ASSOCIATION OF 25-HYDROXY VITAMIN D DEFICIENCY WITH NT-PRO BNP LEVELS IN ACUTE MYOCARDIAL INFARCTION PATIENTS

By

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LIST OF ABBREVIATIONS

25(OH)D – 25 Hydroxy vitamin D
1,25(OH)\textsubscript{2}D – 1,25-dihydroxy vitamin D
VDR – vitamin D receptors
CVS – cardiovascular system
HTN – hypertension
DM-2 – type2 diabetes
AMI – acute myocardial infarction
LVDF – left ventricular dysfunction
ANP – atrial natriuretic peptide
BNP – Brain natriuretic peptide
PTH – parathyroid hormone
SHPT – secondary hyperparathyroidism
NT-proBNP – N-terminal pro-brain natriuretic peptide
CHF – Congestive heart failure
ESRD – end stage renal disease
CKD – chronic kidney disease
GFR – glomerular filtration rate
NSTEMI – non ST segment myocardial infarction
ABSTRACT

Background and objectives: Nutritional vitamin D deficiency is an emerging risk factor for acute myocardial infarction (AMI) and heart failure. The association between 25-hydroxyvitamin D levels and N-terminal pro B-type natriuretic peptide (NT-proBNP), a robust prognostic marker for post-MI mortality and heart failure is unknown and could illuminate a potential pathway for adverse outcomes among post-MI patients with 25-hydroxyvitamin D deficiency.

Design, setting, participants and measurements: In a cross sectional analysis, we studied 238 AMI patients from 21 US centers to test the association of nutritional vitamin D (25-hydroxyvitamin D [25(OH)D]) deficiency with NT-proBNP levels. Patients’ 25(OH)D levels were categorized as normal (≥30 ng/ml), insufficient (>20 – <30 ng/ml), deficient (>10 – ≤ 20 ng/ml), and severely deficient (≤10 ng/ml) groups.

Results: 96% of AMI patients had low 25(OH)D levels, with 75% having 25(OH)D deficiency and 21% having insufficiency. No significant trends for higher mean log NT-proBNP levels in severely deficient (6.9 ± 1.3 pg/ml), deficient (6.9 ± 1.2 pg/ml) and insufficient (6.9 ± 0.9 pg/ml) groups were observed as compared with patients having normal (6.1 ± 1.7 pg/ml) levels, \( P = 0.165 \). In multivariate regression model after adjusting for several covariates, 25(OH)D was not associated with NT-proBNP levels.

Conclusion: Potential associations between nutritional vitamin D deficiency and prognosis in the setting of AMI are unlikely to be mediated through NT-proBNP pathways. Future studies should examine other mechanisms such as inflammation and vascular calcification by which 25(OH)D deficiency could mediate adverse outcomes such as heart failure and mortality post AMI.
CHAPTER 1: BACKGROUND

Introduction

Vitamin D deficiency is highly prevalent in the United States and worldwide.\(^1\) NHANES III reported that the prevalence of 25 hydroxy vitamin D \([25(OH)D]\) deficiency in the US population is between 25%-57%.\(^2\) \(25(OH)D\) is the principal circulating storage form of vitamin D in the human body. Vitamin D in its active form, 1,25-dihydroxy vitamin D \([1,25(OH)_2D]\) is a hormone as it is primarily produced in the kidneys and circulates in blood exerting its effects on various tissues throughout the body via the vitamin D receptors (VDR). VDR’s have a broad tissue distribution that includes vascular smooth muscle, endothelium and cardiomyocytes.\(^1,3\) Although \(1,25(OH)_2D\) is the active form of vitamin D, its serum levels do not correlate with the overall vitamin D status and are generally not clinically useful.\(^4\) On the other hand serum \(25(OH)D\) concentrations reflect both vitamin D intake and endogenous production and are more reflective of an individual’s overall vitamin D status. Hence serum \(25(OH)D\) levels are frequently used in clinical settings to assess vitamin D status. Although a consensus regarding the optimal level of serum \(25(OH)D\) has not been established most experts define \(25(OH)D\) deficiency as a level <20 ng/ml and vitamin D insufficiency as 21-29 ng/ml.\(^5,6\) For studied end points such as incident MI or all cause mortality a level of ≥ 30ng/ml is considered optimal.

It is now increasingly recognized that adequate vitamin D status is not only important for bone health and the prevention of osteoporosis but also for optimal function of many other organs and tissues throughout the body, including the cardiovascular (CV) system.\(^3\) Cardiac myocytes have cytosolic vitamin D receptors
that bind active vitamin D (1,25 dihydroxy vitamin D), but unlike vascular smooth muscle cells, cardiac myocytes lack 1α-hydroxylase activity, an enzyme that converts inactive vitamin D(25 hydroxy vitamin D) to active vitamin D. Hence cardiac muscle is strongly dependent upon circulating active vitamin D or calcitriol levels. In the past, several in vitro studies have shown that calcitriol regulates intracellular calcium metabolism and thus myocardial contractility. Consequently, 25(OH)D deficiency has been associated with aberrant cardiac contractility, cardiomegaly, and increased ventricular mass due to myocardial collagen deposition, independent of its known effects on blood pressure.

Apart from its effects on the myocardium, 25 (OH)D deficiency also leads to enhanced atherosclerosis secondary to vascular smooth muscle cell (VSMC) proliferation and increased production of pro inflammatory cytokines (IL-6 and TNF-alfa).

There is growing body of evidence from clinical studies that 25(OH)D deficiency also plays an important role in the genesis of coronary risk factors, including hypertension (HTN), type-2 diabetes (DM-2) and the metabolic syndrome. Furthermore, large epidemiological studies, including the Health Professionals Study and the Framingham Offspring Study, have shown that low 25(OH)D levels (<15ng/ml) as compared with levels ≥ 30ng/ml, were independently associated with twice the risk of incident myocardial infarction (MI) and a higher risk of incident cardiovascular events including fatal and nonfatal stroke. Moreover, the risk of all-cause mortality was higher among subjects with vitamin D levels <17.8ng/ml in NHANES III as compared with subjects having levels >32. Consequently, circulating
25(OH) D levels do not just represent the vitamin D status of an individual but can potentially serve as a biomarker for cardiovascular risk. Despite these studies emphasizing the importance of 25(OH)D deficiency and CV disease, there is sparse data in the literature about the prevalence of 25(OH)D deficiency in acute myocardial infarction (AMI) patients, who comprise a high risk population. A synopsis of the studies on Vitamin D deficiency in AMI patients is presented below.

**Studies of Vitamin D Deficiency in AMI Patients**

Two small case control studies have examined vitamin D levels in AMI patients.

**Study 1:** Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R: Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. *Int J Epidemiol* 19:559-563, 1990

A study by Scragg and colleagues\(^{22}\) was conducted in the Central Auckland area of New Zealand between March 1986 and 1988. Cases were patients between 35-64 years of age, who were diagnosed with AMI per the WHO-MONICA project criteria.\(^ {23}\) Age and sex matched controls were obtained by random sampling of the general population. About 179 case-control pairs were analyzed. The results indicated that the mean plasma 25(OH)D level in the cases was lower compared to the controls (32 nmol/lit vs. 35 nmol/lit, \(p\) value=0.017). The odds ratio of AMI decreased with increasing quartiles of vitamin D as shown in the table below.
Table 1. Relative risk (95% CI) of myocardial infarction associated with quartile plasma levels of 25-hydroxyvitamin D$_3$

<table>
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<th>Level of 25-hydroxy vitamin D$_3$ (nmol/L)</th>
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<td>&lt;25</td>
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<td>25–32</td>
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<td>33–42</td>
<td>0.33 (0.17, 0.64)</td>
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<td>≥43</td>
<td>0.30 (0.15, 0.61)</td>
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**Study 2:** Lund B, Badskjaer J, Lund B, Soerensen OH: Vitamin D and ischaemic heart disease. *Horm Metab Res* 10:553-556, 1978

In this study by Lund et al.,$^{24}$ serum 25 (OH)D levels were measured in 128 patients with ischemic heart disease admitted to the Frederiksberg Hospital in Denmark. 53 of these patients had a diagnosis of AMI and 75 had angina pectoris. Seasonal variations in the vitamin D levels were compared to 409 controls. The mean 25(OH)D levels in the AMI and angina patients were 24 ± 10ng/ml and 23.5 ± 9.6 ng/ml respectively, which were not statistically different from the level of 28.8 ± 12.3 recorded in controls. However, the mean vitamin D levels in the cases were lower compared to the controls in the months of May-June ($P <0.01$) and July-August ($P <0.05$), indicating that serum vitamin D levels change with sun exposure. In summary, the studies of vitamin D deficiency in AMI patients were not recent and were limited by their small sample sizes and were not based in the U.S., where better nutrition and fortification of milk is common.

