unadjusted log-rank test using the intention-to-treat approach).

The analyses presented herewith were not adjusted for multiplicity and we considered two-tailed P values <0.05 nominally statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Results

Study Population

Of the 3883 randomized patients, calcification propensity (T_{50}) was determined in 2785 (72%) patients ("T_{50} cohort"), who had additional serum samples collected at baseline. Median (10th–90th percentiles) T_{50} was 212 (109–328) minutes in the T_{50} cohort. Median (10th –90th percentiles) age was 54 (34–73) years with 41% women and 57% white participants. Median Quetelet index (BMI) was 26 (20–36) kg/m^2 and dialysis vintage was 47 (10–148) months. One third of patients had a history of diabetes mellitus, and 26% were current smokers. The T_{50} cohort was representative of the overall population of patients randomized in the EVOLVE trial (Supplemental Table 3, Table 1).

Baseline characteristics of the T_{50} cohort were comparable among patients randomized to placebo (n=1366) and cinacalcet (n=1419) (Supplemental Table 4, Table 1). Median (10th–90th percentiles) baseline serum T_{50} was 216 (111–333) minutes and 209 (108–323) minutes in the placebo and cinacalcet groups, respectively (P=0.16).

Factors Associated with T_{50}

Baseline serum T_{50} levels were nearly normally distributed (Supplemental Figure 1). Supplemental Table 1 gives an overview of the distribution of factors associated with demographics, race and region, medical history, laboratory parameters, and medications by quintile of T_{50}. Interestingly, increasing T_{50} quintiles were associated with decreasing quintiles of factors associated with bone resorption (intact PTH, N-telopeptide, phosphorus) and increasing quintiles of factors associated with bone formation (alkaline phosphatase, bone-specific alkaline phosphatase, 1,25-dihydroxy vitamin D). In the multivariate general linear regression model, lower T_{50} values (*i.e.,* lower calcium phosphate crystallization resistance) were associated with older age, men, nonwhite race, lower serum albumin, bicarbonate, creatinine, HDL cholesterol, triglyceride, and 1,25-dihydroxy vitamin D concentrations and higher serum urea nitrogen and phosphate concentrations (Table 2). Notably, variables not associated with serum T_{50} included the traditional CV risk factors: smoking, hypertension, LDL cholesterol, diabetes mellitus, and BMI. Furthermore, the nontraditional risk factors PTH, alkaline phosphatase, and corrected serum calcium had dropped out of the model during the backward selection procedure.

T_{50} and Clinical Outcomes

The primary composite end point (all-cause mortality, MI, hospitalization for unstable angina, heart failure, or PVE) was reached in 671 of 1366 patients (49%) randomized to placebo, and by 679 of 1419 randomized to cinacalcet (48%). Figure 1A shows the Kaplan–Meier curves for the primary composite end point of the placebo group, and Figure 1B for the cinacalcet group, according to quintiles of T_{50}. In both groups, the groups/quintiles with higher T_{50} values had a significantly better outcome than those with a lower T_{50} value. This was also the case when the single components of the composite end point, *i.e.,* all-cause mortality, MI, and PVE, as well as the tertiary CV

Table 1. Baseline demographics of all EVOLVE patients and the T_{50} cohort

Demographics	All EVOLVE Patients (n=3883)	T_{50} Cohort		
		All Patients (n=2785)	Placebo Group (n=1366)	Cinacalcet Group (n=1419)
Age, yr	55 (35–73)	54 (34–73)	54 (34–72)	55 (34–74)
Women, %	41	41	40	41
BMI, kg/m^2	26 (21–37)	26 (20–36)	26 (20–36)	26 (21–37)
Race or ethnic group, %				
White	57.7	57.4	57.0	57.8
Black	21.6	19.8	19.8	19.9
Other	20.8	22.8	23.2	22.3
Dialysis vintage, mo	45 (9–146)	47 (10–148)	48 (11–152)	46 (9–145)
Current tobacco use, %	27.4	26.2	26.5	26.0
Hypertension, %	92.1	91.7	91.9	91.6
Diabetes (types 1 and 2), %	33.6	31.4	31.7	30.9
Coronary artery disease, %	24.5	23.5	22.5	24.5
Dyslipidemia, %	39.6	37.4	36.9	37.9
Calcification propensity (T_{50}), min		212 (109–328)	216 (111–333)	209 (108–323)
iPTH, pg/ml	693 (363–1694)	705 (371–1734)	698 (372–1717)	707 (371–1755)
Serum calcium, mg/dl	9.8 (9.0–10.7)	9.8 (9.0–10.7)	9.8 (9.0–10.7)	9.8 (9.0–10.7)
Serum phosphorus, mg/dl	6.2 (4.9–8.4)	6.3 (4.9–8.4)	6.2 (4.9–8.4)	6.3 (4.9–8.3)
Serum albumin, g/dl	3.7 (3.2–4.1)	3.7 (3.2–4.1)	3.7 (3.2–4.1)	3.7 (3.2–4.1)

