

Low Plasma Level of Cathelicidin Antimicrobial Peptide (hCAP18) Predicts Increased Infectious Disease Mortality in Patients Undergoing Hemodialysis

Adrian F. Gombart,^{1,2,a} Ishir Bhan,^{3,a} Niels Borregaard,⁵ Hector Tamez,³ Carlos A. Camargo, Jr.,⁴ H. Phillip Koefler,¹ and Ravi Thadhani³

¹Department of Medicine, Division of Hematology/Oncology, Cedars-Sinai Medical Center, David Geffen School of Medicine at University of California, Los Angeles; ²Linus Pauling Institute, Department of Biochemistry & Biophysics, Oregon State University, Corvallis; ³Department of Medicine, Division of Nephrology, Massachusetts General Hospital, and ⁴Department of Emergency Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts; and ⁵The Granulocyte Research Laboratory, Department of Hematology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Background. Human cathelicidin antimicrobial protein (hCAP18) is an antimicrobial and immunomodulatory peptide that has pleiotropic effects and is transcriptionally regulated by vitamin D. Because the administration of vitamin D analogues has been linked to decreased mortality among patients with end-stage renal disease, we hypothesized that low hCAP18 levels would identify those who are at increased risk of death attributable to infection while undergoing hemodialysis.

Methods. We performed a case-control study nested in a prospective cohort of patients ($n = 10,044$) initiating incident hemodialysis. Case patients ($n = 81$) were those who died of an infectious disease within 1 year; control patients ($n = 198$) were those who survived at least 1 year while undergoing dialysis.

Results. Mean (\pm SD) baseline levels of hCAP18 in case patients and control patients were 539 ± 278 ng/mL and 650 ± 343 ng/mL, respectively ($P = .006$). hCAP18 levels had a modest correlation with 1,25-dihydroxyvitamin D levels ($r = 0.23$; $P = .053$) but not with 25-hydroxyvitamin D levels ($r = -0.06$; $P = .44$). Patients with hCAP18 levels in the lowest tertile had a 2-fold increased risk (odds ratio, 2.1; 95% confidence interval, 1.2–3.5) of death attributable to infection; after multivariable adjustment, this relationship remained statistically significant (odds ratio, 3.7; 95% confidence interval, 1.2–11.2).

Conclusions. In individuals initiating chronic hemodialysis, low baseline levels of hCAP18, a vitamin D-regulated antimicrobial protein, are independently associated with an increased risk of death attributable to infection.

Traditional immunology research has focused on the adaptive immune system and its ability to generate targeted responses against invading microbes. Innate immunity, the wide-reaching antimicrobial defense that complements more specific responses, has recently garnered increased attention with the discovery of cationic

antimicrobial peptides (AMPs). AMPs comprise a major component of immunologic defense in most multicellular organisms and have activity against gram-positive and gram-negative bacteria, as well as some viruses and fungi [1–7]. AMPs act by disrupting foreign-cell membranes, binding lipopolysaccharide residues, and recruiting leukocytes [1, 8, 9]. The development of resistance is uncommon [10]. These properties have generated excitement about the possibility that AMPs could be used in place of or as a complement to traditional antibiotics, either through exogenous administration or through manipulation of endogenous production [9, 11].

Cathelicidins, one of the major families of AMPs, are characterized by a proprotein with a cathelin-like

Received 9 July 2008; accepted 29 October 2008; electronically published 9 January 2009.

^a A.F.G. and I.B. contributed equally to this article.

Reprints or correspondence: Dr. Ravi Thadhani, Center for D-receptor Activation Research, Massachusetts General Hospital, 55 Fruit St., Bulfinch 127, Boston, MA 02114 (rthadhani@partners.org).

Clinical Infectious Diseases 2009;48:418–24

© 2009 by the Infectious Diseases Society of America. All rights reserved.

1058-4838/2009/4804-0008\$15.00

DOI: 10.1086/596314

N-terminal domain and a C-terminal AMP domain; the pro-protein is cleaved by proteases during activation to release the active peptide [12, 13]. The human cathelicidin antimicrobial proprotein (hCAP18; also known by the name of its active peptide, LL-37) is the only known cathelicidin in humans. It is primarily found packaged in the secondary granules of neutrophils and expressed in other leukocyte populations and a wide range of squamous epithelia, and it is secreted at high levels from myeloid cells in the bone marrow into the systemic circulation [14–17]. In vitro studies with synthetic LL-37 have demonstrated its ability to rapidly kill a broad range of bacteria, including *Mycobacterium tuberculosis* [8, 18]. hCAP18 is able to trigger the release of defensins (the other major class of inducible AMPs) and provide protection in animal models of sepsis [19, 20].