**Studies of Vitamin D Deficiency and Heart failure**
Apart from the paucity of studies on vitamin D deficiency in AMI patients, gaps exist in the literature regarding the role of vitamin D deficiency in the development of left ventricular dysfunction (LVDF) or heart failure, an important complication post MI.

As enumerated in several *in vitro* studies, 25(OH)D deficiency is associated with aberrant cardiac contractility, cardiomegaly, and increased ventricular mass due to myocardial collagen deposition,9;10 all of which ultimately lead to heart failure. Further, it has been shown that activation of nuclear vitamin D receptors by 1,25(OH)$_2$D$_3$ suppresses the expression and secretion of atrial natriuretic peptide (ANP) and Brain natriuretic peptide (BNP) in cardiac myocytes,25-27 both of which are biomarkers of heart failure. In addition to its direct effects on myocardium, vitamin D deficiency may exert indirect effects on the myocardium by elevated serum parathyroid hormone (PTH) levels4 leading to secondary hyperparathyroidism (SHPT). PTH is released from the parathyroid gland in response to low calcium levels from vitamin D deficiency. PTH binds to its receptors on the myocardium and impairs the energy metabolism of myocardial cells28 and induces cardiomyocyte hypertrophy,29 leading to heart failure or left ventricular dysfunction.30

In recent years, it has been established that N-terminal pro-brain natriuretic peptide (NT-proBNP), a pro-hormone of BNP released from cardiac ventricles, is associated with the severity of left ventricular dilatation and dysfunction after MI.31;32 In addition, NT-pro BNP is a sensitive and robust prognostic biomarker of mortality in acute MI,33-35 heart failure(HF)36;37 and chronic hemodialysis38;39 patients. Moreover, NT-pro BNP levels have direct clinical implications, as they are used to guide therapeutic interventions in HF patients, leading to improved outcomes as compared to routine
clinical treatment.\(^{40}\) A synopsis of the studies examining the association of vitamin D and NT-proBNP in other clinical settings is given below.


This was a case-control study that examined the association between plasma NT-proANP, a marker of heart failure severity, and vitamin D metabolites. The study was comprised of 54 cases and 34 controls recruited at the Heart and Diabetes Center in Bonn, Germany from Nov 2000 to March 2001. Among the cases, 20 patients were < 50 years of age and 34 were ≥ 50 years, and all the controls were above 50 years old. All cases had ≥ class II New York Heart Association (NYHA) congestive heart failure. 25 hydroxy vitamin D levels were lower in both patient groups compared to the controls (9 ng/ml and 11ng/ml in cases vs. 18 ng/ml in controls,(p< 0.001). Both groups of CHF patients had markedly elevated NT-proANP levels and parathyroid hormone levels (p <0.001) compared to controls. In a nonlinear regression analysis, 25(OH)D inversely correlated with NT-proANP (r\(^2\) = 0.16, p<0.001).\(^{41}\) This study concluded that low vitamin D levels could contribute to myocardial dysfunction in patients with congestive heart failure.

This was a cross-sectional study to determine the association of Vitamin D deficiency on BNP and other vascular calcification parameters in 223 hemodialysis patients in a single hemodialysis unit in Portugal. The mean serum 25(OH)D levels in these patients was low at 21.6 ± 12.2 ng/ml. In an unadjusted analysis, serum 25(OH)D levels negatively correlated with BNP (r= -0.22, p= .002) and vascular calcification (r=0.26, p<0.001). On multivariate analysis, lower levels of 25(OH)D were independently associated with higher BNP levels (p=0.005) and higher vascular calcification scores (≥3), (p =0.002). This study thus concluded that low 25(OH)D levels are a cardiovascular risk marker in hemodialysis patients and that the effects of its repletion on cardiovascular morbidity and mortality needs to be clarified in large randomized controlled trials.


In humans it is known that the administration of active vitamin D [125(OH)D3] to end stage renal disease (ESRD) patients improves left ventricular function. In this study, 20 pre-dialysis CKD patients (e GFR <30 ml/min/1.73m²) with secondary hyperparathyroidism (SHPT) (i.e.; serum intact PTH levels >180 pg/ml), were
treated with active vitamin D (calcitriol) for 12 weeks and 10 of similar patients were treated with placebo. Echocardiography assessment of cardiac function was performed at baseline and after 12 weeks of treatment. No significant change in LV dimensions or ejection fraction was observed after 12 weeks of treatment, but there was significant improvement in LV diastolic parameters, namely the A wave velocity (0.69 ± 0.089 to 0.68 ± 0.084, \( p = 0.001 \)) and E/A ratio ( \( 1.193 ± 0.21 \) to \( 1.238 ± 0.18, P = 0.001 \)). No change was observed in the placebo group.\(^{46}\) The study concluded that diastolic dysfunction seen in pre-dialysis CKD patients could possibly be improved by active vitamin D therapy, although larger and longer duration studies are warranted to substantiate these findings.


This study included 3,299 patients from the LUDwigshafen RIsk and Cardiovascular Health Study (LURIC). This prospective cohort study included patients routinely referred to coronary angiography from a single tertiary care center in Southwest Germany, and who had baseline 25(OH)D levels measured, between July 1997 and January 2000. The cross sectional associations between baseline 25(OH)D levels and the heart failure marker, NT-proBNP, was studied. In addition the hazard ratio for heart failure deaths according to vitamin D status was studied prospectively. 25(OH)D levels correlated negatively with NT-proBNP levels (\( r = -0.190, P <0.001 \)).
Patients in higher NYHA classes had significantly lower 25(OH)D levels ($P < 0.001$). In multiple linear regression analysis after adjusting for age, race, gender, BMI, smoking status, co-morbidities, renal function (GFR) and C-reactive protein, 25(OH)D remained independently associated with NT–proBNP ($\beta$ coefficient = -0.082, $p < 0.001$).

After a mean follow-up of 7.7 years, 116 patients died due to heart failure and 188 died due to sudden cardiac death (SCD). After adjustment for cardiovascular risk factors, the hazard ratios for death due to heart failure and SCD were 2.84 (95% CI: 1.2 - 6.74) and 5.05 (2.13-11.97), respectively, when comparing patients with severe vitamin D deficiency (<25nmol/l) with persons in normal range ($\geq 75$nmol/l). The study concluded that vitamin D deficiency is associated with prevalent myocardial dysfunction and deaths due to heart failure and sudden cardiac death.