Data are given as median (p10–p90) unless stated otherwise. Mean (SD) T_{50} was 215 (84) minutes in the T_{50} cohort. The "other" race or ethnic group includes Hispanic ethnicity. EVOLVE, Evaluation of Cinacalcet Therapy to Lower Cardiovascular Events; BMI, body mass index; iPTH, intact parathyroid hormone.

Table 2. Factors associated with T_{50}, identified by multivariate linear regression

Parameter	Estimate (min)	95% CI	*P* Value
Intercept	249	(236 to 261)	<0.001
Epidemiologic variables			
Age, yr	−0.46	(−0.67 to −0.25)	<0.001
Women (versus men)	9.95	(3.91 to 15.99)	0.001
Ethnicity (versus white)			
Black	−29.18	(−36.76 to −21.60)	<0.001
Other	−29.54	(−36.30 to −22.77)	<0.001
Blood values per 1 SD increase			
Serum phosphorus, mg/dl	−45.23	(−48.13 to −42.33)	<0.001
Albumin, g/dl	9.08	(6.26 to 11.90)	<0.001
Bicarbonate, mEq/L	6.45	(3.36 to 9.55)	<0.001
1,25(OH)D, pg/ml	3.86	(1.09 to 6.62)	0.006
HDL, mg/dl	6.71	(3.63 to 9.78)	<0.001
Triglycerides, mg/dl	7.97	(4.96 to 10.98)	<0.001
BUN, mg/dl	−6.66	(−10.07 to −3.26)	<0.001
Creatinine, mg/dl	9.41	(5.62 to 13.19)	<0.001

The "estimate" column quantifies the difference in T_{50} value related to an increment of 1 SD in the corresponding parameter value (continuous parameters) or comparing the two groups of a binary parameter. A positive estimate indicates a positive correlation between the parameter and T_{50} value, and a negative estimate indicates a negative correlation between the parameter and T_{50} value. Only variables that were found significant by the backward selection processes were included in the table. Variables assessed: age (years), sex, race group, cause of renal disease, history of diabetes, history of smoking, dialysis vintage (years), weight (kg), BMI (kg/m^2), systolic BP (mmHg), diastolic BP (mm/Hg), pulse pressure (systolic–diastolic BP), parathyroid hormone (pg/ml), corrected serum calcium (mg/dl), serum phosphorus (mg/dl), calcium phosphate product (mg2/dl^2), bone alkaline phosphatase (ng/ml), alkaline phosphatase (U/L), BUN (mg/dl), creatinine (mg/dl), albumin (g/dl), total protein (g/dl), sodium (mEq/L), potassium (mEq/L), chloride (mEq/L), bicarbonate (mEq/L), N-telopeptide (nmol/L), 25 hydroxy vitamin D (ng/ml), 1,25(OH)D (pg/ml), hemoglobin (g/dl), glucose (mg/dl), triglycerides (mg/dl), total cholesterol (mg/dl), HDL (mg/dl), LDL (mg/dl), uric acid (mg/dl), fibroblast growth factor 23 (pg/ml), baseline vitamin D use, baseline calcium phosphate binder use, β-adrenergic antagonists use, Angiotensin-converting enzyme-inhibitors/angiotensin receptor blocker use, antiplatelet use, statin use, erythropoietin use, iron use. Magnitude of SD: albumin 0.35 g/dl, bicarbonate 3.85 mEq/L, creatinine 18.29 mg/dl, HDL 2.85 mg/dl, phosphorus 1.49 mg/dl, triglycerides 1.41 mg/dl, 1,25(OH)$_2$D 10.24 pg/dl. 95% CI, 95% confidence interval; 1,25(OH)D, 1,25 dihydroxy vitamin D.

composite end point (CV mortality, MI, heart failure, and hospitalization for unstable angina) were considered separately in the placebo group. In the cinacalcet group, statistical significance was reached for all-cause mortality and MI, and was close for all-cause mortality (log rank P=0.05) and PVE (log rank P=0.06, Supplemental Figure 2).