The gene encoding hCAP18 is transcriptionally regulated by the vitamin D receptor [21–23]. In vitro, administration of 1,25-dihydroxyvitamin D is able to boost hCAP18 levels in a wide range of human tissues, including keratinocytes, monocytes, neutrophils, and bronchial epithelial cells [21, 24, 25]. Topical vitamin D analogues have been shown to stimulate local production of hCAP18 in the skin [26]. Toll-like receptor 2 stimulation of monocyte and macrophage cells leads to upregulation of both the vitamin D receptor and cytochrome P450 27B1, the enzyme that converts circulating 25-hydroxyvitamin D to the active hormone 1,25-dihydroxyvitamin D [18]. This results in the upregulation of hCAP18 and links vitamin D metabolism to a broad-reaching arm of the innate immune system.

Hemodialysis is the renal replacement modality for over 300,000 patients with end-stage renal disease (ESRD) in the United States; this population is at high risk of both serious infections and vitamin D deficiency. For individuals undergoing hemodialysis, death attributable to infection occurs at a rate 100–300-fold higher than among the general population and represents the second-leading cause of death in this group [27]. A high prevalence of intravenous catheter use likely contributes to the increased risk of infection, but these patients are also at increased risk of non-access-related infections, such as pneumonia [28]. Individuals with ESRD experience a decrease in their ability to produce active 1,25-dihydroxyvitamin D and often have deficient levels of its precursor, 25-hydroxyvitamin D [29–31]. Therefore, we hypothesized that hCAP18 levels at baseline would correlate with vitamin D levels at initiation of hemodialysis and that hCAP18 levels would be strongly linked with subsequent death attributable to infection.

MATERIALS AND METHODS

Accelerated Mortality on Renal Replacement (ArMORR) is a nationally representative, prospective cohort study of patients who initiated chronic hemodialysis from 1 July 2004 through

30 June 2005 at any of the 1056 dialysis centers in the United States that are operated by Fresenius Medical Care. The ArMORR study collected detailed demographic and clinical data, including comorbidities, laboratory results, and serum and plasma samples obtained from all participants at the initiation of dialysis. Clinical data were collected prospectively and entered uniformly into a central database by practitioners at the point of care. All clinical data that were submitted to Fresenius Medical Care underwent rigorous quality assurance—quality control auditing [32, 33]. All blood samples obtained for clinical care were uniformly shipped to and processed by Spectra East (Rockland, NJ), a good clinical practice-accredited central laboratory. After processing for routine clinical testing, remnant samples were shipped on ice to ArMORR study investigators, who aliquotted and stored the samples in liquid nitrogen tanks. This study was approved by the Institutional Review Board of the Massachusetts General Hospital.

Study population. From 1 July 2004 through 30 June 2005, a total of 10,044 patients undergoing incident hemodialysis were prospectively enrolled in ArMORR. To study the association of hCAP18 with death attributable to infection, we performed a nested case-control study. We defined case patients as individuals who died because of infection within 1 year after initiating hemodialysis and control patients as individuals who survived for ≥ 1 year after the initiation of hemodialysis.

Exposures, outcomes, and covariates. The primary exposure variable was hCAP18 level at baseline, measured in samples collected within 14 days after initiation of hemodialysis, before the initiation of intravenous active vitamin D treatment. After collection, blood samples were frozen in liquid nitrogen and underwent a single thaw for this study. Plasma hCAP18 level was measured by ELISA, as described elsewhere [14]. The assay has a detection limit of 0.084 ng/mL with an intra- and inter-assay coefficient of variation of $\leq 6.3\%$. 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels were measured in samples collected at baseline with use of commercially available radioimmunoassay techniques (DiaSorin). The coefficients of variation for 25-hydroxyvitamin D measurements were $< 3\%$ at levels < 30 ng/mL. For 1,25-dihydroxyvitamin D, the coefficients of variation were $< 6.5\%$ at levels < 32.5 pg/mL. 25-hydroxyvitamin D level was measured in 154 control patients and 59 case patients, and 1,25-dihydroxyvitamin D level was measured in 70 control patients and 41 case patients, depending on the quantity of sample available.

