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Association between Proenkephalin A and cardiovascular outcomes in ambulatory Veterans

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ABSTRACT

Proenkephalin (PENK) is a novel biomarker of kidney function associated with cardiovascular risk in patients with cardiovascular disease. Its association with cardiovascular outcomes in ambulatory individuals is less described. In an observational study of 199 ambulatory Veterans enrolled from April to September 2010, we assessed PENK's association with major adverse cardiac events (MACE – cardiovascular death, heart failure [HF] hospitalization, myocardial infarction [MI], or stroke) and individual outcomes of all-cause mortality, incident HF, and cardiovascular death using Cox regression. We also assessed the association of PENK with left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDd), and left ventricular mass index (LVMi) with linear regression. The mean age was 66 ± 12 years, 99 % were men, and 76 % were White, with median follow-up of 12.7 years. Each two-fold higher PENK was associated with a 73 % higher risk of MACE in unadjusted analysis (HR 1.73; 95 % CI 1.00, 2.99; p = 0.043), though this association lost significance after adjusting for confounders (HR 1.69; 95 % CI 0.90–3.15; p = 0.098). PENK was not associated with all-cause mortality, incident HF or cardiovascular death, although risk estimates were elevated with wide confidence intervals for incident HF and cardiovascular death. PENK was not associated with LVMi or LVEDd but had a non-linear relationship with LVEF with low and high PENK associated with lower LVEF. In conclusion, PENK may be associated with a higher risk of MACE in ambulatory Veterans with diverse health statuses; however, further studies are needed.

Abbreviations: PENK: Proenkephalin A; MACE: Major Adverse Cardiac Events.

1. Introduction

Chronic kidney disease (CKD) is a common comorbidity afflicting 14 % of US adults and associated with significant healthcare expenditure and greater risk of death [1]. Kidney disease is associated with a greater risk of cardiovascular disease (CVD) and CVD is the most common cause of death among patients with CKD [1]. Kidney function has been traditionally assessed with serum creatinine or albuminuria, markers of glomerular function and injury, respectively, and higher levels of both markers independently associated with a greater risk of all-cause mortality and cardiovascular mortality [2]. However, serum creatinine has many shortcomings as a marker of glomerular function including variability in creatinine production based on muscle mass, diet, and physical activity, variable secretion by the kidney tubule, and clearance by non-kidney pathways [3]. These shortcomings have prompted interest in alternative biomarkers of glomerular function that also independently capture risk for adverse outcomes and easily integrate into clinical care through routine medical practices such as blood draws. Cystatin C is one such alternate biomarker that has recently been integrated into clinical care and does not share the variability of creatinine and is associated with cardiovascular outcomes [4]; however, it also has disadvantages of reduced accuracy in patients with rapid cell turnover, uncontrolled thyroid disease, or corticosteroid use [3]. Thus, there is potential that additional biomarkers of glomerular function may be

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useful for prognostication of CVD in kidney disease. Proenkephalin A (PENK) is a precursor peptide of metabolites of the endogenous opioid system and is a more stable surrogate biomarker of endogenous opioids [5]. PENK is freely filtered at the glomerulus and plasma levels have been shown to closely reflect glomerular filtration function like creatinine, with higher PENK reflecting lower glomerular filtration rate [6]. Additionally, higher PENK levels have been associated with worse cardiovascular outcomes including worse diastolic dysfunction, greater risk of recurrent myocardial infarction (MI), incident heart failure (HF) [6], higher risk of HF hospitalization [7], and higher risk of death [8]. While these studies suggest PENK may be able to capture risk for CVD, they all recruited specific patient populations with pre-existing CVD with relatively short follow-up. In this study, we evaluated if plasma PENK was associated with incident cardiovascular events and measurements of cardiovascular structure and function on echocardiography in a convenience sample of ambulatory Veterans presenting for an outpatient echocardiogram. We hypothesized that PENK would be associated with cardiovascular outcomes in a general Veteran population independent of serum creatinine.

2. Methods

This analysis utilized data from a prospective observational study of ambulatory Veterans undergoing echocardiography at the San Diego Veterans Affairs Medical Center (SDVAMC) from 2010 to 2017 for clinical indications. The study was approved by the institutional review board at the SDVAMC (IRB # H210144). Ambulatory Veterans presenting to the SDVAMC for an outpatient echocardiogram were approached for participation in the study. Veterans who had echocardiograms performed solely to rule out an intracardiac thrombus or vegetation were excluded. After obtaining written consent, a research blood specimen was collected, immediately centrifuged, and plasma was collected and stored at −80 °C. The participant then completed their echocardiogram after the blood specimen was collected.

