

Original Paper

Association of Growth Differentiation Factor 15 with Mortality in a Prospective Hemodialysis Cohort

Amy S. You^a Kamyar Kalantar-Zadeh^a Lorena Lerner^b Tracy Nakata^a
Nancy Lopez^a Lidia Lou^a Mary Veliz^a Melissa Soohoo^a Jennie Jing^a
Frank Zaldivar^c Jenő Gyuris^b Danh V. Nguyen^c Connie M. Rhee^a

^aHarold Simmons Center for Kidney Disease Research and Epidemiology, University of California Irvine, Orange, CA, ^bAVEO Oncology, Cambridge, MA, and ^cInstitute for Clinical and Translational Science, University of California Irvine, Irvine, CA, USA

Keywords

Growth differentiation factor 15 · Wasting · Cardiovascular disease · Mortality · Dialysis

Abstract

Background/Aims: Cardiovascular disease and protein-energy wasting are among the strongest predictors of the high mortality of dialysis patients. In the general population, the novel cardiovascular and wasting biomarker, growth differentiation factor 15 (GDF15), is associated with decreased survival. However, little is known about GDF15 in dialysis patients. **Methods:** Among prevalent hemodialysis patients participating in a prospective study (October 2011 to August 2015), we examined the association of baseline GDF15 levels with all-cause mortality using unadjusted and case mix-adjusted death hazard ratios (HRs) that controlled for age, sex, race, ethnicity, diabetes, and dialysis vintage. **Results:** The mean age \pm SD of the 203 patients included in the study was 53.2 ± 14.5 years, and the cohort included 41% females, 34% African-Americans, and 48% Hispanics. GDF15 levels (mean \pm SD 5.94 ± 3.90 ng/mL; range 1.58–39.8 ng/mL) were higher among older patients and were inversely associated with serum creatinine concentrations as a surrogate for muscle mass. Each 1.0 ng/mL increase in GDF15 was associated with an approximately 17–18% higher mortality risk in the unadjusted and case mix models ($p < 0.05$). Increments of about 1 SD (a 4.0 ng/mL increase in GDF15) were associated

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Kamyar Kalantar-Zadeh, MD, MPH, PhD
Harold Simmons Center for Chronic Disease Research and Epidemiology
University of California Irvine School of Medicine
101 The City Drive South, City Tower, Suite 400, Orange, CA 92868 (USA)
E-Mail kkkz@uci.edu

with a nearly 2-fold higher death risk. The highest GDF15 tertile was associated with higher mortality risk (reference: lowest tertile): the HRs (95% CI) were 3.19 (1.35–7.55) and 2.45 (1.00–6.00) in the unadjusted and the case mix-adjusted model, respectively. These incremental death trends were confirmed in cubic spline models. **Conclusion:** Higher circulating GDF15 levels are associated with higher mortality risk in hemodialysis patients. Future studies are needed to determine whether GDF15 may represent a novel therapeutic target for cardiovascular disease, wasting, and death in this population.

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Introduction

Hemodialysis patients have an exceedingly high mortality risk compared to the general population, largely due to cardiovascular causes (40% of deaths) [1]. However, conventional risk stratification tools remain imprecise in establishing cardiovascular prognoses in this population [2]. Furthermore, traditional Framingham risk factors explain only a fraction of the high frequency of cardiovascular disease and death among hemodialysis patients [3]. Hence, there has been increasing interest in identifying novel prognostic biomarkers that can more effectively identify high-risk individuals who may benefit from more intensive prevention and treatment.

In the general population, growth differentiation factor 15 (GDF15) is a protein (molecular weight 28 kDa) in the transforming growth factor- β superfamily that has emerged as a potential marker of cardiovascular disease and death [4–7]. Under normal physiologic conditions, GDF15 is weakly expressed by most tissues [8, 9]. However, following injury, ischemia, and other forms of oxidative and/or metabolic stress, its production is potently upregulated by a wide range of tissues including activated macrophages, cardiomyocytes, and vascular smooth muscle cells [10–12]. In large population-based studies, GDF15 has been associated with endothelial dysfunction, atherosclerosis, left ventricular hypertrophy, and impaired systolic function, independent of cardiovascular risk factors such as inflammatory markers (e.g., C-reactive protein) [13, 14]. In studies of healthy community-dwelling adults, as well as of those who have sustained recent myocardial infarction, elevated GDF15 levels have been linked with a higher incidence of congestive heart failure, myocardial infarction, stroke, or other cardiovascular events [15–17]. Furthermore, among patients with preexisting heart failure, higher GDF15 levels have been associated with higher risk of heart failure events and death [18, 19].