The primary outcome was mortality associated with infection within 1 year after initiating hemodialysis. Cause of death was determined from discharge diagnosis reports of the dialysis center. Eighty-one case patients had an adequate residual plasma sample available for hCAP18 analysis. The majority of case patients ($n = 67$) were documented with International Statistical Classification of Diseases and Related Health Prob-

Table 1. Baseline characteristics of case patients and control patients.

Characteristic	Case patients (n = 81)	Control patients (n = 198)	P
Age, mean years	68.7	67.2	
Male sex, %	55.6	53.0	
BMI, mean value	26.6	26.2	
Black race, %	30.9	29.8	.89
ESRD attributable to DM, %	56.8	42.9	.05
Systolic blood pressure, mean mmHg	142	146	.32
Diastolic blood pressure, mean mmHg	73	73	.96
Catheter as primary hemodialysis access, %	65.4	53.3	.08
Hemoglobin level, mean g/dL	10.3	10.3	.12
Albumin level, mean g/dL	3.1	3.5	.02
Parathyroid hormone level, mean pg/mL	262.2	238.6	.47
Phosphorus level, mean mg/dL	4.7	4.7	.84
Calcium level, ^a mean mg/dL	8.8	8.8	.90
Ferritin level, mean ng/mL	355	290	.23
Bicarbonate level, mean mmol/L	23.1	22.7	.41
25-hydroxyvitamin D level, mean ng/mL	19.2	22.2	.10
1,25-dihydroxyvitamin D level, mean pg/mL	22.4	16.8	.11

NOTE. Case patients and control patients were matched according to age and body mass index. BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); DM, diabetes mellitus; ESRD, end-stage renal disease.

^a Calcium levels were corrected for serum albumin level.

lems (ICD)-9 codes of 38 (septicemia), 785.52 (septic shock), and 790.7 (bacteremia). Additional case patients were documented with the ICD-9 codes for HIV (42; n = 3), viral pneumonia (480.9; n = 3), and pneumonia (486; n = 3). The remainder of cases patients were documented to be single cases of echinococcosis, staphylococcal meningitis, acute endocarditis, chronic hepatitis, and gangrene. An additional 198 patients who survived for at least 1 year while undergoing hemodialysis served as control patients. Case and control patients were matched by age and body mass index.

We analyzed several covariates, including age, sex, race, etiology of renal failure, body mass index, use of a catheter for access at initiation of hemodialysis, and systolic and diastolic blood pressure. Race was treated as a dichotomous variable (black vs. not black), as was etiology of renal failure (diabetes vs. other cause). We also analyzed hemoglobin, albumin, parathyroid hormone, phosphorus, calcium, ferritin, and bicarbonate levels and WBC count, as measured at initiation of dialysis. WBC count was analyzed both as a linear variable and categorized into tertiles. When available, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels were also analyzed.

Statistical analysis. We used Fisher's exact test to compare baseline differences in race, cause of ESRD, and catheter-based hemodialysis access. Two-sided *t* tests were used to compare continuous variables.

To identify potential appropriate covariates for multivariate analysis, we examined Spearman correlation coefficients for a

panel of laboratory variables and hCAP18 level. Two-sided *t* tests were used to compare levels of hCAP18 by sex, race, cause of ESRD, and hemodialysis access.

The primary analysis consisted of univariate and multivariate conditional logistic regression to identify the association of hCAP18 level with the primary outcome and to control for potential confounding factors, with significant correlates of hCAP18 levels or case/control status used to determine covariates for inclusion. All regressions were conditioned using age and body mass index (which were used to match case and control patients). hCAP18 levels were examined as continuous data, binary data, and as tertiles. After selection of our final multivariate model, we formally tested for effect modification of the hCAP18-mortality relationship by adding interaction terms for access modality, cause of ESRD, sex, race, and WBC count in separate models.

Data for covariates were missing in <5% of samples. Statistical analyses were performed using SAS, version 9.1.3 (SAS Institute).