A total of 200 Veterans recruited between April and September 2010 had PENK measured. One participant was receiving dialysis and excluded, leaving 199 Veterans as the analytic cohort for this study.

Participant demographics, past medical history, and medications used at time of enrollment were abstracted from the electronic medical record. Medical history (documented history of diabetes mellitus (DM), HF, hypertension, coronary artery disease (CAD), hyperlipidemia, or atrial fibrillation) was collected from review of the medical record. The most recent laboratory value at or before the time of enrollment was also obtained from the electronic medical record, with values allowed up to 3 years prior to enrollment. The estimated glomerular filtration rate (eGFR) was calculated using the 2021 CKD-EPI race free equation. Echocardiographic parameters were measured on the date of enrollment.

The primary outcome was a composite of major adverse cardiovascular events (MACE) which included death from cardiovascular disease, non-fatal cerebral vascular accident (CVA), MI, and hospitalization for HF. The secondary outcomes were the individual outcomes of death from cardiovascular cause, new-onset HF, hospitalization for HF, and MI. Outcomes were determined based on chart review through July 2023. Cause of death was determined via documentation in discharge summaries or physician notes, or death certificates, if available. Only events for which cardiovascular cause of death could be confirmed were marked as cardiovascular death. For new-onset HF, individuals with a history of prevalent HF were excluded from the analysis. Echocardiographic indices assessed included left ventricular ejection fraction (LVEF), left ventricular end-diastolic diameter (LVEDd), and left ventricular mass index (LVMi).

Plasma PENK levels were measured using a chemiluminometric sandwich immunoassay targeted towards amino acids 119–159 of PENK as previously described [9]. Samples were measured in 2014, one to three years after collection and only underwent one freeze–thaw cycle before measurement. PENK concentrations are stable after extended frozen storage and through multiple freeze–thaw cycles, and our storage and measurement are in line with prior studies of PENK [6,7,8,10]. The lower limit of detection for this assay was 5.5 pmol/L. The intra-assay and inter-assay coefficients of variation were 6.4 % and 9.5 % respectively at 50 pmol/L and 4.0 % and 6.5 % at 150 pmol/L. Samples were measured in in duplicate and averaged.

Baseline characteristics of the study population were reported by quartiles of PENK. Differences between quartiles in baseline characteristic were compared with analysis of variance (ANOVA) for normally distributed variables, Mann-Whitney *U* test for non-normally distributed variables, and Chi-square test for categorical variables.

The distribution of PENK was right skewed and values were log base 2 transformed for continuous analyses such that associations should be interpreted as "per two-fold higher" biomarker value. Less than 5 % of data was missing at random. These values were imputed with a total of 5 imputed data sets and results from these datasets were pooled according to Rubin's rule [11]. The association between PENK and longitudinal outcomes was assessed using Cox regression with nested multivariable models. First, the association of PENK with outcomes was evaluated in an unadjusted model. Next, in Model 1 we adjusted for age, race, body mass index (BMI), systolic blood pressure, history of diabetes mellitus (DM), history of hypertension, history of hyperlipidemia, history of coronary artery disease (CAD), history of HF, and blood pressure medication use. Model 2 adjusted for variables in Model 1 plus the serum creatinine value measured closest to enrollment. We chose to adjust for serum creatinine rather than estimated glomerular filtration rate (eGFR) to compare PENK performance more directly as a biomarker with serum creatinine as a biomarker. We also evaluated PENK modeled as quartiles and a restricted cubic spline with 3 knots to assess for non-linear associations. In a sensitivity analysis, we performed a competing risks analysis for any outcomes that did not include all-cause mortality using methods of Fine-Gray to obtain the sub-distribution hazard ratio (sHR) [12].

We assessed the association of PENK with echocardiographic measurements using linear regression with nested multivariable models. Nested models were constructed as above for Cox regression. We again evaluated PENK modeled by quartiles and as a restricted cubic spline with 3 knots to assess for non-linear associations.

Since the cohort included individuals with (n = 113) and without (n = 86) a history of cardiovascular disease (CVD; defined as history of HF, CAD, or CVA), in whom the association of PENK with outcomes may vary, we tested the interaction between PENK and CVD status (PENK*CVD) in fully adjusted models. Given the smaller size of these subgroups, we considered a p-value < 0.10 significant with findings considered hypothesis generating. For all other analyses, a p-value < 0.05 was considered significant. All analyses were performed using R Statistical Software (version 4.2.2, R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.).