Notably, GDF15 has also been identified as a novel appetite regulator that causes anorexia and weight loss when overexpressed in malignancy [20]. Indeed, in cancer-associated cachexia, circulating GDF15 levels correlate with weight loss, lower lean body and fat mass, weaker handgrip strength, and worse survival [21]. These findings bear particular relevance to the hemodialysis population, in whom weight loss and protein-energy wasting are among the most potent predictors of mortality, including cardiovascular death [22–26].

While limited data suggest that circulating GDF15 levels are higher in kidney dysfunction [20], there have been few studies that have examined the prognostic significance of this marker in hemodialysis patients [27, 28]. Thus, to better inform the field, we sought to examine the association between GDF15 levels and mortality risk in a large, racially/ethnically diverse cohort of hemodialysis patients from the prospective, multicenter Malnutrition, Diet, and Racial Disparities in Chronic Kidney Disease (MADRAD) study who underwent rigorous, protocolized measurements of clinical and laboratory characteristics.

Subjects and Methods

Source Population

The study population was recruited from the MADRAD cohort (Clinicaltrials.gov No. NCT01415570) examining racial and ethnic differences in dietary factors and nutritional status among hemodialysis patients [29]. In this substudy, patients were enrolled from 6 outpatient dialysis units in Los Angeles County over the period of October 2011 to November 2011 (i.e., the first semester of the MADRAD study). Patients were included provided that they had undergone serum GDF15 measurements, were aged 18–85 years at the time of study entry (i.e., the date of serum GDF15 measurement), had received thrice-weekly in-center hemodialysis for at least 4 consecutive weeks, and signed a local institutional review board approved consent form. Patients were excluded if they were actively receiving peritoneal dialysis, had a life expectancy of less than 6 months (e.g., stage IV cancer), or were unable to provide consent without a proxy (e.g., suffering from dementia). The study was approved by the Institutional Review Boards of the Los Angeles Biomedical Research Institute at Harbor-UCLA (Torrance, CA, USA) and the University of California Irvine Medical Center (Orange, CA, USA).

Exposure Ascertainment

The exposure of interest was the serum GDF15 level ascertained at study entry. GDF15 levels were measured from thawed serum samples that were obtained before dialysis during weekday hemodialysis treatments at the time of study entry and that chronologically coincided with routine blood tests conducted at outpatient dialysis facilities, and they were immediately stored at -80°C . GDF15 was measured using enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). In primary analyses, we examined the association between serum GDF15 levels, categorized into tertiles (categorized as <4.21 , 4.21 to <6.13 , and ≥ 6.13 ng/mL for tertiles 1, 2, and 3, respectively) and all-cause mortality. In secondary analyses, GDF15 was considered as a continuous variable and scaled to a 1.0 and 4.0 ng/mL (approx. 1 SD) change. To flexibly model the association between continuous GDF15 levels and mortality, we also conducted analyses in which GDF15 was examined as a restricted cubic spline with knots corresponding to the 33rd and 66th percentiles of observed GDF15 values (4.18 and 6.08 ng/mL, respectively).

Outcome Ascertainment

The primary outcome of interest was all-cause mortality. At-risk time began the day after serum GDF15 measurement, and the patients were censored for kidney transplantation, transfer to a nonaffiliated outpatient dialysis unit or peritoneal dialysis, or at the end of the study (August 27, 2015). Each semester, information regarding mortality, censoring events, and associated dates from the preceding 6 months was collected from event forms completed by the MADRAD research coordinators and reviewed by 2 MADRAD study nephrologists (C.M.R. and K.K.-Z.) [29].

Sociodemographic, Comorbidity, and Laboratory Characteristics

Information on sociodemographics, comorbid conditions, and dialysis treatment characteristics were collected at study entry and every semester thereafter by MADRAD research coordinators [29]. Dialysis vintage was defined as the time between the date of study entry and the date of hemodialysis initiation. Routine dialysis laboratory measurements were performed by the outpatient dialysis laboratories on a monthly or quarterly basis using automated methods. In the present study, dialysis laboratory measurements (serum albumin, creatinine, phosphorus, and hemoglobin) were conducted on the date of or within 30 days of GDF15 measurement.