RESULTS

Baseline characteristics. Baseline characteristics of the patients are presented in table 1. Case patient status was associated with lower albumin levels and an increased frequency of diabetes mellitus. Serum calcium level, corrected for baseline albumin level [34], did not differ between case patients and con-

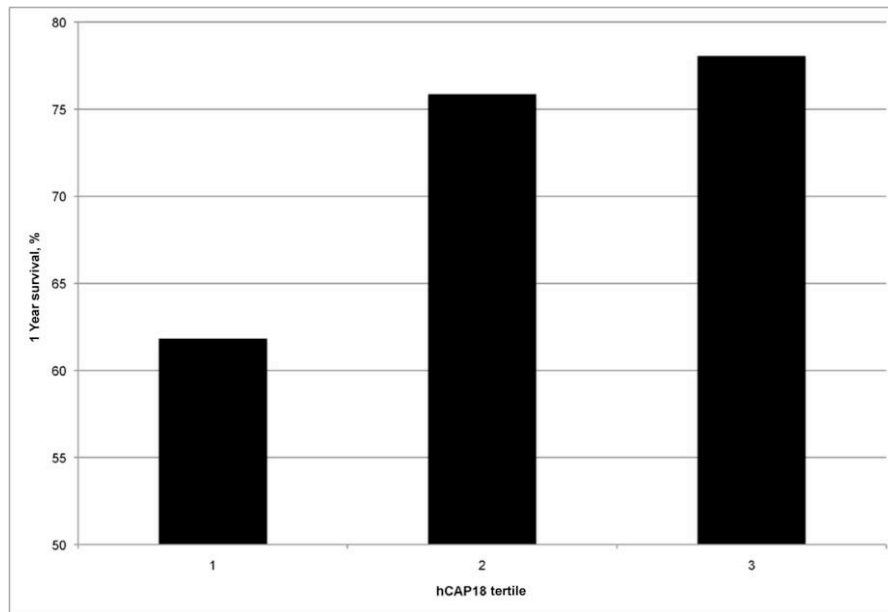


Figure 1. Percentage of individuals in each tertile of human cathelicidin antimicrobial protein (hCAP18) levels who survived for at least 1 year while undergoing hemodialysis (control patients). The remaining subjects died because of infection within 1 year after initiation of hemodialysis (case patients). Individuals within the lowest tertile of hCAP18 levels had a lower chance of survival than did subjects in tertiles 2 or 3 ($P = .007$). There was no significant difference in mortality between tertiles 2 and 3 ($P = .74$). Tertiles are based on the distribution of hCAP18 levels among control patients. Tertile 1 hCAP18 levels, 129–487 ng/mL ($n = 110$); tertile 2 hCAP18 levels, 488–729 ng/mL ($n = 87$); tertile 3 hCAP18 levels, 732–2856 ng/mL ($n = 82$).

control patients. There were no significant differences in race and baseline levels of hemoglobin or parathyroid hormone between case patients and control patients. Hemodialysis catheter use was marginally, although not statistically significantly, more common among case patients than among control patients (66% vs. 53%, $P = .08$).

Association between vitamin D and hCAP18. 25-Hydroxyvitamin D levels were 2–86 ng/mL, with a median level of 18 ng/mL. No correlation was found between 25-hydroxyvitamin D levels and hCAP18 levels ($r = -0.06$; $P = .44$). In contrast, 1,25-dihydroxyvitamin D levels appeared to correlate positively with hCAP18 levels, although this relationship was of borderline statistical significance ($r = 0.23$; $P = .053$). Of note, the majority of patients had insufficient levels of either 25-hydroxyvitamin D (80% of patients had levels <30 ng/mL) or 1,25-dihydroxyvitamin D (66% of patients had levels <18 pg/mL).

hCAP18 levels and 1-year mortality. Sorensen et al. [14] determined the mean (\pm SD) levels of plasma hCAP18 in normal healthy volunteers in Denmark ($n = 58$) to be 1180 ± 200 ng/mL, and individual hCAP18 levels ranged from 400–3200 ng/mL. The mean level of hCAP18 for different populations or disease states has not been determined, thus, a true “normal value” has not been established. In this study, mean (\pm SD) baseline levels of hCAP18 were 619 ± 329 ng/mL for the entire population, which is ~50% lower than that reported

among healthy individuals. In this study, hCAP18 levels were divided into tertiles on the basis of the distribution among control patients. There was no significant difference between hCAP18 tertiles 2 and 3 with respect to death attributable to infection, therefore, hCAP18 was categorized as low (lowest tertile) or elevated (upper 2 tertiles) for subsequent analysis. The proportion of individuals who survived for 1 year after initiating hemodialysis was significantly lower in the bottom tertile versus the upper 2 tertiles (62% vs. 77%; $P = .007$) (figure 1).