3. Results

The mean age of participants was 66 ± 12 years, 99 % of participants were men and 76 % were White (Table 1). The average serum creatinine and eGFR were 1.08 ± 0.26 mg/dL and 79 ± 19 ml/min/1.73 m^2, respectively, and the average PENK was 63.6 ± 25.9 pmoL/L. Comorbid conditions included a history of HF in 23 % of the cohort, history of DM in 30 %, hypertension in 84 %, CAD in 42 %, prior CVA in 11 %, hyperlipidemia in 72 %, and atrial fibrillation in 23 %. When comparing baseline characteristics across quartiles of PENK, the average age and creatinine increased with higher quartiles of PENK while average eGFR declined with higher levels of PENK.

Over a median follow up of 12.7 years (IQR 6.2, 13.0 years), 51 % of participants died, 21 % had MACE, 9 % died from cardiovascular causes, and 1.2 % had a new diagnosis of HF. Each doubling of PENK was

Table 1
Baseline characteristics of participants by quartiles of PENK.

	All (n = 199)	1st Quartile (<46.3 pmoL/L) (n = 50)	Second Quartile (46.3–58.7 pmoL/L) (n = 50)	Third Quartile (58.7–73.7 pmoL/L) (n = 50)	Fourth Quartile (>73.7 pmoL/L) (n = 49)	p-value
Age (years), mean (SD)	66 (12)	62 (12)	63 (12)	66 (12)	71 (11)	0.002
Men, N (%)	197 (99 %)	49 (98 %)	49 (98 %)	50 (100 %)	49 (98 %)	0.572
White, N (%)	151 (76 %)	36 (72 %)	40 (80 %)	82 (64 %)	43 (88 %)	0.037
BMI (kg/m2), mean (SD)	29.4 (5.5)	30.5 (6.3)	30.2 (4.4)	28.6 (5.6)	28.4 (5.5)	0.142
Systolic Blood Pressure (mmHg), mean (SD)	133 (19)	132 (22)	133 (18)	134 (19)	131 (18)	0.848
Creatinine (mg/dL), mean (SD)	1.08 (0.26)	1.02 (0.20)	1.00 (0.21)	1.08 (0.22)	1.22 (0.33)	<0.001
eGFR (mL/min/1.72 m2), mean (SD)	79 (19)	83 (17)	86 (18)	78 (16)	68 (21)	<0.001
Blood Urea Nitrogen (mg/dL), mean (SD)	18 (7)	17 (6)	18 (8)	18 (6)	21 (9)	0.009
Diabetes Mellitus, N (%)	60 (30 %)	16 (32 %)	12 (24 %)	16 (32 %)	16 (32 %)	0.752
Heart Failure, N (%)	46 (23 %)	12 (24 %)	12 (24 %)	7 (14 %)	15 (30 %)	0.269
Hypertension, N (%)	168 (84 %)	41 (82 %)	38 (76 %)	48 (96 %)	41 (82 %)	0.045
Coronary Artery Disease, N (%)	84 (42 %)	26 (52 %)	17 (34 %)	19 (38 %)	22 (44 %)	0.278
Hyperlipidemia, N (%)	143 (72 %)	35 (70 %)	33 (66 %)	38 (76 %)	37 (74 %)	0.641
Atrial Fibrillation, N (%)	45 (23 %)	10 (20 %)	10 (20 %)	8 (16 %)	17 (34 %)	0.126
Beta-Blocker Use, N (%)	119 (59 %)	32 (64 %)	27 (54 %)	30 (60 %)	28 (56 %)	0.524
ACE-I/ARB Use, N (%)	113 (57 %)	30 (60 %)	23 (46 %)	32 (64 %)	28 (56 %)	0.303
Diuretic Use, N (%)	85 (43 %)	18 (36 %)	21 (42 %)	23 (46 %)	23 (46 %)	0.680

ACE-I: Angiotensin-Converting Enzyme-Inhibitor; ARB: Angiotensin Receptor Blocker; BMI: Body Mass Index; eGFR: estimated glomerular filtration rate; PENK: pro-enkephalin

associated with a 73 % greater risk of MACE in unadjusted analysis (Table 3, hazard ratio [HR] 1.73; 95 % confidence interval [CI] 1.00, 2.99; p = 0.043). After adjusting for confounders in Model 1, the HR remained similar, but the association was no longer statistically significant (HR 1.67; 95 % CI 0.92, 3.03; p = 0.090). Similarly, when adjusting for serum creatinine, HR for PENK did not appreciably change but overall association remained not significant (HR 1.69; 95 % CI 0.90, 3.49; p = 0.098). When MACE was analyzed for the competing risk of non-cardiac death in the fully adjusted model, the association was the same as Cox regression (sHR 1.69; 95 % CI 0.92, 3.13; p = 0.092). Assessing the association by quartiles of PENK showed a step-wise increase in risk for MACE with higher quartiles of PENK (Table 2). When modeled as a restricted cubic spline, the spline term was not significant (p = 0.587). There was no significant effect modification by history of CVD (p-interaction = 0.94).

