Vascular Calcification and Mineral Bone Disorder in Chronic Kidney Disease

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This thesis is submitted for a PhD degree in Medicine
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School of Medicine and Health.

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Declaration

This thesis is submitted to University College Cork in accordance with the requirements for the degree of Doctor of Philosophy (PhD) in the Faculty of Medicine.

I declare that this thesis is a record of my own work and has not been submitted for any other academic award. All information sources have been fully acknowledged and referenced.

Parts of this work have appeared in the following peer reviewed publications and presentations:

1. Kinsella S, Chavrimootoo S, Molloy MG, Eustace JA.
   Moderate Chronic Kidney Disease in Women Is Associated with Fracture Occurrence Independently of Osteoporosis.
   Nephron Clin Pract. 2010 Jul 2; 116(3):c256-c262. PMID: 20606487
2. Kinsella S, Moran S, O Sullivan M, Molloy MG, Eustace JA.
   Hyponatraemia independently of Osteoporosis is associated with fracture occurrence. Clin J Am Soc Nephrol 2010 5: 275-280. PMID: 20056759
3. S. M. Kinsella, S. Chavrimootoo, M. G. Molloy, J. A. Eustace
   The Association of MDRD Estimated Glomerular Filtration Rates (eGFR) with Bone Mineral Density, Osteoporosis and Fracture Occurrence.
   JASN. 2007: 18; 830A.
4. S. Kinsella, A. Harrington, S. McDermott, P. O Shea, J.A Eustace.

The Effect of Residual Secondary Hyperparathyroidism on Health Related Quality of Life in Renal Transplant Recipients. Oral Presentation at the Irish Nephrology Society Annual Scientific Meeting, Dublin, May 2012.

5. Sinéad Kinsella, Joseph A. Eustace. The Burden of Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD) in successful Renal Transplant Recipients: The ABC-Heart Study. Presented at the American Society of Nephrology, Renal Week, Philadelphia, November 2011
6. S. Kinsella, J. Coyle, A. Harrington, MGM Molloy, C. Bogue, JA Eustace. Bone Health, Vascular Calcification and Hyperparathyroidism after Renal Transplantation. Presented at the American Society of Nephrology, Renal Week, Denver, November 2010.
7. S. Kinsella, J. Coyle, A. Harrington, J. Brady, B. Murray, MGM Molloy, C. Bogue, JA Eustace. Hyperparathyroidism, bone health, and aortic calcification in renal transplant recipients. Presented at the International Society of Nephrology Nexus Symposium, Kyoto, Japan, April 17th 2010.
8. Sinéad Kinsella. Bone Health and Chronic Kidney Disease. Presented at the Royal College of Physicians William Stokes Award 23rd October 2009
9. Sinéad Kinsella. Bone Health and Chronic Kidney Disease. Presented at the UCC Medical Alumni Annual Scientific Conference 18th September 2009.
10. Kinsella S, Moran S, Molloy M.G, Eustace J.A. Hyponatraemia, independently of Chronic Kidney Disease (CKD), is associated with fracture occurrence among female DXA attendees. Presented at the American Society of Nephrology, Renal Week, Philadelphia, November 2008.

11. Kinsella S, Moran S, Molloy M.G, Eustace J.A. Hyponatraemia independently of Chronic Kidney Disease is associated with fracture occurrence among female DXA attendees. Oral Presentation at the 2nd Annual UCC/Cork University Teaching Hospitals Research Day June 2008.
12. Kinsella S, Chavrimootoo S, Molloy MG, Eustace JA. The Association of MDRD estimated glomerular filtration rates (eGFR) with Bone Mineral Density (BMD) osteoporosis and fracture occurrence. Presented at the American Society of Nephrology, Renal Week, San Francisco November 2007.

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Dedication

This thesis is dedicated to my Dad, Seán Kinsella,
A Legend and Contender,
for his unwavering support and belief in me.
Master of bizarre words of wisdom regarding the eating of elephants and the lighting of candles.

Abstract

Chronic Kidney Disease (CKD), osteoporosis and mild hyponatremia are all prevalent chronic conditions that may coexist and are often under-recognized. Mineral-Bone Disorder begins early in the natural history of CKD and results in complex abnormalities of bone which ultimately confers a well-established increased risk of fragility fractures in End Stage Kidney Disease. Hyponatremia is a novel, usually renal mediated metabolic perturbation, that most commonly occurs independently of the stage of renal dysfunction but which may also predispose to increased fracture risk. The extent -if any- to which either early stages of renal dysfunction or the presence of hyponatremia contribute to fracture occurrence in the general population, independently of osteoporosis, is unclear. Renal transplantation is the treatment of choice for ESKD and although it restores endogenous renal function it typically fails to normalize either the long term cardiovascular or fracture risk. One potential mechanism contributing to these elevated long-term risks and to diminished Health Related Quality of Life is persistent, post-transplant hyperparathyroidism.

In this study we retrospectively examine the association of renal function and serum sodium with Bone Mineral Density and fracture occurrence in a retrospective cohort of 1930 female members of the general population who underwent routine DXA scan. We then prospectively recruited a cohort of 90 renal transplant recipients in order to examine the association of post transplant parathyroid hormone (PTH) level with measures of CKD Mineral Bone Disorder, including, DXA Bone Mineral Density, Vascular Calcification (assessed using both abdominal radiography and CT techniques, as well as indirectly by carotid-femoral Pulse Wave Velocity) and Quality of Life (using the Short Form-12 and a PTH specific symptom score).

In the retrospective DXA cohort, moderate CKD (eGFR 30-59ml/min/1.73m^2) and hyponatremia (<135mmol/L) were associated with fracture occurrence, independently of BMD, with an adjusted Odds Ratio (95% Confidence Interval), of 1.37 (1.0, 1.89) and 2.25 (1.24, 4.09) respectively. In the renal transplant study, PTH was independently associated with the presence of osteoporosis, adjusted Odds Ratio (95% Confidence Interval), 1.15 (per 10ng/ml increment), (1.04, 1.26). The presence of osteoporosis but not PTH was independently associated with measures of vascular calcification, adjusted ß (95% Confidence Interval), 12.45, (1.16, 23.75). Of the eight quality-of-life domains examined, post-transplant PTH (per 10ng/ml increment), was only significantly and independently associated with reduced Physical Functioning, (95% Confidence Interval), 1.12 (1.01, 1.23).

CKD and hyponatremia are both common health problems that may contribute to fracture occurrence in the general population, a major on-going public health concern. PTH and decreased Bone Mineral Density may signal sub-optimal long-term outcomes post renal transplantation, influencing bone and vascular health and to a limited extent long term Health Related Quality of Life.

Chapter 1

Background

Introduction

Chronic Kidney Disease (CKD) is a common condition that remains under-recognized and under-treated. It is associated with increased morbidity and mortality and its outcome is related not only to progression to End Stage Kidney Disease (ESKD), but predominantly to complications of decreased renal function, including increased cardiovascular risk. Disturbances in bone mineral metabolism are almost ubiquitous in progressive CKD and are central to the development of both the bone and vascular complications which are over-represented in this population. Bone Mineral Disorders begin early in CKD and result in complex abnormalities in bone which ultimately confers a markedly increased risk of fragility fractures, with their associated morbidity and mortality. Nevertheless, the extent to which these abnormalities result in an increased fracture risk in subjects with early, as distinct to those with advanced CKD –for whom this relationship is well established- is unclear.

Increased bone fragility is associated with vascular calcification, an association that has been observed in the general population but which is particularly notable in some subjects with advanced CKD, where it is potentially augmented by pathophysiological mechanisms that are markedly upregulated in advanced CKD, such as severe hyperparathyroidism. Renal transplantation restores endogenous renal function in subjects with ESKD and is the treatment of choice for suitable patients with renal failure. However, cardiovascular mortality and the risk of fracture remain chronically elevated despite renal

transplantation, in keeping with the prior history of ESKD and despite the restoration of renal function. The mechanisms underlying this apparently persistent 'metabolic memory' are unknown but one such potential candidate is a state of persistent, relatively autonomous hyperparathyroidism, which is evident in a poorly defined proportion of transplant recipients. In keeping with its described complications in subjects with primary hyperparathyroidism, such persistently elevated parathyroid hormone (PTH) levels may potentially influence not only bone health and vascular risk but also Health Related Quality of Life, although data examining these putative relationships in the transplant setting are sparse.

In this thesis I will seek to quantify some of the above associations, in particular examining the relationship of renal dysfunction with fracture occurrence in women in the general population with early CKD and secondly, the association of persistent post-transplant hyperparathyroidism with abnormalities of bone mineral metabolism, decreased Bone Mineral Density, vascular mineralization and Health Related Quality of Life in renal transplant recipients. In this introductory chapter I outline the relevant background against which these investigations are developed and the current state of knowledge to which the studies described in the subsequent chapters contribute.

Measurement of Renal Function

The National Kidney Foundation (NKF) Clinical Practice Guidelines[1] state that estimates of Glomerular Filtration Rate (GFR) are the best overall indices of the level of renal function. They also advocate the use of prediction equations using serum creatinine and demographic variables to calculate estimated GFR (eGFR). Recommended prediction equations are the Cockcroft-Gault and the Modification of Diet in Renal Disease (MDRD) formulae, (Level A Recommendation). A number of other predication equations such as the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [2] have been developed since the publication of these guidelines, and while they are currently undergoing validation they are not yet in widespread clinical use.

The 4-variable MDRD prediction equation reports an eGFR indexed to body surface area (ml/min/1.73m^2) and is readily calculable with a calculator or computer if age, race, gender and serum creatinine are known. The MDRD prediction equation was developed using a database of 1070 patients with renal disease and validated in an additional cohort of 558 patients.[3] The MDRD equation is more accurate than Cockcroft-Gault in patients with GFR <90 ml/min. While the MDRD equation has been validated in patients with renal transplants[4,5], and in older individuals[6] it has not been validated in people with normal or near normal renal function[7,8] or in individuals at extremes of body weight.[9,10] A recent retrospective study of diabetic patients found that the Cockcroft-Gault prediction equation was a better predictor of renal function

than the MDRD or CKD-EPI prediction equations in the overweight and obese diabetic population with well preserved renal function.[11]

Definition and Classification of CKD

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/DOQI) Clinical Practice Guidelines on Chronic Kidney Disease in 2002 provided the first definition of CKD independent of cause and introduced a classification system of severity based on the GFR level. CKD was defined as: "kidney damage persisting for greater than 3 months (as defined by structural or functional abnormalities of the kidney) with or without decreased GFR, that can lead to decreased GFR, manifest by either pathological abnormalities or markers of kidney damage including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests. CKD may also be defined as GFR<60ml/min/1.73m^2 for greater than 3 months, with or without kidney damage".[1]

The Kidney Disease: Improving Global Outcomes (KDIGO) Group reviewed and modified this definition and classification system in 2005 to include reference to treatment modality (if any), namely the addition of the suffix "T" to denote renal transplant, in addition to the CKD stage and the suffix "D" to denote dialysis treatment.[12] (Table 1.1)

Table 1.1: Classification and Stratification of Chronic Kidney Disease[12]

Stage	Description	eGFR ml/min/1.73m^2	Classification by Treatment
1	Kidney damage with normal or increased GFR	≥ 90	T if Kidney transplant recipient
2	Kidney damage with mild reduction in GFR	60-89	
3	Moderate reduction in GFR	30-59	
4	Severe reduction in GFR	15-29	
5	Kidney Failure	<15 (or dialysis)	D if Dialysis (Haemodialysis or Peritoneal Dialysis)

Prevalence of CKD

The National Health and Nutrition Examination Survey (NHANES) is a health-examination survey of the civilian, non-institutionalized population of the United States conducted by the National Center of Health Statistics. It collects data in order to provide estimates of prevalence of chronic conditions in the United States. Data has been collected for several different time periods since

the early 1960's. These surveys have found that the overall prevalence of CKD in the US population has increased from 11% in the period 1988-1994[13] to 13% in 1999-2004[2], (based on the MDRD prediction equation). The prevalence of CKD, eGFR< 60ml/min/1.73m^2, has increased from 4.7% to 8.2% over the same period. The reported prevalence of CKD Stage 3 in those over 70 years has increased from 25% to 37% between 1988-1994 and 1999-2004.[2, 13]

The vast majority of patients with CKD die before progressing to ESKD. In one study of 28,000 patients, Keith et al followed patients with an eGFR of <90ml/min/1.73m^2 for over 5 years. This study found that even among patients with advanced Stage 4 CKD, death prior to renal replacement therapy was more than twice as likely as progression to ESKD.[14] Higher CKD stage is also associated with a graded increase in complications of CKD such as hypertension, anemia, malnutrition and disordered mineral metabolism.[1] Go et al demonstrated an independent graded association between reduced eGFR and the risk of death, cardiovascular events and hospitalization[15], confirming that CKD is an undisputed risk factor for all-cause and cardiovascular mortality which increases with declining GFR.

Bone Mineral Density, Osteoporosis and Fracture Risk in the General Population

Osteoporosis is defined as "a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture: bone strength reflects the integration of two main features: bone density and quality".[16] There is no readily available clinical measure of bone strength. Bone Mineral Density is used as a surrogate measure and accounts for approximately 70% of bone strength.[17] Therefore the most commonly used non-invasive tool for the diagnosis of osteoporosis (and prediction of fracture risk) is based on measurement of areal (mass per area) Bone Mineral Density by DXA (Dual energy x-ray absorptiometry), which is determined by the mineral content of bone. Bone Mineral Density measurements have been shown to correlate with the risk of fracture at several skeletal sites. The World Health Organization (WHO) diagnosis of osteoporosis is based on Bone Mineral Density measurement, where osteoporosis is defined as a T-score of at least minus 2.5 standard deviations (SD) below the average Bone Mineral Density. A T-score is defined as the number of SDs above or below the average BMD for young, healthy white females. This is in distinction to a Z-score, which is the number of SDs above or below the average Bone Mineral Density for age and gender matched controls.[18] Recognized risk factors for decreased Bone Mineral Density include advancing age, female gender, oestrogen deficiency, low body mass index, immobility, family history of osteoporosis, low calcium intake, smoking, alcohol consumption, co-morbid

conditions such as thyroid disease and medications (including corticosteroids, anti-convulsants and calineurin inhibitors).[16]

In the post menopausal population, decreased Bone Mineral Density is strongly associated with fracture risk. Age related changes in bone quality are also associated with increased fracture risk. For each additional decade over the age of 50 years, fracture risk doubles, independently of Bone Mineral Density. Thus, while Bone Mineral Density is a good predictor of bone strength, it remains a surrogate determinant as it does not evaluate aspects of bone quality, which contribute to overall bone strength. In addition, bone quality (and therefore strength) is adversely affected by the normal aging process and consequently measures of Bone Mineral Density do not fully predict fracture risk even in the healthy population.

Hyponatremia and Bone Health

Recent attention has focused on serum sodium level as a novel marker of bone disease and fracture risk. Evidence suggests that mild chronic hyponatremia (serum sodium levels of 130- 134mmol/L) is associated with subtle central nervous system impairment including gait and attention deficits that may lead to an increased risk of falls.[19] Several case control studies have reported the association of mild asymptomatic hyponatremia with bone fracture in the ambulatory elderly,[20,21] although neither study was able to control for the presence of osteoporosis, a major potential confounder of this relationship, as

the prevalence of both osteoporosis and hyponatremia increase with older age. Evidence also suggests that hyponatremia itself contributes to bone loss. In 1954, Bergstrom et al. demonstrated that acute hyponatremia in experimental animals resulted in release of sodium from bone[22, 23], analogous to bone resorption and calcium efflux from bone in settings of hypocalcaemia. More recent studies by Verbalis et al. confirmed that chronic hyponatremia (>3 months) significantly reduced Bone Mineral Density compared with normonatremic rats. Using the NHANES III dataset, Verbalis et al. also reported a significant association between mild hyponatremia (<135mmol/L) and increased odds ratio of osteoporosis at the hip (OR 2.85, 95% CI 1.03-7.86, $p<0.01$).[24] Mild hyponatremia therefore may be a readily identifiable and potentially modifiable risk factor for fracture both in the CKD and general population.

The major clinical consequence of osteoporosis and decreased Bone Mineral Density is increased incidence of fracture. As Bone Mineral Density decreases and fracture risk increases with advancing age, rates of fragility fractures, most notably of the hip, increases. The lifetime risk of a hip fracture above the age of 50 years has been estimated to be between 17 and 19.5% for females and 6 and 8% for males.[25, 26] Fragility fractures therefore are a major public health concern and are associated with prolonged hospitalization, disability and in the case of hip fracture, with an increased mortality at one year, which is more

pronounced in males. (RR 3.3 for females and 4.2 for males under the age of 75 years relative to controls without hip fracture).[27]

Bone Health and Fracture in CKD

The prevalence of bone fracture is even higher in patients with CKD than in the general population and is not limited to patients with End Stage Kidney Disease. A 2.3 fold increase in risk of hip fracture has been reported in patients with eGFR <60mls/min/1.73m^2 compared to those without CKD.[28] Fracture risk increases further with declining renal function and patients with Stage 5 CKD on dialysis have a reported 4.5 fold increased risk of hip fracture relative to the general population.[29] The occurrence of hip fracture in patients with End Stage Kidney Disease is associated with an increase in mortality ranging from 50 to 64% at one year[30, 31], compared to a one year mortality of 20% in the general population.[31]

Renal Osteodystrophy and Limitations of DXA

The utility of Bone Mineral Density measurement by DXA in predicting fracture risk in patients with CKD remains controversial. Disorders of mineral metabolism and bone turnover begin early in the course of CKD and become more severe with progressive loss of renal function. These abnormalities lead to a wide range of disorders of bone quality and turnover, leading to an increase in fracture risk independent of Bone Mineral Density. The bone

abnormalities seen in CKD are collectively termed renal osteodystrophy. A histological classification system for renal osteodystrophy was introduced by Sherrard et al in 1993, describing the spectrum of bone disorders in CKD as mild, osteitis fibrosa (high turnover), mixed uremic osteodystrophy, osteomalacia, and adynamic (low turnover) bone disease. These bone lesions were found to associate with PTH levels.[32]

In 2005, KDIGO introduced a new classification system for renal osteodystrophy and recommended that the term "renal osteodystrophy" be used exclusively to describe the histological abnormalities of bone seen in CKD. This classification system is based on evaluation of bone turnover, mineralization and volume (TMV Classification System) and was felt to provide additional information based on indicators other than just bone turnover.[33] (Table 1.2)

Table 1.2: 2005 TMV Classification System for Renal Osteodystrophy.[33]

Turnover	**Mineralization**	**Volume**
Low	Normal	Low
Normal		Normal
High	Abnormal	High

The gold standard for the diagnosis of renal bone disease remains quantitative bone histomorphometry and correlations of Bone Mineral Density with bone histology are poor. Bone Mineral Density gives no information on the rate of bone turnover and cannot distinguish between abnormalities in cortical and trabecular bone. Therefore Bone Mineral Density measurement in the setting of CKD can be misleading, depending on the site of measurement, as high turnover bone disease may lead to thickened sclerotic trabecular bone, while simultaneously causing bone loss at cortical bone sites, such as the distal radius.[34] Falsely elevated or false normal results may also be seen, depending on the site of measurement, as all mineralization along the path of the X-Ray beam will contribute to the Bone Mineral Density result, for example, DXA performed at the lumbar-sacral spine will also detect mineralization of the abdominal aorta, unless assessed laterally, which is common among patients with CKD.[35] Similarly, low Bone Mineral Density by DXA does not reliably distinguish the type of renal bone disease which may be present, for example, low Bone Mineral Density measured by DXA may be present in both high and low turnover bone disease states. Therefore while fracture risk is increased in patients with CKD, fracture risk does not correlate well with Bone Mineral Density as measured by DXA, as numerous other factors influencing bone strength are equally, if not more important and exert more influence on fracture risk as renal function declines. In addition, CKD is associated with increased levels of frailty, Vitamin D deficiency, deterioration in muscle

function and general deconditioning which predispose to an increased risk of falls with subsequent fragility fracture.[36-38] Given these limitations of DXA the KDIGO practice guidelines recommended in 2009 that patients with CKD Stage 3-5D, with evidence of abnormal mineral metabolism, should not undergo routine measurements of Bone Mineral Density.[39] However, this recommendation has been called into question following a recent study by Yenchek et al. accompanied by an editorial commentary by Nickolas.[40] Yenchek et al. in a longitudinal study, analyzed the effect of CKD on fracture risk prediction by DXA in a cohort of 2754 community dwelling older adults, followed for a median of 11.3 years. This study demonstrated that low Bone Mineral Density by DXA predicted fracture occurrence in patients with and without CKD, even following adjustment for mineral and bone abnormalities (elevated PTH and low Vitamin D levels). The majority of patients with CKD in this cohort had CKD Stage 3, eGFR 45-59.9mls/min.[41] Additionally, Jamal et al have suggested that in early CKD (Stage 1-3), in the absence of abnormal mineral metabolism, Bone Mineral Density measurements by DXA may be useful in determining fracture risk, but that this cannot be recommended in more advanced CKD.[42]

Quantitative Computed Tomography (QCT) may have more utility in the prediction of fractures and assessment of bone health in patients with CKD. Its main advantages are that it can distinguish between cortical and trabecular Bone Mineral Density, a distinction that is particularly important in renal bone

disease. Secondly it provides an assessment of bone volume, (reported as mg/cm^3), rather than the areal Bone Mineral Density (g/cm^2) measured by DXA. The WHO definition of osteoporosis (T-score less than 2.5) does not apply to QCT; instead osteoporosis is defined as a QCT Bone Mineral Density score less than $80mg/cm^3$. [43]

Chronic Kidney Disease - Mineral Bone Disorder

The term renal osteodystrophy was previously used to describe the spectrum of mineral abnormalities and bone disease that occur in CKD. These disorders included abnormalities in serum calcium, phosphate, parathyroid hormone (PTH) and Vitamin D metabolism. As mentioned previously, KDIGO recommended in 2005 that the term renal osteodystrophy be reserved for the description of the range of morphological changes seen on bone biopsy in CKD. It was observed that these biochemical and bone abnormalities contributed to the excessive cardiovascular risk and significantly increased morbidity and mortality seen in patients with renal disease. It was recognized that abnormalities in mineral metabolism, bone health and cardiovascular outcomes were closely inter-related. The term Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD) was introduced by KDIGO in 2005 to describe the clinical syndrome linking the clinical, biochemical and imaging abnormalities seen in CKD.[33]

CKD-MBD is defined as a systemic disorder of mineral and bone metabolism manifested by either one, or a combination of the following;

- Abnormalities of calcium, phosphate, PTH or Vitamin D metabolism
- Abnormalities in bone turnover, mineralization, volume, growth or strength
- Calcification of the vasculature or soft tissues.[33]

Pathophysiology of CKD-MBD

The development of secondary hyperparathyroidism is central to the pathophysiology of CKD-MBD. The aetiology of secondary hyperparathyroidism is complex and multifactorial. The main parameters responsible for continued PTH secretion in CKD are hypocalcaemia, decreased 1, 25 OH Vitamin D and hyperphosphatemia. Calcium and phosphate levels are preserved within the normal range until late stage CKD, [44] while elevations in PTH levels and Vitamin D deficiency develop early in the course of the disease.[44-46] These mechanisms are initially compensatory, serving to maintain a normal serum calcium and phosphate. However as CKD progresses, these mechanisms become overwhelmed and pathophysiological.

Parathyroid Hormone – Normal Physiology

PTH is one of 3 major hormones modulating calcium and phosphate homeostasis, (the others being Vitamin D and FGF 23). PTH synthesis and secretion are regulated through an exquisitely sensitive calcium sensing receptor (CaSR) [47] expressed widely, including in parathyroid tissue. The CaSR senses minute changes in serum calcium concentrations and PTH is responsible for calcium homeostasis on a minute to minute basis, maintaining serum calcium concentration within a very narrow range.[48] The PTH receptor is heavily expressed in bone and kidney and is also found in the skin, heart and blood vessels.

PTH and Calcium homeostasis

In response to decreases in serum calcium, there is immediate exocytosis of stored PTH from secretory vesicles into the extracellular fluid which occurs over seconds to minutes.[48] PTH acts acutely by increasing renal tubular reabsorption and releasing calcium from skeletal stores. Sustained hypocalcaemia results in a reduction in intracellular degradation of PTH which occurs within minutes to hours. In response to chronic hypocalcaemia, PTH stimulates increased renal hydroxylation of 25-OH Vitamin D to active 1, 25-OH Vitamin D (calcitriol), causing increased intestinal calcium absorption and osteoclastic resorption of bone and further calcium release. Prolonged hypocalcaemia increases PTH gene expression (over hours to days) and

increases the number of parathyroid cells resulting in gland hyperplasia (over days to weeks).[48]

PTH and Phosphate homeostasis

Factors influencing calcium homeostasis also influence phosphate homeostasis. PTH has a number of effects on phosphate metabolism. Both calcium and phosphate are released from skeletal stores and absorbed from the intestine in response to PTH secretion. In the renal proximal tubule, Type II sodium-phosphate transporters are heavily expressed and are responsible for the reabsorption of 80% of filtered phosphate. PTH, Fibroblast Growth Factor 23 (FGF23) and dietary phosphate are the major regulators of these transporters and their expression is rapidly reduced in response to PTH and dietary phosphate loading. Intestinal phosphate transport is less well elucidated, but Type II sodium phosphate transporters have been identified in the small intestine. These intestinal sodium phosphate transporters are regulated by 1, 25-OH Vitamin D and dietary phosphate load. Unlike the regulation of renal proximal tubular sodium phosphate transporters, current evidence suggests that PTH does not regulate sodium phosphate transporter expression directly, but that it affects intestinal transport of phosphate indirectly via its stimulatory effect on 1, 25-OH Vitamin D.[49] In health, the net effect of these mechanisms is a correction of serum calcium concentration to normal with little or no effect on serum phosphate concentration.