Statistical Analyses

Baseline characteristics between exposure groups were compared using p for trend tests calculated by ANOVA or Cochran-Armitage trend tests. We first examined the relationship of relevant clinical characteristics to high serum GDF15 levels at study entry (defined as GDF15 >66 th percentile) using logistic regression. We then estimated the association between serum GDF15 tertiles and all-cause mortality using Cox proportional-hazard models. Logistic regression and the Cox regression models were analyzed using 2 incremental levels of covariate adjustment:

1. Unadjusted model: included serum GDF15 level as the primary exposure of interest
2. Case mix analyses: adjusted for covariates in the unadjusted model, as well as for age, sex, race, ethnicity, diabetes, and dialysis vintage

Table 1. Baseline characteristics according to growth differentiation factor 15 (GDF15) levels categorized as tertiles in maintenance hemodialysis patients

	Overall	Tertile 1 <4.21 ng/mL	Tertile 2 4.21 to <6.13 ng/mL	Tertile 3 ≥6.13 ng/mL	<i>p</i> value ^a
Patients, % (<i>n</i>)	100.0 (203)	33.0 (67)	33.5 (68)	33.5 (68)	N/A
<i>Case mix characteristics</i>					
Mean age ± SD, years	53.2 ± 14.5	47.0 ± 14.1	53.7 ± 14.9	58.9 ± 11.9	<0.001
Female, %	41	45	43	35	0.26
Black race, %	34	42	38	21	0.009
Hispanic ethnicity, %	48	40	47	57	0.05
Diabetes, %	54	46	54	62	0.07
Mean dialysis vintage ± SD, months	55.6 ± 53.4	46.8 ± 49.2	68.7 ± 64.3	51.1 ± 42.6	0.65
<i>Laboratory tests^b</i>					
Serum albumin, g/dL	4.1 (3.8, 4.3)	4.1 (3.9, 4.3)	4.1 (3.8, 4.3)	4.0 (3.8, 4.3)	0.25
Creatinine, mg/dL	9.5 (7.5, 11.9)	10.1 (7.6, 13.3)	9.6 (8.0, 11.7)	8.9 (7.3, 10.6)	0.01
Phosphorus, mg/dL	4.8 (4.1, 5.7)	4.9 (4.1, 6.3)	4.8 (4.0, 5.8)	4.7 (4.1, 5.5)	0.28
Hemoglobin, g/dL	10.6 (9.9, 11.0)	10.4 (9.9, 10.9)	10.6 (9.9, 11.0)	10.7 (10.0, 11.2)	0.18
Body mass index	26.2 (23.5, 31.0)	27.1 (23.1, 34.5)	26.9 (23.6, 31.5)	25.5 (23.6, 28.7)	0.003
Normalized protein catabolic rate, g/kg/day	1.04 (0.85, 1.22)	0.97 (0.83, 1.16)	1.06 (0.11, 0.86)	1.05 (0.86, 1.23)	0.27

^a *p* for trend estimated by ANOVA or Cochran-Armitage trend test. ^b Laboratory test results are presented as median (IQR).

Given that higher GDF15 levels may be representative of both cardiovascular disease and wasting, we sought to parse out these potential pathways by conducting sensitivity analyses that incrementally adjusted for body mass index (BMI) and normalized protein catabolic rate (nPCR) in addition to case mix covariates in sensitivity analyses.

The proportional-hazards assumption was confirmed graphically. There were no missing values for age, sex, race, ethnicity, diabetes, and dialysis vintage. The analyses and figures were generated using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), Stata version 13.1 (Stata Corporation, College Station, TX, USA), and SigmaPlot version 12.5 (Systat Software, San Jose, CA, USA).

Results

Study Population

Among 203 patients meeting the eligibility criteria, the mean ± SD, median (IQR), and minimum-to-maximum of the obtained GDF15 values were 5.94 ± 3.90, 5.05 (3.84, 6.67), and 1.58–39.8 ng/mL, respectively. Compared with patients in the lowest GDF15 tertile, patients in the highest tertile were older, were less likely to be of Black race, were more likely to be of Hispanic ethnicity, and had lower serum creatinine levels (Table 1). Patients in the highest tertile were also more likely to be male and to have underlying diabetes, a longer dialysis vintage, and lower serum phosphorus and higher hemoglobin levels, although the differences across tertiles were not statistically significant.