In unadjusted analysis, higher PENK was associated with a higher risk of death (HR 1.69; 95 % CI 1.15, 2.47; p-value 0.009) but PENK was not associated cardiovascular death or incident HF, though HR for these outcomes were higher than for MACE or all-cause death (Fig. 1). After adjusting for confounders and serum creatinine (Model 2), PENK was no longer associated with death (HR 0.94; 95 % CI 0.50, 1.38; p-value 0.802). PENK remained not associated with cardiovascular death or incident HF, though HRs only mildly attenuated for cardiovascular death while they were numerically higher for incident HF (Fig. 1). Quartiles of PENK showed a step-wise higher risk of cardiovascular death and incident HF with higher quartiles of PENK in the fully adjusted models (Supplemental Table S1). There were no significant non-linear associations between PENK and the outcomes when PENK was modeled as a spline (p-value > 0.15 for all). There was no significant effect modification by history of CVD (p-interaction > 0.1) for all secondary outcomes. When PENK's association with cardiovascular death and incident HF were assessed with competing risks analysis, the findings were similar to Cox regression (Supplemental Table S2).

PENK was not associated with LVEF, LVEDd or LVMi when PENK was evaluated as a continuous variable (Table 3). When evaluated by quartiles of PENK, there appeared to be an inverted U-shaped relationship between PENK and LVEF such that both high and low PENK values were associated with lower LVEF (Table 3). A similar relationship was also seen when PENK was modeled as a spline (Fig. 2A) with the non-linear spline component significant in the model (p-value 0.010). Quartiles and splines for LVEDd and LVMi showed a linear association between PENK and these measures. There was no significant effect modification for PENK by history of CVD with LVMi or LVEDd (p-interaction > 0.10). There was a significant difference in the association of PENK with LVEF in those with and without a history of CVD (p-interaction = 0.05); thus, we evaluated the association of PENK with LVEF by splines and quartiles in individuals with and without a history of CVD. In individuals without a history of CVD, only the lowest quartile of PENK had a lower LVEF, and the top three quartiles had similar LVEFs (Supplemental Table S3, Supplemental Figure S1A). In individuals with a history of CVD, there again appeared to be an inverted U-shaped relationship, though this was less prominent than the relationship seen in the whole cohort (Supplemental Table S3, Supplemental Figure S1B).

Table 2
Association of PENK with major adverse cardiovascular events by Cox regression in Ambulatory Veterans.

Model	Per two-fold higher HR (95 % CI)	p-value	1st Quartile (<46.3 pmoL/L) (n = 50)	Second Quartile (46.3–58.7 pmoL/L) (n = 50)	Third Quartile (58.7–73.7 pmoL/L) (n = 50)	Fourth Quartile (>73.7 pmoL/L) (n = 49)	p-value trend
Unadjusted	1.73 (1.00, 2.99)	0.043	1 (ref)	1.42 (0.55, 3.62)	1.55 (0.61, 3.89)	2.13 (0.88, 5.15)	0.096
Model 1	1.67 (0.92, 3.03)	0.090	1 (ref)	1.86 (0.69, 4.98)	1.52 (0.57, 4.01)	2.21 (0.85, 5.72)	0.148
Model 2	1.69 (0.90, 3.15)	0.098	1 (ref)	1.87 (0.70, 4.99)	1.52 (0.57, 4.02)	2.22 (0.83, 5.90)	0.160
Competing Risks	1.69 (0.92, 3.13)	0.092	1 (ref)	1.85 (0.68, 5.07)	1.52 (0.57, 4.08)	2.23 (0.86, 5.79)	0.147

Model 1 include adjustments for age, race, BMI, systolic blood pressure, or past medical history of diabetes, hypertension, coronary artery disease, hyperlipidemia, heart failure, or blood pressure medication use.
Model 2 included the variables in Model 1 plus serum creatinine.
CI: Confidence Interval; HR: hazard ratio; MACE: Major Adverse Cardiac Events; PENK – pro-enkephalin

Table 3
Association of PENK with LVEF, LVEDd, LVMi assessed with linear regression in Ambulatory Veterans.