PTH response to Vitamin D

Parathyroid cells express Vitamin D receptors (VDR) and the PTH gene has a Vitamin D response element. Calcitriol (1, 25-OH Vitamin D) binds to the VDR and inhibits PTH gene expression and PTH synthesis.[50] Calcitriol also inhibits parathyroid cell proliferation.[51]

Metabolism of Phosphate in CKD

With progressive CKD, single nephron phosphate excretion increases but due to the reduced number of functioning nephrons, the kidney becomes increasingly unable to excrete phosphate and maintain a neutral phosphate balance, resulting eventually in hyperphosphatemia. This positive phosphate balance initiates a cascade of responses in an attempt to maintain a normal serum phosphate level.

Phosphate and Fibroblast Growth Factor 23

Fibroblast Growth Factor 23 (FGF23) is a potent phosphaturic peptide, secreted by osteocytes. It was originally identified in diseases associated with phosphate wasting such as tumour induced osteomalacia[52] and autosomal dominant hypophosphatemic rickets.[53] Further studies established FGF23 as an important regulator of phosphate and Vitamin D metabolism, both in health and CKD.[54-56]

FGF23 binds to FGF Receptor (FGFR) with the highest affinity in the presence of its co-receptor Klotho[57] and down regulates expression of the sodium-phosphate co-transporter in the renal proximal tubule, thereby inhibiting

phosphate reabsorption.[58] FGF23 is secreted by osteocytes in response to dietary phosphate loading and increases in serum phosphate via unclear signalling pathways. It exerts a potent phosphaturic effect serving to maintain normal serum phosphate in the presence of adequate renal excretory capacity.[57]

FGF23 also directly inhibits renal 1-alpha hydroxylase expression, preventing hydroxylation of 25-OH Vitamin D to its active metabolite 1, 25-OH Vitamin D. In addition, FGF23 increases the activity of the catabolic enzyme 24-hydroxylase (CYP24A), which degrades existing 1, 25-OH Vitamin D.[59] FGF23 has also been shown to suppress PTH secretion, which in turn leads to a reduction in 1, 25-OH Vitamin D thereby reducing intestinal absorption of phosphate.[60] FGF23 affects intestinal phosphate indirectly, mediated through its effects on circulating 1, 25-OH Vitamin D levels. The combination of these effects may explain why elevations in PTH levels and Vitamin D deficiency develop early in the course of CKD while calcium and phosphate levels remain relatively normal until late CKD. FGF23 levels begin to rise early in the course of CKD and have been shown to be an important predictor of 1, 25-OH vitamin D levels, independently of eGFR and serum phosphate level.[56]

Phosphate and PTH

PTH is an extremely important modulator of phosphate metabolism. PTH is a phosphaturic hormone and acts on the proximal tubules to suppress sodium-phosphate co-transporter activity, inhibiting phosphate reabsorption.[58] PTH

also stimulates osteoclastic bone resorption and phosphate release and promotes the activity of 1-alpha hydroxylase (resulting in production of 1, 25-OH Vitamin D and increased intestinal absorption of phosphate).[61] On a cellular level, hyperphosphatemia inhibits the release of calcium ions from intracellular structures resulting in decreased intracellular calcium, stimulating further PTH secretion.[62] Prolonged hyperphosphatemia, such as occurs in the setting of reduced renal function results in increased PTH synthesis, secretion and parathyroid cell proliferation and gland hyperplasia.[62] In CKD this response becomes a pathophysiological vicious cycle as the kidney cannot mount a phosphaturic response to PTH.

Phosphate Homeostasis and the Renal-Gastrointestinal Axis

It has generally been accepted that phosphate homeostasis is regulated via control of phosphate reabsorption in the renal proximal tubule. How dietary phosphate intake and increases in serum phosphate signals a phosphaturic response is less well understood. It has been proposed by Berndt et al. that the small intestine mucosa senses an increase in phosphate concentrations and secretes a circulating substance that exerts a rapid phosphaturic effect in the kidney. This group demonstrated in animals that infusion of sodium phosphate, but not sodium chloride increased renal phosphate excretion within twenty minutes. This response was not attributable to alterations in serum phosphate, PTH, FGF23 or an increase in GFR. In addition, renal

denervation did not alter the phosphaturic response.[63] Marks et al. have suggested that the circulating substance proposed by Berndt et al. may be matrix extracellular phosphoglycoprotein, (MEPE), which is a phosphatonin with an inhibitory effect on intestinal phosphate absorption independently of 1, 25-OH Vitamin D. It is expressed in duodenum and jejunum and also in the renal proximal tubule.[49] Further elucidation of these pathways is ongoing and will provide important insights and possibly novel therapeutic targets in disorders of phosphate homeostasis.

Vitamin D metabolism in CKD

CKD has a number of adverse effects on Vitamin D metabolism. As mentioned previously, FGF23 inhibits 1-alpha hydroxylase and stimulates 24-hydroxylase resulting in decreased levels of 1, 25-OH Vitamin D. This has both direct and indirect effects which contribute to the development of secondary hyperparathyroidism. Firstly, decreased VDR binding of 1, 25-OH Vitamin D directly results in increased PTH gene expression, PTH synthesis, parathyroid cell proliferation and gland hyperplasia, independent of the effect of hypocalcaemia.[51] Parathyroid hyperplasia is associated with important abnormalities of parathyroid cell function, most notably the development of nodular parathyroid tissue with reduced expression of both the VDR and CaSR. The net result of these changes are a hyperplastic nodular gland, autonomously secreting PTH and no longer responsive to circulating calcium or

Vitamin D levels.[64] In addition, decreased 1, 25-OH Vitamin D leads to decreased intestinal calcium absorption resulting in hypocalcaemia and increased PTH secretion.

In summary, secondary hyperparathyroidism develops as an initial compensatory response via complex mechanisms but it ultimately becomes maladaptive and contributes to the pathophysiology of CKD. (Figure 1.1) Abnormalities in these parameters, both individually and collectively are associated with the increased fracture rate, cardiovascular and all-cause mortality seen in the clinical syndrome of CKD-MBD.

Figure 1.1: Pathogenesis of Secondary Hyperparathyroidism in CKD

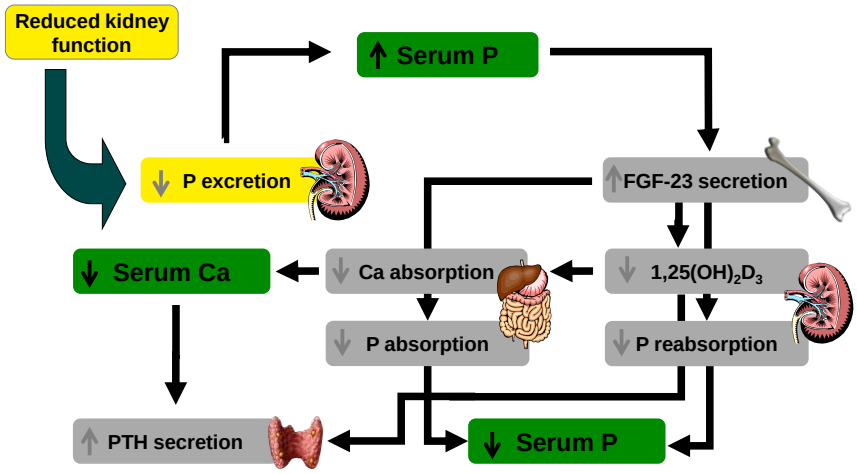


Figure 1.1: In normal conditions, the system provides an adaptive mechanism that normalises serum phosphorus in early kidney disease mediated by fibroblast growth factor (FGF-23). FGF-23 is a bone-derived hormone that inhibits phosphorus reabsorption, thereby increasing phosphorus excretion in the kidney. An increase in serum phosphorus levels also leads to a decrease in the conversion of vitamin D to active vitamin [1, $25(OH)_2D_3$] by the kidneys via FGF-23, causing a decrease in serum $1,25(OH)_2D_3$ levels. An increase in serum phosphate stimulates the secretion of FGF-23 by bone osteocytes. The kidney is the major target organ for FGF-23. FGF-23 inhibits phosphate reabsorption in the proximal tubule, leading to phosphaturia. Inhibition of 1α-hydroxylase by FGF-23 leads to decreased levels of 1, $25(OH)_2D_3$. The phosphaturic effects of FGF-23 along with suppression of 1, $25(OH)_2D_3$ formation provide a means to lower serum phosphate levels. In CKD, the reduction in kidney function leads to diminished response to FGF-23 such that serum Phosphate remains high. Reduced kidney function also leads to low serum calcium and calcitriol (activated vitamin D), both of which increase PTH secretion from the parathyroid gland. CKD-derived hyperphosphataemia thus drives SHPT development through FGF-23 and hypocalcaemia. Adapted with permission from "Calcimimetics or Vitamin D analogs for suppressing parathyroid hormone in end-stage renal disease: time for a paradigm shift?" James B Wetmore & L Darryl Quarles. *Nature Reviews Nephrology* **5**, 24-33[61].

Non-invasive assessment of Bone Health in CKD.

Quantitative bone histomorphometry is the gold standard for diagnosis of bone disorders in CKD[39]. However, bone biopsy is not widely utilized or available in clinical practice. It is an invasive and specialized procedure and importantly, there is a paucity of pathologists able to analyze the specimens. As such, non-invasive methods, mainly biochemical parameters are used as - albeit imperfect- surrogate measures of bone health, frequently in combination with radiological investigations.

Calcium

In health, the adult human body contains approximately 1kg (25,000 mmol) of calcium, 99% of which comprises the mineral compartment of bone. Only 1% (20mmol) of total body calcium is measurable in the extracellular compartment. Homeostatic mechanisms regulate concentration of calcium in the ECF rather than total body calcium content.[65] Serum calcium concentrations are tightly regulated within a narrow range, predominantly by PTH and do not reflect total calcium balance.[65] Total serum calcium is the usual measure of calcium status. Ionized calcium, which represents 40-50% of total calcium, is physiologically active. The remainder is bound to albumin or to anions such as phosphate, citrate and bicarbonate and is physiologically not active. Total calcium levels are affected by protein binding. In the presence of hypoalbuminaemia, there is an increase in ionized calcium, relative to total

calcium and total calcium measurement in this situation will underestimate the physiologically active ionized calcium. In clinical practice the serum calcium is typically corrected for the serum albumin level using the following formula: the addition of 0.02mmol/L to the measured calcium for each 1g/L decrease in serum albumin below 40g/L.[66] Using the albumin corrected serum calcium value has not been shown to be as accurate as ionized calcium and may not offer any additional benefit over using the total serum calcium value in patients with CKD[67]. Measurement of ionized calcium is not routinely available, but may be preferable if serum albumin or plasma pH are abnormal.[68]

Phosphate

Phosphate represents approximately 1% of total body weight. Mineralized bone contains 85% of total body phosphate; of the remainder the intracellular compartment contains 14% and extracellular fluid less than 1%. Phosphate circulates mainly in its free form (85%) with the remainder being protein bound. The majority of circulating phosphate therefore is freely filtered by the glomerulus. [69] This filterable fraction becomes important as with progressive CKD (and decreased filtration rate), the overall phosphate balance becomes positive in patients with CKD stages 4-5D.[70] Serum phosphate levels may be influenced by diurnal variation, post-prandial state and changes in pH, (increased with acidosis, decreased with alkalosis). However, in health the day-

to-day variation is approximately 5-10%. Due to the high intracellular phosphate concentration, levels may also be affected by haemolysis.

Both high serum calcium levels and phosphate levels are associated with increased mortality in patients with CKD and on dialysis.[71, 72] It is recommended therefore that serum calcium and phosphate levels be measured regularly in patients with eGFR <60ml/min/1.73m^2 and more frequently as CKD progresses.[39]

Vitamin D

25-hydroxy Vitamin D (25-OH Vitamin D) is the parent compound of Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol). It is metabolized in the liver to 25-OH Vitamin D3 (calcidiol) or 25-OH Vitamin D2 (25-hyrdroxyergocalciferol or ercalcitriol). These compounds undergo further 1-alpha hydroxylation in the kidney to become the active metabolite, 1, 25 Vitamin D. Quantification of 25-OH Vitamin D is the best measure of Vitamin D status as it includes measurement of nutritional sources and skin synthesis of Vitamin D. It is also a stable compound with a half life of approximately 2-3 weeks. 25-OH Vitamin D can be measured by radioimmunoassay, chemiluminescence assay or liquid-chromatography-tandem mass spectrometry. The latter has the advantage of being able to distinguish between 25-OH Vitamin D2 and 25-OH Vitamin D3. However, this technique is

expensive and requires specialized training and is therefore not widely used.[39] 25-OH Vitamin D levels show seasonal variation due to increased cholecalciferol production during summer months following sun exposure. Optimal values of 25-OH Vitamin D have not been well validated in CKD but are taken to be above 50nmol/L (20ng/ml) in the general population.[73]

1, 25-OH Vitamin D

This is the active metabolite of Vitamin D and this term describes both hydroxylated Vitamin D2 (25-hydroxyergocalciferol/ercalcitriol) and Vitamin D3 (calcitriol). 1, 25-OH Vitamin D has a very short half-life of 4-6 hours and levels are approximately one-thousandth of the 25-OH Vitamin D level. The measured level of 1, 25-OH Vitamin D is affected by prevailing 25-OH Vitamin D stores, 1-alpha hydroxylase and 24-hydroxylase enzyme levels. Due to its short half-life and the multiple factors affecting interpretation of the result, routine measurement of 1, 25-OH Vitamin D in clinical practice is not recommended.[39]

Parathyroid Hormone

Secondary hyperparathyroidism is an almost ubiquitous complication of CKD and the measurement of PTH is a widely used non-invasive surrogate marker for the assessment of bone turnover and bone health. PTH is an 84-amino acid single-chain polypeptide, synthesized and secreted from the chief cells of the parathyroid gland in response to reductions in extra-cellular calcium concentrations. The majority of secreted PTH is as its intact or biologically active form, 1-84 PTH. The remainder is compromised of PTH fragments namely C-terminal truncated and N-terminal truncated PTH fragments, of which the N-terminal 7-84 PTH fragment is the most abundant. This molecule usually represents approximately 15% of circulating PTH fragments.[74] It has however been shown to be over-represented in patients with severe primary[75] and secondary[76] hyperparathyroidism. Once released into the circulation 1-84 PTH is degraded peripherally to C-terminal fragments. These fragments have a longer half life than 1-84 PTH and represent the majority of total circulating PTH in health. CKD and ESKD can result in accumulation of these PTH fragments.[74]

The first generation of PTH radioimmunoassay consisted of a single antibody directed against the entire PTH molecule and measured fragments of PTH along with the intact 1-84 PTH molecule. Second generation PTH assays, which are the most widely used in clinical practice, use two separate antibodies. One is directed against the C-terminal fragment of the PTH

molecule and the second is directed against the N-terminal fragment. These assays were initially thought to quantify only the full-length biologically active 1-84 PTH molecule. It is now known that they also measure other large PTH fragments, predominantly 7-84 PTH. While the activity of these PTH fragments remains uncertain, they have been suggested to have, as yet, unconfirmed direct biological actions in skin and bone.[74] Third generation PTH assays also use a two-antibody technique but in these assays one antibody is radio-labelled and directed only against the first six N-terminal amino acids of 1-84 PTH, while the second antibody binds to the C-terminal region of 1-84 PTH. These assays are more sensitive and specific for the measurement of biologically active 1-84 PTH. Typically mean PTH levels are 30-60% lower using the third generation PTH assays compared to second generation assays[77]. Wide variations between the different methods of PTH measurement have been demonstrated.[78] This had led to concerns that patients may be incorrectly diagnosed with either low turnover or high turnover bone disease, based on PTH cutoffs, depending on the PTH assay used and demonstrates that results from different centres are not interchangeable. Some investigators suggest that the ratio of intact PTH to N-terminal PTH fragments may be useful in distinguishing between these disorders. It has been proposed that a ratio of less than one indicates low-turnover bone disease, although this remains controversial.[79]

Biochemical Markers of Bone Turnover

Bone Formation

Bone Specific Alkaline Phosphatase

Alkaline Phosphatase is a ubiquitous enzyme that is present in high concentrations in the intestine, kidney tubules, bone, liver and placenta. Although similar, distinct isoenzymes exist in each of these tissue sites. The vast majority of circulating alkaline phosphatase originates from either the liver or bone. The bone isoenzyme of alkaline phosphatase (Bone specific Alkaline Phosphatase (BAP)), is produced by osteoblasts and is a marker of bone mineralization and maturation. BAP levels are elevated in the circulation during active periods of bone formation and bone growth, particularly during infancy and puberty. BAP is not filtered by the kidneys and is not dialyzed, therefore plasma concentrations are not affected by variations in GFR and levels depend solely on the rate of release from osteoblasts and on its rate of degradation. Total circulating alkaline phosphatase has been used for many years as a marker of bone metabolism, but in the presence of liver disease levels may be unhelpful. Radioimmunoassays have been developed using monoclonal antibodies that can reliably detect the bone specific form of alkaline phosphatase and have been shown to be more sensitive than total alkaline phosphatase in the evaluation of bone turnover in CKD.[80] High levels of BAP in CKD correlate with PTH levels and histological evidence of high bone

turnover.[81] However, low levels of BAP alone do not reliably exclude high turnover bone disease. When evaluated in combination with PTH levels, the sensitivity and specificity of predicting the type of bone turnover is improved.[82]

Osteocalcin

Osteocalcin, which is also known as Bone Gla protein, is the most important non-collagen protein in the bone matrix. It is a 49 amino acid peptide synthesized by mature osteoblasts. Osteocalcin is produced during bone formation and is dependent on Vitamin K and is stimulated by Vitamin D. Both intact osteocalcin and the large N-terminal mid-molecule fragment circulate in the blood. Intact osteocalcin is much less stable than the N-terminal fragment. Electrochemiluminescence immunoassay ELICA assays have been developed using monoclonal antibodies directed against the N-terminal mid-molecule fragment and the N-terminal of the intact molecule. The assay therefore detects both intact osteocalcin along with the cleaved fragments. Serum osteocalcin is primarily cleared by the kidney and the molecule can accumulate in uraemia. This occurs when eGFR falls below 20-30mls/min/1.73m^2.[83] Osteocalcin has been shown to correlate positively with PTH in pre-dialysis CKD, independent of eGFR.[84]

Procollagen Type 1 N-Terminal extension Peptide

More than 90% of bone matrix consists of Type 1 collagen. Type 1 collagen is derived from Type 1 Procollagen which is synthesized in osteoblasts. Type 1 Procollagen has N (amino) and C (carboxy) terminal extensions. These extensions are cleaved off during the conversion of procollagen to collagen. The Procollagen Type 1 N-terminal extension peptide (PINP) can be measured in the serum and is a marker of Type 1 collagen deposition in the bone matrix and therefore a marker of bone formation. PINP is measured using an ELISA assay, using an antibody directed against the alpha-chain of Type 1 Procollagen.[85] PINP correlate positively with PTH and BAP and negatively with forearm BMD in haemodialysis patients.[86]

Bone Resorption

Tartrate Resistant Acid Phosphatase (TRAP)

TRAP is an enzyme released by osteoclasts during bone resorption. It has a number of different isoforms and specific antibodies have been developed that can distinguish the different sub-types, using enzyme-linked immunoabsorbant assay (ELISA). The isoform TRACP5b is exclusively associated with bone resorption rate and has been shown to be elevated in patients with osteoporosis and is negatively correlated with Bone Mineral Density in post-menopausal women.[87] TRACP5b has been evaluated in

patients with CKD and is unaffected by GFR or by haemodialysis.[88] Higher levels of TRACP5b have also been shown to be associated with rapid rates of cortical bone loss in patients receiving haemodialysis.[89] This enzyme is relatively unstable, and samples must be separated rapidly and frozen within 2 hours before analyzing, which limits the utility of TRACP5b as a useful non-invasive marker of bone resorption in clinical practice.

Type 1 Collagen N-Terminal Cross-Link Telopeptide

When osteoclasts resorb bone, minerals and collagen fragments are released into the circulation. The N-Terminal cross-link telopeptide (NTX) is preferentially released from Type 1 collagen by osteoclastic activity.[90] Serum and urine concentrations of NTX can be measured by ELISA using a monoclonal antibody against the N-Telopeptide of mature collagen chains in Type 1 collagen. Urine assays are corrected for urine concentration by indexing to urine creatinine levels and are performed on second void urine samples. Serum NTX levels have been shown to correlate negatively with forearm Bone Mineral Density and positively with PTH in haemodialysis patients.[91]

C-Terminal Cross-Link Telopeptide

Similar to NTX, C-Terminal Cross-Link Telopeptides (CTX) are degradation products of Type 1 collagen and are generated by bone resorption. In C-terminal telopeptides, α-aspartic acid is isomerised to the ß-aspartic form as the bone ages. The ß isomerised telopeptides are specific for the degradation of type 1 collagen in bone. CTX assays are based on the Crosslaps antibodies which recognize the ß isomerised aspartate residue. CTX can be measured using an automated analyser. CTX has been shown to be correlated with forearm bone loss in post-menopausal women and serum levels decrease significantly following treatment with anti-resorptive therapy.[92] Serum CTX was also shown to correlate with cortical boss loss and with BAP and osteocalcin in haemodialysis patients.[93]

Sclerostin

Sclerostin, a product of the SOST gene, is a protein expressed by osteocytes. The main action of sclerostin is to decrease bone formation by inhibiting osteoblast proliferation and promoting osteoblastic apoptosis.[94] Whether sclerostin also has a role in bone resorption is unclear. Sclerostin levels are higher in patients with CKD Stage 5 on dialysis than in healthy controls and post-menopausal women.[95, 96] In a bone biopsy study of 60 haemodialysis patients, Cejka et al. found sclerostin to be a strong predictor of bone turnover

and osteoblast number and in this study low serum sclerostin levels were a better predictor than PTH for high bone turnover and osteoblast number.[96] Administration of anti-sclerostin anti-bodies has been show to increase Bone Mineral Density in humans.[97]

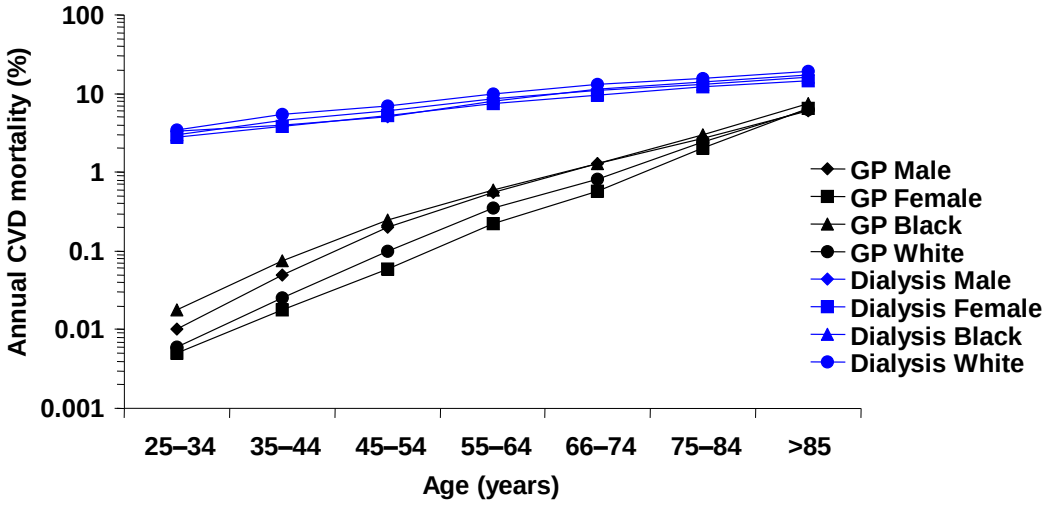
In summary, bone biopsy is the gold standard for the assessment of bone turnover and bone health in CKD. As bone biopsy is not widely available in clinical practice, PTH levels have been used as a surrogate to diagnose high and low turnover bone disease. Recent evidence suggests that PTH used alone is not a reliable marker of bone turnover in patients with CKD. The use of biochemical markers of bone formation and resorption, in combination with PTH may potentially improve the diagnosis and monitoring of CKD-MBD.