Predictors of GDF15 Level

In the unadjusted and case mix-adjusted logistic regression analyses, patients of older age had a higher risk of having a high GDF15 level at study entry, whereas patients with a higher BMI had a lower likelihood of high GDF15 levels (Table 2). In the unadjusted analyses, patients of Black race and with higher serum creatinine levels had a lower likelihood of high

Table 2. Clinical characteristics associated with high growth differentiation factor 15 (GDF15) levels (>66th percentile) using logistic regression

Variable	Unadjusted		Case mix adjusted	
	OR (95% CI)	p value	OR (95% CI)	p value
<i>Case mix characteristics</i>				
Age (Δ10 years)	1.57 (1.25–1.98)	<0.001	1.58 (1.23–2.02)	<0.001
Female	0.70 (0.39–1.28)	0.25	0.62 (0.33–1.19)	0.15
Black race	0.39 (0.20–0.77)	0.007	0.43 (0.17–1.07)	0.07
Hispanic	1.73 (0.96–3.12)	0.07	1.12 (0.48–2.58)	0.80
Diabetes	1.59 (0.88–2.88)	0.13	1.00 (0.49–2.02)	0.99
Dialysis vintage (Δ1 year)	0.97 (0.90–1.04)	0.40	0.99 (0.92–1.07)	0.79
<i>Laboratory tests</i>				
Serum albumin, Δ0.5 g/dL	0.76 (0.49–1.17)	0.21	0.77 (0.47–1.26)	0.30
Creatinine (mg/dL)	0.89 (0.80–0.98)	0.02	0.96 (0.85–1.10)	0.56
Phosphorus (mg/dL)	0.92 (0.75–1.11)	0.37	1.03 (0.84–1.27)	0.75
Hemoglobin (g/dL)	1.18 (0.89–1.57)	0.25	1.21 (0.88–1.68)	0.24
Body mass index	0.75 (0.58, 0.96)	0.02	0.75 (0.56, 0.99)	0.02
Normalized protein catabolic rate (g/kg/day)	1.05 (0.86, 1.28)	0.61	0.99 (0.78, 1.24)	0.90

Case mix analyses adjusted for age, sex, race, ethnicity, diabetes, and dialysis vintage. Bold values indicate significant associations with the highest GDF15 levels.

GDF15 levels, but these associations were attenuated to null following adjustment for case mix covariates.

Association of GDF15 Levels with All-Cause Mortality

The patients contributed a total of 7,633 person-months of follow-up, during which time 40 all-cause death events occurred. The median (IQR) at-risk time was 45.8 (29.7, 46.0) months. In the unadjusted analyses, incrementally higher tertiles were associated with increasingly higher numerical risk, but this did not reach statistical significance for the middle tertile (reference: lowest tertile; hazard ratios [HRs] [95% CI] 1.94 [0.77–4.86] and 3.19 [1.35–7.55] for the middle and highest tertiles, respectively) (Fig. 1; Table 3). Upon adjustment for case mix covariates, incrementally higher tertiles were again associated with higher numerical risk, but this did not achieve statistical significance for the middle tertile (reference: lowest tertile; adjusted HRs [95% CI] 1.40 [0.54–3.59] and 2.45 [1.00–6.00] for the middle and highest tertiles, respectively). A similar pattern of findings was observed in the sensitivity analyses that were adjusted for case mix covariates + BMI + nPCR (adjusted HRs [95% CI] 1.57 [0.60–4.10] and 2.62 [1.03–6.63] for the middle and highest tertiles, respectively; *p* for trend = 0.03).