	Change per two-fold higher PENK (95 % CI)	p-value	1st Quartile (<46.3 pmoL/L) (n = 50)	Second Quartile (46.3–58.7 pmoL/L) (n = 50)	Third Quartile (58.7–73.7 pmoL/L) (n = 50)	Fourth Quartile (>73.7 pmoL/L) (n = 49)	p-value trend
LVEF							
Unadjusted	0.53 (−3.42, 4.48)	0.792	ref	9.41 (3.54, 15.28)	9.16 (3.35, 14.97)	3.03 (−2.87, 8.93)	0.351
Model 1	2.47 (−0.99, 5.93)	0.161	ref	10.00 (5.13, 14.87)	7.49 (2.66, 12.32)	5.17 (0.10, 10.23)	0.108
Model 2	2.41 (−1.25, 6.07)	0.195	ref	10.03 (5.16, 14.91)	7.36 (2.51, 12.21)	4.67 (−0.59, 9.93)	0.179
LVEDd							
Unadjusted	−0.13 (−0.36, 0.11)	0.283	ref	−0.15 (−0.50, 0.20)	−0.45 (−0.80, −0.10)	−0.23 (−0.58, 0.12)	0.081
Model 1	−0.13 (−0.36, 0.10)	0.269	ref	−0.16 (−0.49, 0.17)	−0.32 (−0.65, 0.01)	−0.23 (−0.58, 0.12)	0.122
Model 2	−0.13 (−0.37, 0.11)	0.292	ref	−0.16 (−0.49, 0.18)	−0.32 (−0.65, 0.01)	−0.23 (−0.59, 0.13)	0.143
LVMi							
Unadjusted	−3.90 (−16.35, 8.56)	0.538	ref	−13.53 (−32.18, 5.12)	−15.00 (−33.35, 3.35)	−13.41 (−23.07, 5.24)	0.163
Model 1	−6.42 (−19.00, 6.17)	0.315	ref	−13.53 (−31.88, 4.83)	−13.11 (−31.10, 4.89)	−14.48 (−33.06, 4.11)	0.147
Model 2	−10.15 (−23.24, 2.95)	0.128	ref	−12.15 (−30.45, 6.16)	−13.93 (−31.84, 3.97)	−18.38 (−37.36, 0.61)	0.063

Model 1 included adjustments for age, race, BMI, systolic blood pressure, or past medical history of diabetes, hypertension, coronary artery disease, hyperlipidemia, heart failure, or blood pressure medication use.
Model 2 included adjustments for the variables in Model 1 plus serum creatinine.
CI: Confidence Interval; LVEDd: Left Ventricular End Diastolic Diameter; LVEF: Left ventricular ejection fraction; LVMi: Left Ventricular Mass Index.