Several demographic and baseline laboratory factors were examined to determine if they had any influence on hCAP18 levels among control patients. Older patients had slightly lower hCAP18 levels ($r = -0.16$; $P = .03$). There was an inverse association between hCAP18 and bicarbonate levels ($r = -0.22$; $P < .01$). An increased hCAP18 level was positively associated with higher body mass index ($r = 0.17$; $P = .01$) and increased phosphorus levels ($r = 0.34$; $P < .01$). The strongest association was between higher hCAP18 levels and higher WBC counts ($r = 0.51$; $P < .01$). Levels of hCAP18 were higher in individuals for whom diabetes was a cause of ESRD (mean hCAP18 level [\pm SD], 709 ± 380 vs. 607 ± 308 ng/mL; $P = .05$), but levels did not differ according to sex, race, or presence of a catheter for dialysis access. There was no association between hCAP18 levels and diastolic or systolic blood pressures or albumin, parathyroid hormone, or ferritin levels (data not

shown). In multivariate analysis of all significant predictors of hCAP18, the only independent predictors were bicarbonate level ($\beta = -13$; $P = .03$) and WBC count ($\beta = 43$; $P < .01$).

In univariate analysis of the clinical outcome, lower baseline hCAP18 levels were predictive of an increased risk of death attributable to infection (OR, 2.1; 95% CI, 1.2–3.5). On multivariate analysis (adjusting for sex, race, cause of ESRD, baseline albumin levels, and baseline laboratory factors shown to correlate with hCAP18 level [WBC count and phosphorus and bicarbonate levels]), low hCAP18 levels remained independently predictive of 1-year mortality (OR, 2.6; 95% CI, 1.4–5.0). Of the other covariates in the model, only albumin level was independently predictive of mortality (OR, 0.28 per 1 g/dL increase; 95% CI, 0.16–0.48). Catheter use did not meet the criteria for inclusion in the multivariate model and, when tested, did not act as a significant confounder of the hCAP18-mortality relationship (change in OR, <10%). When the multivariate model was further adjusted for 1,25-dihydroxyvitamin D level (which necessitated use of the smaller sample of patients for whom these values were available), hCAP18 level remained independently predictive of mortality (OR, 3.7; 95% CI, 1.2–11.2) (table 2).

As WBC count was the most closely linked of all covariates with hCAP18 level, we screened demographic and laboratory factors for their ability to predict WBC count in a multivariate linear regression model with hCAP18 level. Catheter-based hemodialysis access was the only additional predictor of WBC count that was identified (with an effect independent of hCAP18 level).

There was no evidence of effect modification for this relationship by access modality, race, sex, or cause of ESRD (diabetes vs. other causes). Despite the strong association between WBC counts and hCAP18 levels, WBC counts themselves were not predictive of mortality ($P = .62$) and there was no evidence of effect modification on the relationship between hCAP18 level and mortality (for interaction, $P = .67$).

DISCUSSION

We performed a nested case-control study of 279 individuals initiating hemodialysis, to study the relationship between baseline levels of the human cathelicidin hCAP18 and infection-associated 1-year mortality. Prior to our prospective study, several observations in human and animal studies had suggested that higher hCAP18 levels might offer protection against infection. A deficiency of salivary hCAP18 in patients with Kostmann syndrome has been associated with severe periodontal disease, and transfected mice that are induced to systemically overexpress hCAP18 are protected from septic death [35, 36]. We thus suspected that individuals with lower baseline hCAP18 levels would be at increased risk of death attributable to infection, the second leading cause of death in this population. Indeed, we found that, after adjusting for potential demographic and clinical confounding factors, individuals in the lowest tertile of hCAP18 levels had an approximately 3-fold increase in the odds of death attributable to an infectious cause. To our knowledge, this is the first report of systemic plasma levels of hCAP18 being associated with mortality in any human population.