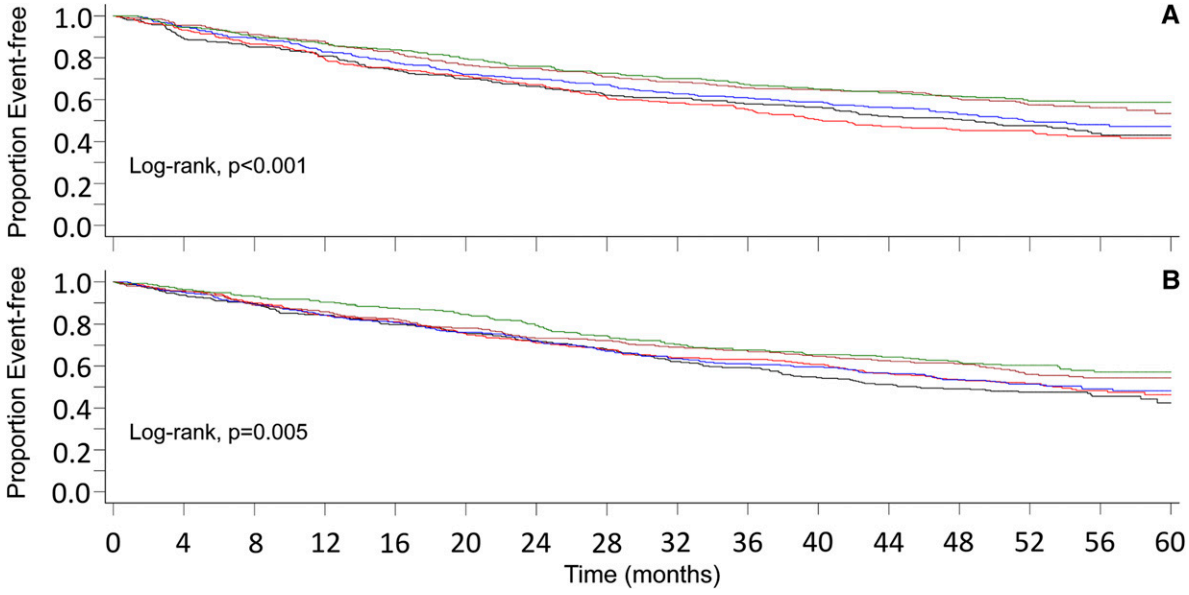

Figure 1. | Kaplan–Meier curves of the primary composite end point demonstrate that quintiles of T_{50} are significantly associated with outcome. (A) Placebo and (B) cinacalcet treatment group. Colors of lines representing T50 quintiles: lower: black, lower mid: red, middle: blue, upper mid: brown, upper: green.