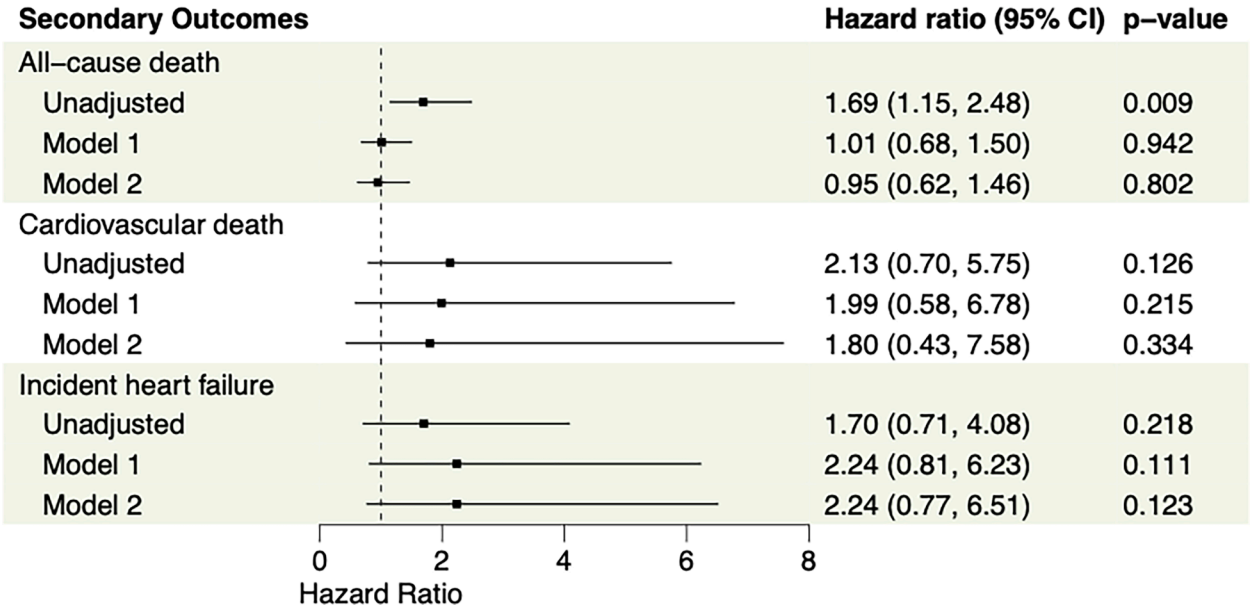

Fig. 1. Forest Plot showing the hazard ratios per doubling of PENK for secondary outcomes in nested multivariable models. Model 1 adjusted for age, race, body mass index, systolic blood pressure, and past medical history of diabetes, hypertension, coronary artery disease, hyperlipidemia, heart failure, or blood pressure medication use. Model 2 adjusted for variables in Model 1 and serum creatinine.

4. Discussion

In this study, we evaluated the association of PENK with cardiovascular outcomes in a convenience sample of ambulatory Veterans with widely varying medical conditions with median follow up of more than 12 years. We found higher levels of PENK were associated with a greater than 70 % risk of MACE per two-fold higher PENK levels in unadjusted analysis. This association was no longer significant after adjusting for confounders, though the point estimate for risk did not appreciably change with adjustment. Conversely, while PENK was associated with all-cause mortality in an unadjusted analysis, this association was attenuated and no longer significant after adjusting for confounders. PENK was also not associated with other secondary outcomes of cardiovascular death or incident HF, though the risk estimates were elevated and did not substantially attenuate with adjustment as it did for all-cause mortality. Lastly, PENK had a unique relationship with LVEF with both individuals with high and low PENK values having a lower LVEF, though relationship may only be for individuals with CAD. Overall, these findings suggest that PENK could be associated with risk of MACE, independent of serum creatinine, if evaluated in a larger sample size with adequate power.

We have previously reported an association between higher PENK

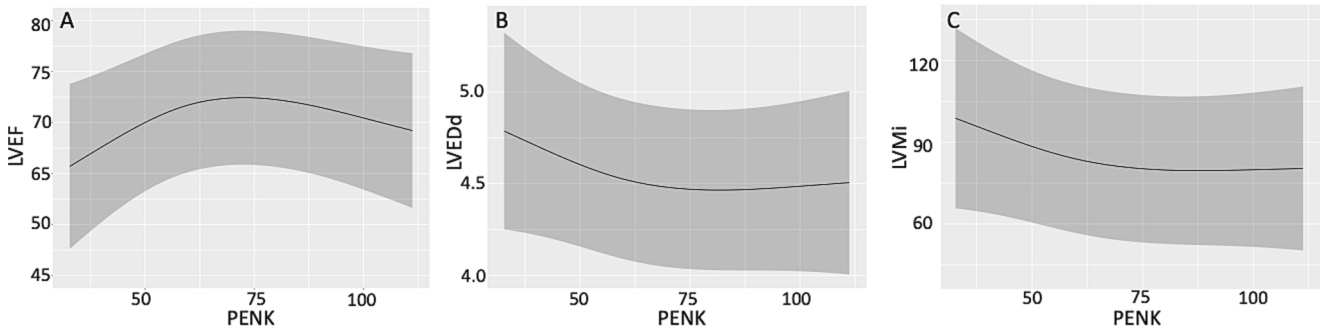

Fig. 2. Estimated echocardiographic measurements and 95% confidence intervals for PENK values modeled as restricted cubic spline in the fully adjusted linear regression models. There was a significant non-linear association between PENK and LVEF, with LVEF having an inverted U-shaped relationship with PENK (A). There were no significant non-linear relationships between PENK and LVEDd and LVMi (B and C).

and MACE, defined as cardiovascular hospitalization or death, in this analytic cohort. However, this prior analysis only had 4 years of follow up leading to a limited number of events and was only able to evaluate the unadjusted association of PENK [9]. We have now expanded these prior findings by extending follow up beyond 12 years, identifying more deaths and cardiovascular events, and adjusting for relevant confounders. Thus, our current analysis better contextualizes the clinical utility of PENK with regards to an individual's health status.