We recognize that our observational study cannot prove causation. It is possible that hCAP18 level is a marker for susceptibility to infection or risk of death attributable to infection but does not play a direct biological role in the outcome. It may be that another factor related to hCAP18 level is more central to determining survival. We cannot exclude the possibility that unmeasured factors, such as residual renal function, may be associated with 1,25-dihydroxyvitamin D or hCAP18 production. Nonetheless, the independent relationship between hCAP18 levels and ultimate outcome, in combination with biology that supports a causative mechanism, suggests that further investigation is warranted to more directly study the potential role of hCAP18 in reducing infectious complications in patients undergoing dialysis and other high-risk populations.

The role of vitamin D in the biology of this antimicrobial

Table 2. Human cathelicidin antimicrobial protein (hCAP18) and 1-year mortality associated with infection.

Model	No. of patients	OR ^a (95% CI)
hCAP18 level (univariate)	279	2.1 (1.2–3.5)
hCAP18 level, sex, race, and diabetes mellitus status	279	2.3 (1.3–4.1)
hCAP18 level, sex, race, diabetes mellitus status, albumin level, phosphorus level, bicarbonate level, and WBC count	265	2.6 (1.4–5.0)
hCAP18 level, sex, race, diabetes mellitus status, albumin level, phosphorus level, bicarbonate level, WBC count, and 1,25-dihydroxyvitamin D level	103	3.7 (1.2–11.2)

NOTE. In univariate and multivariate conditional regression models, hCAP18 levels in the lowest tertile were independently predictive of death attributable to infection within 1 year after initiation of hemodialysis.

^a The OR represents the risk of death for patients in tertile 1 vs. patients in tertiles 2 and 3 of hCAP18 levels.

protein is most intriguing. Several previous studies have demonstrated the ability of 1,25-dihydroxyvitamin D to directly regulate the expression of hCAP18 on a genetic level [21, 23, 26]. Stimulation of Toll-like receptor 2 causes upregulation of both 1- α -hydroxylase and the vitamin D receptor, leading to increased local production of 1,25-dihydroxyvitamin D and, consequently, hCAP18 [18]. Thus, hCAP18 levels might be linked to vitamin D stores, underlying infection, or some combination of the 2. Our findings suggest that the effect of pre-existing infection is unlikely in the study population, because individuals with higher levels of hCAP18 had a reduced, rather than elevated, risk of mortality attributable to infection. Some individuals with elevated hCAP18 levels may have had an underlying clinical or subclinical infection, which would have tended to blunt the protective effect seen in this study. Indeed, studies that manipulate hCAP18 levels directly might reveal a more powerful effect on outcomes than that seen in this observational study.

The link between vitamin D and hCAP18 suggests that vitamin D therapy might be one potential mechanism to boost hCAP18 levels and potentially reduce the risk of infection. Although we did not find an association with 25-hydroxyvitamin D levels and hCAP18 in our study, there was a borderline positive association with 1,25-dihydroxyvitamin and hCAP18, which may be more relevant in the ESRD population (in which 1- α -hydroxylase activity is significantly reduced). Our ability to detect these associations was limited by the high prevalence of vitamin D deficiency, which was present in the majority of our patients. Furthermore, *in vitro* studies have demonstrated that the relationship between vitamin D levels and hCAP18 is strongest during active inflammation [18]; in our study, only baseline (preinfection) hCAP18 levels were measured. Despite the limitations of observational studies, the regulation of hCAP18 by vitamin D has been well established in controlled experiments [18, 21, 23, 26].

In several well-conducted observational studies, the administration of active vitamin D analogues to patients undergoing hemodialysis was associated with reduced mortality [33, 37–39]. The results of this study raise the possibility that this effect may be mediated in part through hCAP18. Importantly, the association of hCAP18 with mortality in multivariate models was not eliminated by adjustment for 1,25-dihydroxyvitamin D, which suggests that hCAP18 is not merely a marker of vitamin D status. Although it is possible that vitamin D may also influence immunity through factors other than hCAP18, hCAP18 appears to be more directly related to survival. The monitoring of hCAP18 levels may be important in future randomized trials of vitamin D analogues to elucidate underlying mechanisms of disease prevention and to identify potential differences in the properties of various vitamin D analogues.