Cardiovascular Disease in CKD

Cardiovascular Disease is a common complication of CKD and ESKD and is an undisputed risk factor for mortality, the incidence of which is greatly increased, even in young patients with CKD. Go et al have shown a graded increase in all-cause and cardiovascular mortality in CKD patients as renal function decreased. This study showed age standardized cardiovascular mortality rates of 2.1 per 100 person years for patients with GFR 45-59 ml/min/1.73m^2 which increased to 36.6 per 100 person years at GFR less than 15ml/min/1.73m^2.[15] Similarly, Foley et al. showed that mortality rates from

cardiovascular disease for patients on dialysis are approximately 10 – 20 times higher in patients with ESKD than in the general population, regardless of age. In particular, even in young patients on dialysis, mortality from cardiovascular disease was similar to that of octogenarians without kidney disease. (Figure 1.2) In this study cardiovascular mortality was defined as death due to arrhythmia, cardiomyopathy, cardiac arrest, myocardial infarction, atherosclerotic heart disease or pulmonary oedema.[98] After successful renal transplantation this risk of cardiovascular death, while attenuated remains markedly elevated.[99]

Figure 1.2: Cardiovascular mortality in the General Population compared to ESKD treated with dialysis. [98] Reproduced with permission from "Clinical epidemiology of cardiovascular disease in chronic renal disease." Foley RN, Parfrey PS, Sarnak MJ. *Am J Kidney Dis* 1998; **32:** S112-119.



Vascular Calcification in CKD

Calcification of the blood vessel wall occurs at two sites, the intima and the media. Intimal calcifications are associated with atherosclerosis and are due to calcification of atherosclerotic plaques. Intimal calcification occurs preferentially in the aorta, coronary, carotid and femoral arteries and lesions tend to be discontinuous. In contrast, calcification of the arterial media, also known as Monckeberg sclerosis occurs as diffuse sheet-like calcification of the tunica media without intimal involvement. Medial calcification occurs in the elastic lamina of large and medium sized blood vessels and is common in patients with CKD. It is also associated with advancing age and diabetes. Medial and intimal calcifications may be present simultaneously in the patient with CKD, but their clinical consequences are different. Intimal lesions are associated with plaque rupture and distal occlusion of the vessel lumen, while medial calcification is associated with increased arterial stiffness, increased pulse pressure,[100] increased cardiac afterload, left ventricular hypertrophy and predisposition to heart failure and sudden cardiac death.[101] Medial vascular calcification was originally thought to represent passive mineral deposition; however, it is now recognized as being a highly ordered maladaptive process, influenced by several systemic and local inhibitors and promoters of calcification.

Pathogenesis of Vascular Calcification

Vascular smooth muscle cells (VSMCs) are central to the development of both intimal and medial arterial calcification. VSMC's retain their ability to differentiate and proliferate in order to repair the vessel wall after injury. However, they also have the ability to undergo phenotypic change and can differentiate into other cell types such as osteoblasts, chrondrocytes and adipocytes.[102] This phenotypic change is induced by injury to the VSMC which in atherosclerosis is due to lipid-induced inflammation. In CKD, the injury to the VSMC is due to abnormal mineral homeostasis, (where elevations in calcium and phosphate have a synergistic effect), [103] chronic volume overload and hypertension. Once the VSMC is injured, apoptosis of the cell and vesicle formation ensues.[104] This induces phenotypic change of the VSMCs into osteoblast-like cells. The apoptotic bodies also act as a nidus for calcification in the vessel wall. Calcification is accelerated in the presence of high levels of PTH, Vitamin D, calcium, and mostly significantly, phosphate.[105]

While vascular calcification is common in CKD and ESKD, not all patients calcify, despite persistent abnormalities in mineral homeostasis. This has led to the identification of a number of local and systemic inhibitors of vascular calcification which protect against calcification in health and in a certain patients with CKD.

Fetuin A

Fetuin A is an extracellular protein which is a potent inhibitor of calcium-phosphate precipitation. It is produced in the liver and is a negative acute phase protein, with reduced circulating levels during inflammation. Fetuin A acts both systemically and locally to prevent vascular calcification. Systemically it binds calcium and phosphate and prevents VSMCs from exposure to high levels of these minerals. Locally, Fetuin A inhibits VSMC apoptosis and promotes phagocytosis of vesicles by VSMCs, thereby removing the potential nidus for calcification. In addition, VSMCs can secrete Fetuin A and protect the cell from calcium and phosphate mediated injury. Fetuin A levels are lower in patients with ESKD compared to the general population, [106] and lower levels are associated with increased cardiovascular mortality.[107]

Matrix Gla Protein

Matrix Gla protein is derived from bone and vascular smooth muscle cells and is a potent local inhibitor of vascular calcification. It is dependent on carboxylation by Vitamin K in order to become fully biologically active. Matrix Gla protein inhibits vascular calcification directly by preventing precipitation of calcium and phosphate. It also acts to prevent differentiation of VSMCs into osteoblast-like cells. Decreased levels of uncarboxylated matrix Gla protein are associated with declining renal function and increased vascular

calcification. Vitamin K antagonists, such as warfarin prevent the carboxylation of matrix Gla protein to its active form.[108] Nutritional Vitamin K intake has been shown to be inversely associated with vascular calcification and mortality.[109] The use of Vitamin K antagonists such as warfarin has been associated with increased coronary artery and valvular calcification, [110] although other studies have not confirmed these findings.[111]

Osteoprotegerin

Osteoprotegerin is a glycoprotein member of the tumour necrosis factor (TNF) receptor family. It acts as a soluble inhibitor that prevents activation of nuclear factor κB (RANK) ligand (RANKL) with its receptor RANK. Osteoprotegerin is an important modulator of bone remodelling and by inhibiting activation of RANK it inhibits osteoclast formation, differentiation, activation and survival, preventing bone resorption. It is expressed by endothelial cells, VSMC's and osteoblasts.[112] In experimental animal models, osteoprotegerin deficiency is associated with accelerated medial vascular calcification and these animals also develop severe osteoporosis. Replacement of osteoprotegerin leads to reversal of the osteoporosis, but had no effect on the vascular calcification.[113] Recently an anti RANKL antibody has been licensed for the treatment of post menopausal osteoporosis.[114] In humans, osteoprotegerin levels are significantly higher in patients with CKD and ESKD than in those without CKD and osteoprotegerin levels are higher at lower levels of renal function.[115] The

role of osteoprotegerin in vascular calcification in CKD is unclear. Observational studies in patients with normal renal function[116] and in haemodialysis patients have shown a positive association between osteoprotegerin levels and severity and progression of vascular calcification[117, 118] and osteoprotegerin levels were predictive of future cardiovascular events.[119, 120] In a large cohort of renal transplant recipients osteoprotegerin has recently been independently associated with renal events, cardiovascular events and mortality.[121] The role of osteoprotegerin in the pathogenesis of vascular calcification in these studies is unclear but it has been suggested that osteoprotegerin levels increase as a defensive response to rapidly progressive mineral deposition in the vessel wall.[122]

Pyrophosphate

Pyrophosphate is a potent inhibitor of vascular calcification and exerts its effect by directly preventing hydroxyapatite formation. It is produced by arterial smooth muscle and is hydrolysed and inactivated by alkaline phosphatase.[123] O' Neill et al. have demonstrated that plasma pyrophosphate is negatively associated with vascular calcification in ESKD and CKD.[124] Experimental studies have shown that administration either subcutaneously[125] or intraperitoneally[126] of pyrophosphate can inhibit vascular calcification in the uremic setting without adversely affecting bone formation.

In summary, vascular calcification in CKD is a highly regulated process, initiated by injury to the VSMC, and involves a complex interplay between promoters and inhibitors of vascular calcification.[127] (Figure 1.3) This process shares many similarities with the process of bone mineralization.

Figure 1.3: Pathogenesis of Vascular Calcification in Chronic Kidney Disease.

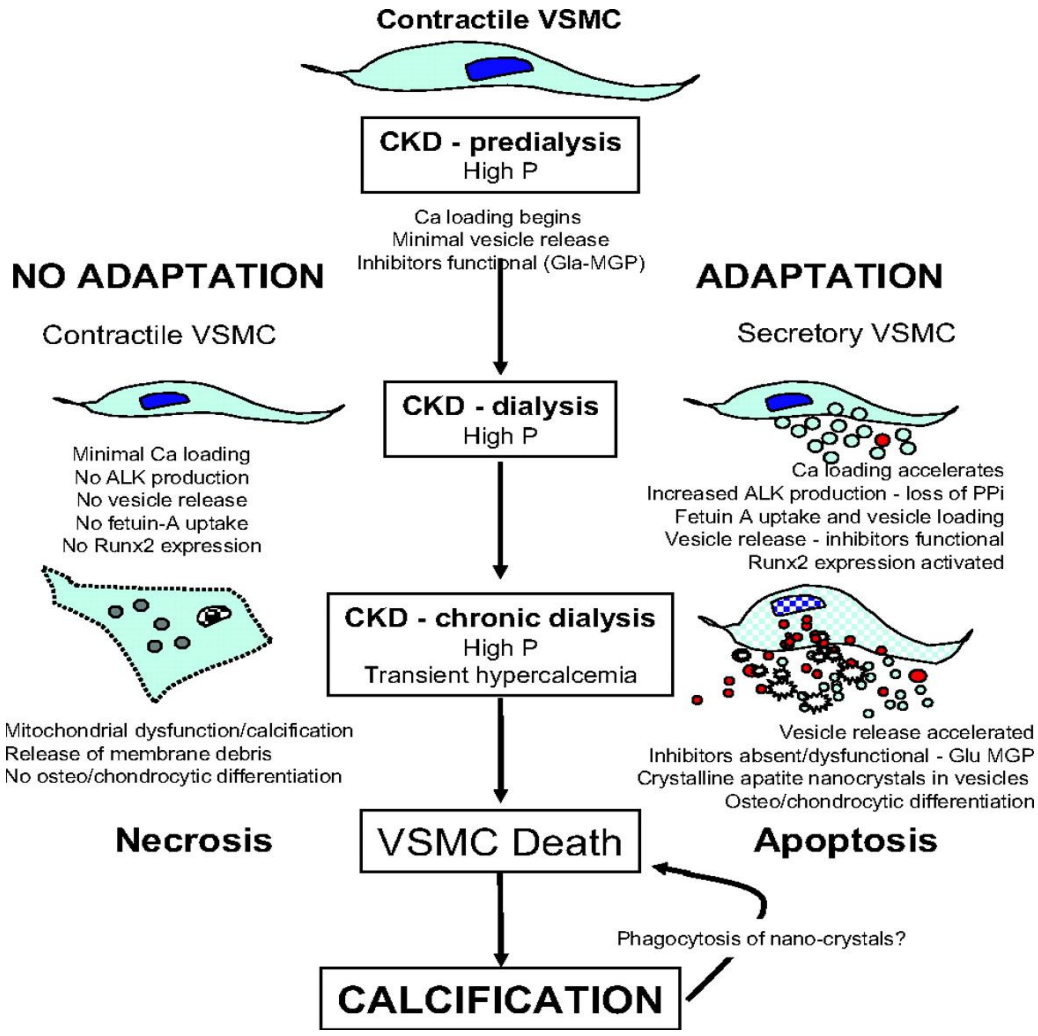


Figure 1.3: Model shows VSMC phenotypic adaptation in response to mineral dysregulation in CKD. VSMCs that fail to adapt to a synthetic phenotype and release vesicles to protect against Calcium overload will eventually undergo necrosis. In contrast, VSMCs that release vesicles do not succumb to intracellular Calcium overload but deposit Calcium in the extra cellular matrix, which eventually calcifies. This process eventually results in apoptosis of VSMCs. Reproduced with permission from "Chronic mineral dysregulation promotes vascular smooth muscle cell adaptation and extracellular matrix calcification." Shroff R C et al. JASN 2010; 21:103-112[103]

VSMC, Vascular Smooth Muscle Cell, CKD, Chronic Kidney Disease, P, Phosphate, Gla-MGP, Matrix Gla protein, ALK, Alkaline Phosphatase, PPi, Pyrophosphate, Runx2, Runt -related transcription factor 2, required for osteoblast differentiation.

Assessment of Vascular Calcification

A number of non-invasive methods are available to assess vascular calcification and arterial stiffness. The presence of vascular calcification or increased arterial stiffness using each of these modalities is associated with an increased risk of adverse consequences and cardiovascular mortality both in CKD and ESKD.

Plain Radiography

Plain radiography is a useful non-invasive and inexpensive tool for detecting the presence of vascular calcification, both in the general population and in CKD. The pattern of calcification seen on the radiograph can help to differentiate between intimal and medial calcification, with the former typically demonstrating patchy, discontinuous lesions. Medial calcifications are seen as linear "tram-track" lesions along the course of the vessel wall. However, the pattern is not sufficiently diagnostic to allow any of the available imaging modalities to reliably distinguish between the two forms.

Both types of calcification are associated with increased mortality. Blacher et al showed an association between arterial calcification and survival in patients on haemodialysis, with mortality increasing with higher number of arterial sites demonstrating calcification on plain radiograph.[128] Kauppila at al introduced a semi-quantitative scoring system for vascular calcification using

lateral radiographs of the lumbar spine. Using this system, calcifications along the anterior and posterior margins of the aortic contour, adjacent to the 4 lumbar vertebrae are scored 0 to 3. A score of zero represents no evidence of calcification, 1 represents calcification extending less than one third the length of the adjacent vertebra, a score of 2 represents calcification extending for more than one third, but less than two thirds the length of the vertebra and a score of 3 represents calcification extending more than two thirds the length of the vertebra. This system, applied to each of the lumbar aortic segments, results in a composite score of between 0 and 24.[129] This scoring system was used in a study of 515 dialysis patients which showed the presence of aortic calcification to be predictive of all cause and cardiovascular mortality.[130] The KDIGO group recommended in 2006 that lateral abdominal radiography be used as a screening tool for the detection of vascular calcification.[33]

Aortic Pulse Wave Velocity

Carotid – Femoral (Aortic) Pulse Wave Velocity measures the speed with which the arterial pulse travels down the aorta and thus is a measure of aortic stiffness. Using this technique, Doppler waveforms are obtained transcutaneously over the common carotid and femoral arteries, in sequence. Pulse Wave Velocity is calculated as the distance between the suprasternal notch and the femoral artery recording site, measured over the body surface. This distance is divided by the time taken for the pulse wave to travel between

the two arterial sites.[131] Time delay is measured using the R wave on continuous ECG monitoring as a timing reference. Pulse Wave Velocity is expressed as meters/second. This method is supported by a large number of epidemiological studies which show that aortic stiffness is an independent predictor of all cause and cardiovascular mortality in patients with various levels of cardiovascular risk, [132] including ESKD.[133]

Computed Tomography

While plain radiographs provide a semi-quantitative assessment of vascular calcification and Pulse Wave Velocity provides an assessment of the resultant aortic stiffness, Electron Beam (EBCT) and Multi Slice Computed Tomography represent the gold standard in the detection of vascular calcification although they are not universally available. The extent and degree of calcification can be precisely quantified using Agatston and volumetric scoring systems. Multi Slice CT scanners can acquire up to 64 simultaneous slices with thickness of the slices as low as 0.5mm, allowing precise quantification of vascular calcification. Newer software systems can facilitate low radiation dose protocols with similar diagnostic results. While CT scanning cannot distinguish between intimal and medial calcification, the detection of vascular calcification by CT is associated with reduced survival in dialysis patients.[134] Due to its precision, CT has also been used to monitor vascular calcification and to assess the effect of therapeutic interventions in slowing its progression.[135]

Relationship between Bone Abnormalities and Vascular Calcification

Bone disease and vascular calcification are both multifactorial, complex processes. Vascular calcification is a highly regulated process which resembles bone mineralization. Several epidemiological studies in the general population have demonstrated a relationship between decreased bone mineral density and increased vascular calcification. In the Framingham Heart Study, Kiel et al. demonstrated that there was a significant association between percentage change in Bone Mineral Density (measured by metacarpal relative cortical area (MCA)) and progression of abdominal aortic calcification (measured by lateral lumbar radiograph) in women though not men over a 25 year follow up period.[136] Similarly, in a cross-sectional study of 2348 post-menopausal women, Schluz et al. found aortic calcification scores were inversely related to bone mineral density (both parameters determined by CT). This study also reported that the Odds Ratios (95% Confidence Interval) for vertebral and hip fractures in those women with calcification were 4.8 (3.6 – 6.5) and 2.9 (1.8 – 4.8) respectively, compared to those without evidence of calcification. In a subgroup analysis of 228 women with available longitudinal data, this study also demonstrated a significant graded association between progression of vascular calcification and bone loss, where women with the greatest increase in vascular calcification had 4 times greater yearly bone loss than women of similar age in the lowest vascular calcification quartile.[137] In a larger prospective study of 624 men and women over the age of 50 years, Naves et

al. showed that progression of aortic calcification (determined by lateral lumbar radiograph) was associated with the rate of decline in Bone Mineral Density (determined by DXA) over 4 years of follow-up. This relationship remained significant following adjustment for age, gender, smoking history and diabetic status.[138]

This relationship has also been demonstrated in patients on dialysis, a population with markedly elevated risks of bone fracture and cardiovascular events. London et al. showed an inverse relationship between arterial calcification and bone activity, determined by bone biopsy. This study found that subjects with adynamic bone disease on bone biopsy had the greatest degree of arterial calcification, while active bone was associated with less vascular calcification.[131] This finding is consistent with the theory that low turnover bone disease impairs the ability of the bone to buffer minerals and they are then deposited in soft tissues and vasculature. It was also noted in this study that patients with a greater degree of arterial calcification had lower PTH levels, indicating that over-suppression of PTH predisposes to vascular calcification. Similarly the Treat to Goal study showed that treatment with the non-calcium containing phosphate binder sevelemar slowed progression of aortic calcification [139] and vertebral bone loss, [140] compared to calcium based phosphate binders (both p = 0.01).

The pathogenesis of both bone disease and vascular calcification in CKD is closely inter-related. Both conditions are stimulated by abnormal mineral homeostasis, particularly abnormally high levels of phosphate. In addition a number of proteins involved in bone formation have been identified at sites of arterial calcification, such as alkaline phosphatase, bone sialoprotein, osteocalcin, along with hydroxyapatite, the same crystal found in bone. FGF 23 which is secreted by osteocytes and whose primary function is the regulation of phosphate homeostasis has been associated with increased risk of Left Ventricular Hypertrophy,[141] cardiovascular events and mortality across a wide range of renal function.[142, 143] As discussed previously, abnormal mineral metabolism also stimulates differentiation of the VSMC into osteoblast-like cells, capable of mineralisation. While the mechanisms underlying the relationship between abnormal bone health and vascular calcification in CKD are incompletely understood, based on current evidence it appears likely, though it remains unproven, that minimizing abnormalities in phosphate, calcium, PTH and Vitamin D metabolism may help to prevent bone loss and cardiovascular morbidity and mortality.

Post Transplant Bone Disease and Vascular Calcification.

Successful renal transplantation restores effective endogenous renal function to patients with ESKD and improves survival, but it fails to restore either normal longevity or health.[98, 144] Cardiovascular disease accounts for up to

50% of all-cause mortality in renal transplant recipients[145] and mortality rates, while substantially lower than that of the dialysis population,[144] are approximately four times that of the age matched general population.[146] Risk factors for cardiovascular disease after renal transplant include traditional risk factors common to the general population, such as older age, pre-existing diabetes mellitus, hypertension, hypercholesterolemia and Left Ventricular Hypertrophy. Renal transplant recipients also accrue additional non-traditional risk factors specific to their post-transplant status namely, often suboptimal levels of renal function provided by the allograft, de-novo hyperglycaemia and hyperlipidemia due to immunosuppressive agents and residual disturbances in mineral metabolism.[147] Post-transplant hyperparathyroidism improves over the first year post transplant but PTH levels stabilize at elevated concentrations in over 50% of patients and in a substantial minority of cases are associated with hypercalcaemia.[148-151] It is typically assumed that with normalization of bone minerals and improvement in PTH levels, vascular calcification stabilizes or improves though few studies have examined the natural history of arterial calcification post-renal transplant. Several small cross-sectional studies have reported the prevalence of vascular calcification in renal transplant recipients, determined by EBCT, to be between 65% and 92%, which was similar to the comparative dialysis population.[152, 153] One prospective study of 23 transplant recipients found no evidence of progression of coronary artery or aortic calcification scores,

determined by spiral CT over 15-20 months of follow-up.[154] Similarly, several small studies (n=36 to 41) have shown improvement in vascular function and vascular stiffness following renal transplant.[155-157] Conversely, in a recent study of 281 renal transplant recipients, Marechal et al. demonstrated a significant increase in spiral CT measures of coronary artery and thoracic aorta calcification scores over 3.5 years of follow-up.[158]

While cardiovascular risk improves post renal transplantation (compared to continuing on dialysis), fracture risk remains elevated and in fact the risk of fracture increases further post transplant. [159-161] In contrast to vascular calcification, numerous studies have evaluated fracture risk and its predictors in the post transplant population. Nikkel et al, in a study of 69,000 renal transplant recipients, reported that 22.5% of patients suffered a fracture within 5 years.[162] The cumulative incidence of any fracture at 15 years post transplant was reported by Vautour to be 60%.[159] Overall, renal transplant recipients have an adjusted incidence ratio for fracture of 4.59 (95% CI 3.29 to 6.31) compared to the general population, along with an increased risk of all-cause mortality of 1.6 (95% CI 1.13 to 2.26).[163] A high prevalence of fractures affecting the extremities has been observed in renal transplant recipients, at sites containing a high proportion of cortical bone. [164, 165]

Risk factors for fracture post renal transplant include dialysis vintage prior to transplant, severity of pre-transplant hyperparathyroidism, duration since transplant, female gender, diabetes mellitus, age greater than 45 years,

decreased bone mineral density, prior history of fracture and immunosuppressive therapies (corticosteroids and calcineurin inhibitors).[165] Evaluation of Bone Mineral Density by DXA after renal transplant in most studies shows rapid bone loss particularly in the first 6 to 12 months post transplant. Sprague et al, in a literature review, reported that 35% of renal transplant recipients had low lumbar spine Bone Mineral Density and 21% had low Bone Mineral Density at the femoral neck within the first 6 years post transplant. After 6 years, the proportion of patients with low lumbar Bone Mineral Density was 22%, while the proportion with low femoral neck Bone Mineral Density was essentially unchanged at 22%.[150]

Unlike in the ESKD population, Bone Mineral Density assessment by DXA is associated with fracture risk in the post transplant population. In a study of 238 renal transplant patients who underwent DXA scanning of the lumbar spine and hip, 13.9% of the participants had osteoporosis, defined as a T-score less than -2.5. The authors found that osteopenia and osteoporosis were independent risk factors for fracture with relative risks of 2.7 (95% CI 1.6 to 4.6) and 3.5(95% CI 1.8 to 6.4) respectively.[166] Interestingly, the majority of fractures occurred at peripheral sites such as wrist, foot and leg. Assessment of a predominantly cortical bone site –such as distal radius- was not included in the study; this may have resulted in a higher prevalence of osteoporosis. Roe at al. in a study of 134 renal transplant patients reported that the proportion of patients diagnosed as having osteoporosis increased from 30%

to 41% when DXA examination of the distal forearm was included. In this study, the level of PTH, which has preferential catabolic action on cortical bone, correlated strongly with Bone Mineral Density at the radial site.[167]

While individual components of CKD-MBD, such as residual mineral disturbances, fracture risk and vascular calcification, have been examined in the post transplant population, whether the complex inverse relationship between bone health and vascular calcification seen in CKD and dialysis patients persists in the post transplant setting remains poorly described, but may influence the long term health of the successful transplant recipient.

Hyperparathyroidism and Health Related Quality of Life

Primary Hyperparathyroidism

With increased use of routine screening of serum calcium levels, the clinical presentation of primary hyperparathyroidism has changed. The classic symptoms of bone pain, nephrolithiasis and significant neuropsychiatric disturbance are seen in only 20% of patients presenting with primary hyperparathyroidism.[168] In a seminal Consensus Conference statement in 1991, the National Institute of Health (NIH) stated that evidence of mineral bone loss, a decrease in renal function and classical symptoms were indications for surgical correction of primary hyperparathyroidism.[169] However

most patients with primary hyperparathyroidism exhibit vague, non specific manifestations of the disease such as mood swings, irritability, fatigue and increased absenteeism from work.[170] These symptoms often develop insidiously and are under appreciated or attributed to the effects of aging.

Recognition of these non-classical symptoms has led to the development of a disease specific outcome tool for hyperparathyroidism which has been validated by a number of studies in symptomatic and asymptomatic patients.[171, 172] These studies and others[173, 174] also demonstrated an improvement in symptom score and Health Related Quality of Life following surgical treatment of primary hyperparathyroidism. In light of this more recent data, the original NIH consensus statement has been revised. A consensus statement from the proceedings of the Third International Workshop on Asymptomatic Primary Hyperparathyroidism (2008) stated that "the balance of available evidence suggests that surgery is appropriate in the majority of patients with asymptomatic hyperparathyroidism".[175]

Post Renal Transplant Hyperparathyroidism and Quality of Life.