Our findings align with prior studies of PENK and cardiovascular outcomes but supplement prior studies by evaluating associations in a population with more diverse health statuses [6,7,8]. In a single-center study in the United Kingdom of over 1100 patients hospitalized with an acute MI (AMI) with PENK measured within 36 h of AMI, each $\log_{10}$ increase in PENK was associated with a 76 % greater risk of recurrent MI and 67 % greater risk of subsequent HF [6]. Subsequently, a multi-center study in Europe of over 1900 patients hospitalized with acute HF with PENK measured within 12 h of presentation to the emergency department found each $\log_{10}$ increase in PENK level was associated with a 27 % higher risk of HF mortality independent of kidney function [7]. Lastly, a multicenter study in Europe that enrolled 522 hospitalized or outpatients with HFpEF found each $\log_{10}$ higher PENK was associated with a 45 % higher risk of HF hospitalization or death independent of serum creatinine [8]. Compared to our study though, these studies focused on specific populations with pre-existing cardiovascular disease and their follow up was shorter. While our analysis focused on the Veteran population, it enrolled ambulatory individuals with a range of medical comorbidities; thus, allowing an evaluation of PENK's prognostic utility in a setting more akin to patients seen in a clinic setting. Our findings support continued evaluation of PENK as a prognostic marker for cardiovascular outcomes in ambulatory patient populations.

While we did not find PENK was associated with cardiac structures and function on echocardiography when evaluated as a linear association, we did find PENK had a significant non-linear relationship with LVEF such that both the lowest and highest PENK quartiles were associated with lower LVEF. This association also differed depending on the presence or absence of CVD in an individual. It is not clear why PENK has this inverted U association; however, this may stem from limited knowledge of the cardiovascular actions of PENK. Activation of opioid receptor pathways by enkephalins has been associated with both cardioprotective measures and adverse cardiac effects including negative inotropy [13] and cardiac hypertrophy [14]. Potentially, PENK has a constitutive endocrine effect in the cardiovascular system where too much or too little activation of opioid receptor pathways can have deleterious effects on cardiac function analogous to other endocrinological pathways such as the adverse cardiac effects of hypo- and hyperthyroidism. We also cannot exclude that our small sample size resulted in greater LVEF variation in certain quartiles and other unmeasured confounders influencing this relationship. Interestingly, the relationship appeared to vary depending on an individual's history of CVD, with an inverted U shape association persisting for individuals without CVD but more linear for individuals with CVD. The cause of this differential relationship is unclear, but potentially alterations in enkephalin signaling precede subsequent CVD and lower PENK levels reflect a deficiency enkephalin system that has detrimental effects on cardiac tissues. These findings are hypothesis generating though given our limited sample size but suggest the relationship of PENK with cardiovascular structure and function can vary based on prevalent CVD and comorbidities.

Key strengths of our study include a population with widely varying degrees of health and a longer follow up than prior studies of PENK at almost 13 years. Our study also has important limitations. First, our sample size is small and heterogenous, and as a result we are underpowered for many of our outcomes. Though the demographics of the Veteran population are changing with increasing women serving, our study still enrolled primarily men. Second, although studies have shown measurement of PENK is stable after storage at −80° C and multiple freeze–thaw cycles, we cannot exclude potential degradation of PENK during the period of storage in this analysis and this may have altered results. Third, we lacked data on other biomarkersn of cardiac function and inflammation such as BNP and CRP; thus, we cannot exclude the associations in this analysis would further attenuate with adjusting for other contemporary risk biomarkers. Lastly, we lacked measures of albuminuria and Cystatin C which are other important measures of kidney function and whose levels have been associated with cardiovascular outcomes.

5. Conclusion

In a population of ambulatory Veterans with widely varying comorbidities, we found PENK levels may be associated with LVEF measured on echocardiography and with the risk of MACE and other cardiovascular outcomes regardless of the presence or absence of CVD. Further studies are needed to characterize the prognostic ability of PENK in the general population.

CRediT authorship contribution statement

Shreya Banerjee: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pranav S. Garimella:** Writing – review & editing, Methodology, Conceptualization. **Kimberly N. Hong:** Writing – review & editing, Methodology, Conceptualization. **Alexander L. Bullen:** Writing – review & editing. **Lori B. Daniels:** Writing – review & editing, Investigation. **Nicholas Wettersten:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Dr. Nicholas Wettersten and this work was supported (or supported in part) by Carerr Development Award Number IK CX002105 from the United States (U.S.) Department of Veteans Affairs Clinical Sciences R&D (CSRD) Service. The contents do not represent the view of the U.S. Department of Veterans Affairs or the United States Government.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2024.101557.