**Summary and Specific Aims**

There are no studies in the U.S. that have examined the vitamin D status of patients admitted with acute myocardial infarction. Studies completed outside of the Untied States indicate that both 25(OH)D and NT-proBNP are associated with LV dysfunction, and that low circulating levels of 25(OH)D could potentially contribute to, or potentiate, the development of left ventricular dysfunction (LVDF) and heart failure. However, all of the above studies were not based on patients living in the U.S., where better nutrition and fortification of milk is common.$^{47}$

Given the adverse health implications of 25(OH)D deficiency and the lack of studies in patients who have had a MI (a particularly high-risk group), we will examine the prevalence of vitamin D deficiency and the association of 25(OH)D deficiency with
NT-pro BNP levels in a multicenter cohort of AMI patients. To study these important gaps in knowledge, we will use a unique multicenter prospective registry called TRIUMPH (Translational Research Investigating Underlying disparities in recovery from acute Myocardial infarction: Patients' Health status). This data base has a nationally representative sample of AMI patients with detailed socio-demographic and clinical data in addition to laboratory values (including 25(OH)D levels) and clinical health status. These data were collected between June and December 2008. The specific aims for the thesis research project are:

**Specific aim 1:** To describe the prevalence and patient characteristics associated with 25(OH)D deficiency in AMI patients. Using the TRIUMPH database we will describe the distribution of 25(OH)D levels both as a continuous measure and categorized as those with levels below 10 ng/ml, 10 to ≤ 20 ng/ml, >20 and <30ng/ml and ≥ 30 ng/ml.

**Specific aim 2:** To determine the association of 25(OH)D deficiency with NT-pro BNP levels in AMI patients. We hypothesize that patients with lower vitamin D levels will have higher NT–pro BNP levels and that vitamin D deficiency is independently associated with NT-proBNP levels.

**Significance of the Proposed Research**

The current analyses will add to our understanding of the prevalence of 25(OH)D deficiency in AMI patients, a high risk population and also provide novel insights into those clinical characteristics which are most strongly associated with 25(OH)D deficiency in AMI patients. For example, if 25(OH)D deficiency in AMI patients is found
to be significantly associated more with non ST segment elevation myocardial infarction (NSTEMI) than ST segment elevation myocardial infarction (STEMI) in our cross-sectional analysis, it might form the basis for future studies to determine the protective role of 25(OH)D in preventing plaque rupture leading to partial occlusion of a coronary artery which is the physiology behind a NSTEMI. Apart from the potential mechanistic insights that might be inspired by our findings, our data will define the prevalence of a novel adverse risk factor among AMI patients and could lead to studies that seek to develop innovative strategies for the recognition and treatment of 25(OH)D deficiency at the time of AMI and prior to hospital discharge.

In addition, the discovery of an association between circulating levels of 25(OH)D and NT-proBNP would not only suggest a potential pathway for adverse outcomes among post-MI patients with 25(OH)D deficiency, but could identify a potentially novel therapeutic target (i.e., nutritional vitamin D supplementation) to reduce NT-proBNP levels in the hopes of improving prognosis after MI.

Finally, with longer follow-up of this patient population (beyond the scope of this proposed project) we will be able to determine whether 25(OH)D deficiency further risk stratifies long-term clinical outcomes post MI after adjusting for currently used prognostic schemes.
REFERENCES


CHAPTER 2

Association of 25-hydroxyvitamin D deficiency with NT-proBNP levels in AMI patients

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ABSTRACT

Background and objectives: Nutritional vitamin D deficiency is an emerging risk factor for acute myocardial infarction (AMI) and heart failure. The association between 25-hydroxyvitamin D levels and N-terminal pro B-type natriuretic peptide (NT-proBNP), a robust prognostic marker for post-MI mortality and heart failure is unknown and could illuminate a potential pathway for adverse outcomes among post-MI patients with 25-hydroxyvitamin D deficiency.

Design, setting, participants and measurements: In a cross sectional analysis, we studied 238 AMI patients from 21 US centers to test the association of nutritional vitamin D (25-hydroxyvitamin D [25(OH)D]) deficiency with NT-proBNP levels. Patients’ 25(OH)D levels were categorized as normal (≥30 ng/ml), insufficient (>20 – <30 ng/ml), deficient (>10 – ≤ 20 ng/ml), and severely deficient (≤10 ng/ml) groups.

Results: 96% of AMI patients had low 25(OH)D levels, with 75% having 25(OH)D deficiency and 21% having insufficiency. No significant trends for higher mean log NT-proBNP levels in severely deficient (6.9 ± 1.3 pg/ml), deficient (6.9 ± 1.2 pg/ml) and insufficient (6.9 ± 0.9 pg/ml) groups were observed as compared with patients having normal (6.1 ± 1.7 pg/ml) levels, P = 0.165. In multivariate regression model after adjusting for several covariates, 25(OH)D was not associated with NT-proBNP levels.

Conclusion: Potential associations between nutritional vitamin D deficiency and prognosis in the setting of AMI are unlikely to be mediated through NT-proBNP pathways. Future studies should examine other mechanisms such as inflammation and vascular calcification by which 25(OH)D deficiency could mediate adverse outcomes such as heart failure and mortality post AMI.
**Keywords**: vitamin D, N-terminal proBNP, acute myocardial infarction

**Introduction**

Nutritional vitamin D deficiency is highly prevalent, occurring in approximately 30%-50% of the general population (1,2). In several studies, 25-hydroxyvitamin D [25(OH)D] deficiency has been independently associated with both incident acute myocardial infarction (AMI) (3) and heart failure (HF) (4,5), suggesting that 25(OH)D plays an important role in cardiac function. Supporting this hypothesis, several *in vitro* studies have shown that calcitriol (1,25(OH)$_2$D$_3$), an active form of vitamin D, regulates intracellular calcium metabolism and myocardial contractility through specific vitamin D receptors on cardiac myocytes (6-8). Consequently, 25(OH)D deficiency has been associated with aberrant cardiac contractility, cardiomegaly, and increased ventricular mass due to myocardial collagen deposition (7,8), independent of its known effects on blood pressure (9). In studies of experimental animals, activation of nuclear vitamin D receptors by 1,25(OH)$_2$D$_3$ suppresses the expression and secretion of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in cardiac myocytes (10-12), while in clinical studies of predialysis chronic kidney disease patients, individuals treated with 1,25(OH)$_2$D$_3$ for 12 weeks were observed to have improved left ventricular diastolic function as compared with placebo (13). Collectively, these findings suggest that low circulating levels of 25(OH)D could potentially contribute to, or potentiate, the development of left ventricular dysfunction (LVDF) and heart failure after AMI.

In recent years, N-terminal pro-brain natriuretic peptide (NT-proBNP), a prohormone of BNP released from cardiac ventricles, has been associated with the
severity of left ventricular dilatation and dysfunction after AMI (14,15). In addition, NT-proBNP is a sensitive and robust prognostic biomarker of mortality in AMI (16-18), HF (19,20) and chronic hemodialysis (21,22) patients. Moreover, NT-proBNP levels have direct clinical implications, as they are used to guide therapeutic interventions in HF patients, leading to improved outcomes as compared with routine clinical treatment (23).

Thus, while both 25(OH)D and NT-proBNP are both known to be associated with LV dysfunction after AMI, it is not known whether there is a correlation between levels of 25(OH)D and NT-proBNP. In clinical studies, inverse associations between 25(OH)D levels and NT-proANP in HF (4) and between 25(OH)D and BNP in dialysis patients have been suggested (24,25). However, these studies had small sample sizes and were not based on patients living in the U.S., where better nutrition and fortification of milk is common (26). Given the adverse health implications of 25(OH)D deficiency and the lack of studies in AMI patients (a particularly high-risk group), we examined the association of 25(OH)D deficiency with NT-proBNP levels in a multicenter cohort of AMI patients. Discovery of an association between circulating levels of 25(OH)D and NT-proBNP would not only suggest a potential pathway for adverse outcomes among post-AMI patients with 25(OH)D deficiency, but could also identify a potentially novel therapeutic target (i.e., nutritional vitamin D supplementation) to reduce NT-proBNP levels in the hopes of improving prognosis after MI.