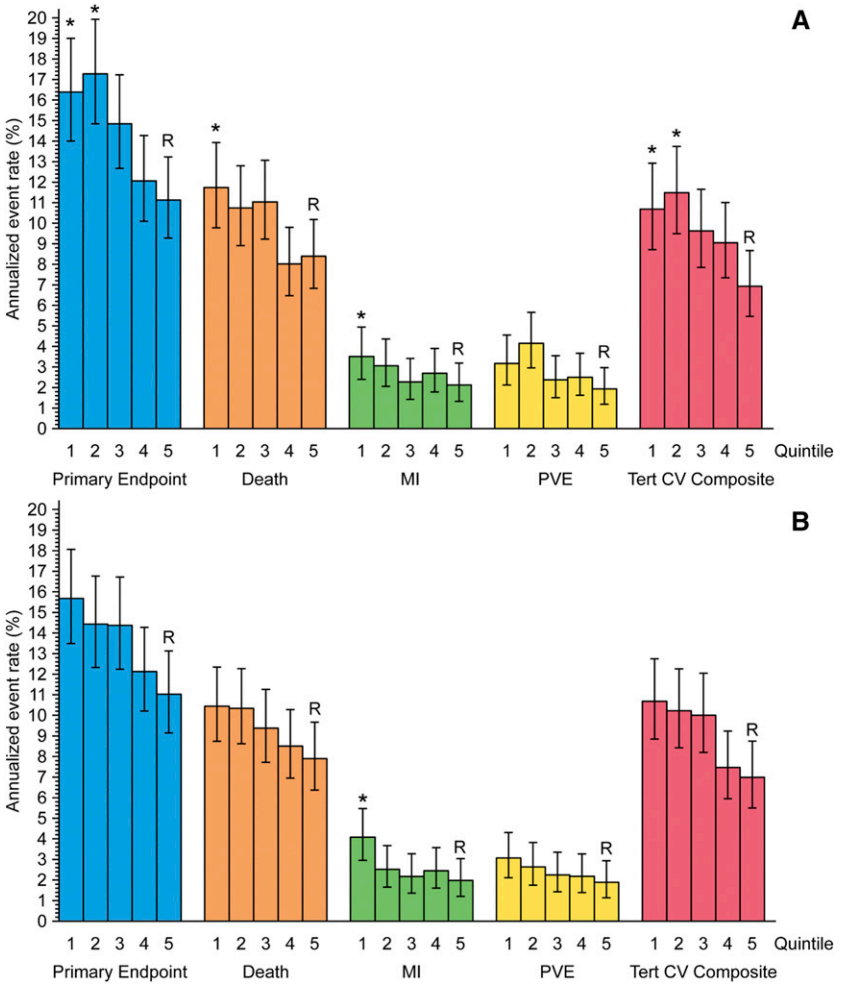

Figure 2. | **T_{50} is associated with annualized event rates according to quintiles of T_{50}.** (A) Placebo and (B) cinacalcet group. | represent 95% confidence intervals. Asterisks indicate *P* values <0.05 from a multivariable adjusted Cox regression analysis in which T_{50} was included as a categoric variable (quintiles, Supplemental Table 6). Quintile 5 was the reference group. "*R*" means the reference group. "*" indicates that the subjects in this T_{50} quintile have significantly different annualized event rate compared with subjects in the 5th quintile. The T_{50} quintile ranges for the placebo group were, q1: 49–138 minutes; q2: 139–189 minutes; q3: 190–236 minutes; q4: 237–289 minutes; q5: 290–521 minutes. The T_{50} quintile ranges for the cinacalcet group were, q1: 42–134 minutes; q2: 135–184 minutes; q3: 185–234 minutes; q4: 235–285 minutes; q5: 286–550 minutes. MI, myocardial infarctions; PVE, peripheral vascular events; Tert CV Composite, tertiary composite end point of the EVOLVE study.

Figure 2 shows graphical representations of the annualized event rates by end point and quintiles of T_{50} in the placebo (Figure 2A) and the cinacalcet group (Figure 2B) where T_{50} was put into the adjusted Cox regression analysis as a categoric variable. With reference to quintile 5, significant differences were found in the placebo group for the primary composite end point (first and second quintile), death (first quintile), MI (first quintile), and the tertiary CV composite (first and second quintile) and in the cinacalcet group for MI (first quintile). Supplemental Table 2 provides general information about the variables and Supplemental Table 5 detailed information about the significant variables in the final Cox regression model for the primary composite end point.

When T_{50} was considered a continuous variable in the pooled data, which had of note been adjusted for planned cinacalcet treatment, T_{50} was associated with the primary composite end point independent of other important baseline factors (HR per 1 SD decrease, 1.15; 95% CI, 1.08 to 1.22). The association was also observed for all-cause mortality (HR per 1 SD decrease, 1.10; 95% CI, 1.02 to 1.17), myocardial infarction (HR per 1 SD decrease, 1.38; 95% CI, 1.19 to 1.60), and PVE (HR per 1 SD decrease, 1.22; 95% CI, 1.05 to 1.42) (Figure 3). The Harrell C statistics calculated from the Cox regression model were 0.65 (95% CI, 0.60 to 0.70) both adjusting and not adjusting for baseline T_{50}. Harrell C statistic is limited in its ability to quantify improvement in model performance after addition of a new marker (28). The IDI at 3-year risk was 0.004 (95% CI, 0.003 to 0.008; $P<0.001$) and NRI at 3-year risk was 0.02 (95% CI, 0.01 to 0.04; $P=0.001$).