Secondary hyperparathyroidism is a frequent complication of Chronic Kidney Disease (CKD) and End Stage Kidney Disease (ESKD). After successful renal transplantation, serum calcium and phosphate values typically normalise. However whether hyperplastic parathyroid tissue ever spontaneously

involutes is controversial.[176] Regardless, the secretion of PTH decreases over several weeks to months and usually falls to within the normal reference range. However in up to one quarter of renal transplant recipients the restoration of adequate endogenous renal function does not lead to normalisation of PTH levels. Approximately one third to one half of these patients also demonstrates mild to moderate hypercalcaemia.[148, 165]

The disease specific Parathyroid Assessment of Symptoms tool has been utilised in a study of patients with secondary and tertiary hyperparathyroidism due to ESKD, where tertiary hyperparathyroidism was defined as persistently elevated PTH six months post successful renal transplantation. This study compared the pre and post-operative disease specific tool scores and Quality of Life scores of patients with primary, secondary and tertiary hyperparathyroidism with those of a control group with non-toxic thyroid disease. Although the group with tertiary hyperparathyroidism (post transplant), was too small (n=10) for meaningful statistical analysis, they showed resolution of their symptoms similar to those in the primary hyperparathyroidism group.[53]

The non-specific symptoms of hyperparathyroidism such as fatigue irritability and mood swings are generally assumed to be due to associated hypercalcaemia. However studies have shown that patients with primary hyperparathyroidism have significant functional health status impairment

independent of the level of serum calcium. The relative impact of elevated PTH levels independent of serum calcium values on the health status of renal transplant recipients has not previously been evaluated.

In summary, abnormalities in mineral metabolism are common complications of CKD and are central to the development of bone disorders and vascular calcification, which is an undisputed risk factor for cardiovascular mortality, the incidence of which is greatly increased even in young people with CKD. Disorders of bone health in CKD are closely related to the development and progression of vascular calcification and confer a substantially increased risk of fragility fractures, with their attendant increases in morbidity and mortality. Cardiovascular mortality and fracture risk remain markedly elevated following renal transplantation. The role played by persistent abnormalities in mineral metabolism, particularly residual hyperparathyroidism, in these inter-related pathologies has not been evaluated, nor has the effect of elevations in PTH post transplant on the Health Related Quality of Life and overall well-being of the transplant recipient been examined.

This study was undertaken to examine firstly, risk factors for decreased Bone Mineral Density and fracture occurrence in the general population, namely renal function and serum sodium levels and secondly to determine if post renal transplant hyperparathyroidism and hypercalcaemia are associated with increased measures of vascular calcification, abnormal bone turnover,

decreased Bone Mineral Density, and decreased measures of Health Related Quality of Life.

Study Aims and Hypotheses

Specific Aim 1a (Chapter 2): To examine the cross-sectional association of CKD Stage 2-5 with decreased Bone Mineral Density and fracture occurrence within the general population by cross-linking Bone Mineral Density measurements from the DXA database at Cork University Hospital (CUH) Department of Rheumatology with estimated GFRs obtained from Cork University Hospital central biochemistry laboratory.

Specific Aim 1b (Chapter 3): To examine the cross-sectional association of hyponatremia (serum sodium <135mmol/L) with fracture occurrence in the general population and to assess if this relationship was associated with decreased Bone Mineral Density.

Hypothesis 1 That the presence of mild to moderate quantitative (e.g. a reduction in Glomerular Filtration Rate) or qualitative (e.g. reduced serum sodium concentration) abnormalities in renal function are independently associated with decreased Bone Mineral Density and occurrence of bone fracture in the general population.

Specific Aim 2: To conduct a cross-sectional observational study of successful first renal transplant recipients between 4 months and 12 years post-transplantation to determine the association of persistent post-transplant hyperparathyroidism with CKD Mineral Bone Disorder, in particular:

Specific Aim 2A (Chapter 4): Prevalent Post-Transplant Measures of Bone Health including DXA Bone Mineral Density and indices of bone formation and resorption.

Specific Aim 2B (Chapter 5): Measures of Vascular Calcification as measured by Pulse Wave Velocity and radiographic calcification scores.

Specific Aim 2C (Chapter 6): Measures of Health Related Quality of Life as measured by parathyroid disease specific and SF 12 Quality of Life surveys.

***Hypothesis 2*:** That persistent post-transplant hyperparathyroidism is associated with decreased Bone Mineral Density, abnormal bone turnover, increased measures of vascular calcification, and reduced Health Related Quality of Life, all of which are likely to be injurious to the long term health of the transplant recipient.

Chapter 2

Moderate CKD in Women is Associated with Fracture Occurrence Independently of Osteoporosis

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Introduction

Several recent studies have reported the association of increased fracture risk with stage 3 and 4 Chronic Kidney Disease (CKD)[177-182], an association that is well established in subjects with End Stage Kidney Disease[183, 184]. Fragility fractures are a major public health concern[185] and result in prolonged hospitalization, disability and substantial mortality.[184] Given the prevalence of mild to moderate CKD in the general population,[186] an independent association of CKD with fragility fractures would add substantially to the Public Health burden attributable to CKD.

Fragility fractures are strongly associated with age related osteoporosis, a condition that shares many risk factors with CKD.[187] Moreover, advanced CKD leads to a combination of quantitative and qualitative abnormalities of bone, referred to as Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD).[188] The aetiology of CKD-MBD is complex and includes hyperphosphatemia, hyperparathyroidism, hypovitaminosis D and uncontrolled acidosis.[184, 187, 189] These factors may co-exist with unrelated pathologies such as age related decreased bone mineral density, though distinguishing between these processes in advanced CKD is extremely difficult in the absence of a bone biopsy.[190] A progressive deterioration in many of the factors that predispose to CKD-MBD is evident at earlier stages of CKD[191] but the extent to which the risk of fragility fracture in moderate CKD is mediated by or, alternatively, is

independent of the presence of osteoporosis is unclear. We therefore conducted the following retrospective study of subjects who had undergone routine elective bone mineral density measurement to test the hypothesis that stage 3 CKD is associated with fracture occurrence independently of the presence of osteoporosis.

Methods

Study population. Female patients, aged over 18 years who underwent a DXA scan at Cork University Hospital between 1st September 2006 and 11th April 2007 were eligible for inclusion. To avoid referral bias, as a result of enriching the sample with subjects with known decreased renal function who were referred for DXA scanning due to a concern for osteoporosis, we excluded patients who had been referred from nephrology services.

Bone Densitometry measurement: Dual energy x-ray absorptiometry (DXA) measurements were performed at the lumbar sacral spine and both hips using a Lunar IDXA™ scanner (General Electric) and expressed as T-scores indicative of the number of SDs by which the bone mass value deviated from the mean of a group of young normal controls. In keeping with the World Health Organization definition, osteopenia was defined as a lowest reported T-score of between -1.0 and -2.5 and osteoporosis as the lowest T-score of < -2.5.[185]

Data regarding patient demographics, risk factors, current and prior treatment regimes for osteoporosis, history of prior fracture and details of co-existent medical conditions were obtained from a standardized self-report questionnaire, completed at the time of the DXA scan.

Fracture Validation Subgroup: The definition of fracture used in our primary analysis was purely based on subjects self report obtained at time of DXA scanning. In an attempt to at least partially validate fracture occurrence we cross checked self reported fracture occurrence against radiology reports and/or fracture clinic attendance at Cork University Hospital. As local patients who were referred to Cork University Hospital for DXA scan would also be expected to be referred here for fracture care this would be expected to confirm many but certainly not all patients with an actual past history of fracture. Moreover subjects with spinal collapse fracture would not have been referred to the fracture clinic and could have obtained radiological diagnosis at a number of other regional radiology units, while alternative fracture services are also provided at two other regional public hospitals as well as at a private hospital, the records of which we were not able to cross reference. As a result, while the absence of fracture confirmation at Cork University Hospital does not reliably refute the possibility of a fracture, confirmation does strongly support the diagnosis.

Calculation of eGFR: The DXA database was manually cross referenced against the central laboratory database using agreement of at least 2 of 3 potential patient identifiers (name, date of birth, medical record number) to identify those subjects who had a serum creatinine within 1 year of the index DXA scan. Laboratory results obtained during in-patient admissions were not included unless these were similar to previously measured outpatient levels. Serum creatinine was assayed at either our main biochemistry laboratory (90.4% of samples) or at one of two regional laboratories using the modified Jaffe reaction and reported in umol/l. The estimated Glomerular Filtration rate (eGFR) in ml/min/1.73 m^2 body surface area was calculated using the 4 variable Modification of Diet in Renal Disease (MDRD) prediction equation.[186] Due to concerns regarding the precision of eGFR estimates at near normal levels of renal function we a priori excluded those subjects whose eGFR was greater than 90 ml/min/1.73m^2.[192,193] Subjects were categorized using eGFR cut-offs of 75-89, 60-74, 30-59 and <29 ml/min/1.73m^2 body surface area. In keeping with National Kidney Foundation Clinical Practice Guidelines subjects with an eGFR of 30-60 ml/min/1.73m^2 were considered to have moderate CKD. We were unable to determine to what extend subjects in the 60-89 group may have met the definition of CKD as we had no other routinely available data regarding the presence of kidney damage such as presence of proteinuria.

Statistical Considerations: Outlying and clinically implausible values were double checked against the original clinical data. The distribution, central tendency and variance of each variable was examined using standard tabular and graphical methods. We examined the correlation between the lowest recorded T-score per subject – as is used in the clinical definition of osteoporosis - and the eGFR using the non-parametric Spearman rank method. Tests for linear trend were performed using eGFR expressed as a 3-level ordinal dependant variable within the appropriate linear or logistic regression model. We examined the presence, strength, significance and independence of the association of eGFR category with reduced bone mineral density using logistic regression adjusting sequentially for age, osteoporotic risk factors and treatments. All regression analyses were also adjusted for the laboratory used in order to control for potential inter-laboratory variability. We repeated the above analytic strategy to examine the relationship between eGFR and self-reported fracture occurrence with the T-score being used as an additional explanatory variable. Due to their small number (n=13), data from subjects with an eGFR <30 ml/min per 1.73m^2 are provided for descriptive purposes but are not used in statistical tests. Routine model diagnostics included assessment of points of high leverage and influence and measurement of the overall model 'Goodness of Fit' using the method of Hosmer and Lemeshow. Analyses were performed using SPSS Version 12.0 software, (Chicago, Illinois,

USA) with a 2-sided type 1 error rate of 0.05. The study protocol was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Results

Clinical Characteristics: Of the 2900 potential subjects who underwent DXA scanning during the study period, 1930 had an available creatinine measurement. Subjects with eGFR greater than 90ml/min per 1.73m^2 (n = 228) were excluded due to concerns regarding the accuracy of the MDRD equation at these levels, resulting in a sample size of 1702 participants, Figure 2.1. Patients with an available serum creatinine were older, less likely to have been referred for DXA by their primary care physician, and had less exposure to steroids and alcohol; however their occurrence of osteoporosis and fractures was similar to those who did not have an available creatinine measurement, Table 2.1. Median interval from time of DXA scanning to measurement of renal function was 4 weeks, with 69.4% of measurements being within 3 months.

Figure 2.1: Study Population, 1702 subjects with available DXA and creatinine measurement.

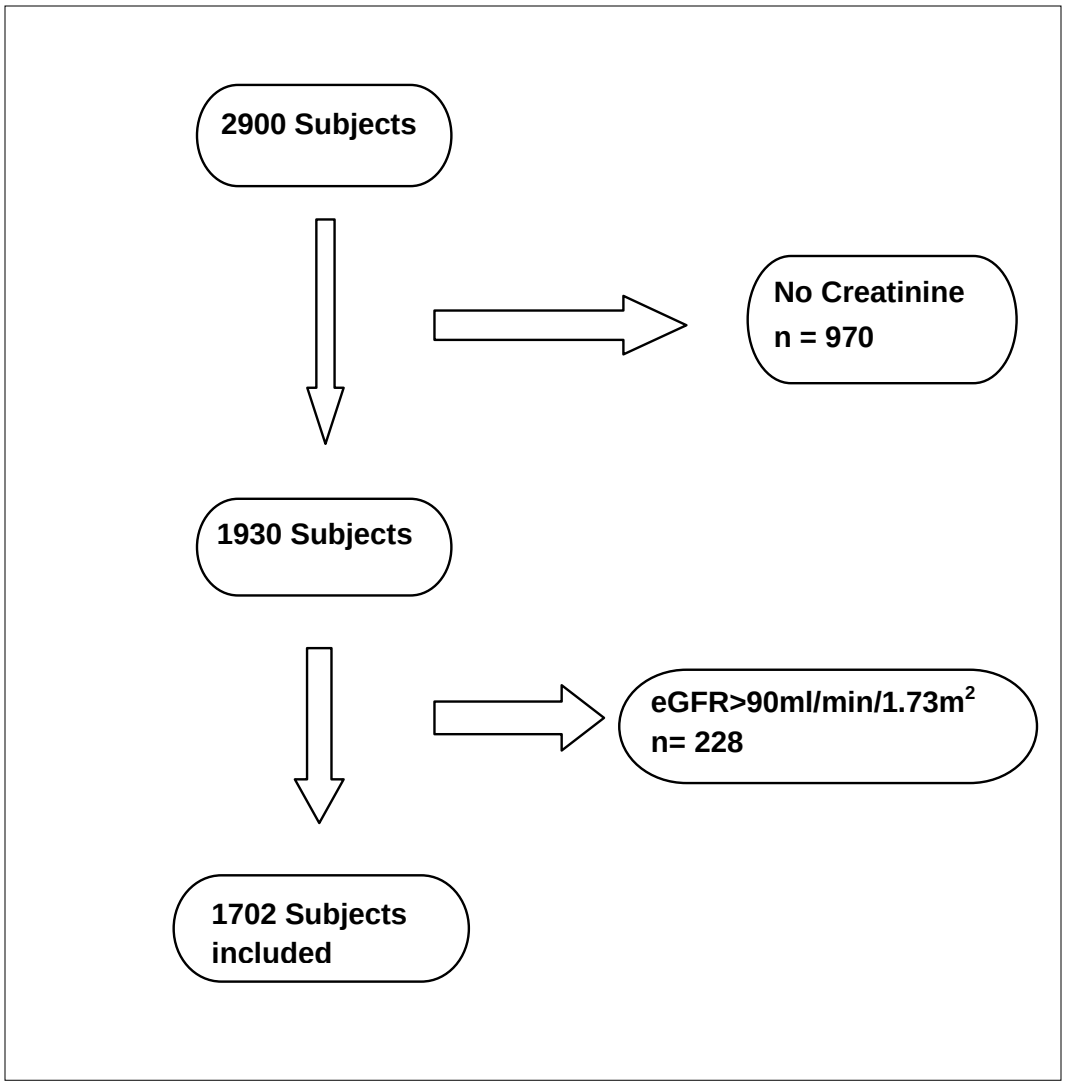


Table 2.1: Patient Characteristics of women undergoing routine DXA scan, with or without an available serum creatinine measurement within 1 year of the scan.

Characteristic	Creatinine Available	No Creatinine Available	p value
Number of Patients	1930	970	
Age (years), Mean (SD)	61.7 (10.8)	58.6 (11.8)	<0.001
Range	20 to 97	20 to 88	
T-score, Mean (SD)	-2.3 (1.1)	-2.3 (1.1)	0.47
Range	-6.5 to 0.9	-6.4 to 0.9	
Osteoporosis %	46.8	44.0	0.16
Risk Factors for Osteoporosis			
Amenorrhea %	83.7	76.7	0.01
Dairy <3 serving/wk %	42.4	40.0	0.34
Alcohol >5units/wk %	18.2	23.0	0.01
Steroid Use %	6.3	11.2	<0.01
Ever Smoked %	18.3	19.6	0.64
Family History %	10.7	11.3	0.56
Treatment for Osteoporosis			
Calcium/Vitamin D %	34.7	34.6	0.95
Antiresorptive %	23.1	19.7	0.06
HRT %	9.5	10.1	0.6
Prior Fracture			
None %	71.1	74.0	0.27
Single %	25.4	23.1	
Multiple %	3.5	2.9	
Referral Source			
General Practitioner %	82.3	91.5	<0.001
Hospital %	17.7	8.5	

Abbreviations: HRT, Hormone Replacement Therapy

The mean (range) age of study participants was 61.7 (23 - 97) years; 29 subjects (1.7%) were aged less than 35 years. The mean (SD) eGFR was 68.8 (12.2) ml/min per 1.73m^2. The percentage of subjects with eGFR of 75-89, 60-74, and 30-59 and <30 ml/min per 1.73 m^2 was approximately 34%, 45%, 20% and 1% respectively, Table 2.2. Eighty-two percent of subjects had been referred for the DXA by their primary care physician. Risk factors for osteoporosis included amenorrhoea (84%), low dietary intake of dairy products (42%), ever having smoked (18%) and a high alcohol intake (18%). Over one third of subjects were taking vitamin D / calcium supplementation, 23% were treated with bisphosphonates or other anti-resorptive agents and 10% were on hormonal replacement therapy. As expected, there was a strong relationship between older age and reduced renal function, with those in the 30-59 ml/min per 1.73m^2 category being on average 10 years older than those in the 75-89 ml/min per 1.73m^2 category. In keeping with their older age, lower levels of renal function were also associated with more amenorrhoea and with treatment with anti-resorptives agents, and with less alcohol use and a lower proportion with a family history of osteoporosis. (Table 2.2) Self-reported fracture was associated with older age, more amenorrhoea and more calcium and Vitamin D supplementation and anti-resorptive treatment. (Table 2.3)

Table 2.2: Patient Characteristics of women who met study entry criteria by eGFR Category.

Characteristic	Total	eGFR (ml/min per 1.73m^2)				p trend*
		75-89	60-74	30-59	*<30**	
Number of Patients (%)	1702	583 (34.3)	772 (45.4)	334(19.6)	*13 (0.8)*	
Age (yr) Mean (SD)	61.7(10.8)	57.5(10.6)	62.3(10.1)	67.6(9.6)	*67.7(11.9)*	<0.001
Range	23 - 97	23 – 88	24 - 97	40 – 89	*38 - 83*	
Risk Factors for Osteoporosis						
Amenorrhoea %	83.7	77.5	85.8	90.1	*76.9*	<0.001
Dairy < 3/week %	42.4	40.8	44.3	41.0	*38.5*	0.75
Alcohol intake >5units/week %	18.2	23.5	16.7	12.6	*15.4*	<0.001
Steroid Use %	6.3	5.7	5.4	9.3	*7.7*	0.06
Ever Smoked %	18.3	19.9	18.8	15.9	*23.1*	0.31
Family History %	10.7	12.9	11.3	5.7	*7.7*	0.001
Treatment for Osteoporosis						
Calcium/Vitamin D %	34.7	35.3	33.7	36.2	*23.1*	0.91
Antiresorptive %	23.1	18.7	25.3	26.0	*23.1*	0.004
HRT %	9.5	10.1	9.1	9.6	*0.0*	0.71
Referral Source						
General Practitioner %	82.3	82.3	84.1	79.6	*38.5*	0.46
Hospital %	17.7	17.7	15.9	20.4	*61.5*	

Abbreviations: HRT: Hormone Replacement Therapy. eGFR: 4 variable MDRD estimated Glomerular Filtration Rate.

* Due to its small sample size data from the eGFR <30 category is provided for descriptive purposes only and is not used in calculation of p-values.

Table 2.3: Patient Characteristics by Self-Reported Fracture Status.

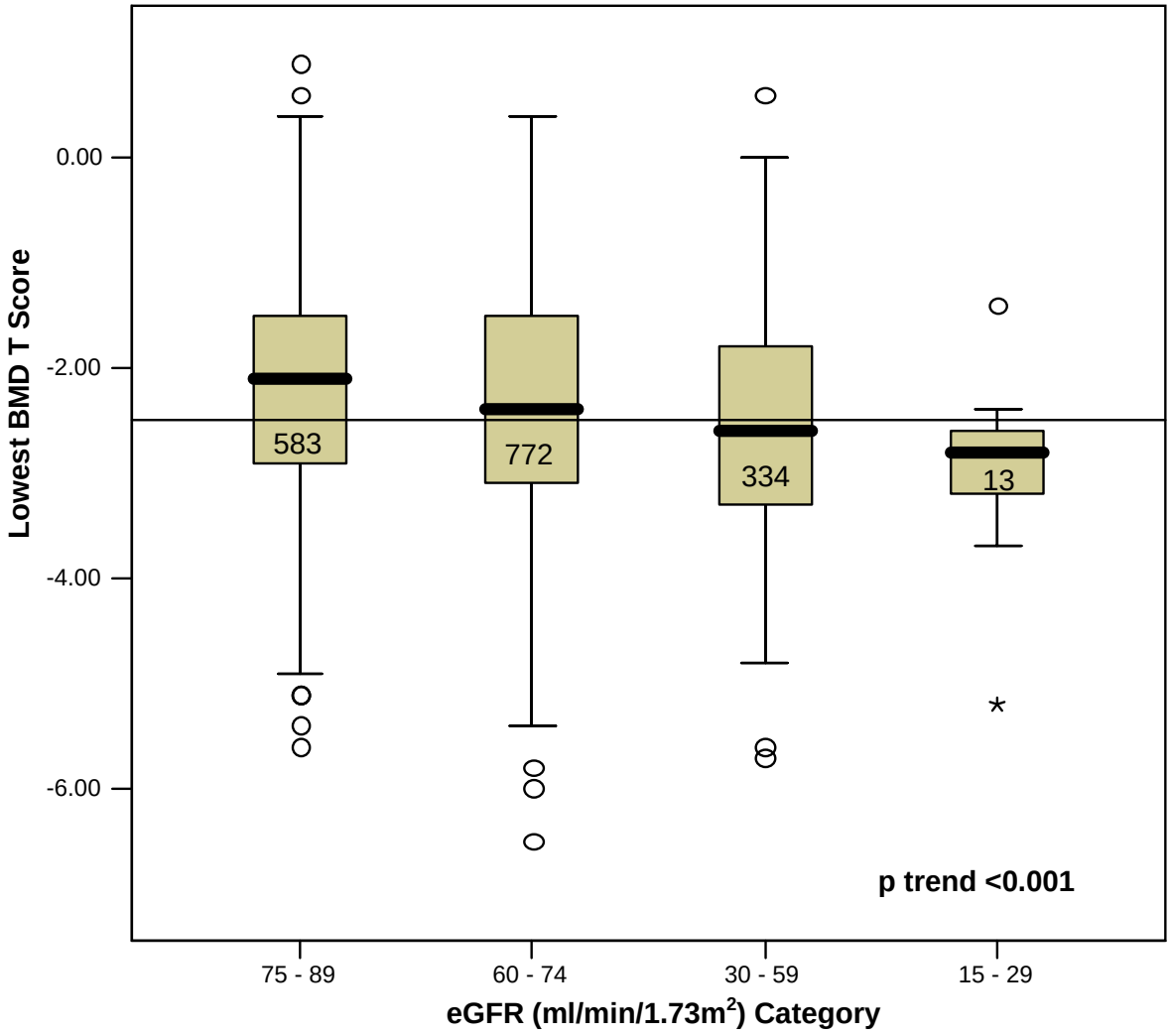
Characteristic	Total	Fracture	No Fracture	p
Number of Patients (%)	1702	492	1210	
Age (yr) Mean (sd)	61.7(10.8)	65.4 (10.9)	60.3 (10.4)	<0.001
Range	23 - 97	27 - 97	23 - 89	
Risk Factors for Osteoporosis				
Amenorrhoea %	83.7	88.6	81.7	<0.001
Dairy < 3/week %	42.4	44.9	41.4	0.194
Alcohol intake >5units/week %	18.2	16.5	18.9	0.240
Steroid Use %	6.3	6.1	6.4	0.912
Ever Smoked %	18.3	16.5	19.0	0.240
Family History %	10.7	11.6	10.3	0.489
Treatment for Osteoporosis				
Calcium/Vitamin D %	34.7	44.9	30.5	<0.001
Antiresorptive %	23.1	31.5	19.8	<0.001
HRT %	9.5	8.1	10.0	0.237
Referral Source				
General Practitioner %	82.3	76.8	84.5	<0.001
Hospital %	17.7	23.2	15.5	

Association of eGFR with osteoporosis: The mean T-score (SD) was -2.3 (1.1); 47% of subjects had osteoporosis (T-score <-2.5). The measured site of lowest bone mineral density was spinal in 809 (47.5%). There was no association between site of lowest bone mineral density and eGFR category. The eGFR was weakly though significantly correlated with T-score, Spearman rank r=0.14, p<0.001. The risk of osteoporosis was significantly higher at lower eGFR, p linear trend <0.001, Table 2.4 and Figure 2.2. Although an eGFR of 30-59 and 60-74 as compared to the reference category of 75-89 ml/min per 1.73 m^2 were associated with osteoporosis on univariate logistic regression, with adjustment for age, the strength of this relationship was substantially attenuated and no longer significant, Table 2.5. Subsequent sequential models, adjusting simultaneously for risk factors and treatments for osteoporosis did not alter this finding and eGFR remained non significant (data not shown).