**Materials and Methods**

*Study Population*
This study is a cross sectional analysis of a cohort study. Participants were drawn from the TRIUMPH (Translational Research Investigating Underlying disparities in recovery from acute Myocardial infarction: Patients' Health status) study, a prospective multicenter cohort study of AMI patients across 21 U.S. centers. Patients were eligible for TRIUMPH if they were ≥18 years of age and had a diagnosis of AMI. AMI was diagnosed by the presence of either a CK–MB elevation greater than twice normal or Troponin-I elevation of >0.1mg/ml within 24 hours of arrival to the hospital with a clinical presentation suggestive of an AMI (e.g., prolonged ischemic signs or chest pain symptoms, at least one EKG with ST-wave elevation or ST-wave depression in 2 or more consecutive leads, and no alternative explanation for the presence of elevated serum cardiac markers). Patients were excluded if they transferred to the participating hospital from another facility greater than 24 hours after their original AMI presentation, if they refused or could not provide informed consent, or if they were receiving hospice care. For this study, we included the last 250 TRIUMPH patients, in each of whom vitamin D levels were assessed in addition to the standard data collected. TRIUMPH complied with the Declaration of Helsinki and was approved by the institutional review boards of each participating institution. Written informed consent was obtained from all participants.

Data Collection

During index hospitalization, trained data collectors performed a patient interview and detailed chart abstraction within 24-72 hours of admission. Patient data at AMI presentation, including demographic features, socioeconomic status, co-morbidities,
severity of AMI (ST elevation vs. non-ST elevation AMI), Killip class, Rose dyspnea index, vital signs, and laboratory values were abstracted. The Rose dyspnea score is a validated self-reported question in AMI patients that is scored from 0-4, with higher scores indicating worse dyspnea (27). Regional and seasonal data were collected, given their association with vitamin D levels (3,28,29). Patients were classified by their residing states into 5 geographic regions, namely northeast (NE), southeast (SE), southwest (SW), mid-west (MW) and west (W). Months of April-June, July-August and September-December were classified as summer, fall, and early winter seasons, respectively.

Left ventricular systolic function was classified as normal, mild, moderate or severe as assessed by echocardiography, angiography or nuclear imaging and as documented in the hospital record. Finally, reperfusion therapy (coronary angiography, percutaneous intervention [PCI], and/or coronary artery bypass surgery [CABG]) and other acute therapies as well as medications prescribed at discharge were recorded. Blood samples from all consenting patients were sent to a core laboratory (Clinical Reference Laboratories, Lenexa, KS) for NT-proBNP measurement (Roche Diagnostics, Indianapolis, IN) and 25(OH)D assays, as described below.

**Laboratory Measurements**

An *in vitro* radioimmunoassay (RIA) assay (DiaSorin, Stillwater, MN) was used for quantitative determination of 25(OH)D and other hydroxylated vitamin D metabolites in human serum. The DiaSorin 25(OH)D assay comprises a two-step procedure involving both a rapid extraction of 25(OH)D and other hydroxylated metabolites from
serum or plasma with acetonitrile and a 25(OH)D-specific antibody and tracer while incubating for 90 minutes at 20-25°C, and phase separation with a second antibody-precipitating complex. The total (intra- and inter-assay) precision of the assay has a coefficient of variation (CV) of 9.4% and 11% for control values of 8.6ng/ml and 49.0ng/ml, respectively.

For measurement of circulating NT-proBNP levels, the Roche electrochemiluminescence immunoassay for the in vitro quantization was used (ECLIA Roche diagnostics GmbH, Mannheim, Germany). The precision of this assay was represented by a CV of 3.2% and 2.3% for control values of 175pg/ml and 4962 pg/ml, respectively.

Blood samples for NT-proBNP were drawn prior to hospital discharge. The mean time for NT-proBNP blood draw was 3.5 ± 4 days in this cohort. Glomerular filtration rate (eGFR) was estimated using the four variable Modified Diet in Renal Disease (MDRD) study equation (30).

**Statistical analysis**

Participants were classified into clinically-relevant categories on the basis of 25(OH)D levels. AMI patients with levels ≥30 ng/ml were classified as normal, while levels >20ng/ml and <30ng/ml were considered insufficient, levels of >10 to ≤ 20ng/ml were deficient, and levels ≤10ng/ml were severely deficient. Median NT-proBNP levels were compared using the non-parametric Kruskal-Wallis test, due to the positively skewed distribution of NT-proBNP. Log NT-proBNP and quartiles of NT-proBNP were compared across the four 25(OH)D strata using ANOVA and chi-square tests, respectively. Spearman rank correlation was obtained between NT-proBNP and
25(OH)D levels. Multivariable linear regression analysis to determine the association of 25(OH)D levels with log NT-proBNP was performed, adjusting for relevant covariates and confounders. A \( p \) value of <0.05 was considered statistically significant. All analyses were conducted using SAS v9.1 software (Cary, NC, U.S.).

**Results**

Characteristics of participants across 25(OH)D groups are shown in Table 1. Of the 238 enrolled patients, the median 25(OH)D concentration was 16 ng/ml (interquartile range [IQR] 12-21). Classifying 25(OH)D levels into clinically-interpretable ranges, 40 (16.8%) were found to be severely deficient, 138(57.9%) deficient and 50 (21.0%) insufficient. Only 4.2% of participants in the study had normal 25(OH)D levels, with none of the African-American participants having normal 25(OH)D levels.

No statistically significant differences by age or gender were noted across 25(OH)D groups. Deficiency in 25(OH)D was associated with a history of recent smoking, low physical activity, lack of medical insurance, and poor social support. No difference in those with or without 25(OH)D deficiency were observed for hypertension, diabetes, history of MI, HF, or the type of AMI (STEMI vs. NSTEMI). Similarly, Killip class at arrival and Rose dyspnea scores did not differ by 25(OH)D levels. During the study period, no statistically significant regional variations were observed in patients’ enrollment across 25(OH)D groups (\( p = 0.79 \)), nor did estimated glomerular filtration rates differ (\( p = 0.24 \)). There was a trend towards seasonal variation in 25(OH)D levels (\( p = 0.064 \)). As expected, lower serum calcium and higher parathyroid hormone levels (PTH) were significantly associated with 25(OH)D deficiency (\( p \) values of 0.03 and
Likewise the use of Omega 3 supplements was higher in patients with normal 25(OH)D levels (50%) compared to those who had insufficient (36%), deficient (22.5%) and severely deficient (7.5%) levels (p value = 0.002).

No statistically significant correlation between 25(OH)D and log NT-proBNP levels was observed (\( \rho = -0.0025, p = 0.97; \) Figure 1). No significant trends for higher mean log NT-proBNP levels in severely deficient (6.9 ± 1.3 pg/ml), deficient (6.9 ± 1.2 pg/ml) and insufficient (6.9 ± 0.9 pg/ml) groups were observed as compared with patients having normal (6.1 ± 1.7 pg/ml) levels, \( p = 0.165. \) (Table 2).

In the multivariable linear regression model, after adjusting for age, race, gender, BMI, social support, medical insurance, smoking status, seasons, co morbidities such as diabetes and history of prior MI, in-hospital percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG), high sensitivity C reactive protein, e GFR and omega 3 supplements use, 25(OH)D levels were not significantly associated with log NT-proBNP levels. (Table 3). However, NT-proBNP levels remained independently associated with hs CRP, a marker of inflammation, even after adjusting for 25(OH)D levels.

**Discussion**

In this cross sectional observational study, we found no evidence of an association between 25(OH)D levels and NT-proBNP levels, nor other clinical markers of acute LV dysfunction, including Killip class or Rose dyspnea score. To our knowledge, this is the first study to compare levels 25(OH)D and NT-proBNP in an AMI population. Thus, while both 25(OH)D levels and NT-proBNP are associated with
cardiovascular disease and heart failure, they appear to impact prognosis through different mechanisms in the setting of AMI.