References

[1] K.L. Johansen, G.M. Chertow, D.T. Gilbertson, A. Ishani, A. Israni, E. Ku, S. Li, S. Li, J. Liu, G.T. Obrador, I. Schulman, K. Chan, K.C. Abbott, A.M. O'Hare, N.R. Powe, N.S. Roetker, J.S. Scherer, W. St. Peter, J. Snyder, W.C. Winkelmayer, S.P.Y. Wong, J.B. Wetmore, US renal data system 2022 Annual data report: epidemiology of kidney disease in the united states, *Am J Kidney Dis* 2023 (81) (2022) A8–A11.

[2] K. Matsushita, M. van der Velde, B.C. Astor, M. Woodward, A.S. Levey, P.E. de Jong, J. Coresh, R.T. Gansevoort, Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality: a collaborative meta-analysis of general population cohorts, *Lancet* 375 (2010) 2073–2081.

[3] M.G. Shlipak, M.D. Mattes, C.A. Peralta, Update on cystatin C: incorporation into clinical practice, *Am J Kidney Dis* 62 (2013) 595–603.

[4] M.G. Shlipak, M.J. Sarnak, K. Ronit, L.F. Fried, S.L. Seliger, A.B. Newman, D. S. Siscovick, S.-B. Catherine, Cystatin C and the risk of death and cardiovascular events among elderly persons, *N. Engl. J. Med.* 352 (2005) 2049–2060.

[5] A. Ernst, J. Köhrle, A. Bergmann, Proenkephalin A 119–159, a stable proenkephalin A precursor fragment identified in human circulation, *Peptides* 27 (2006) 1835–1840.

[6] L.L. Ng, J.K. Sandhu, H. Narayan, P.A. Quinn, I.B. Squire, J.E. Davies, A. Bergmann, A. Maisel, D.J.L. Jones, Proenkephalin and prognosis after acute myocardial infarction, *J. Am. Coll. Cardiol.* 63 (2014) 280–289.

[7] L.L. Ng, I.B. Squire, D.J.L. Jones, T.H. Cao, D.C.S. Chan, J.K. Sandhu, P.A. Quinn, J. E. Davies, J. Struck, O. Hartmann, A. Bergmann, A. Mebazaa, E. Gayat, M. Arrigo, E. Akiyama, Z. Sabti, J. Lohrmann, R. Twerenbold, T. Herrmann, C. Schumacher, N. Kozhuharov, C. Mueller, Proenkephalin, renal dysfunction, and prognosis in patients with acute heart failure: A GREAT network study, *J. Am. Coll. Cardiol.* 69 (2017) 56–69.

[8] P. Kanagala, I.B. Squire, D.J.L. Jones, T.H. Cao, D.C.S. Chan, G. McCann, J. K. Sandhu, P.A. Quinn, J. McAdam, A.-M. Marsh, J.E. Davies, J. Struck, A. Bergmann, Z. Sabti, R. Twerenbold, T. Herrmann, N. Kozhuharov, C. Mueller, L. L. Ng, Proenkephalin and prognosis in heart failure with preserved ejection fraction: a GREAT network study, *Clin Res Cardiol* 108 (2019) 940–949.

[9] B. Arbit, N. Marston, K. Shah, E.L. Lee, H. Aramin, P. Clopton, A.S. Maisel, Prognostic usefulness of proenkephalin in stable ambulatory patients with heart failure, *Am J Cardiol* 117 (2016) 1310–1314.

[10] L.J. Donato, J.W. Meeusen, J.C. Lieske, D. Bergmann, A. Sparwaßer, A.S. Jaffe, Analytical performance of an immunoassay to measure proenkephalin, *Clin Biochem.* 58 (2018) 72–77, https://doi.org/10.1016/j.clinbiochem.2018.05.010.

[11] D.B. Rubin, *Multiple imputation for nonresponse in surveys*, Wiley-Interscience, Hoboken, N.J, 2004.

[12] J.P. Fine, R.J. Gray, A proportional hazards model for the subdistribution of a competing risk, *J. Am. Stat. Assoc.* 94 (1999) 496–509.

[13] S. Pepe, O.W.V. van den Brink, E.G. Lakatta, R.-P. Xiao, Cross-talk of opioid peptide receptor and β-adrenergic receptor signalling in the heart, *Cardiovasc. Res.* 63 (2004) 414–422.

[14] O.W.V. van den Brink, L.M. Delbridge, F.L. Rosenfeldt, D. Penny, D.S. Esmore, D. Quick, D.M. Kaye, S. Pepe, Endogenous cardiac opioids: Enkephalins in adaptation and protection of the heart, *Heart Lung Circ.* 12 (2003) 178–187.