Table 2.4: Bone Mineral Density and prior history of fracture occurrence among 1702 women undergoing routine outpatient DXA measurement, by MDRD eGFR category

eGFR (ml/min per 1.73 m2)						
	Total	**75-89**	**60-74**	**30-59**	*15-30**	**p-value***
Bone Mineral Density						
T-Score: Mean (SD)	-2.3 (1.1)	-2.2 (1.1)	-2.3 (1.1)	-2.5 (1.1)	*-3.0 (0.9)*	<0.001
Osteoporosis (%)	47.1%	47.1%	48.8%	54.2%	*84.6%*	<0.001
Number of Fractures						
None: n (%)	1210 (71.1%)	449 (77%)	540 (69.9%)	211 (63.2)	*10 (76.9%)*	<0.001
One: n (%)	432 (25.4%)	119 (20.4%)	203 (26.3%)	107 (32.0%)	*3 (23.1%)*	
Multiple: n (%)	60 (3.5%)	15 (2.6 %)	29 (3.8%)	16 (4.8%)	*0 (0%)*	

Figure 2.2: Box plot of lowest measured Bone Mineral Density by MDRD eGFR category.



Boxplot: showing median (solid heavy line), intra quartile range (box) and outliers (open circles). Number within box = sample size.

Table 2.5: Crude and age adjusted OR (95% CI) for Association of osteoporosis with eGFR (ml/min/1.73m^2)

	Unadjusted Model	Age Adjusted Model
eGFR 75 - 89	1 (Reference)	1 (Reference)
eGFR 60 - 74	1.44 (1.16 - 1.80) ***	1.02 (0.80 - 1.29)
eGFR 30 - 59	1.79 (1.36 - 2.35) ***	0.83 (0.61 - 1.12)
Age (10 yrs)	-	2.33 (2.07 – 2.6) ***

***p < 0.001

Fracture Occurrence: Overall, 492 patients (28.9%) reported a total of 554 fractures; 60 (3.5%) subjects had 2 or more prior fractures, Table 2.4**.** The percentage of patients with a prior self-reported fracture was significantly higher at lower levels of eGFR, p linear trend <0.001 The percentage of patients with multiple prior fractures also increased across lower eGFR categories from 2.6% in 75-89 ml/min per 1.73m^2 category to 4.8% in the 30-59 ml/min per 1.73m^2 category, p chi square <0.001. In 94 cases, the site of fracture was not recorded; of the remainder, 125 (27%) were lower limb, 243 (53%) were upper limb and 92 (20%) involved the axial skeleton. The site of fracture did not significantly differ by eGFR category.

Patients with eGFR 60-74 and 30-59 ml/min per 1.73m^2 had a significantly increased crude Odds Ratio (95% CI) of fracture occurrence, 1.4 (1.1-1.8) and 1.9 (1.4-2.5) respectively, as compared with the reference group (eGFR 75-89ml/min per 1.73m^2), Table 2.6. In the 60-74 ml/min per 1.73m^2 category the OR, adjusting sequentially for age and T-score (model 2), osteoporosis risk factors (model 3) and osteoporosis treatments (model 4) remained persistently elevated at 1.2 but it was no longer statistically significant. However, the 30-59 ml/min per 1.73m^2 category was both significantly and independently associated with fracture occurrence in the fully adjusted model (model 4, Table 2.6), having a statistically significant (p=0.03) approximately 40% increased odds of fracture relative to the 75-89 ml/min per 1.73m^2 category. T score (per unit decrease), adjusted OR (95% CI) 1.26 (1.12, 1.41) and age (per decade) adjusted OR 1.38 (1.21, 1.57) were both significant and independent predictors of fracture occurrence. In order to examine whether the association of chronic kidney disease on fracture varied according to the site of lowest bone mineral density (spinal vs. non spinal) we repeated the above regression model adding the relevant multiplicative interaction term, however there was no evidence of a significant interaction (p interaction =0.42)

Table 2.6: Crude and adjusted OR (95% CI) for Association of Fractures with eGFR Category

eGFR (ml/min per 1.73 m^2)	Model 1 Crude OR	Model 2 Adjusted OR	Model 3 Adjusted OR	Model 4 Adjusted OR
75 – 89	1 (Reference)	1(Reference)	1 (Reference)	1 (Reference)
60 – 74	1.44 (1.13-.84)**	1.20 (0.93-1.55)	1.20 (0.93-1.55)	1.20 (0.93-1.55)
30 – 59	1.95 (1.46-.62)***	1.34 (0.97-1.84) †	1.37 (1.0-1.89)*	1.37 (1.0-1.89)*

† p<0.1, *p <=0.05, **p <= 0.01

Model 1: Unadjusted.

Model 2: Adjusted for age (decades), and T-score.

Model 3: Adjusted for age (decades), T-score, amenorrhoea, fewer than 3 diary servings per week, corticosteroid use, ever having smoked, known family history of osteoporosis.

Model 4: Adjusted as per model 3 above plus treatment with calcium/vitamin D, use of bisphosphonate / other anti-resorptive agents or hormonal replacement therapy.

In the validated fracture subgroup, 431 fractures could be confirmed by review of radiology reports or by fracture clinic attendance at Cork University Hospital. The adjusted odds ratio (95% CI) for fracture occurrence at eGFR of 75-89, 60-74 and 30-59 ml/min per 1.73m^2 were similar to the main analysis, namely 1 (reference), 1.18 (0.89-1.55) p=0.26, and 1.34 (0.95-1.89) p=0.09 with age and the presence of osteoporosis both remaining significant independent predictors of fracture.

Discussion

We report that amongst Irish women referred for routine DXA, moderate CKD is associated with fracture occurrence independently of bone mineral density. The utility of DXA scanning in predicting fractures in the dialysis population has been questioned, however in our study of patients at earlier stages of CKD bone mineral density remained unambiguously associated with fracture risk. These data also suggest that within the population that we studied, at least 1 in 5 subjects had an additional independent risk factor for fracture, in the form of CKD, which in most cases was probably neither recognized nor actively managed. The improved recognition of this association may provide important opportunities to improve both renal and long-term bone health in this patient group.

Several analyses have suggested an association between diminished renal function and decreased Bone Mineral Density.[194-197] In an analysis of 885 women in the Cardiovascular Health Project, renal function defined by quartiles of Cystatin C, was associated with a decrease in bone mineral density over time on univariate but not multivariate analyses.[196] Hsu et al. using the Third National Health And Nutrition Examination Survey (NHANES III) 1988-1994 dataset similarly found that Cockcroft-Gault estimated creatinine clearance, though associated with femoral bone mineral density on univariate analysis, was attenuated and no longer significant following adjustment for subject demographics.[197] We also find that CKD is associated with low BMD on

univariate analysis but not following adjustment for age. Thus, while chronic kidney disease is associated with osteoporosis, this is mainly due to confounding by age, with patients who have CKD being older.

End Stage Kidney Disease has been independently associated with increased fracture risk and with worse patient outcomes post fracture in several studies.[183, 198] Several [177-182] though not all [199] recent reports have suggested that earlier stages of renal dysfunction are also associated with increased fracture risk. The extent to which this observation may be the result of coexistent osteoporosis remains inadequately explored. In an analysis of the NHANES III dataset the adjusted odds ratio (95% CI) for self reported fracture for subjects with an eGFR below as compared to those above 60 ml/min per $1.73m^2$ was 2.32 (1.13 – 4.74) and was independent of history of osteoporosis.[180] In a case cohort study nested within a large observational cohort of osteoporosis, baseline bone mineral density was measured at the calcaneus using single photon absorptiometry and at the lumbar spine and femoral neck using standard DXA scanning on average 2.2 years later.[177] Decreased renal function was associated with fracture risk when adjusting for the calcaneal but not for femoral bone mineral density.[177] There remains therefore substantial uncertainty as to the role that osteoporosis plays in fracture risk in patients with CKD. Yenchek et al. in a recent longitudinal study, analyzed the effect of CKD on fracture risk prediction by DXA in a cohort of 2754 community dwelling older adults, followed for a median of 11.3 years.

This study demonstrated that low BMD by DXA predicted fracture occurrence in patients with and without CKD, even following adjustment for mineral and bone abnormalities (elevated PTH and low Vitamin D levels). The majority of patients with CKD in this cohort had CKD Stage 3, eGFR 45-59.9mls/min.[41] In distinction to the above, our data suggests that while part of the increased fracture risk seen in the unadjusted analysis results from decreased bone mineral density, a significant component of it is independent of this. The reasons for these disparate results in the limited literature that has to date examined this issue are unclear and may relate to different study designs or populations studied. Additional prospective studies are required to better delineate the independent role of early CKD in fragility fractures.

The cause of increased fracture risk in CKD is speculative; it may be related to progressive development of bone mineral disorders in subjects with CKD. As serum PTH and vitamin D levels are not routinely measured in general practice we were unable to examine to what extent the association with CKD is mediated by derangements in these parameters. Alternatively part of this risk may be mediated not only by increased bone fragility but also by an increased risk of suffering a fall or injury. Decreasing renal function is associated with increased levels of fragility, anaemia and deconditioning as well as specific alterations in muscle function all of which may predispose an osteoporotic subject to fall and suffer an injury resulting in a fragility fracture.[185]

Several limitations apply to our study. Due to its cross sectional nature it is susceptible to incidence-prevalence bias, especially given the association of hip fracture with increased mortality[200], however if present, survival bias would tend to enrich the prevalent population with subjects who had not suffered from a fracture and would thus tend to attenuate the association between CKD and fracture. Only two thirds of DXA screenees had an available serum creatinine within 1 year of DXA measurement and were eligible for inclusion in our study. These subjects were significantly older and more likely to have been referred for DXA scanning from hospital and may thus have had a higher burden of comorbidity and possibly more CKD than in those with no available creatinine measurement. This may have contributed to the relatively high prevalence of osteoporosis and of fracture within the study population though this possibly also represents the historically limited availability and resulting underutilization of DXA scanning in Ireland. The prevalence of CKD amongst DXA screenees may be less in countries where there is a greater utilization of DXA screening in the general healthy population. Our diagnosis of fracture in our primary analysis was based on patient recall; however patient recall has been shown to be reasonably reliable with regard to fractures of hip, wrist and upper arm[201], while we found a similar relationship in the subgroup in which a history of fracture could be confirmed. Parathyroid hormone has differing effects on trabecular than on cortical bone, however we did not detect any interaction between CKD category and site of lowest bone mineral

density on fracture risk. Strengths of our study include its large sample size and the fact that it is predominantly based on a primary care rather than a hospital based or formal research setting.

In conclusion, our data supports that CKD is present in over 1 in 5 Irish women who had a creatinine measurement within the previous year and who underwent routine DXA scanning. CKD was an independent risk factor for fracture occurrence and its presence should potentially influence the decision to treat decreased bone mineral density. The nature of the association of CKD with fracture risk requires confirmation and warrants further evaluation into its mechanism(s). A better understanding of its pathogenesis may allow for focused secondary preventative measures and may thereby serve to reduce the risk of fragility fracture in CKD with all of its attendant complications.

Chapter 3

Hyponatremia Independently of Osteoporosis is Associated with Fracture Occurrence

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Introduction

In the previous chapter we found that moderate CKD in women was associated with fracture occurrence, independently of Bone Mineral Density. Numerous factors in addition to bone mineral density influence fracture risk as renal function declines. Additionally, CKD is associated with increased levels of frailty, Vitamin D deficiency, deterioration in muscle function and general deconditioning which predispose to an increased risk of falls with subsequent fragility fracture.[36-38] In this chapter we examine hyponatremia as a potential novel risk factor for falls which may also exert direct effects on bone mineral density and thus predispose to fragility fracture by both direct and indirect mechanisms.

Background

Severe hyponatremia is a well recognised cause of increased patient morbidity and mortality, although milder degrees of hyponatremia -serum sodium levels of 130-134 mmol/L- are usually devoid of obvious symptoms.[202] However recent evidence suggests that mild chronic hyponatremia is associated with subtle central nervous system impairment including gait and attention deficits that may lead to an increased risk of falling.[203, 204] Furthermore, two case control studies have reported the association of mild asymptomatic hyponatremia with bone fracture in the ambulatory elderly,[20,21] although neither study was able to control for the presence of osteoporosis, a major

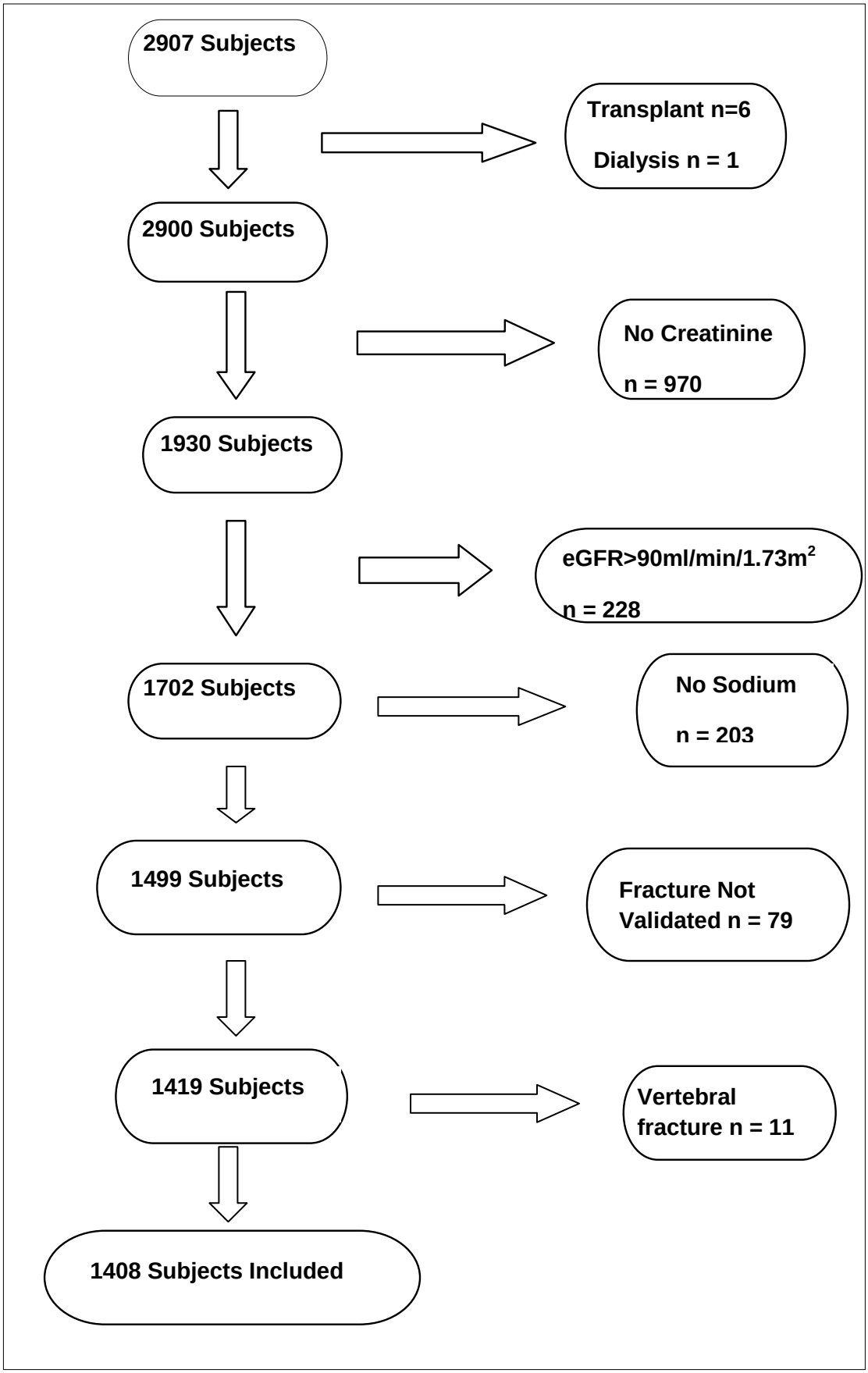
potential confounder of this relationship, as the prevalence of both osteoporosis and hyponatremia increase with older age. Evidence also suggests that hyponatremia itself contributes to bone loss. Approximately one third of total body sodium is stored in bone. Bergstrom et al. demonstrated in 1954 that acute hyponatremia in experimental animals resulted in release of sodium from bone[22, 23], analogous to bone resorption and calcium efflux from bone in settings of hypocalcaemia. More recent studies by Verbalis et al. confirmed that chronic hyponatremia (>3 months) significantly reduced Bone Mineral Density compared with normonatremic rats. Histomorphometric analyses in these animals indicated that hyponatremia markedly increased both cortical and trabecular bone resorption and decreased bone formation. [24] Using the NHANES III dataset, Verbalis et al. also reported a significant association between mild hyponatremia (<135mmol/L) and increased odds ratio of osteoporosis at the hip (OR 2.85, 95% CI 1.03-7.86, p<0.01).[24]

We therefore conducted the following analysis in order to quantify the presence, strength and significance of the association of hyponatremia with fracture occurrence -based on the hypothesis that hyponatremia predisposes to falls with an attendant increase in fracture risk- and in particular to assess whether this relationship is associated with osteoporosis.

Methods

This is a secondary analysis of data collected for a retrospective study examining the association of chronic kidney disease with self reported fracture occurrence. Concise methods have been published elsewhere [205] and are described in detail in Chapter 2. Briefly, female patients, aged over 18 years who underwent a DXA scan at Cork University Hospital between 1st September 2006 and 11th April 2007 and who had an available serum creatinine level measured within 1 year of the DXA scan were eligible for inclusion. Subjects who were referred from nephrology services (in order to avoid referral bias) and those with an eGFR greater than 90 ml/min per 1.73m^2 (due to concerns regarding the accuracy of the MDRD equation at these levels) were excluded. For the current analysis we additionally a priori excluded 203 subjects who did not have an available serum sodium, 79 patients whose fracture could not be validated (see below) and 11 subjects who had a vertebral collapse fracture, resulting in a sample size of 1408 participants. (Figure 3.1) Patients with vertebral collapse fractures were excluded as they are frequently non traumatic and our hypothesis centred on hyponatremia leading to an increased risk of falls with resulting fracture.

Figure 3.1: Study Population, 1408 subjects with available DXA and sodium



Dual energy x-ray absorptiometry (DXA) measurements were performed at the lumbar sacral spine and both hips using a Lunar IDXA™ scanner (General Electric) and expressed as T-scores indicative of the number of SDs by which the bone mass value deviated from the mean of a group of young normal controls. In keeping with the World Health Organization definition, osteoporosis was defined as a T-score of less than -2.5.[185] Data regarding patient demographics, risk factors, current and prior treatment regimes for osteoporosis, history of prior fracture and details of co-existent medical conditions were obtained from a standardized self-report questionnaire, completed by the subject at the time of the DXA scan.

The DXA database was manually cross referenced against the central laboratory database at Cork University Hospital, as outlined in Chapter 2 to identify those subjects who had a serum creatinine measured within 1 year of the index DXA scan. Serum creatinine was assayed using the modified Jaffe reaction and reported in umol/l. The estimated Glomerular Filtration Rate (eGFR) in ml/min per 1.73 m^2 body surface area was calculated using the 4 variable Modification of Diet in Renal Disease (MDRD) prediction equation.[186] Subjects were categorized using a modified National Kidney Foundation staging system with eGFR cut-offs of 75-89, 60-74, 30-59 and <29 ml/min per 1.73m^2. Serum sodium concentrations were measured by an ion selective electrode and reported in mmol/l. Hyponatremia was defined as $[Na^+]$ < 135mmol/L and mild hyponatremia as a value between 130-134 mmol/L.

In an attempt to partially validate fracture occurrence we cross checked self reported fracture against radiology reports and/or fracture clinic attendance at Cork University Hospital.

Statistical Considerations: Outlying and clinically implausible values were double checked against the original clinical data. The distribution, central tendency and variance of each variable was examined using standard tabular and graphical methods. The study population was categorised by serum sodium levels and characteristics were compared using the Mann-Whitney test for continuous variables and chi-square for categorical variables. We examined the presence, strength, significance and independence of the association of hyponatremia with fracture occurrence using logistic regression, adjusting sequentially for age, osteoporotic risk factors (amenorrhoea, family history, regular steroid use, smoking history, alcohol use, history of liver disease and low calcium diet) and osteoporosis treatments (calcium and vitamin D supplements, antiresorptives and hormonal replacement therapy). To examine if there was a relationship between values within or above the normal reference range with fracture occurrence, we additionally repeated the above multivariate logistic regression model, expressing the serum sodium as a 6 level categorical variable, namely <135, 136-137, 138-140 (reference), 141-142, 143-145 and > 145 mmol/L. All analyses were performed using SPSS Version 12.0 software, (Chicago, Illinois, USA) with a 2-sided type 1 error rate

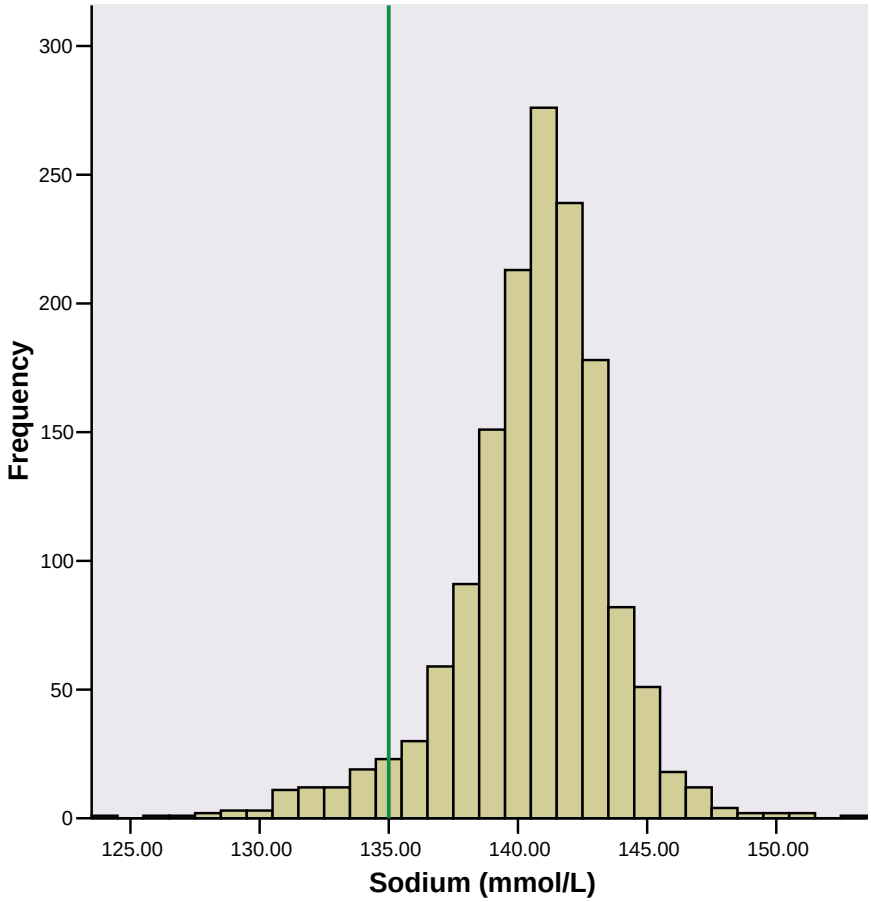
of 0.05. The study protocol was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Results

Clinical Characteristics:

The mean (sd) age of the 1408 subjects analyzed was 61 (11) years and mean (sd) eGFR was 69 (12) ml/min per 1.73m^2, Table 3.1. The mean T-score (SD) was -2.3 (1.1); 45% of subjects had osteoporosis (T-score < -2.5). Serum sodium was normally distributed with a mean (sd) of 140.6 (3.0) mmol/L, Figure 3.2. Hyponatremia was present in 59 patients (4.2%) and was mild in 53 (3.8%).

Figure 3.2: Distribution of Serum Sodium Values in 1408 women undergoing DXA scanning.



Subjects with, as compared to those without, hyponatremia were significantly older, had a lower bone mineral density and a higher prevalence of osteoporosis but similar eGFR. The use of antiresorptive agents, calcium and vitamin D and hormonal replacement therapy was not significantly different between the two groups, Table 3.1.