Importantly, however, we found exceedingly low levels of nutritional vitamin D in this cohort of AMI patients, such that few patients had normal 25(OH)D levels. In fact, 74.7% of these AMI patients had significant 25(OH)D deficiency with another 21% having insufficiency, a prevalence much higher than the national estimates of 25%-57% in the general population (31-33). Consistent with previous reports, we also found that 25(OH)D deficiency was associated with older age, higher BMI, smoking, low physical activity and lesser use of omega 3 supplements (1,28,34). Moreover, we were able to extend these previously known clinical correlations by finding that 25(OH)D deficiency is associated with low social support and lack of medical insurance, both of which are associated with higher morbidity and mortality after MI (34-36). Thus although our data do not support an association between 25(OH)D deficiency and a biomarker of LV dysfunction in the setting of AMI, the high prevalence of 25(OH)D deficiency in this cohort of AMI patients is noteworthy. Given that 25(OH)D deficiency has been associated with incident MI (3) and CV events (47) in prior observational studies, it would be important for future investigators to examine whether rectifying vitamin D levels in post-AMI patients is associated with an improvement in subsequent outcomes.

Our findings extend and confirm the preliminary insights provided by Pilz et al, who studied patients with established CAD referred to coronary angiography. While they found that 25(OH)D deficiency was significantly associated with higher NYHA functional HF classes in unadjusted analysis, this association was no longer significant after adjustment for other clinical variables (37). In contrast, our findings are dissimilar from
prior observations that in hemodialysis patients, where lower 25(OH)D levels were found to be associated with significantly higher log BNP levels (24). NT-proBNP is a more stable form of BNP and correlates well with BNP levels in heart failure patients (38). Similar reports in chronic HF patients have also suggested an inverse, nonlinear association between 25(OH)D and NT-proANP levels ($r^2 = 0.16$, $p<0.001$) (4), although a clinical trial of nutritional vitamin D supplementation failed to alter patients’ NT-proBNP levels (39). More recently, a large cohort of patients referred to coronary angiography in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study suggested that 25(OH)D levels remained independently associated with NT-proBNP levels in a multivariable model ($\beta = -0.180$, $p<0.001$) (37). It is unknown whether the discrepancy in our findings and these previous studies is due to unmeasured confounding in prior reports, or whether the lack of an association is due to the impact of acute myocardial injury on NT-proBNP levels (40,41).

In recent years there has been growing interest in the role of 25(OH)D and optimal organ function, including that of the cardiovascular (CV) system. Cardiac myocytes have cytosolic vitamin D receptors (VDR) (42) that bind active vitamin D, but lack 1α-hydroxylase activity (43). Hence cardiac muscle is strongly dependent upon circulating calcitriol level, underscoring the importance of studying 25(OH)D levels in patients with cardiovascular disease. For example, several studies have shown in-vitro 25(OH)D deficiency to be associated with impaired cardiac inotropy, increased left ventricular mass due to myocardial collagen deposition (7,8), enhanced atherosclerosis secondary to vascular smooth muscle cell (VSMC) proliferation (44,45) and pro-inflammatory cytokines (IL-6 and TNF-alfa) (46). Furthermore, large epidemiological
studies, including the Health Professionals study (3) and Framingham offspring study (47) have shown low 25(OH)D levels (<15ng/ml vs. ≥30ng/ml) to be independently associated with a doubling of risk for incident MI, both fatal and nonfatal events. Moreover, the risk of all-cause mortality was higher among subjects with 25(OH)D levels <17.8ng/ml in NHANES III as compared with subjects having levels >32ng/ml. Finding no association between vitamin D levels and NT-proBNP in our study suggests that the association of 25(OH)D with AMI mortality is unlikely to be mediated through NT-proBNP, despite the prognostic association between both biomarkers and survival.

The results of our study should be interpreted in the context of some potential limitations. Our sample size was small, and therefore was underpowered for some analyses. For example, we did not find an association with hypertension (HTN) or diabetes (28,47), chronic kidney disease (42,48) or heart failure history (4,5) as reported in prior studies. However, in this study of 238 patients we had 80% power to observe an unadjusted correlation of 0.18 between 25(OH)D and NT-proBNP. Importantly, there were very few patients (n =10) who had normal 25(OH)D levels, and if there is a non-linear association between lower and normal 25(OH)D levels, we may have been underpowered to detect this. It is also possible that the association between the biomarkers we tested might have been stronger at a time of clinical stability or at the time of acute presentation, since our samples for both 25(OH)D and NT-proBNP were taken prior to discharge; however the prognostic importance of NT-proBNP at the time of MI has consistently been shown to be strong.(17,18)

In conclusion, the mechanism by which nutritional vitamin D deficiency mediates outcomes in AMI patients does not appear to be through its effects on, or a relationship
with, NT-pro BNP. Future studies should better clarify the clinical mechanism by which 25(OH)D deficiency is associated with outcomes in AMI patients. Potential candidates might include more long-term processes such as inflammation and vascular calcification. While we did not observe an association between levels of 25(OH)D and NT-proBNP, we did find a remarkably high prevalence of 25(OH)D deficiency among AMI patients. Hospitalization for an AMI offers clinicians an opportunity to not only identify modifiable risk factors and optimize medications for secondary prevention but also to address other co morbidities, such as 25(OH)D deficiency. Given that nutritional vitamin D is readily available, inexpensive, and has a good safety profile, future studies should investigate whether addressing 25(OH)D deficiency might improve outcomes in AMI patients, regardless of the mechanism of its known association with post-MI risk.

Acknowledgments

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Disclosures

None
Figure 1. Spearman correlation between 25(OH)D (ng/ml) and NT-proBNP levels (pg/ml)