Discussion

The T_{50} test is a novel *in vitro* blood test, which enumerates the propensity of individual sera to resist mineralization by measuring the timing of the transformation from primary to secondary CPP (21). Supplemental Figures 3 and 4 show the factors associated with the transformation from primary to secondary CPP found in this study and a

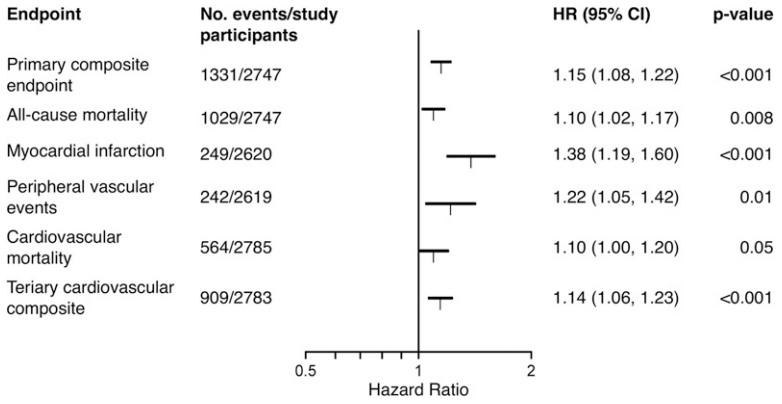

Figure 3. | **T_{50} is associated with clinical outcomes.** Forest plot showing the magnitude of the association between lower T_{50} values and worse outcome per SD of T_{50}, (84 minutes). T_{50} was not significantly associated with unstable angina or heart failure, therefore these end points are not shown here. 95% CI, 95% confidence interval; HR, hazard ratio.

schematic illustration of the T_{50} test principle, respectively. Here we observed an association between the results of the T_{50} test and the primary composite end point of the EVOLVE study and several of its individual components. Primarily nontraditional CV risk factors related to disorders of mineral metabolism appear to play an important role in patients receiving hemodialysis (29–32). Previous reports have linked these factors individually to vascular calcifications (12,33,34) and to survival (10,34,35) in cohorts with CKD. It has been speculated that several of these factors may serve as surrogate markers (*e.g.*, albumin for inflammation [36]), or may be directly involved in active cellular processes (*e.g.*, phosphate as a trigger of the osteogenic transformation of vascular smooth muscle cells [37]), or the mechanism has remained elusive (*e.g.*, bicarbonate has not been attributed a specific role other than serving as a buffer involved in acid-base disturbances). Interestingly, BUN and creatinine had opposing effects in the multivariate regression analysis. Although the reason for these opposing effects is not known, we speculate that it might rather reflect a negative association with protein catabolism (BUN) and a positive association with muscle mass (creatinine) than with the urea and creatinine molecules themselves.

The individual associations of factors like phosphate, bicarbonate, and albumin with vascular calcifications and survival have been thoroughly studied, but the possibility of a direct functional interplay of these factors has not been tested because of the lack of a suitable *in vitro* test system. Such a direct interplay is however apparent in the calcification cascade, which proceeds in aqueous solutions from the formation of amorphous calcium phosphate to the transient formation of octacalcium phosphate, *i.e.*, a hydroxyapatite precursor, and the final product hydroxyapatite (38,39), the main constituent of physiologic (bone and teeth) and pathologic (soft tissue and vasculature) calcifications. This sequence of events also occurs in body fluids like serum, where it is however a vastly delayed and regulated process integrated into a system of colloidal chemistry (Supplemental Figure 3) (40,41). Besides phosphate (destabilizing), also magnesium, pyrophosphate, and bicarbonate (all stabilizing) and likely other yet unidentified factors influence this process. Also pointing toward a functional interplay, a recent study reported that the serum magnesium concentration modifies the CV mortality risk associated with hyperphosphatemia (42). Lending plausibility to the major factors we found to be associated with T_{50} in our study, these are largely congruent with factors identified in previous *in vitro* experiments (*i.e.*, phosphate, albumin [21]) and/or have been identified in previous clinical studies (phosphate, albumin, bicarbonate [22,23,43]).