In addition to its association with reduced all-cause mortality, vitamin D therapy has also been linked to reduced cardiovascular mortality among patients undergoing hemodialysis [33, 38]. Interestingly, hCAP18 has been detected in atherosclerotic lesions and thus has the potential to affect cardiovascular outcomes [40]. The link between cardiovascular disease and hCAP18 remains a topic for future study.

Of all measured covariates, hCAP18 was most strongly and independently associated with WBC count. The source of circulating hCAP18 is still not definitively known. This relationship may reflect myelopoietic activity because bone marrow is the major source of hCAP18 in plasma [14]. Despite this association, hCAP18 levels remained predictive of mortality independent of WBC counts, which suggests that hCAP18 levels are not merely a marker of leukocyte production.

Despite the fact that hCAP18 levels were measured at the initiation of dialysis, differences in survival according to hCAP18 level were evident after 1 year of follow-up. Baseline hCAP18 level, measured prior to the onset of known infection, might represent a measure of immune system health that reflects future susceptibility to infection. If confirmed by future studies, hCAP18 levels might serve to guide the treatment of patients undergoing dialysis (e.g., minimizing catheter-based access or lowering the threshold for antibiotic use).

Further assessment of hCAP18 will benefit from the identification of agents that can modulate systemic hCAP18 levels. Identification of hCAP18 levels in other groups of patients who are at high risk of infection is essential to gauge the scope of hCAP18-related treatments. If hCAP18 is directly responsible for increases in survival, therapy with hCAP18 or agents that influence hCAP18 levels might be attractive adjuncts to traditional antibiotic therapy in an era where antibiotic resistance is becoming increasingly prevalent.

Acknowledgments

We thank Charlotte Horn (Rigshospitalet; Copenhagen, Denmark) and Wai Man Lio (Cedars-Sinai Medical Center; Los Angeles, CA) for their technical assistance.

Financial support. National Kidney Foundation (to I.B.) and the National Institutes of Health (5R01AI065604–02 to A.E.G.).

Potential conflicts of interest. All authors: no conflicts.

References

1. Zasloff M. Fighting infections with vitamin D. *Nat Med* **2006**; *12*: 388–90.
2. Zasloff M. Antimicrobial peptides in health and disease. *N Engl J Med* **2002**; *347*:1199–200.
3. Ganz T. Immunology: versatile defensins. *Science* **2002**; *298*:977–9.
4. Hancock RE, Diamond G. The role of cationic antimicrobial peptides in innate host defences. *Trends Microbiol* **2000**; *8*:402–10.
5. Lehrer RI, Ganz T. Antimicrobial peptides in mammalian and insect host defence. *Curr Opin Immunol* **1999**; *11*:23–7.
6. Hancock RE, Scott MG. The role of antimicrobial peptides in animal defenses. *Proc Natl Acad Sci U S A* **2000**; *97*:8856–61.