Spearman's rho = -0.0025, P = 0.97
Table 1. Baseline characteristics of study participants, by 25(OH)D groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>0 – 10 (n= 40)</th>
<th>&gt;10 – ≤20 (n = 138)</th>
<th>&gt;20 – &lt;30 (n = 50)</th>
<th>≥30 (n = 10)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)²</td>
<td>55.0±11.2</td>
<td>57.8±11.8</td>
<td>58.8±10.2</td>
<td>62.4±11.3</td>
<td>0.209</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>26 (56)</td>
<td>104 (75.4)</td>
<td>38 (76)</td>
<td>8 (80)</td>
<td>0.569</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>23 (57.5)</td>
<td>103 (74.6)</td>
<td>41 (82)</td>
<td>10 (100)</td>
<td>0.012</td>
</tr>
<tr>
<td>BMI³ (kg/m²)</td>
<td>32.1 ± 5.6</td>
<td>30.9 ± 6.9</td>
<td>29.5 ± 5.6</td>
<td>26.5 ± 4.6</td>
<td>0.066</td>
</tr>
<tr>
<td>Socio-economic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low social support, n (%)</td>
<td>11 (27.5)</td>
<td>20 (14.9)</td>
<td>3 (6)</td>
<td>0 (0)</td>
<td>0.023</td>
</tr>
<tr>
<td>No health insurance, n (%)</td>
<td>14 (35)</td>
<td>28 (20.9)</td>
<td>5 (10.4)</td>
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<td>0.036</td>
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<tr>
<td>Lifestyle</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Smoked within 1 month, n (%)</td>
<td>24 (60)</td>
<td>49 (35.5)</td>
<td>15 (30)</td>
<td>0 (0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mainly sedentary, n (%)</td>
<td>20 (50)</td>
<td>56 (40.9)</td>
<td>19 (38)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td>Mild exercise</td>
<td>13 (32.5)</td>
<td>49 (35.8)</td>
<td>11 (22)</td>
<td>3 (30)</td>
<td></td>
</tr>
<tr>
<td>Moderate exercise</td>
<td>6 (15)</td>
<td>27 (19.7)</td>
<td>17 (34)</td>
<td>4 (40)</td>
<td></td>
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<tr>
<td>Strenuous exercise</td>
<td>1 (2.5)</td>
<td>5 (3.6)</td>
<td>3 (6)</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>Geographical region, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td>Midwest</td>
<td>18 (56.3)</td>
<td>53 (45.3)</td>
<td>15 (34.1)</td>
<td>5 (62.5)</td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>3 (9.4)</td>
<td>15 (12.8)</td>
<td>5 (11.4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td>5 (15.6)</td>
<td>23 (19.7)</td>
<td>9 (20.5)</td>
<td>1 (12.5)</td>
<td></td>
</tr>
<tr>
<td>Southwest</td>
<td>4 (12.5)</td>
<td>22 (18.8)</td>
<td>12 (27.3)</td>
<td>2 (25)</td>
<td></td>
</tr>
<tr>
<td>West</td>
<td>2 (6.3)</td>
<td>4 (3.4)</td>
<td>3 (6.8)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Season, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.064</td>
</tr>
<tr>
<td>Apr-Jun</td>
<td>1 (2.5)</td>
<td>1 (0.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Jul-Sep</td>
<td>10 (25)</td>
<td>56 (40.6)</td>
<td>24 (48)</td>
<td>7 (70)</td>
<td></td>
</tr>
<tr>
<td>Oct-Dec</td>
<td>29 (72.5)</td>
<td>81 (58.7)</td>
<td>26 (52)</td>
<td>3 (30)</td>
<td></td>
</tr>
<tr>
<td>Co-morbidities, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>13 (32.5)</td>
<td>41 (29.7)</td>
<td>8 (16)</td>
<td>2 (20)</td>
<td>0.209</td>
</tr>
<tr>
<td>Hypertension</td>
<td>26 (65)</td>
<td>89 (64.5)</td>
<td>33 (66)</td>
<td>6 (60)</td>
<td>0.99</td>
</tr>
<tr>
<td>Prior MI</td>
<td>2 (5)</td>
<td>36 (26.1)</td>
<td>9 (18)</td>
<td>2 (20)</td>
<td>0.019</td>
</tr>
<tr>
<td>CKD⁵</td>
<td>5 (12.5)</td>
<td>4 (2.9)</td>
<td>2 (4)</td>
<td>1 (10)</td>
<td>0.06</td>
</tr>
<tr>
<td>Chronic heart failure</td>
<td>3 (7.5)</td>
<td>6 (4.3)</td>
<td>1 (2)</td>
<td>1 (10)</td>
<td>0.373</td>
</tr>
<tr>
<td>LVH on admission EKG, n (%)</td>
<td>7 (17.9)</td>
<td>9 (6.7)</td>
<td>4 (8.2)</td>
<td>0 (0)</td>
<td>0.171</td>
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<tr>
<td>Killip class on arrival, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.347</td>
</tr>
<tr>
<td>I</td>
<td>36 (94.7)</td>
<td>128 (94.8)</td>
<td>43 (86)</td>
<td>9 (90)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>2 (5.3)</td>
<td>4 (3)</td>
<td>5 (10)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>0 (0)</td>
<td>2 (1.5)</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0 (0)</td>
<td>1 (0.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Rose dyspnea score</td>
<td>1.0 ± 0.9</td>
<td>0.9 ± 0.9</td>
<td>0.8 ± 0.8</td>
<td>0.6 ± 1.0</td>
<td>0.43</td>
</tr>
<tr>
<td>Final MI diagnosis, n (%)</td>
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<td></td>
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<td>0.352</td>
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<tr>
<td>STEMI⁶</td>
<td>15 (37.5)</td>
<td>64 (46.4)</td>
<td>23 (46)</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>25 (62.5)</td>
<td>74 (53.6)</td>
<td>27 (54)</td>
<td>8 (80)</td>
<td></td>
</tr>
<tr>
<td>LV systolic function, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.533</td>
</tr>
<tr>
<td>Normal</td>
<td>24 (61.5)</td>
<td>88 (63.8)</td>
<td>36 (72)</td>
<td>6 (60)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>8 (20.5)</td>
<td>24 (17.4)</td>
<td>10 (20)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>4 (10.3)</td>
<td>16 (11.6)</td>
<td>4 (8)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>3 (7.7)</td>
<td>10 (7.2)</td>
<td>0 (0)</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>Laboratory values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troponin I (ng/ml)⁷</td>
<td>1.4 ±1.9</td>
<td>1.7 ±1.8</td>
<td>1.6 ±1.7</td>
<td>0.7 ±1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)⁷</td>
<td>150.6 ±47</td>
<td>157.3 ±29</td>
<td>150.5 ±29</td>
<td>189.7 ±47</td>
<td>0.011</td>
</tr>
</tbody>
</table>
Table 2: Association of 25(OH)D with NT-proBNP levels

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>0−10</th>
<th>&gt;10−≤20</th>
<th>&gt;20−&lt;30</th>
<th>≥30</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D group, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median NT-proBNP (pg/ml)</td>
<td>1094.5</td>
<td>897.5</td>
<td>1065.5</td>
<td>757.5</td>
<td>0.457</td>
</tr>
<tr>
<td>Log NT-proBNP&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9 ± 1.3</td>
<td>6.9 ± 1.2</td>
<td>6.9 ± 0.9</td>
<td>6.1 ± 1.7</td>
<td>0.165</td>
</tr>
<tr>
<td>NT-pro BNP&lt;sup&gt;b&lt;/sup&gt;, n (%)</td>
<td>3 (7.5)</td>
<td>31 (22.5)</td>
<td>18 (36)</td>
<td>5 (50)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data shown as mean ± standard deviation.
<sup>b</sup>NT-proBNP, N terminal pro brain natriuretic peptide.

Table 2. Association of 25(OH)D with NT-proBNP levels

<table>
<thead>
<tr>
<th>25(OH)D group, ng/ml</th>
<th>Median NT-proBNP (pg/ml)</th>
<th>Log NT-proBNP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>NT-pro BNP&lt;sup&gt;b&lt;/sup&gt;, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0−10 (n=40)</td>
<td>1094.5</td>
<td>6.9 ± 1.3</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>&gt;10−≤20 (n=138)</td>
<td>897.5</td>
<td>6.9 ± 1.2</td>
<td>34 (24.6)</td>
</tr>
<tr>
<td>&gt;20−&lt;30 (n=50)</td>
<td>1065.5</td>
<td>6.9 ± 0.9</td>
<td>10 (20)</td>
</tr>
<tr>
<td>≥30 (n=10)</td>
<td>757.5</td>
<td>6.1 ± 1.7</td>
<td>3 (30)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data shown as mean ± standard deviation.
<sup>b</sup>NT-proBNP, N terminal pro brain natriuretic peptide.
Table 3. Results of Multivariable regression analysis