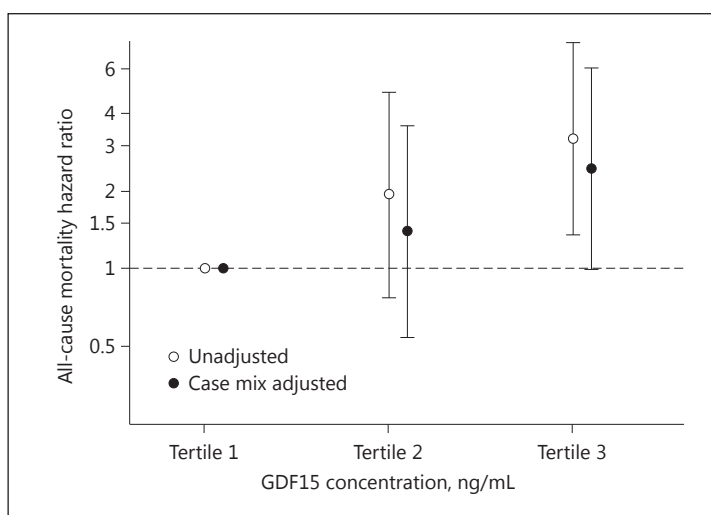
In additional analyses, we observed that a 1.0 ng/mL increase in GDF15 level was associated with an approximately 17–18% higher mortality risk in the unadjusted and case mix models (Table 3). Increments of approximately 1 SD (4.0 ng/mL increase in GDF15 level) were associated with a nearly 2-fold higher death risk. Similar estimates were observed in sensitivity analyses that adjusted for case mix covariates + BMI + nPCR.

In analyses examining the association between continuous GDF15 levels and all-cause mortality using a cubic spline function, we observed that there was a monotonic increase in death risk across higher GDF15 levels exceeding 10 ng/mL in both unadjusted and case mix-adjusted analyses (Fig. 2).

Table 3. Association of growth differentiation factor 15 (GDF15) levels with all-cause mortality

	Unadjusted		Case mix adjusted		Case mix + BMI + nPCR adjusted	
	HR (95% CI)	p value ^a	HR (95% CI)	p value ^b	HR (95% CI)	p value ^b
<i>Categorical GDF15 analyses</i>						
GDF15 tertile 1 (<4.21 ng/mL)	1 (Ref.)	N/A	1 (Ref.)	N/A	1 (Ref.)	N/A
GDF15 tertile 2 (4.21 to <6.13 ng/mL)	1.94 (0.77–4.86)	0.16	1.40 (0.54–3.59)	0.49	1.57 (0.60–4.10)	0.36
GDF15 tertile 3 (≥6.13 ng/mL)	3.19 (1.35–7.55)	0.008	2.45 (1.00–6.00)	0.05	2.62 (1.03–6.63)	0.04
<i>Continuous GDF15 analyses</i>						
ΔGDF15 by 1.0 ng/mL	1.18 (1.11–1.26)	<0.001	1.17 (1.09–1.26)	<0.001	1.18 (1.10–1.27)	<0.001
ΔGDF15 by 4.0 ng/mL	1.96 (1.51–2.55)	<0.001	1.90 (1.43–2.52)	<0.001	1.92 (1.44–2.57)	<0.001

Case mix-adjusted models adjusted for age, sex, race, ethnicity, diabetes, and dialysis vintage. BMI, body mass index; nPCR, normalized protein catabolic rate.

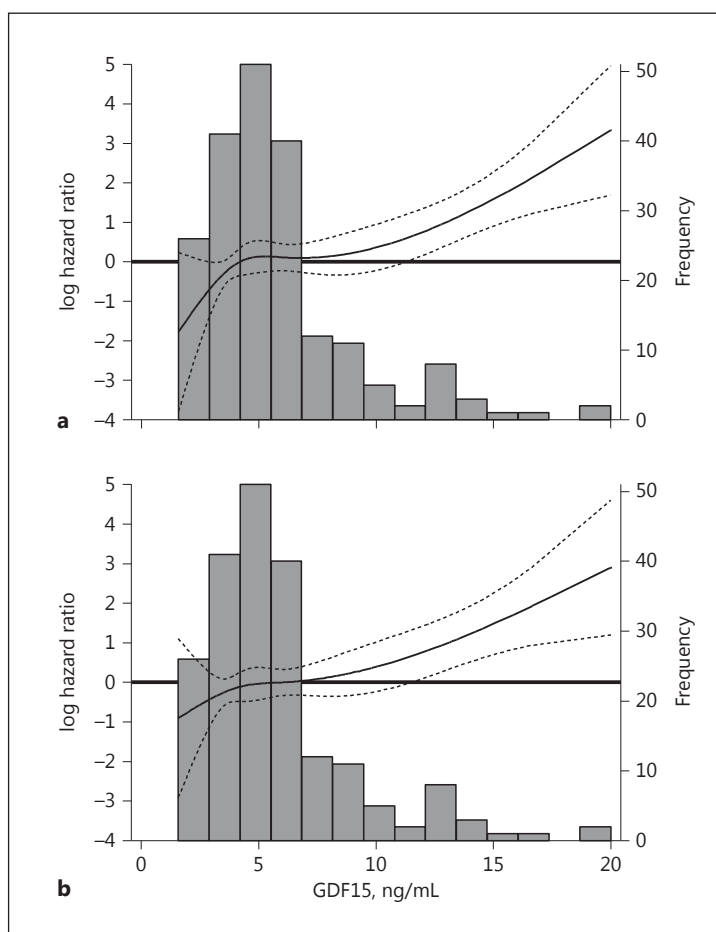
^a p for trend = 0.006. ^b p for trend = 0.03.**Fig. 1.** Association of growth differentiation factor 15 (GDF15) levels with all-cause mortality. Case mix analyses adjusted for age, sex, race, ethnicity, diabetes, and dialysis vintage.