Table 3.1: Subject Characteristics by Serum Sodium Level

Characteristic	Total	Na < 135mmol/l	Na >135mmol/l	p
No. of Patients (%)	1408	59 (4.2)	1349 (95.8)	
Mean (sd), Age (years)	61.4 (10.7)	67.8 (13.0)	61.1 (10.5)	<0.001
Range	23 to 97	32 to 97	23 to 89	
Mean (sd), T-score	-2.3 (1.1)	-2.6 (1.2)	-2.3 (1.1)	0.03
Range	-6.5 to 0.9	-5.6 to -0.2	-6.5 to 0.9	
Osteoporosis (%)	44.9%	57.6%	44.3%	0.04
(T-score < -2.5)				
OP Risk Factors (%)				
Amenorrhoea	83.9%	81.4%	84.0%	0.59
Dairy Servings<3/wk	42.3%	25.4%	43.0%	<0.01
Alcohol >5units/week	18.0%	8.5%	18.5%	0.05
Maintenance Steroids	6.4%	3.4%	6.5%	0.34
Ever Smoked	18.3%	18.6%	18.3%	0.95
Family History	10.3%	5.1%	10.5%	0.18
Liver Disease	0.7%	1.7%	0.7%	0.36
Treatment for OP (%)				
Calcium	33.0%	39.0%	32.8%	0.32
Vitamin D	8.3%	13.6%	8.1%	0.14
Antiresorptive	21.9%	28.8%	21.6%	0.19
HRT	9.6%	11.9%	9.5%	0..54
Mean (sd)	140.6 (3.0)	132.2 (1.8)	141.0 (2.4)	<0.001
$[Na^+]$(mmol/l)	127 to 153	127 to 134	135 to 153	
Range				
Mean (sd), eGFR				
(ml/min/1.73m^2)	68.8 (12.3)	66.9 (14.4)	68.9 (12.2)	0.43
Range	20 to 89	29 to 88	20 to 89	

Abbreviations: OP, Osteoporosis, HRT, Hormone Replacement Therapy, eGFR, estimated Glomerular Filtration Rate.

Overall 254 (18.0%) subjects had at least one prior fracture. Hyponatremia was present in 8.7% of those with a fracture versus 3.2% of those without a fracture, p (chi square) <0.001. Patients with hyponatremia had a 2.86 fold increased unadjusted Odds Ratio for fracture occurrence as compared to non hyponatremic subjects, p<0.001, Table 3.2. On adjusting simultaneously for age and T-score the Odds Ratio (95% CI) for fracture occurrence remained significantly elevated at 2.06 (1.14 – 3.65). In the fully adjusted model (simultaneously adjusting for age, T-score, Chronic Kidney Disease stage, osteoporotic risk factors and osteoporosis treatments, as listed in Table 3.1), there continued to be a significantly increased Odds Ratio (95% CI) of fracture occurrence in the hyponatremic group of 2.25 (1.24 - 4.09). When serum sodium was entered into the above multivariate model as a 6 level categorical variable and using the 138-140 mmol/L category as the reference group, the < 135mmol/L category was independently associated with fracture while the confidence intervals for categories above 135mmol/L largely overlapped each other and were not significantly different to reference, Figure 3.3.

Table 3.2: Crude and Adjusted Odds Ratio for Association of Hyponatremia with Fracture Occurrence.

	Odds Ratio	**95% CI**	**p**
Model 1	2.86	1.66 – 4.94	<0.001
Model 2	2.06	1.14 – 3.65	0.02
Model 3	2.06	1.15 – 3.71	0.02
Model 4	2.25	1.24 – 4.09	0.01

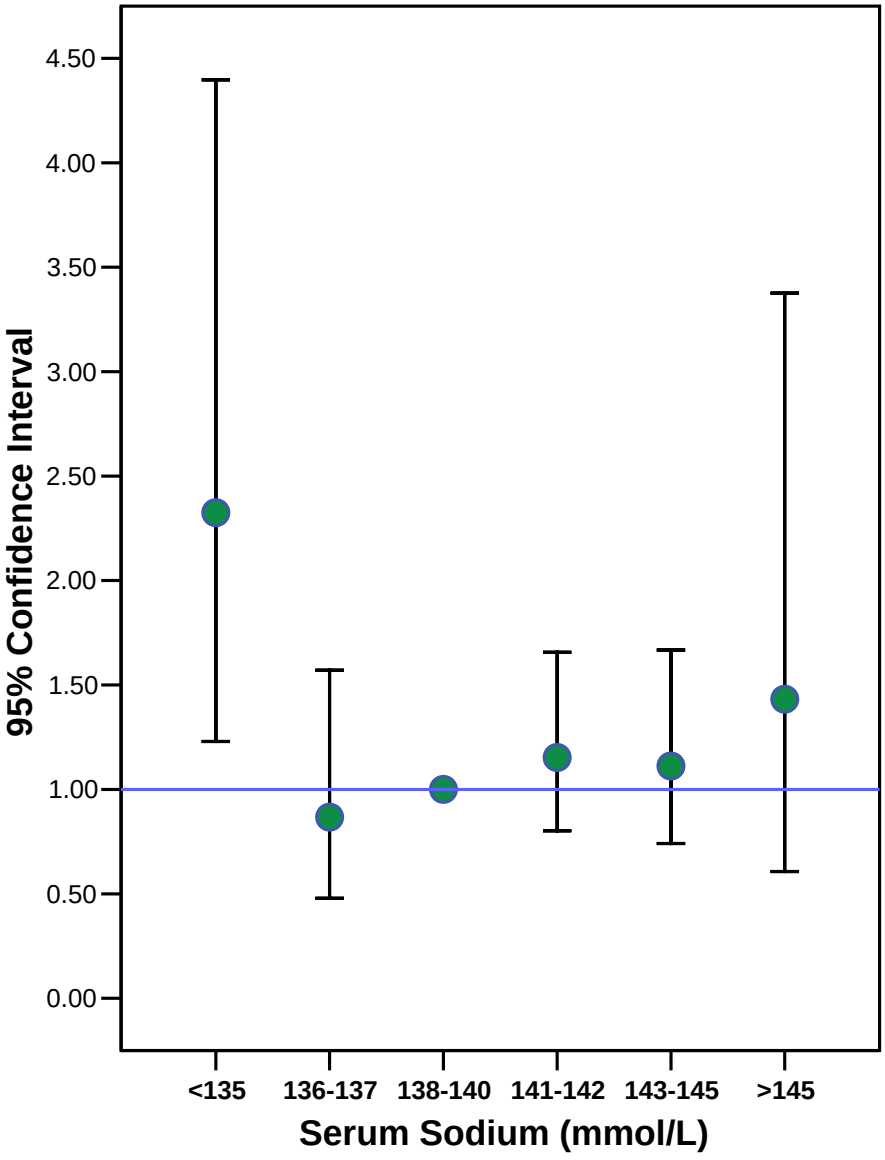
Model 1: unadjusted

Model 2: Adjusted for age (years), and T-score.

Model 3: Adjusted for age (years), T-score and CKD stage.

Model 4: Adjusted for age (years), T-score, CKD stage, osteoporotic risk factors (amenorrhoea, low dietary diary intake, high alcohol intake, maintenance steroids, ever having smoked, family history of osteoporosis and history of liver disease) and treatment (use of calcium, vitamin D , anti-resorptive therapy, hormonal replacement therapy).

Figure 3.3: Odds Ratio (95% Confidence Interval) of fracture occurrence by [Na^+] category, adjusting simultaneously for age (years), T-score, CKD stage, osteoporotic risk factors amenorrhea, low dietary calcium intake, high alcohol intake, maintenance steroids, ever having smoked, family history of osteoporosis, and history of liver disease), and osteoporosis therapy (use of calcium, Vitamin D, antiresorptive therapy, hormonal replacement therapy).



In a sensitivity analysis, we excluded hyponatremic subjects with serum sodium below 130mmol/L in order to examine whether the observed association with fracture was driven by the small number of extreme values. The unadjusted OR (95% CI) for mild hyponatremia (130-134mmol/L) versus those with serum sodium of 135mmol/L or greater was 2.92 (1.65 – 5.18), p<0.001. This remained significant following sequential models with the adjusted OR (95% CI) as per model 4, table 3.2 being 2.2 (1.18 – 4.13) p= 0.014

Discussion

We report that mild hyponatremia, of a degree that is often ignored in clinical subjects is significantly associated with fracture occurrence independently of bone mineral density. Renneboog et al. have shown that mild chronic hyponatremia is associated with gait and attention deficits and with an increased risk of falls. They also found that hyponatremia caused more attention deficits than did a serum alcohol level of 0.6g/L in an age and sex matched control group.[203] In a preliminary report of an additional study the threshold for gait and attention deficits induced by hyponatremia were 134 and 132 mEq/L respectively. [204] Furthermore, a case control study by Kengne et al demonstrated that mild asymptomatic hyponatremia (mean serum sodium 131 mmol/L) was associated with bone fracture following incidental fall in the ambulatory elderly, with an adjusted OR of 4.16 (95% CI 2.24 – 7.71)[20] with a similar significant association reported in a recent additional

study.[21] A limitation of both these studies was the inability to fully adjust for bone mineral density, which as demonstrated in our analysis partially -though incompletely- attenuates the association seen in unadjusted analyses. Advanced chronic kidney disease is associated with a range of qualitative abnormalities of bone turnover. However the majority of subjects in our analysis had well preserved renal function (mean eGFR 69 ml/min per 1.73 m^2), while the association of hyponatremia with fracture was independent of the presence of Chronic Kidney Disease. As well as predisposing to falls through disturbance of balance and gait, hyponatremia has been shown to contribute to abnormalities in underlying bone health by a direct effect on bone turnover.[24] Unfortunately we were unable to examine this issue in this retrospective study.

The term 'symptomatic hyponatremia' has been used to refer to the presence of overt complications of hyponatremia, typically gastrointestinal and neuro-psychiatric in nature. This is on occasion associated with seizures and indeed the initial report of hyponatremia associated fracture occurred in a subgroup of post-menopausal women with severe hyponatremia who presented with fracture following seizure activity.[206] Mild chronic hyponatremia, which lacks these overt symptoms, is typically considered asymptomatic; however subtle alterations in gait and balance are unlikely to be volunteered by patients and

may often be attributed to concomitant conditions such as old age. Whether the range of clinical complications associated with mild chronic hyponatremia is limited to disturbances of gait and balance or is more extensive is unknown, as is its impact on quality of life. In view of these uncertainties the suitability of the term 'asymptomatic hyponatremia' is open to question.

Fragility fractures, occurring after minor trauma or falls, are a major public health concern due to their attendant morbidity and mortality.[207] Recurrent incidental falls result in bone fracture in 4 – 6% of cases, with up to 2% of these patients dying.[208] Effective treatment and preventative strategies for recurrent falls remains highly limited.[209] Mild chronic hyponatremia is a common electrolyte imbalance with a reported prevalence of 2-4% in the general population, rising to 7-11% in the ambulatory elderly, [210,211] and to 42% in hospitalized subjects. [211] The potential importance of the association of mild hyponatremia with fracture relates to this high prevalence of the condition, especially in groups who have a high risk of fracture following falls, such as the elderly. In addition, hyponatremia commonly complicates advanced cardiac and liver failure, in both of which conditions it has well recognized prognostic import, [212, 213] though the hyponatremia per se is usually considered to be asymptomatic. It is of interest that advanced cardiac failure has been recently associated with an increased incidence of fracture,[214] and it

has been speculated that this may be mediated in part through the development of hyponatremia. [215]

Two common reversible causes of hyponatremia are the use of thiazide type diuretics[216] and Selective Serotonin Reuptake Inhibitor (SSRI)[217] antidepressants, both of which are in widespread clinical use. Thiazide diuretics are widely used as first line agents in the treatment of hypertension but are complicated in up to 14% of subjects by hyponatremia.[218, 219] Among subjects aged 65 years and older resident in a nationally representative sample of US nursing homes, thiazide use was associated with increased fracture risk over 1 year of follow-up.[220] However, thiazide type diuretics in addition to their antihypertensive effect, also decrease urinary calcium excretion and promote a positive calcium balance, thereby helping to maintain and improve bone mineral density.[221,222,223] It is possible that the potential uses of thiazide diuretics for this indication may have led to their selective use in subjects with pre-existing osteoporosis and thus confound the association of thiazide induced hyponatremia with fracture in ours and in other studies examining this relationship. However, against this, thiazide type diuretics have not been widely used in this indication in Irish clinical practice (Prof MG Molloy, Professor of Rheumatology, Cork University Hospital, personal communication). Pending further studies, our results would urge caution in

the use of thiazide type diuretics for bone protection in those subjects who develop hyponatremia, as the benefits of a positive calcium balance may be offset by the potential increase in falls risk. In addition, it may be prudent to routinely screen serum sodium levels following initiation of thiazide diuretics, especially in those at high risk for developing hyponatremia such as elderly females.

Depression and the use of centrally acting agents are recognized as being associated with increased risk of falls and of fracture. Available evidence suggests that some of this increased risk is due to the agents independently of the presence of depression.[224] Of the studied agents the highest risk of falls has been associated with selective serotonin reuptake inhibitors (SSRI's), [225] with peak fracture risk occurring in the first 2 weeks after initiation of therapy. This risk of fracture mirrors the increased risk of hyponatremia in SSRIs as compared to alternative agents and the time course in which hyponatremia typically occurs[226,224] however the potential role of hyponatremia in predisposing to fracture in this population remains unstudied.

The mainstay of treatment for persistent hyponatremia, where the syndrome is either irreversible or where the iatrogenic cause cannot be readily altered, centres on fluid restriction, the severity of which depends on the degree of the

diluting defect; however this is difficult to maintain and patient adherence is often incomplete. As a result, the goal of such therapy is often to limit the severity of the dysnatremia rather than to fully correcting it, mild hyponatremia being seen as an acceptable clinical compromise between the dangers of severe hyponatremia and the inconvenience of aggressive therapy. Thus the perception of mild hyponatremia as being devoid of clinical significance in many cases tempers the aggressiveness of therapy. Of the available medical adjuncts to fluid restriction, loop diuretics are of limited efficacy, oral urea is unpalatable and demeclocycline is potentially nephrotoxic.[227] The recent development of selective oral vasopressin V2 receptor antagonists may facilitate the more complete correction of hyponatremia in patients with persistent hyponatremia,[228] but whether such therapy would reduce the occurrence of falls and low impact fractures in susceptible individuals is to date unknown.[229]

Several limitations apply to our analysis, serum sodium measurement was not available at the actual time of fracture but was instead related to the bone density measurement, while this may have resulted in a degree of non differential misclassification, this would tend to reduce the observed association toward the null hypothesis and attenuate the effect size of any association. Indeed the magnitude of the association we found is less than that described by other authors, this may relate in part to different populations examined, ours was relatively young and may therefore be less severely

affected by mild gait disturbances as compared to older subjects. Moreover serum sodium levels measured following a fall, would be potentially influenced by both complications of the fall (such as development of dehydration) or to initial therapeutic measures such as intravenous fluid infusion initiated prior to venupuncture, either of which may systematically distort the measurement serum sodium level relative to the true pre-fall level. In addition our effect size is also partially attenuated as it is adjusted for bone mineral density. A major limitation on the inferences that can be drawn from this study is that, as with all observational research, it can at best only provide support for the above hypothesis but cannot prove it. Both unmeasured confounders such as unreported co-morbid conditions, sedative drug use or the presence of malignancy may impact on the described relationship of hyponatremia with fracture. Nevertheless, there exists a plausible mechanism to explain this putative association and as in our work the observed relationship has been independent of a wide range of relevant confounders, including the presence of osteoporosis. In an editorial commentary, Ayus et al. summarise that bone abnormalities should be viewed as an additional complication of chronic hyponatremia.[230] The increased risk of falls and subsequent fracture in hyponatremic patients is magnified by hyponatremic-induced bone loss; therefore chronic hyponatremia should be viewed as a modifiable risk factor for fracture. This view is supported by an increasing body of literature from epidemiological and experimental studies.[231-234]

Nevertheless, controlled interventional trials are essential in order to reliably estimate the true attributable risk of hyponatremia with falls and fracture occurrence.

In conclusion, mild chronic hyponatremia is a significant independent risk factor for bone fracture. In keeping with recent studies our data suggests that mild chronic hyponatremia is neither a benign nor an inconsequential condition. If confirmed in prospective studies the prevention, identification and effective management of this relatively common condition may provide an important opportunity to reduce the risk of recurrent falls and repeated fractures and lead to alterations in a wide range of current clinical practices.

Chapter 4

The Burden of Chronic Kidney Disease - Mineral Bone Disorder (CKD-MBD) in Successful Renal Allograft Recipients: The ABC-HEART Study.

Introduction

Chronic Kidney Disease - Mineral Bone Disorder (CKD-MBD) is the term introduced by the Kidney Disease–Improving Global Outcomes (KDIGO) international consensus group to encompass the closely interrelated processes of abnormal bone mineral homeostasis, Vitamin D and parathyroid hormone (PTH) dysregulation, derangements of bone turnover, mineralization and volume and aberrant vascular and soft tissue calcification that develop as complications of CKD.[33] These complex interrelated processes have been extensively studied in subjects with End Stage Kidney Disease treated with dialysis, while in recent years there has been increased awareness that the genesis of the disorder occurs from early on in the natural history of CKD.[46]

Successful renal transplantation restores effective endogenous renal function to patients with End Stage Kidney Disease and improves survival but it fails to restore either normal longevity or health.[144, 147, 235] Furthermore, it is associated with persistent and on occasion progressive morbidities that result from the combined sequelae of prior native CKD, de novo transplant-related complications and consequences of the often suboptimal level of kidney function provided by the allograft.[150, 151, 167, 236-239] To date several reports have examined the prevalence of individual components of CKD-MBD in transplant recipients. Post-transplant hyperparathyroidism improves over the first year post transplant but remains highly prevalent occurring in over 50% of patients

and in a substantial minority of cases is associated with hypercalcaemia.[148-151] Vitamin D levels are typically low due to ultraviolet light avoidance –resulting from concerns regarding cutaneous malignancy- and limited use of vitamin D supplements – resulting from concerns over exacerbating hypercalcaemia and promoting nephrolithiasis.[149, 240, 241] In addition, transplant recipients have unambiguously been shown to have increased fracture risk relative to the general population,[159, 161, 163, 165] while the limited available bone biopsy data suggests widespread derangements of normal bone architecture, turnover and mass.[151, 242-245] Despite its acknowledged limitations, areal bone density measurement is widely measured in renal transplant recipients and is recommended by current renal transplant guidelines.[246, 247] There is only sparse available data regarding the presence and natural history of vascular mineralization post transplantation using either radiographic or functional measurements such as pulse wave characteristics.[156, 157, 248-250]

However, to date the overall burden of CKD-MBD experienced by successful renal allograft recipients in routine medical practice has not been comprehensively examined in the same transplant population. With improved allograft and recipient survival rates, the presence and severity of CKD-MBD is likely to prove to be increasingly important in influencing the long term health and quality of life of successful renal allograft recipients and to assume an increasing priority in both the medical management and research agenda within this population. As most interventions simultaneously influence

multiple components of CKD-MBD it is relevant, and indeed necessary, to evaluate the occurrence of the disorder in its entirety. We therefore conducted the following observational study to comprehensively examine the occurrence and severity of CKD-MBD within successful renal allograft recipients and to examine the relationship of severity of the disorder with their level of transplant function.

Methods

We enrolled a convenience sample of 90 prevalent renal transplant recipients into a prospective cohort study the working title of which was; the 'Association of Bone and Cardiovascular HEalth After Renal Transplantation' (ABC HEART) study, in order to examine the interaction of bone and vascular health within this population. The current report uses baseline data at study entry. Subjects who were waiting to be reviewed at their routine follow-up clinic were approached and invited to participate, only 4 potential subjects declined, citing time constraints. To be eligible subjects had to be between 0.5 and 12 years post-transplant and to have a current transplant eGFR >30 ml/min per $1.72m^2$ and to be in their usual state of health. All study procedures were performed on an outpatient basis. One subject had a prolonged hospitalization closely following enrolment and subsequently died without undergoing any of the study specific procedures and was therefore excluded from analysis. Informed written consent was obtained from all subjects; the study was

approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Information on demographics, clinical details and past medical and fracture history were obtained by patient self-report and abstracted from the medical record. Serum creatinine was measured using the Jaffé reaction on the Olympus 5400 analyser. The method was changed to an IDMS traceable form in October 2009. eGFR (ml/min per 1.73 m^2 body surface area) was calculated using the 4-variable MDRD equation[1] and categorized as Transplant CKD Stage 1T-2T for eGFR >60 ml/min, Stage 3AT for 45-59 ml/min and Stage 3BT for 30-44 ml/min Serum calcium was measured by the Arsenazo 111 method on the Olympus 5400 analyser. Serum albumin was measured by the bromocresol purple method up to October 2009. Bromocresol green was then used. The laboratory participates in the UK national external quality assessment (UKNEQAS) scheme for clinical chemistry analytes Albumin adjusted serum calcium was calculated as the measured serum calcium plus 0.2 *(40-serum albumin). Uncuffed venous samples for ionised calcium and venous pH were collected anaerobically in a heparinised syringe and immediately analysed on the GEM 4000 blood gas analyser in the main biochemistry laboratory. An additional urine sample for measurement of calcium-creatinine ratio and simultaneous serum calcium was measured on 85 subjects several months after the study entry blood draw and so was not contemporaneous with the other study tests.

Blood samples and second void urine samples were collected following an 8 hour fast. The blood was centrifuged and serum and urine samples aliquoted and frozen at -80^{0}C and -20^{0}C respectively, within 1 hour of collection and subsequently analysed in batch for biochemical markers of bone turnover that have been validated in CKD.[251]. Bone Specific Alkaline Phosphatase (Bone ALP), a marker of bone mineralisation and maturation was measured by an enzyme-linked immunosorbent assay (ELISA) (Immunodiagnostic Systems Ltd, Boldon, Tyne and Wear, UK) with inter-assay coefficients of variation of 2.3% (at 10.95 ug/L and 39.77 ug/L) and intra-assay coefficients of variation of -6.9% (at -12.43 µg/L) and -5.1% (at -35.9 µg/L) respectively. Tartrate- Resistant Acid Phosphatase 5b(TRACP5b) a marker of bone resorption was determined by ELISA (IDS Ltd) with - inter-assay coefficients of variation of 1.58% (at 1.46 U/L) and 0.74% (at 4.18 U/L) and intra-assay coefficients of variation of -2.53% (at -2.37 U/L), 3.42% (at 4.62 U/L and -1.44% (at 13.36 -U/L) respectively. Urinary N-Terminal cross-linking telopeptide of Type 1 collagen (NTx-I) was measured using ELISA (Osteomark, Ostex International Inc., Seattle, USA-) with an inter-assay coefficient of variation of 4.7% (at 1503.9 nmol BCE) and intra-assay coefficients of variation of 9.54% (at 457 nmol BCE) and 8.18% (at 1142 nmol BCE) respectively. Assay values are corrected for urine concentration by indexing to urinary creatinine level and expressed in nanomoles of bone collagen equivalents per millimole of creatinine per litre (nM BCE per mM/L). Serum 25-hydroxyvitamin D (25(OH) D) was measured by a competitive

radioimmunoassay (IDS). Interassay coefficients of variations were 6.2% and 7.7% at concentrations of 28.8 nmol/l and 105.4 nmol/l, respectively. The intra-assay coefficients of variations were 3.0% and 2.7% at concentrations of 28.9 nmol/l and 73.9 nmol/l, respectively. Optimal values were considered to be over 50 nmol/l[73]. Biochemical markers of bone turnover and 25-OH Vitamin D assays were performed in the Metabolism Laboratory in St Vincent's University Hospital under the supervision of Dr. Jennifer Brady. Intact PTH was measured using a Roche Elecsys assay. This is a second generation PTH assay, which is the most widely used in clinical practice in Europe. These assays use 2 separate antibodies, one is directed against the C-terminal fragment of the PTH molecule and the second is directed against the N-terminal fragment. These assays were initially thought to quantify only the full-length, biologically active 1-84 PTH molecule. It is now known that they also measure other large PTH fragments, predominantly 7-84 PTH, the biological activity of which is uncertain.

Areal Bone Mineral Density was measured at the lumbar sacral spine, the femoral neck and total femur of both hips and the non-dominant forearm, using a Lunar IDXA scanner (General Electric) and expressed as a T-score. Osteoporosis was defined as a lowest T-score of -2.5 or less and osteopenia as a T-score of 1.5-2.49. Carotid Femoral Pulse Wave Velocity was performed by a single investigator (SK) experienced in the performance of the technique using a dedicated Pulse Trace 400 PWV system (Viasys Healthcare) following the

method described by London.[131] Aortic calcification and vertebral collapse fractures were independently assessed by 2 radiologists blinded to the clinical study detail. Aortic vascular calcification was quantified from lateral lumbar spine radiographs using the Framingham method, scoring on a scale of 1-3 the presence of calcification along the anterior and posterior margin of the expected aortic contour adjacent to the 4 lumbar vertebrae, resulting in a composite score of between 0-24.[129]

Statistical analysis:

Clinically implausible and outlying data were checked against the original clinical record. Normality was examined using boxplots and Shapiro-Wilks test and distribution described using mean (sd) or median (intra quartile range [IQR]) as appropriate. Non-parametric analytic methods were exclusively used including Spearman rank correlation and Mann-Whitney U test. The relationship of variables across eGFR stage was assessed using a hierarchical approach initially testing for a linear trend across groups, which if not significant was followed by comparison between groups using the non-parametric Kruskal-Wallis test. The relationship of vitamin D with iPTH stratified by the presence or absence of an elevated total serum calcium was modelled using restricted cubic spline plots. The association of osteoporosis (lowest DXA T-score less than -2.5) with iPTH was examined using multivariate

logistic regression analysis, adjusting sequentially and simultaneously for age, gender, ESKD vintage (cumulative time spent on dialysis and post transplant), current corticosteroid use, serum 25-OH Vitamin D and TCO2 level. Routine model diagnostics included assessment of points of high leverage and influence and measurement of overall "Goodness of Fit", using the model of Hosmer and Lemeshow. Analysis was conducted using SPSS (Chicago, Illinois) V16 with a 2 sided type one error rate of 0.05.