7. Andreu D, Rivas L. Animal antimicrobial peptides: an overview. *Bio-polymers* **1998**;47:415–33.
8. Turner J, Cho Y, Dinh NN, Waring AJ, Lehrer RI. Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils. *Antimicrob Agents Chemother* **1998**;42:2206–14.
9. Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature* **2002**;415:389–95.
10. Boman HG. Antibacterial peptides: basic facts and emerging concepts. *J Intern Med* **2003**;254:197–215.
11. Fernandez-Lopez S, Kim HS, Choi EC, et al. Antibacterial agents based on the cyclic D,L-alpha-peptide architecture. *Nature* **2001**;412:452–5.
12. Zanetti M, Gennaro R, Romeo D. Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. *FEBS Lett* **1995**;374:1–5.
13. Sorensen OE, Follin P, Johnsen AH, et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* **2001**;97:3951–9.
14. Sorensen O, Cowland JB, Askaa J, Borregaard N. An ELISA for hCAP-18, the cathelicidin present in human neutrophils and plasma. *J Immunol Methods* **1997**;206:53–9.
15. Agerberth B, Charo J, Werr J, et al. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. *Blood* **2000**;96:3086–93.
16. Frohm Nilsson M, Sandstedt B, Sorensen O, Weber G, Borregaard N, Stahle-Backdahl M. The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. *Infect Immun* **1999**;67:2561–6.
17. Bals R, Wang X, Zasloff M, Wilson JM. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc Natl Acad Sci U S A* **1998**;95:9541–6.
18. Liu PT, Stenger S, Li H, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* **2006**;311:1770–3.
19. Zheng Y, Niyonsaba F, Ushio H, et al. Cathelicidin LL-37 induces the generation of reactive oxygen species and release of human alpha-defensins from neutrophils. *Br J Dermatol* **2007**;157:1124–31.
20. Mookherjee N, Rehaume LM, Hancock REW. Cathelicidins and functional analogues as antiseptic molecules: expert opinion on therapeutic targets **2007**;11:993–1004.
21. Wang TT, Nestel FP, Bourdeau V, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* **2004**;173:2909–12.
22. Martineau AR, Wilkinson KA, Newton SM, et al. IFN-gamma- and TNF-independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. *J Immunol* **2007**;178:7190–8.
23. Gombart AF, Borregaard N, Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. *Faseb J* **2005**;19:1067–77.
24. Yim S, Dhawan P, Ragunath C, Christakos S, Diamond G. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). *J Cyst Fibros* **2007**;6:403–10.
25. Gombart AF, O’Kelly J, Saito T, Koeffler HP. Regulation of the CAMP gene by 1,25(OH)2D3 in various tissues. *J Steroid Biochem Mol Biol* **2007**;103:552–7.
26. Weber G, Heilborn JD, Chamorro Jimenez CI, Hammarsjo A, Torma H, Stahle M. Vitamin D induces the antimicrobial protein hCAP18 in human skin. *J Invest Dermatol* **2005**;124:1080–2.
27. US Renal Data System (USRDS). *USRDS 2007 Annual Data Report*. **2007**.
28. Sarnak MJ, Jaber BL. Pulmonary infectious mortality among patients with end-stage renal disease. *Chest* **2001**;120:1883–7.
29. Wolf M, Shah A, Gutierrez O, et al. Vitamin D levels and early mortality among incident hemodialysis patients. *Kidney Int* **2007**;72:1004–13.
30. Levin A, Bakris GL, Molitch M, et al. Prevalence of abnormal serum vitamin D, parathyroid hormone, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int* **2007**;71:31–8.
31. Ishimura E, Nishizawa Y, Inaba M, et al. Serum levels of 1,25-dihydroxyvitamin D, 24,25-dihydroxyvitamin D, and 25-hydroxyvitamin D in nondialyzed patients with chronic renal failure. *Kidney Int* **1999**;55:1019–27.
32. Teng M, Wolf M, Lowrie E, Ofsthun N, Lazarus JM, Thadhani R. Survival of patients undergoing hemodialysis with paricalcitol or calcitriol therapy. *N Engl J Med* **2003**;349:446–56.
33. Teng M, Wolf M, Ofsthun MN, et al. Activated injectable vitamin D and hemodialysis survival: a historical cohort study. *J Am Soc Nephrol* **2005**;16:1115–25.
34. Correcting the calcium. *Br Med J* **1977**;1:598.
35. Putsep K, Carlsson G, Boman HG, Andersson M. Deficiency of antibacterial peptides in patients with morbus Kostmann: an observation study. *Lancet* **2002**;360:1144–9.
36. Bals R, Weiner DJ, Moscioni AD, Meegalla RL, Wilson JM. Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. *Infect Immun* **1999**;67:6084–9.
37. Kalantar-Zadeh K, Kuwae N, Regidor DL, et al. Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. *Kidney Int* **2006**;70:771–80.
38. Shoji T, Shinohara K, Kimoto E, et al. Lower risk for cardiovascular mortality in oral 1alpha-hydroxy vitamin D3 users in a haemodialysis population. *Nephrol Dial Transplant* **2004**;19:179–84.
39. Melamed ML, Eustace JA, Plantinga L, et al. Changes in serum calcium, phosphate, and PTH and the risk of death in incident dialysis patients: a longitudinal study. *Kidney Int* **2006**;70:351–7.
40. Edfeldt K, Agerberth B, Rottenberg ME, et al. Involvement of the antimicrobial peptide LL-37 in human atherosclerosis. *Arterioscler Thromb Vasc Biol* **2006**;26:1551–7.