<table>
<thead>
<tr>
<th>Covariate</th>
<th>β Coefficient</th>
<th>$P$ -Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age per 10yr increment</td>
<td>0.256</td>
<td>0.001</td>
</tr>
<tr>
<td>Caucasian race</td>
<td>0.374</td>
<td>0.03</td>
</tr>
<tr>
<td>Male sex</td>
<td>-0.179</td>
<td>0.27</td>
</tr>
<tr>
<td>BMI$^a$ per 5 units increment</td>
<td>-0.115</td>
<td>0.06</td>
</tr>
<tr>
<td>Lack of social support</td>
<td>-0.205</td>
<td>0.33</td>
</tr>
<tr>
<td>No health insurance</td>
<td>0.275</td>
<td>0.17</td>
</tr>
<tr>
<td>Smoked within 1 month</td>
<td>0.027</td>
<td>0.87</td>
</tr>
<tr>
<td>Seasons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nov-Feb vs. Mar-Oct</td>
<td>0.170</td>
<td>0.25</td>
</tr>
<tr>
<td>History of Diabetes</td>
<td>0.006</td>
<td>0.97</td>
</tr>
<tr>
<td>History of prior MI$^a$</td>
<td>0.244</td>
<td>0.16</td>
</tr>
<tr>
<td>In hospital PCI$^a$</td>
<td>0.164</td>
<td>0.35</td>
</tr>
<tr>
<td>In hospital CABG$^a$</td>
<td>-0.152</td>
<td>0.55</td>
</tr>
<tr>
<td>hs CRP$^a$</td>
<td>0.439</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>e GFR$^a$</td>
<td>-0.040</td>
<td>0.008</td>
</tr>
<tr>
<td>Omega 3 supplements</td>
<td>-0.217</td>
<td>0.2</td>
</tr>
<tr>
<td>25(OH)D$^a$ ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&lt;10$ vs. $\geq 30$</td>
<td>0.907$^b$</td>
<td>0.06</td>
</tr>
<tr>
<td>$&gt;10$- $\leq 20$ vs. $\geq 30$</td>
<td>0.848</td>
<td>0.05</td>
</tr>
<tr>
<td>$&gt;20$- $&lt;30$ vs. $\geq 30$</td>
<td>0.764</td>
<td>0.08</td>
</tr>
</tbody>
</table>

$^a$BMI, body mass index; MI, myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; hs CRP, high sensitivity c reactive protein; e GFR, estimated glomerular filtration rate; $^{25}$ (OH)D, 25 hydroxy vitamin D.

$^b$ log BNP change for 1ng/m change in 25(OH)D


CHAPTER 3: DISCUSSION

Project Summary

In this study we evaluated the association of nutritional vitamin D deficiency with NT-proBNP levels in acute myocardial infarction patients. Nutritional vitamin D [25-hydroxyvitamin D 25(OH)D] deficiency is highly prevalent, occurring in approximately 30%-50% of the general population.\textsuperscript{1,2} In several studies, 25-hydroxyvitamin D [25(OH)D] deficiency has been independently associated with both incident acute myocardial infarction (AMI)\textsuperscript{3} and heart failure (HF)\textsuperscript{4,5}, suggesting that 25(OH)D plays an important role in cardiac function. As shown in several in-vitro studies, 25(OH)D deficiency has been associated with aberrant cardiac contractility, cardiomegaly, and increased ventricular mass due to myocardial collagen deposition.\textsuperscript{6,7} On the other hand, N-terminal pro-brain natriuretic peptide (NT-proBNP), a prohormone of BNP released from cardiac ventricles, has been shown to be a sensitive and robust prognostic biomarker for post-MI mortality and heart failure.\textsuperscript{8-12} Thus, low circulating levels of 25(OH)D could potentially contribute to, or potentiate, the development of left ventricular dysfunction (LVDF) and heart failure after AMI. We therefore hypothesized that low levels of 25(OH)D would be associated with higher NT-proBNP levels which is a marker of heart failure.

We prospectively studied 238 acute myocardial infarction (AMI) patients from 21 US centers to test the association of nutritional vitamin D deficiency (25-hydroxyvitamin D [25(OH)D]) with NT-proBNP levels. Patients’ 25(OH)D levels were categorized in to clinically interpretable groups such as normal ($\geq 30\text{ng/ml}$), insufficient (<30ng/ml and
Surprisingly 96% of AMI patients were found to have low 25(OH)D levels, of which 40 (16.8%) were severely deficient, 138 (57.9%) deficient and 50 were (21.0%) insufficient. The median 25(OH)D concentration was 16 ng/ml (interquartile range [IQR] 12-21). Notably, none of the African American patients in the study had normal vitamin D levels. Patient reported indicators of heart failure such as Killip class at arrival and Rose dyspnea scores did not differ by 25(OH)D levels.

The main finding of the study was that no statistically significant correlations between 25(OH)D and log NT-proBNP levels were observed ($\rho = -0.0025$, $p = 0.97$). Similarly, no significant associations between the log-transformed NT-proBNP levels and 25(OH)D categories (6.9 ± 1.3 pg/ml in severely deficient vs. 6.1 ± 1.7 pg/ml in replete group, $P = 0.165$) were found. In multivariable regression model after adjusting for several covariates, 25(OH)D was not associated with NT-proBNP levels.

**Additional analyses**

Bivariate associations between NT-proBNP and patient characteristics were obtained to determine the variables that needed adjustment in the multivariable model (Table 4). These results are being presented as additional analyses that were completed for this thesis project, but were not appropriate to include in the manuscript (Chapter 2). This analysis was a precursor to the multivariable model that is presented in Table 3 of Chapter 2.
Table 4. Association of NT-proBNP with patient characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quartile 1 (5 - &lt;488)</th>
<th>Quartile 2 (488 - &lt;924.5)</th>
<th>Quartile 3 (924.5 - &lt;1882)</th>
<th>Quartile 4 (1882 - 31230)</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NT-proBNP levels</strong></td>
<td>n= 58</td>
<td>n= 61</td>
<td>n= 59</td>
<td>n= 60</td>
<td></td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr) supb</td>
<td>54.2 ± 10.4</td>
<td>56.1 ± 10.8</td>
<td>57.2 ± 10.8</td>
<td>63.3 ± 11.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>44 (75.9)</td>
<td>51 (83.6)</td>
<td>42 (71.2)</td>
<td>39 (65)</td>
<td>0.122</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>40 (69)</td>
<td>47 (77)</td>
<td>41 (69.5)</td>
<td>49 (81.7)</td>
<td>0.317</td>
</tr>
<tr>
<td>BMI kg/m^2 supb</td>
<td>32.4 ± 6.2</td>
<td>29.9 ± 6.1</td>
<td>31.4 ± 7.3</td>
<td>28.8 ± 5.9</td>
<td>0.017</td>
</tr>
<tr>
<td>Smoked within 1 month, n (%)</td>
<td>20 (34.5)</td>
<td>27 (44.3)</td>
<td>21 (35.6)</td>
<td>20 (33.3)</td>
<td>0.586</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial Fibrillation, n (%)</td>
<td>4 (6.9)</td>
<td>0 (0)</td>
<td>3 (5.1)</td>
<td>9 (15)</td>
<td>0.006</td>
</tr>
<tr>
<td>Chronic Heart Failure, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (10.2)</td>
<td>5 (8.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>39 (67.2)</td>
<td>31 (50.8)</td>
<td>41 (69.5)</td>
<td>43 (71.7)</td>
<td>0.066</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>19 (32.8)</td>
<td>9 (14.8)</td>
<td>16 (27.1)</td>
<td>20 (33.3)</td>
<td>0.077</td>
</tr>
<tr>
<td>Prior MI supb, n (%)</td>
<td>11 (19)</td>
<td>12 (19.7)</td>
<td>9 (15.3)</td>
<td>17 (28.3)</td>
<td>0.34</td>
</tr>
<tr>
<td>Prior PCI supb, n (%)</td>
<td>12 (20.7)</td>
<td>13 (21.3)</td>
<td>11 (18.6)</td>
<td>13 (22)</td>
<td>0.986</td>
</tr>
<tr>
<td>Prior CABG supb, n (%)</td>
<td>5 (8.6)</td>
<td>6 (9.8)</td>
<td>7 (11.9)</td>
<td>9 (15)</td>
<td>0.71</td>
</tr>
<tr>
<td>Final MI diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td>STEMI supb</td>
<td>17 (29.3)</td>
<td>32 (52.5)</td>
<td>32 (54.2)</td>
<td>23 (38.3)</td>
<td></td>
</tr>
<tr>
<td>NSTEMI supb</td>
<td>41 (70.7)</td>
<td>29 (47.5)</td>
<td>27 (45.8)</td>
<td>37 (61.7)</td>
<td></td>
</tr>
<tr>
<td>Arrival Killip Class, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.928</td>
</tr>
<tr>
<td>I</td>
<td>53 (94.6)</td>
<td>55 (93.2)</td>
<td>53 (89.8)</td>
<td>55 (93.2)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>2 (3.6)</td>
<td>4 (6.8)</td>
<td>3 (5.1)</td>
<td>3 (5.1)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 (1.8)</td>
<td>0 (0)</td>
<td>2 (3.4)</td>
<td>1 (1.7)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.7)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Rose dyspnea score</td>
<td>0.7 ± 0.8</td>
<td>0.7 ± 0.7</td>
<td>0.9 ± 0.9</td>
<td>1.2 ± 1.0</td>
<td>0.007</td>
</tr>
<tr>
<td>LV Systolic Function, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Normal</td>
<td>47 (81)</td>
<td>45 (73.8)</td>
<td>35 (60.3)</td>
<td>27 (45)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>9 (15.5)</td>
<td>9 (14.8)</td>
<td>14 (24.1)</td>
<td>11 (18.3)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>2 (3.4)</td>
<td>6 (9.8)</td>
<td>5 (8.6)</td>
<td>12 (20)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>0 (0)</td>
<td>1 (1.6)</td>
<td>4 (6.9)</td>
<td>10 (16.7)</td>
<td></td>
</tr>
<tr>
<td>In hospital PCI supb, n (%)</td>
<td>32 (55.2)</td>
<td>50 (82)</td>
<td>45 (76.3)</td>
<td>40 (66.7)</td>
<td>0.009</td>
</tr>
<tr>
<td>In hospital CABG supb, n (%)</td>
<td>9 (15.5)</td>
<td>2 (3.3)</td>
<td>4 (6.8)</td>
<td>8 (13.3)</td>
<td>0.085</td>
</tr>
<tr>
<td>Laboratory values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troponin I (ng/ml) supa</td>
<td>0.6 ± 0.7</td>
<td>1.7 ± 1.6</td>
<td>2.0 ± 1.8</td>
<td>2.0 ± 2.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>e GFR supb</td>
<td>89.4 ± 22.3</td>
<td>91.3 ± 17.7</td>
<td>85.8 ± 27.4</td>
<td>76.2 ± 31.3</td>
<td>0.006</td>
</tr>
<tr>
<td>Calcium (mg/dl) supb</td>
<td>9.0 ± 0.6</td>
<td>9.0 ± 0.6</td>
<td>8.9 ± 0.5</td>
<td>8.6 ± 0.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intact PTH supb</td>
<td>32.3 ± 24.8</td>
<td>40.6 ± 27.5</td>
<td>39.9 ± 29.2</td>
<td>54.1 ± 56.4</td>
<td>0.016</td>
</tr>
<tr>
<td>Phosphate (mg/dl) supb</td>
<td>3.8 ± 0.8</td>
<td>3.4 ± 0.8</td>
<td>3.6 ± 0.7</td>
<td>3.3 ± 0.8</td>
<td>0.007</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl) supa</td>
<td>158.7 ± 35.4</td>
<td>165.1 ± 31.0</td>
<td>154.3 ± 36.0</td>
<td>146.0 ± 33.4</td>
<td>0.024</td>
</tr>
<tr>
<td>hsCRP (mg/l) supb</td>
<td>1.7 ± 2.1</td>
<td>2.7 ± 2.7</td>
<td>4.3 ± 5.4</td>
<td>7.0 ± 7.0</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Implications of the research project