Results

Of the 141 transplant clinic attendees who met study entry criteria, 89 participated in the study. The demographics of the study sample are shown in Table 4.1. Our study sample was broadly representative of our overall transplant population, the latter being 56% male, with a mean (sd) age of 42.8 (17.8) years, mean (sd) eGFR of 55.5 (16.7) ml/min/1.73m^2 and a median (IQR) duration of transplantation of 3.5 (1.6, 6.6) years. The proportion of post-transplant hyperparathyroidism (available from routine clinical records in 128 patients) and of hypercalcaemia in the overall transplant population was 77.9% and 37.4% respectively.

Table 4.1: Patient Characteristics of 89 enrolled renal transplant recipients by CKD-T Stage

	Enrolled Study Population				
	All subjects	**Stage T1-2**	**Stage T3A**	**Stage T3B**	**p-value**
N	**89**	**32**	**27**	**30**	
Mean (sd) eGFR (ml/min per 1.73m^2)	53.9 (16.5)	72.2 (10.0)	51.2 (4.3)	36.8 (4.6)	<0.001†
Age, years, Mean (sd)	46.8 (12.7)	39.9 (14.2)	48.9 (10.4)	52.3 (9.5)	<0.001
Sex, Male, n (%)	53 (59.6%)	21 (65.6%)	19 (70.4%)	13 (43.3%)	0.08
Cause ESKD, n (%)					
GN	29 (34.9)	15 (55.6)	8 (29.7)	6 (20.7)	
PCKD/Alports	22 (26.6)	3 (11.1)	9 (33.3)	10 (34.5)	0.05
DM	7 (8.4)	2 (7.4)	4 (14.8)	1 (3.4)	
Other	25 (30.1)	7 (26.0)	6 (22.2)	12 (41.1)	
Duration pre-ESKD renal care (yrs), Median (IQR)	4.8 (1.9 - 9.0)	3.3 (1.1 -8.8)	4.0 (1.6 -9.1)	5.7 (2.9 – 12.8)	0.30
Dialysis Modality n (%):					
Haemodialysis	49 (55.1%)	19 (59.4%)	11 (40.7%)	19 (63.3%)	
Peritoneal dialysis	34 (38.2%)	11 (34.4%)	15 (55.6%)	8 (26.7%)	0.23
Pre-emptive transplant	6 (6.7%)	2 (6.2%)	1 (3.7%)	3 (10.0%)	
Duration Dialysis (yrs)[1], Median (IQR)	2.3 (1.5 – 3.2)	2.1 (1.3 – 2.9)	2.6 (1.7 – 3.6)	2.3 (1.4 – 3.8)	0.45
Duration Transplant (yrs), median (IQR)	2.6 (1.0, 6.3)	2.4 (1.0 – 7.3)	3.0 (2.2 – 5.2)	2.1 (0.7 – 5.9)	0.52
Acute cellular rejection n (%)	10 (11.2)	2 (6.2)	5 (18.5)	3 (10)	0.43
ASCVD	8	2	2	4	N/A[2]
Tacrolimus n (%) [Median serum level, mg/dl]	75 (84.3) [8.9]	26 (81.2) [9.0]	25 (92.6) [8.5]	24 (80.0) [9.1]	0.36 0.77
Mycophenolate n (%) [median total dose, g/day]	71 (79.8) [1g/day]	23 (71.9) [1g/day]	24 (88.9) [1g/day]	24 (80.0) [0.75 g/day]	0.27
Corticosteroid: n (%) [median total dose, mg/day]	34 (38.2%) [5mg/d]	12 (37.5%) [5mg/day]	8 (29.6%) [3mg/day]	14 (46.7%) [5mg/day]	0.42
Parathyroidectomy n (%)	7 (7.9)	2 (7.4)	3 (11.1)	2 (7.1)	N/A[2]
Cinacalcet n (%)	11 (12.4)	4 (12.5)	1 (3.7)	6 (20)	0.175
One alpha Vitamin D n (%)	6	0	1	5	N/A[2]
Bisphosphonates n (%)	7 (7.9)	0 (0)	2 (7.4)	5 (17.2)	N/A[2]

Abbreviations: eGFR, estimated Glomerular Filtration Rate, ESKD, End Stage Kidney Disease, IQR, Intra Quartile Range, GN, Glomerulonephritis, PCKD, Polycystic Kidney Disease, DM, Diabetes Mellitus, ASCVD, Atherosclerotic Cardiovascular Disease.

[1]Excluding 6 pre-emptive transplants who were never dialyzed.

[2]Variables with fewer than 10 events are provided for information purposes only and are not compared statistically

p values for continuous variables denoted †were calculated using linear test for trend. All other continuous variables were examined using Kruskal Wallis non-parametric test. Categorical variables were examined using Pearson's Chi square test

The mean (sd) eGFR of the 89 study subjects at enrolment was 53.9 (16.5) ml/min/1.73m^2; allograft function was stage 1T-2T in 36%, stage 3AT in 30% and stage 3BT in 34%. Reduced levels of kidney function were associated with older age and female gender (Table 4.1). The commonest cause of ESKD was chronic glomerulonephritis, occurring in over one third of subjects**.** Thirteen subjects (15%) had diabetes; the attributed cause of ESKD in 7, while it developed post-transplantation in 5. Six subjects had undergone pre-emptive transplantation. The median duration of dialysis was 2.3 years and was similar for haemodialysis and peritoneal dialysis subjects. Eighty subjects received a single cadaveric allograft, 2 a dual cadaveric transplant, 3 a combined kidney-pancreas transplantation and 4 a living related transplant. The majority of subjects were initially treated with triple immunosuppressive therapy, including tacrolimus in 84% and mycophenolate mofetil in 88%. Sixty-two percent of subjects had had their corticosteroids successfully withdrawn post transplantation; the median dose in the remainder was 5mg/day. Eight subjects had a past history of atherosclerotic cardiovascular disease, including 3 who had undergone a coronary revascularization procedure. Nine subjects had undergone a total parathyroidectomy prior to transplant - all of whom had a forearm parathyroid implant. Medication use of note included Cinacalcet® (n=9), loop diuretics (n=9), bisphosphonates (n=7), calcium supplementation (n=6) and 1-OH vitamin D supplementation (n=5).

Eighty-two percent of subjects had a post-transplant iPTH level >65ng/ml; the median (IQR) iPTH was 100ng/ml (75-148). Subjects in stage T3B CKD had significantly higher iPTH than those in stage T1-2; moreover the majority of patients in all 3 CKD categories had an iPTH that was above the normal reference range, Table 2, Figure 4.1. iPTH was positively correlated with serum calcium (*vide infra),* and negatively correlated with serum phosphate ($r= -0.43$, $p<0.001$).

Table 4.2: Bone Turnover Parameters of Study Population by CKD-T Stage

	All subjects	Stage T1-2	Stage T3A	Stage T3B	p-value
N	89	32	27	30	
PTH and Vitamin D					
iPTH ng/ml, Median (IQR)	100 (75, 148)	99 (76,119)	91 (69, 109)	144 (82, 197)	0.003†
iPTH >65ng/ml, n (%)	73 (82%)	27 (84.4%)	21 (77.8%)	25 (83.3%)	0.84
25 (OH) Vitamin D nmol/l Median (IQR)	41.3 (29., 52.5)	40 (28.4, 51.5)	42.1 (33.1, 52.3)	39.7 (30.2, 52.1)	0.51
25 (OH) Vitamin D <50nmol/l n (%)	63 (70.8%)	22 (68.8%)	20 (74.1%)	21 (70%)	0.9
Calcium and Phosphate					
Serum Calcium, mmol/L Mean, sd	2.57 (0.16)	2.58 (0.13)	2.53 (0.18)	2.61 (0.2)	0.4
Albumin Corrected Serum Calcium mmol/L Mean (sd)	2.63 (0.16)	2.60 (0.12)	2.59 (0.17)	2.61 (0.16)	0.14
Serum Calcium >2.62mmol/l n (%)	34 (38.2%)	11 (34.4%)	9 (33.3%)	14 (46.7%)	0.52
Urine Ca:Cr ratio (mmol/ mmol), Median (IQR)	0.16 (0.09, 0.24)	0.18 (0.8, 0.32)	0.16 (0.1, 0.22)	0.12 (0.09, 0.29)	0.68
Serum Phosphate (mmol/L), Mean (sd)	1.02 (0.2)	1.02 (0.23)	0.98 (0.17)	1.1 (0.2)	0.24
Serum Phosphate <0.7 mmol/L, n (%)	11 (12.4%)	5 (15.6%)	4 (14.8%)	2 (6.7%)	0.52
TCO_2 and pH					
Serum TCO_2 (mmol/L) Mean (sd)	24.6 (2.7)	25.2 (2.7)	25 (2.3)	23.6 (3.0)	0.03†
Serum TCO_2 <24mmol/L, n (%)	29 (33%)	8 (25%)	6 (22%)	15 (50%)	0.05
pH, Mean (sd)	7.34 (0.04)	7.36 (0.06)	7.33 (0.03)	7.33 (0.03)	0.03†
Bone Turnover					
Alkaline Phosphatase (mmol/L), Mean (sd)	103.3 (45.8)	117.4 (53.1)	91.6 (33.5)	98 (44.3)	0.12
Bone ALP (ug/L)[1] Median (IQR)	20.3 (14.5, 30.3)	26.1 (20.5, 37.5)	15.7 (13.4, 21.9)	18.9 (13.1, 32.5)	0.01
TRACP5b (U/L)[2] Mean (sd)	4.1 (1.7)	4.8 (1.8)	3.3 (1.1)	4.0 (1.7)	0.01
NTx-I (nM BCE per mM/L)[3] Median (IQR)	49.0 (31.6, 92.6)	71.0 (41.7, 119.3)	40.4 (29.3, 55.5)	72.3 (28.6, 105)	0.01

Abbreviations: iPTH, intact parathyroid hormone, Bone ALP, Bone Specific Alkaline Phosphatase, TRACP5B, Tartrate Resistant Acid Phosphatase 5b, NTx-I, Urinary N-Terminal cross-linking telopeptide of Type 1 collagen.

p values for continuous variables denoted †were calculated using linear test for trend. All other continuous variables were examined using Kruskal Wallis non-parametric test. Categorical variables were examined using Pearson's Chi square

[1]Reference Range, Male (7.2 – 15.0ug/L), Female (6.1 -11.8ug/L)

[2]Reference Range, Male (1.3 – 4.82 U/L), Female (1.03 – 4.15U/L)

[3]Reference Range (25.5 – 72.4 nM BCE per mM/L)

Figure 4.1: Boxplot of iPTH level by National Kidney Foundation stage of Chronic Kidney Disease

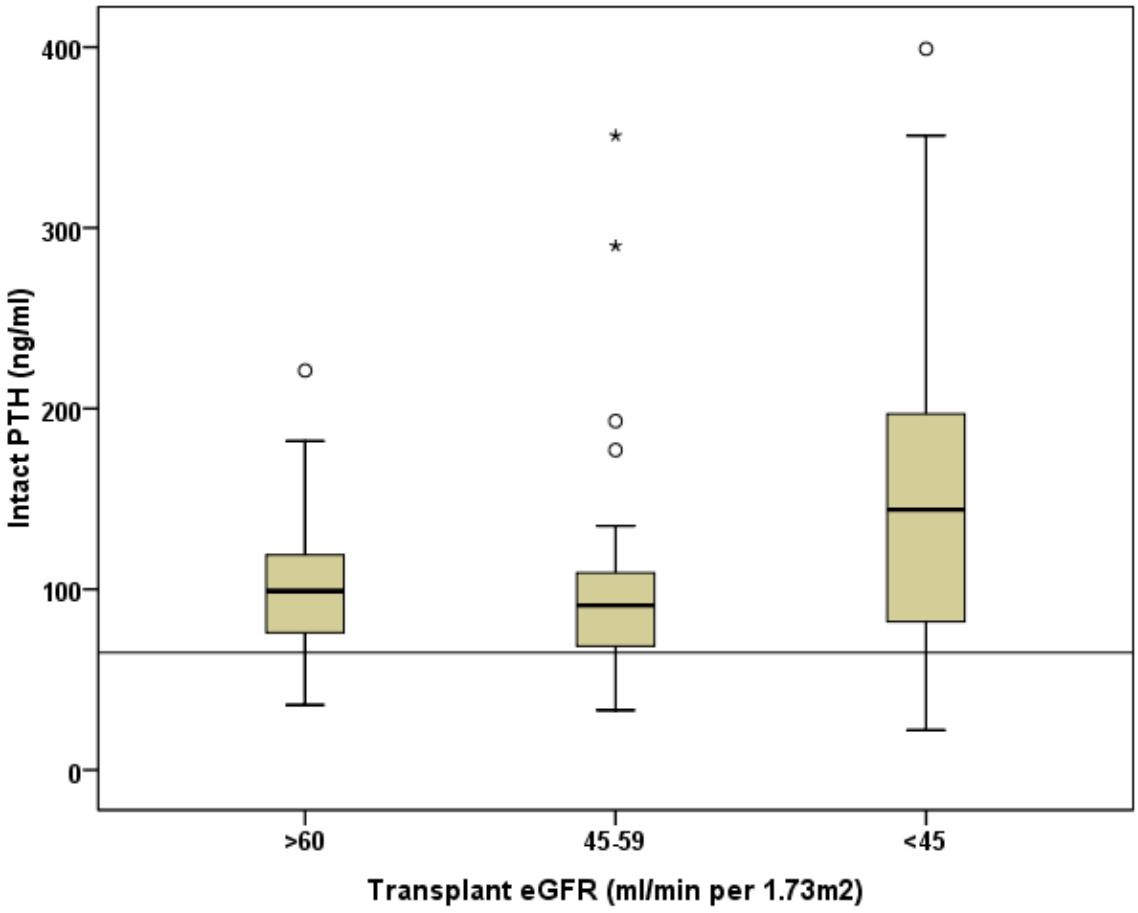


Figure 4.1: Boxplot of iPTH level by National Kidney Foundation stage of Chronic Kidney Disease in 89 prevalent renal transplant recipients. The solid line within the box indicates the median value, the box the intra-quartile range and open circles outliers. The horizontal line denotes the upper limit of normal of iPTH (65ng/ml).

Seventy-one percent of subjects had suboptimal 25(OH) vitamin D levels, which were uniformly low across the 3 CKD categories - median values of 40-42nmol/l, Table 4.2, Figure 4.2. Eighty-seven percent of subjects with a suboptimal Vitamin D had an elevated iPTH >65ng/ml vs. 68% of those with adequate vitamin D, p=0.06.

Figure 4.2: Boxplot of 25-OH Vitamin D level by National Kidney Foundation stage of Chronic Kidney Disease

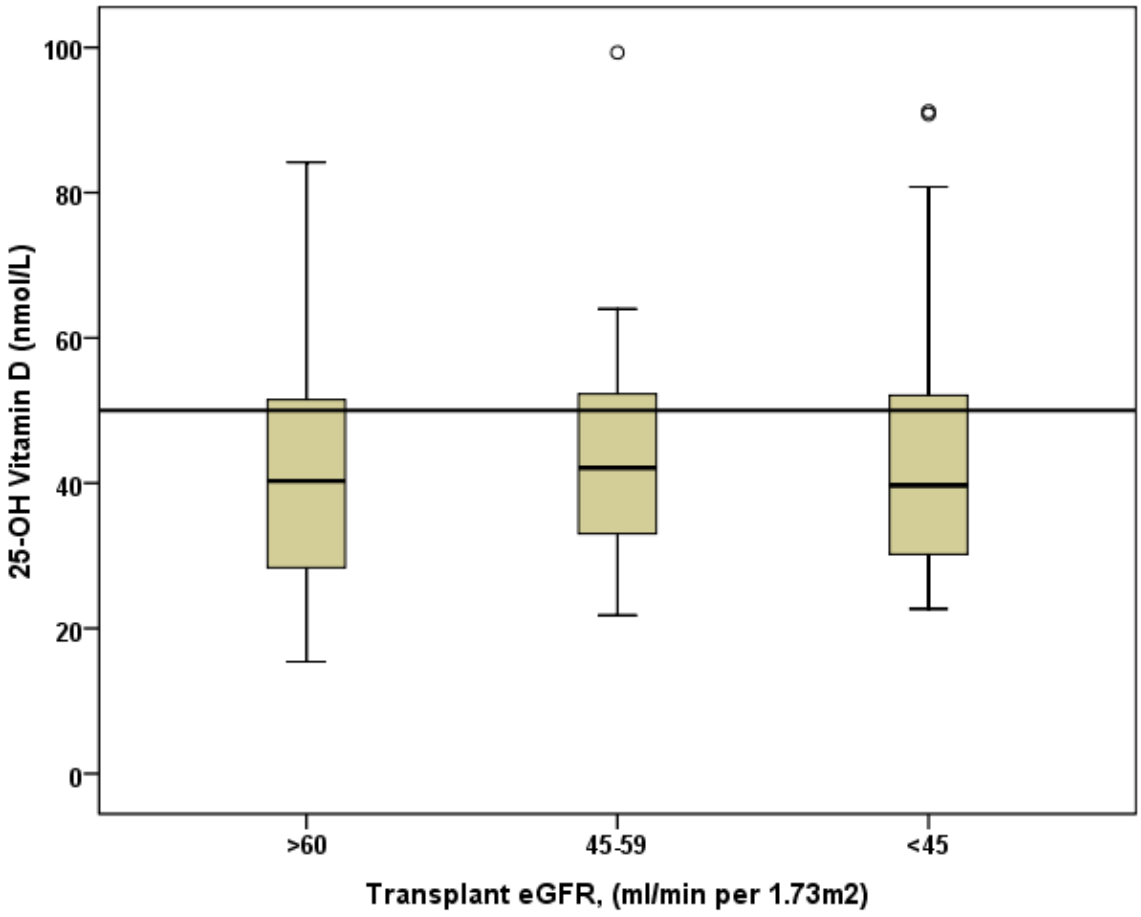


Figure 4.2: Boxplot of 25-OH Vitamin D level by National Kidney Foundation stage of Chronic Kidney Disease in 89 prevalent renal transplant recipients. The solid line within the box indicates the median value, the box the intra-quartile range and open circles outliers. The horizontal line denotes a 25-OH Vitamin D level of 50nmol/L

We further examined the relationship of 25-OH vitamin D with iPTH stratified by the presence or absence of hypercalcaemia having first excluded the 5 patients treated with activated vitamin D. In those subjects with an elevated total serum calcium there was no association between iPTH and 25-OH vitamin D level (r^2=0.06, p=0.35). However, in those subjects who were normocalcaemic the 25-OH Vitamin D level was significantly associated with iPTH, in keeping with a tendency to secondary hyperparathyroidism (p=0.003; r^2 =0.31) Figure 4.3.

Figure 4.3: Restricted Cubic Splines plot of iPTH with 25-OH vitamin D in successful renal allograft recipients with (panel A) or without (panel B) hypercalcaemia.

Figure 4.3A

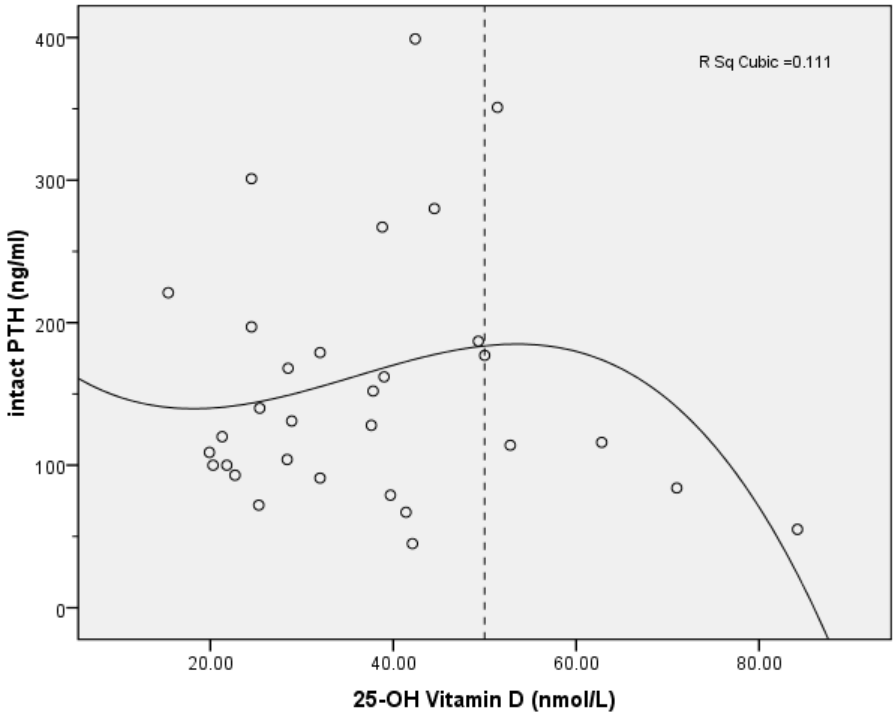


Figure 4.3B

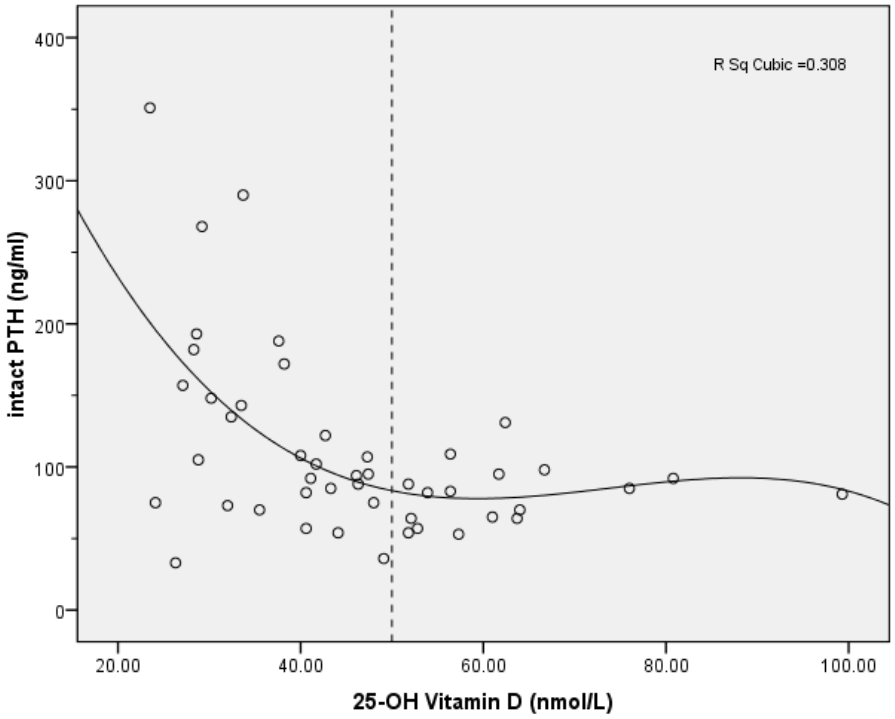


Figure 4.3: Restricted Cubic Splines plot of iPTH with 25-OH vitamin D in successful renal allograft recipients with (panel A) or without (panel B) hypercalcaemia. Five subjects who were treated with activated vitamin D are excluded. The vertical dashed line denotes a 25 OH Vitamin D level of 50nmol/L

The mean (sd) serum calcium and albumin corrected serum calcium was 2.57 (0.16) mmol/l and 2.63 (0.16) mmol/L respectively. Ionized calcium was available for 83 study subjects for whom the median (IQR) was 1.16 (1.08, 1.22) mmol/L. The median (IQR) of ionized calcium expressed as a proportion of total calcium and of albumin corrected serum calcium was 0.45 (0.43, 0.47) and 0.44 (0.42, 0.46). Whereas no subject had a subnormal serum calcium by either total or albumin corrected calcium measurement, 25% had a subnormal ionized calcium (< 1.15mmol/l) level. The percentage of subjects with hypercalcaemia by the 3 methods was: total calcium (38.2%), albumin corrected calcium (49.4%) and ionized calcium (10.8%). The Spearman Rank correlation of iPTH with the above 3 measures of calcium was r=0.29 ($p < 0.01$), r=0.23 (p=0.03) and r=0.44 ($p<0.001$) respectively.