The T_{50} test is a functional test, which can conceptually best be compared with functional clotting tests. While these tests give an estimate of the efficiency of an individual's clotting system to form fibrin cross-links, the T_{50} test provides an estimate of the efficiency of an individual's anticalcification system to inhibit the formation of calcium phosphate nanocrystals. Both tests are not representative of any single "independent" factor but instead depend as composite functional tests on the presence and interaction of multiple factors. Therefore, adjusting for factors already known to affect T_{50} will weaken the association between the T_{50} test result and the clinical outcome. For this reason, we did not include bone and mineral-related factors associated with T_{50} in our regression analyses.

Patients receiving hemodialysis suffer from an excess morbidity and mortality largely related to CV events (44). Unfortunately, controlled clinical outcome trials investigating the effects of intensified dialysis (45), lipid-lowering (46), or phosphate-binder choice (47) in patients receiving dialysis did not enhance survival or reduce the risk of CV events. The recognition, description, and characterization of the functional humoral mineralization system appears relevant, as it may help to better understand the pathophysiology and mechanisms involved in CV events in patients with CKD (22,23). Of note, the T_{50} test improved prediction despite the inclusion of clinical variables like "history of myocardial infarction" and "history of PVEs" which are strongly associated with future events and could therefore have considerably weakened the predictive value of the T_{50} test, as CV events may be in its "causal pathway." It is tempting to speculate that going forward, the T_{50} test may guide multimodal and personalized interventions with the aim to *e.g.*, co-ordinate meaningful phosphate-lowering and magnesium- and bicarbonate-increasing interventions.

Although the precise mechanism(s) which links T_{50} with outcome has not been elucidated in our study, the nature

of the test indicates a role of the propensity of serum to form calcium phosphate nanocrystals.

Limitations of our analysis include that the EVOLVE trial only studied patients with moderate-to-severe secondary hyperparathyroidism receiving hemodialysis; hence, the findings reported here may not extend to other clinical settings, including patients new to dialysis, patients with mild-to-moderate CKD, or patients with only mild disturbances of mineral metabolism. This *post hoc* analysis also precludes conclusions concerning the causality of our findings. A further shortcoming is that vascular calcification itself was not evaluated in EVOLVE. Although the NRI and IDI were statistically significant, their magnitude as well as the magnitude of the c-statistic was modest. Finally, we only evaluated the predictive value of T_{50} testing on baseline samples. Longitudinal T_{50} testing could improve diagnostic accuracy. Major strengths of our study include the large sample size, diverse by age, sex, race/ethnicity, and geographic origin; detailed clinical characterization; and adjudication of CV end points.

In summary, we observed associations between lower T_{50} and higher risk of death, MI, and PVE in patients of the EVOLVE trial. The nature of the T_{50} test indicates that pathologic disturbances of serum-based calcification resistance provide a mechanism which links T_{50} to the pronounced morbidity and mortality in patients receiving hemodialysis. Prospective interventional studies are needed to determine whether these associations can be causally linked.

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Disclosures

A.P. is an employee and stock holder of Calciscon. W.J.-D. is a stock holder of Calciscon; G.M.C. reports to receive grant support from Amgen; E.R.S. reports to hold stock in Calciscon and to receive grant support from Amgen and Baxter. J.F. reports consulting fees from Chugai, Bayer, Fresenius, Vifor, and lecture fees from Amgen, Vifor, Fresenius, Shire. P.P. reports lecture fees from Amgen. G.A.B. reports consulting fees from Amgen and to own stock in Ardelyx; X.M. is an employee of Amgen. M.B. is a part-time employee of Calciscon. S.A. has nothing to disclose. Rheinisch-Westfälische-Technische Hochschule Aachen, University of Aachen, Germany has submitted a patent regarding the T_{50} test, licenced to Calciscon. This study was supported by a grant from Amgen for the measurement of T_{50}, which was carried out in the research lab of A.P. Ethical approval: None required for this ancillary *post hoc* analysis.

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