The results of this study have several important implications. This is the first study in the US to report the prevalence of nutritional vitamin D deficiency in a multicenter cohort of AMI patients. Vitamin D deficiency was highly prevalent in this cohort of AMI patients with only 4.2% of participants in the study having normal 25(OH)D levels. Given that 25(OH)D deficiency has been associated with incident MI and CV events in prior observational studies, it would be important for future investigators to examine whether rectifying vitamin D levels in post-AMI patients is associated with an improvement in subsequent outcomes.

Secondly, this study is unique as it identifies a potential mechanism by which 25(OH)D deficiency mediates worse prognosis after AMI, which is currently an area of intense research. If the findings of this study could be replicated in a larger cohort of AMI patients or post-AMI heart failure patients, especially during the recovery period, it would further strengthen our conclusion that nutritional vitamin D deficiency may not be mediating prognosis after AMI through effects on NT-proBNP pathways. This could lead to studies that would explore other potential mechanisms such as inflammation and
vascular calcification by which 25(OH)D deficiency could mediate adverse outcomes in AMI patients.

**Future Directions**

Future studies with longer follow-up of AMI patients could help determine whether 25(OH)D deficiency further risk stratifies long-term clinical outcomes post-MI after adjusting for currently used prognostic schemes such as Thrombolysis in Myocardial Infarction (TIMI) or Global registry of Acute Coronary Events (GRACE) risk scores. Also given that nutritional vitamin D is readily available, inexpensive, and has a good safety profile, future randomized controlled trials should investigate whether treating 25(OH)D deficiency might improve hard outcomes, such as mortality and health status in AMI patients, regardless of the mechanism of its known association with post-MI risk.

Currently I am a 3rd year nephrology fellow. With the experience that I have gained through this thesis project, I plan to pursue a career development award on a related subject. One of the areas of my interest is the role of 25-hydroxy vitamin D deficiency in the progression of chronic kidney disease and its impact on cardiac dysfunction in predialysis patients. Chronic kidney disease (CKD) affects nearly 20 million individuals in the US and is associated with significant cardiovascular morbidity and all-cause mortality. In addition an estimated 300,000 patients have end stage renal disease (ESRD) and are currently undergoing dialysis in the US. Although it has been known for several decades that hypertension, diabetes and albuminuria are the risk factors for progression to ESRD, gaps exist in our knowledge.
about the role of novel risk factors such as vitamin D, inflammation and uric acid that
could effect progression of CKD to ESRD. Vitamin D has anti-proliferative and
immunomodulatory properties, and functions as an endocrine regulator of the renin-
angiotensin system. Animal and cell culture studies have shown that vitamin D
suppresses the transcription of renin, decreased circulating angiotensin II levels,
preventing podocyte loss and glomerulosclerosis, and thus decreasing albuminuria
which is an established predictor of CKD progression. As such, low levels of vitamin D
are a potential novel risk factor for progression of CKD. In addition, nutritional vitamin
D deficiency has been associated with incident MI and heart failure both of which
are highly prevalent in CKD patients. Thus, understanding the renal and cardio-
protective role of vitamin D in cohort studies would not only provide mechanistic insights
but also lay the foundation for future randomized controlled trials with vitamin D, a safe
and inexpensive treatment.
References


17. de Araujo GP, Ferreira J, Aguiar C, Seabra-Gomes R: TIMI, PURSUIT, and GRACE risk scores: sustained prognostic value and interaction with revascularization in NSTE-ACS. *Eur Heart J* 26:865-872, 2005


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Rajyalakshmi Gadi

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06/2004 – 06/2007: Internship and Residency at University of Arkansas for Medical Sciences (UAMS)
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08/2002 -- 05/2003: House officer at Care cardiac hospital,
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06/2001-- 06/2002: Internship and House Officer,
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03/2003: Passed USMLE Step 1 (99 Percentile)
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11/2003: Passed CSA (clinical skills examination)
04/2004: Passed USMLE Step 3 (85 Percentile)
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08/2007: Certified in ABIM examination (American Board of Internal Medicine)

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RESEARCH INTERESTS

Cardiovascular outcomes in chronic kidney disease patients.

Role of vitamin D deficiency in acute myocardial infarction patients and outcomes.

Hypertension control in chronic kidney disease patients and outcomes.

PUBLICATIONS


SOCIETY MEMBERSHIPS

American College of Physicians

American Society of Nephrology