Discussion

In this prospective contemporary cohort of maintenance hemodialysis patients from the multicenter MADRAD study with a median follow-up of approximately 4 years, we found that higher circulating levels of GDF15 were associated with higher mortality risk independent of sociodemographic and comorbidity characteristics. In cross-sectional analyses adjusted for case mix covariates, we also observed that older age was directly associated with higher GDF15 levels, whereas higher BMI was inversely associated with higher GDF15 levels.

To date, there have been 2 studies that have examined the relationship between GDF15 levels and mortality in end-stage renal disease patients [27, 28]. In one study of 381 prevalent US hemodialysis patients by Breit et al. [27], incrementally higher GDF15 levels (i.e., every 10 ng/mL increase) were independently associated with higher mortality risk. In the same study, among 98 incident hemodialysis patients from Sweden, higher GDF15 levels were independently associated with higher mortality in the first 3 years of dialysis. In the Swedish subcohort, higher GDF15 levels were also associated with worse self-reported nutrition as ascertained by Subjective Global Assessment questionnaires, as well as with lower BMIs (<25). In a subsequent study of 87 hemodialysis patients from Turkey without preexisting cardiovascular disease by Yilmaz et al. [28], higher GDF15 levels (i.e., every 10 ng/mL increase) were inde-

Fig. 2. Association between continuous growth differentiation factor 15 (GDF15) gradations and all-cause mortality using unadjusted (**a**) and case mix-adjusted (**b**) restricted cubic splines. The figures present log hazard ratios (short-dashed lines indicate 95% CIs) for GDF15 analyzed as a spline with knots at the 33rd and 66th percentiles of observed values (GDF15 values of 4.18 and 6.08 ng/mL, respectively). A histogram of the observed GDF15 values and a log hazard reference ratio of 0 (horizontal solid line) is overlaid. The case mix-adjusted model is adjusted for age, sex, race, ethnicity, diabetes, and dialysis vintage.



pendently associated with higher mortality risk as well as subclinical atherosclerosis as assessed by carotid intima media thickness.

Our study expands upon the existing literature on GDF15 in end-stage renal disease patients across several fronts. In this racially/ethnically diverse cohort of hemodialysis patients (approx. 33% Black and 50% Hispanic patients), we observed that Black patients were less likely to have high GDF15 levels compared to those of non-Black race in unadjusted cross-sectional analyses. Conversely, there was a trend towards Hispanics having a higher likelihood of high GDF15 levels, although the estimates did not achieve statistical significance. In addition, we observed a persistent association between higher GDF15 levels and higher mortality risk in this racially and ethnically heterogeneous study population. While the small sample size of our cohort precluded us from separately examining GDF15-mortality associations across individual racial/ethnic subgroups, our findings indicate that GDF15 is an adverse prognostic marker even among minority kidney disease patients despite their established differences in underlying cardiovascular risk [30–32] and body anthropometry [33–35].

Another noteworthy finding of our study was the remarkably similar distribution of GDF15 levels in comparison to that found in the aforementioned study by Breit et al. [27] (ranges of 1.4–34.4 and 3.0–25.1 ng/mL and medians of 7.1 and 7.4 ng/mL in the Swedish and US cohorts, respectively). In non-ESRD populations, an incrementally higher prevalence of elevated GDF15 levels has been observed across the cardiovascular continuum, including those patients with stable coronary artery disease, congestive heart failure, and terminal

heart failure [10]. Our data add to the growing body of literature showing that, similar to cardiovascular and cancer patients, the hemodialysis population displays higher GDF15 concentrations [28]. While a consistent association between higher GDF15 levels and kidney dysfunction has been observed [10], further studies are needed to establish the sources of GDF15 production in hemodialysis patients. Although the renal clearance of GDF15 has not yet been documented, animal studies suggest that GDF15 may undergo hepatic clearance via scavenger receptors expressed on liver sinusoidal cells [36].

At this time, the specific pathways by which GDF15 adversely impacts the survival of hemodialysis patients remain undefined. In terms of cardiovascular mechanisms, GDF15 is a product of activated macrophages [33] – which plays a prominent role in the pathogenesis of atherosclerosis and vascular thrombosis, as the final endpoint of atherosclerotic disease [37, 38]. Two recent studies have in fact shown an antiatherogenic feature of GDF15-deficient mice, suggesting that low levels of GDF15 may be cardioprotective [39, 40]. Cardiomyocytes also produce and secrete GDF15 in response to oxidative stress, ischemia, mechanical stretch, angiotensin II, and proinflammatory cytokines [41]. As cardiovascular disease models have shown that GDF15 may have apoptotic, antihypertrophic, and anti-inflammatory actions, it remains unclear as to whether GDF15 may have a counterregulatory role in cardiac injury [10]. With respect to protein-energy wasting pathways, GDF15 acts directly upon the hypothalamus to reduce food intake and energy expenditure [20]. In animal models, GDF15 administration has been shown to lead to satiety and weight reduction, which has been reversed or prevented by the administration of anti-GDF15 neutralizing antibodies [20, 26, 42]. Notably, in the sensitivity analyses we found that the relationship between higher GDF15 levels and higher mortality risk persisted following adjustment for body size (i.e., BMI) and protein intake (i.e., nPCR), suggesting that cardiovascular pathways may be implicated. However, given the large body of evidence showing the interrelationships between higher GDF15 levels, cardiovascular disease, anorexia, muscle and fat wasting, and mortality across multiple chronic disease cohorts, prospective controlled studies are needed to elucidate the causal implications of GDF15, and whether GDF15 may represent a novel therapeutic target for the fatal complications of cardiovascular disease and protein-energy wasting in these populations.

The strengths of our study include the following: its examination of a racially/ethnically diverse, multicenter study population; the uniform collection of GDF15 serum specimens in an outpatient setting and measurements within a single laboratory; and the rigorous, protocolized collection of sociodemographic, comorbidity, and laboratory data. However, several limitations of our study should be acknowledged. First, GDF15 levels were based upon measurements obtained at a single point in time upon study entry, and changes in levels over time were not considered. However, it bears mentioning that in certain analogous chronic disease populations (i.e., acute coronary syndrome patients without acute heart failure), GDF15 levels have been shown to remain stable over time [15, 43]. Second, due to data limitations, we lacked information on cause-specific death. Third, due to the modest sample size of our cohort, we had limited power to examine associations within subgroups or to adjust for a large number of confounders. Lastly, given the observational nature of our study, our findings do not confirm a causal association between elevated GDF15 levels and higher death risk.

In conclusion, our study supports an independent association between higher GDF15 levels and mortality risk in a racially/ethnically diverse cohort of hemodialysis patients. As an emerging biomarker, further studies are needed to confirm the findings, to define the pathways by which GDF15 adversely impacts survival among hemodialysis patients, and to determine whether the reduction of GDF15 concentrations favorably impacts cardiovascular and metabolic health in this population.

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Statement of Ethics

This study was approved by the Institutional Review Boards of the Los Angeles Biomedical Research Institute at Harbor-UCLA (Torrance, CA, USA) and the University of California Irvine Medical Center (Orange, CA, USA). All participants provided written informed consent.

Disclosure Statement

K.K.-Z. has received honoraria/support from AVEO Oncology.

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