The median (IQR) spot urine calcium-creatinine ratio was 0.16 (0.09, 0.24) mmol/mmol. The ratio was not correlated with the concomitant total serum calcium (r=0.12, p=0.27). Spot urine calcium creatinine ratios were higher in subjects treated with cinacalcet at the time of measurement (n=9) vs. not so treated: median (IQR) 0.32 (0.20 - 0.68) vs. 0.14, (0.08 - 0.24) mmol/mmol, p=0.01. The median ratio for those on 30mg cinacalcet (n=6) was 0.27 mmol/mmol and for those on 60mg/day (n=3) 0.67 mmol/mmol. Spot urine calcium creatinine ratios also tended to be higher in subjects treated vs. not treated with loop diuretics (n=9): 0.39 (0.98, 0.63) vs. 0.15 (0.08, 0.24)

mmol/mmol, p= 0.07 respectively. Three subjects were on both cinacalcet and a loop diuretic.

Mean (sd) phosphate levels were 1.02 (0.2) mmol/L; only 1 subject was hyperphosphatemic (with a value of 1.51 mmol/L), whereas 11 subjects had hypophosphatemia of whom 10 had concomitant suboptimal 25(OH) vitamin D and 9 an iPTH >100 ng/mL. The median iPTH was significantly higher in subjects with vs. those without hypophosphatemia (177ng/ml vs. 94ng/ml, p=0.007) whereas measures of calcium were similar.

Acidosis was present in up to one third of study subjects with a venous TCO_2 below 24 mmol/L being present in 29 (33%) and a level of below 22 mmol/L in 11 (12.4%); venous pH was below 7.33 in 22/69 subjects (32%). Venous pH correlated with eGFR (r=0.25, p=0.04) and was negatively correlated with iPTH (r= -0.36, p<0.005) and albumin corrected serum calcium (r= -0.37, p<=0.005).

Of the 9 subjects with a past history of parathyroidectomy, the median (range) iPTH was 94 ng/ml (22 – 197); 5 had post transplant hyperparathyroidism and 8 had suboptimal vitamin D levels. Of 8 subjects treated with cinacalcet the median (IQR) iPTH was 151 ng/L (82-290), 3 had persistent hypercalcaemia and none were hyperphosphatemic.

Eight-five subjects had an available areal bone mineral density measurement, of whom 22 (26%) had osteoporosis and 28 (31%) had osteopenia. In 8 cases the distal one third of the radius was the only site in which the T-score was

below 2.5. T-scores did not significantly differ across different stages of CKD, Table 4.3. There were significant differences in T-scores by site examined (Friedman p <0.001), with the lumbar spine being considerably higher than other sites, Figure 4.4. Significant correlations were found between T-score at the neck of femur and distal radius with age (r=-0.28 and -0.22 respectively) and iPTH r= -0.20 and -0.31 respectively. 25(OH) vitamin D level only correlated with T-score at the distal forearm (r=0.36, p=0.001).

Table 4.3: Bone and Vascular Health Parameters by CKD-T Stage

	All subjects	Stage T1-2	Stage T3A	Stage T3B	p-value
N	**89**	**32**	**27**	**30**	
Areal Bone Mineral Density					
T-Score, L1-4, Mean (sd)	-0.22 (1.6)	-0.44 (1.55)	0.42 (1.34)	-0.55(1.82)	0.06
T-Score, Total femur, Mean (sd)	-0.73 (1.4)	-0.6 (1.57)	-0.39 (1.29)	-1.14 (1.26)	0.16
T-Score Neck of femur, Mean (sd)	-0.83 (1.29)	-0.56 (1.52)	-0.55 (1.0)	-1.28 (1.17)	0.03†
T- score Distal 1/3 radius, Mean (sd)	-1.2 (1.36)	-1.08 (1.3)	-0.95 (1.0)	-1.52 (1.64)	0.4
Lowest T-score, Mean (sd)	-1.73 (1.19)	-1.67 (1.25)	-1.28 (0.9)	-2.2 (1.22)	0.01
Osteoporosis, n (%)	22 (24.7)	8 (28.6)	2 (7.7)	12 (41.4)	0.15
Osteopenia, n (%)	27 (30.3)	9 (32.1)	9 (34.6)	9 (31)	1.0
Fractures					
Appendicular Fracture, n (%)	14 (15.7)	7 (21.9)	3 (11.1)	4 (13.3)	0.54
Spinal collapse fracture, n (%)	8 (9)	3 (11.1)	4 (16.7)	1 (3.8)	0.34
Vascular Health					
Pulse Wave Velocity, m/s median (IQR)	5.5 (3.1, 9.5)	6.5 (4.0, 9.9)	5.7 (2.8, 9.3)	4.7 (3.2, 7.8)	0.38
Pulse pressure, mmHg, Mean (sd)	57.3 (13.3)	52.4 (10.7)	58.8 (12.4)	60.8 (15.7)	0.01†
Aortic calcification n (%)	43 (48)	11 (39.3)	14 (56)	18 (69.2)	0.09
Calcification score, Median (IQR)	1.0 (0,6)	0.0 (0, 1)	1.0 (0, 3)	3.0 (0, 9)	0.04

p values for continuous variables denoted †were calculated using linear test for trend. All other continuous variables were examined using Kruskal Wallis non-parametric test. Categorical variables were examined using Pearson's Chi square test

Figure 4.4: Boxplot of areal Bone Mineral Density by site studied

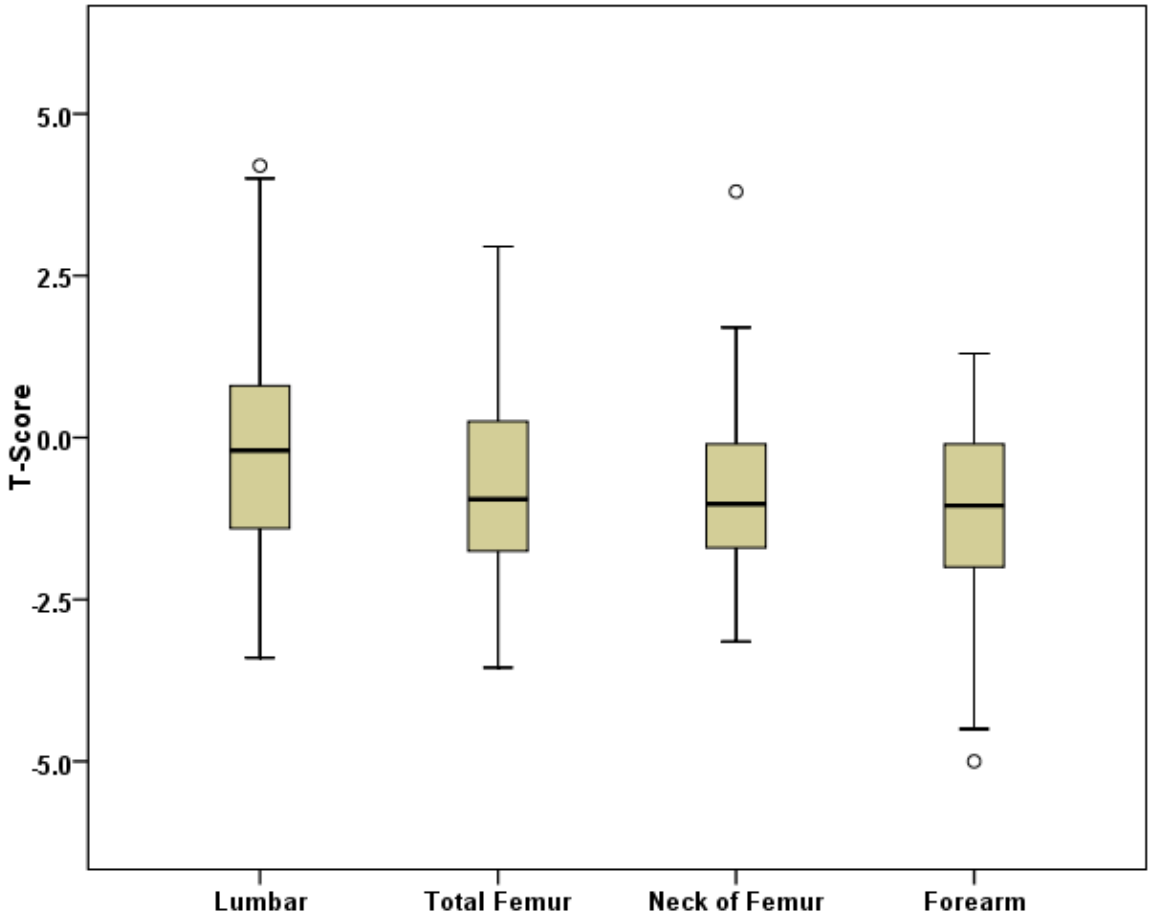


Figure 4.4: Boxplot of areal Bone Mineral Density at different sites in 85 prevalent renal transplant recipients. The solid line within the box indicates the median value, the box the intra-quartile range and open circles outliers.

The 3 markers of bone turnover, Bone ALP, TRACP5b, and NTx-I were closely correlated with each other (correlation coefficients of 0.6 - 0.77, all $p<0.001$). None of the markers correlated with age and only TRACP5b correlated with eGFR, ($r=0.23$, $p=0.04$). Bone ALP and NTx-I both correlated with iPTH ($r=0.52$ and 0.53 respectively, both $p<0.001$) and negatively with 25(OH) vitamin D ($r=-0.42$ and -0.38 respectively, both $p<0.001$); the above correlations for TRACP5b were weaker but similar in direction, (iPTH: $r= 0.26$, $p=0.02$ and 25(OH) Vitamin D: $r= -0.20$, $p=0.08$ respectively). All 3 bone turnover markers correlated negatively with lowest measured T score, with correlations of -0.32 for Bone ALP, -0.45 for TRACP5b and -0.43 for NTx-I, all $p< 0.005$, Figure 4.5.

Figure 4.5: Scatterplots of 3 biomarkers of bone turnover with areal Bone Mineral Density

Figure 4.5a: Scatterplot of Bone Specific Alkaline Phosphatase (ug/L) with areal Bone Mineral Density

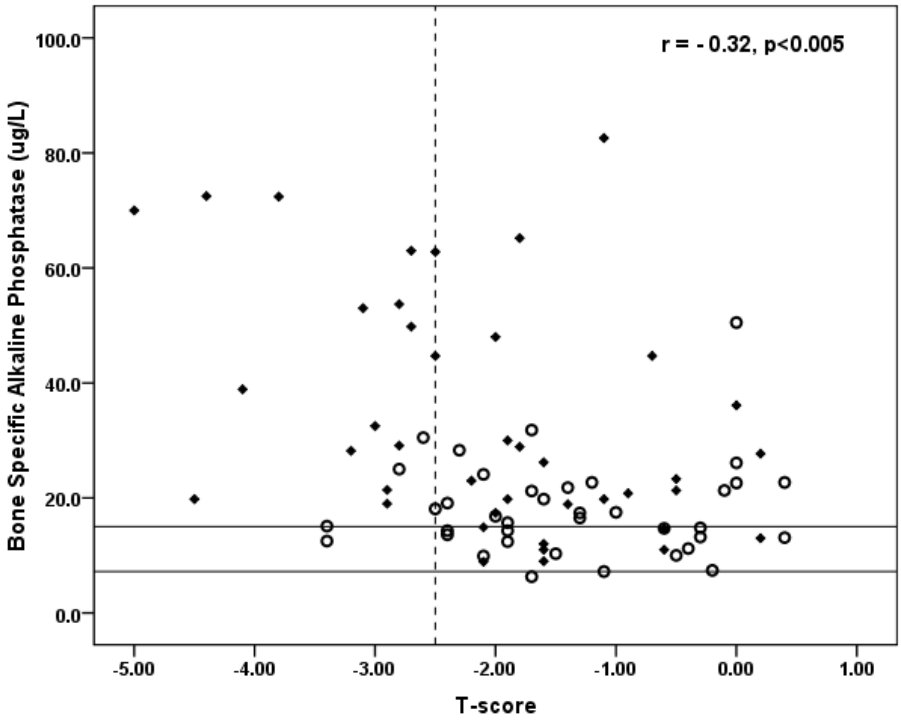


Figure 4.5b: Scatterplot of Urinary N-Telopeptide (nMBCE per mM/L) with areal Bone Mineral Density

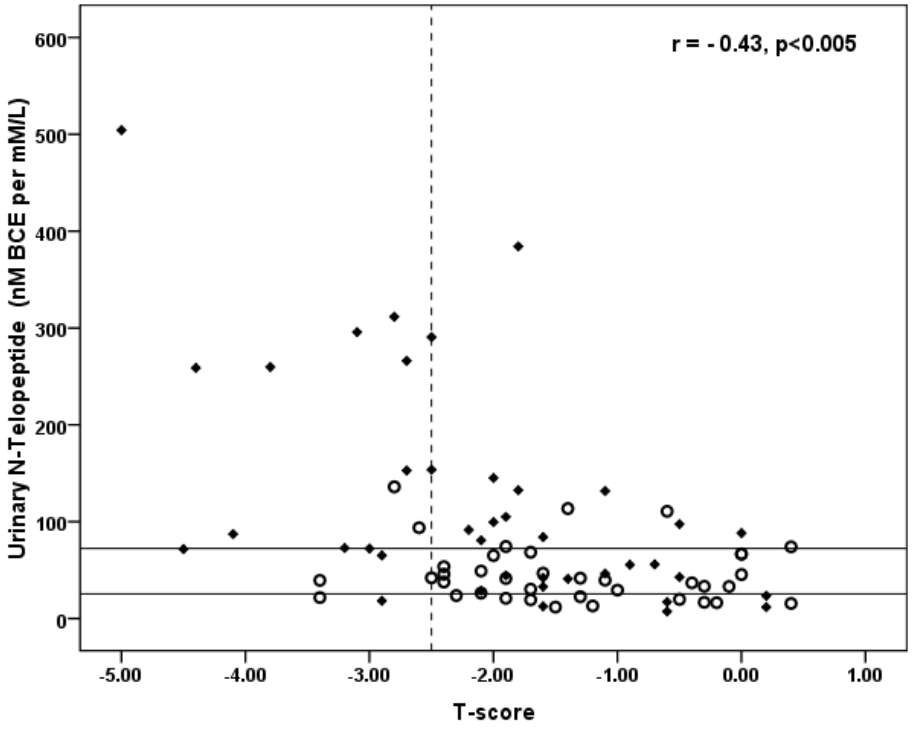


Figure 4.5c: Scatterplot of Tartrate Resistant Acid Phosphatase (TRACP5b) (U/L) with areal Bone Mineral Density

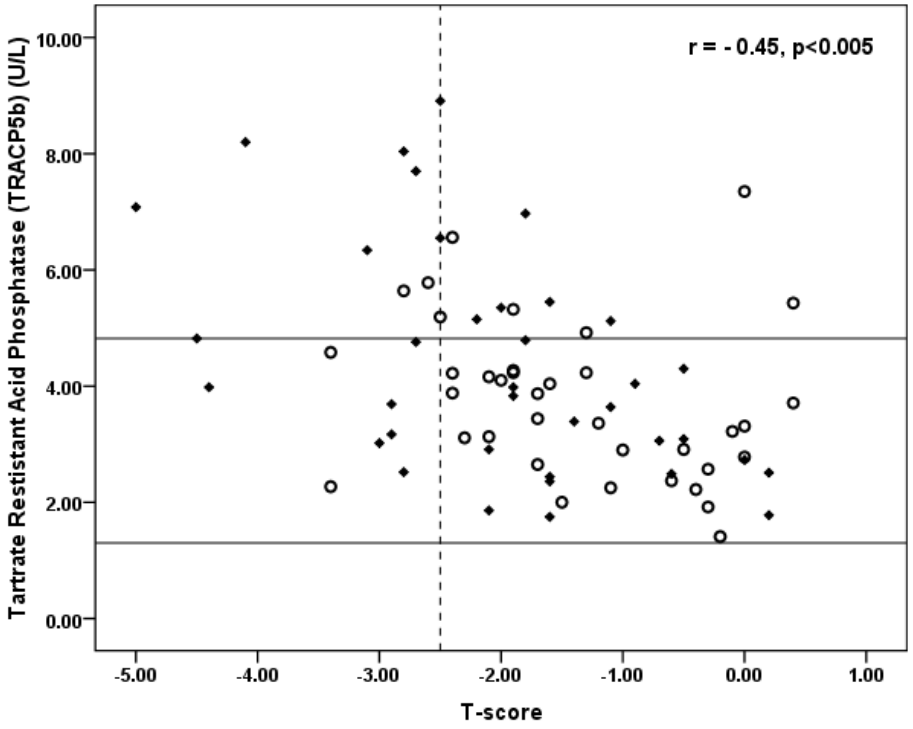


Figure 4.5: Scatterplots of 3 biomarkers of bone turnover with areal bone mineral density in 85 prevalent renal transplant recipients. Open circles indicate those subjects with an iPTH <=100ng/ml, asterisks those >100 ng/ml. The dashed line represents the cut-off for osteoporosis (T-score <-2.5), the solid horizontal lines represent the biomarker reference range within the general population.

Forty-two (47%) of subjects had evidence of calcification based on lateral lumbar radiographs; 39%, 56% and 69% in stage T1-2, T3A and T3B respectively, p trend = 0.03. In those with detectable aortic calcification the severity of the calcification also tended to be higher at higher CKD stage, with median calcification score of 1, 3 and 6.5 respectively. Vascular calcification was associated with a history of prior atherosclerotic cardiovascular events (p=0.06). Median pulse wave velocity was 5.5m/sec; 19.1% had a pulse wave velocity of ≥ 10m/sec. Pulse wave velocity did not significantly differ by aortic calcification status but was significantly higher in those with past history of atherosclerotic cardiovascular disease than in those without such a history, 11.3 (6.2 – 17.8) vs. 5.1 (3.1 – 8.9) m/sec, p=0.02.

Fourteen subjects (15.7%), 9 men and 5 women, suffered from a low impact peripheral fracture post transplantation. The fracture occurred at the wrist (5 cases), digit (4 cases), knee (2 cases), tibia (1 case), ankle (1 case), and rib (1 case). Fracture sufferers' median age was 42.4 years, median eGFR was 59 ml/min/m^2; their median duration of transplantation and of dialysis was 3.2 and 2.2 years respectively. Their median lowest T score (-1.9), median iPTH (119ng/ml) and 25(OH) Vitamin D levels (33nmol/l) were not significantly different compared to the overall study population. Eight subjects had a prevalent lumbar collapse fracture evident on lateral lumbar radiograph which involved 2 or more vertebrae in 4 patients.

Overall 91% of renal transplants with eGFR >30ml/min/m^2 had an elevated iPTH and/or suboptimal vitamin D level, 54% had aortic calcification and 42% osteoporosis, a prevalent vertebral collapse fracture or a post-transplant peripheral fragility fracture, Figure 4.6 . Of the 75 subjects with complete data 27% had all 3 types of abnormalities, while 97% had at least one element of CKD-MBD present. Even in those with an eGFR >60 ml/min/m^2 23% had all 3 and 96% at least 1 abnormality present.

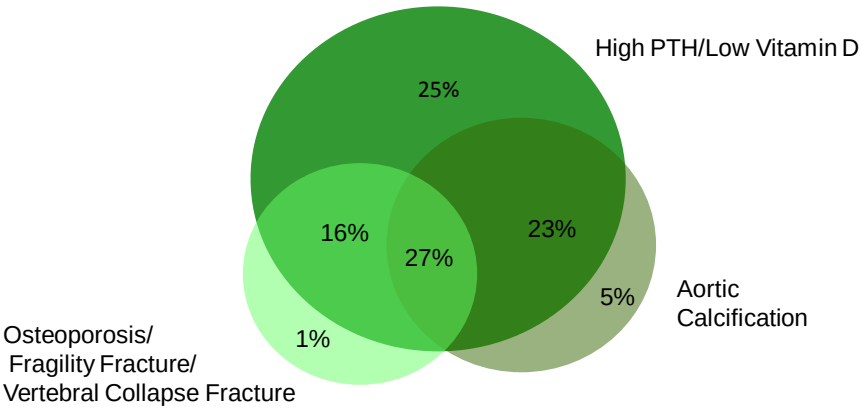


Figure 4.6: Venn diagram of the prevalence of either hormonal dysregulation (iPTH > 65 ng/L and/or 25 hydroxy Vitamin D <50nmol/L), aortic calcification and bone pathology (osteoporosis, prevalent vertebral collapse fracture or post-transplant peripheral fragility fracture) in 89 prevalent renal allograft recipients with an eGFR > 30 ml/min/1.73m^2

On univariate logistic regression analysis the presence of osteoporosis (DXA T-score less than -2.5) was significantly associated with iPTH (per 10ng/ml increment), Odds Ratio (95% Confidence Interval), 1.13 (1.05, 1.22). On multivariate logistic regression analysis, adjusting simultaneously for age, gender, ESKD vintage (cumulative time on dialysis and post transplant), eGFR, current corticosteroid use, 25-OH Vitamin D and TCO2 level, the presence of osteoporosis remained independently associated with PTH. (Table 4.4).

Table 4.4: Logistic Regression Crude and Adjusted Association of Osteoporosis with iPTH (per 10ng/ml increment), Odds Ratio (OR), (95% Confidence Interval)

	OR	**95% Confidence Interval**	**p**
Model 1	1.13	1.05, 1.22	0.001
Model 2	1.15	1.06, 1.26	0.001
Model 3	1.15	1.05, 1.25	0.002
Model 4	1.15	1.04, 1.27	0.006

Model 1: Crude Association of Osteoporosis (Lowest DXA T-score <-2.5) and intact PTH (per 10ng/ml increment).
Model 2: Adjusted for Age (decades), Gender, ESKD vintage (years) and MDRD eGFR (ml/min/1.73m^2)
Model 3: Adjusted for Age (decades) and Gender, ESKD vintage (years), MDRD eGFR (ml/min/1.73m^2) and current corticosteroid use
Model 4: Adjusted for Age (decades), Gender, ESKD vintage (years), MDRD eGFR (ml/min/1.73m^2), current corticosteroid use, 25-OH Vitamin D level (nmol/L) and TCO2 level (mmol/L)

Discussion

In this chapter we report that CKD-MBD remains highly prevalent post renal transplantation; 91% of our study population had an elevated iPTH and/or suboptimal Vitamin D level. We found iPTH to be independently associated with the presence of osteoporosis, a well recognized risk for fracture occurrence. The burden of CKD-MBD in successful renal allograft recipients is overwhelming and may be a major mechanism contributing to the persistent elevation in morbidity and mortality in this population. The prevalence of CKD-MBD differs substantially post transplantation to that reported in native kidney CKD at an equivalent level of renal function.[44] Unfortunately the successful restoration of renal function fails to restore normal bone and vascular health. The clinical consequences of this are underscored by high prevalence of both lumbar collapse fractures and peripheral fragility fractures as described previously and as evident in our population.[159, 161, 163, 165, 167]

Post-transplant hyperparathyroidism appears central to the presence of deranged bone mineral homeostasis. Although higher than in several reports, the prevalence of post transplant hyperparathyroidism in our study is similar to that found by Marcen who described a prevalence of 77% amongst 509 renal transplant recipients.[238] The pathophysiology of post transplant hyperparathyroidism may relate to both persistent autonomous PTH production from hyperplastic parathyroid glands and/or from a degree of secondary hyperparathyroidism in setting of diminished renal function,

vitamin D deficiency and diminished vitamin D receptor activation as occurs in the setting of native CKD.[236] Alternatively, in the setting of hypercalcaemia the condition more closely resembles primary hyperparathyroidism with associated hypophosphatemia and is relatively independent of the 25(OH) Vitamin D level. Given the literature of often subtle complications of minor elevations in PTH and calcium seen in patients with primary hyperparathyroidism[252], there is surprisingly little data, particularly on the non skeletal consequences, of such autonomous hyperparathyroidism post transplantation. Borchhardt et al reported that -the off label- use of cinacalcet post transplantation was associated with a persistently increased urinary calcium excretion[253], a finding that could theoretically predispose to transplant nephrolithiasis and which may therefore support prospective monitoring of urinary calcium excretion in this setting.

The clinical relevance of post-transplant hyperparathyroidism even with regard to skeletal health remains to date uncertain. The extent and time scale over which the skeletal resistance to PTH that exists in CKD stage 5D resolves following transplantation is unknown, as is the optimal iPTH target post transplantation and the extent to which factors such as the type and severity of dialysis associated CKD MBD should influence this target.

PTH is known to reduce bone mineral density especially at sites with a high proportion of cortical bone such as the distal forearm. It is thus noteworthy that while iPTH did not correlate with lumbar BMD, it strongly correlated with the forearm site and to a lesser extent with femoral site. In keeping with current recommendations for primary hyperparathyroidism –though not specifically discussed in current transplant guidelines- the assessment of bone mineral density post transplantation if conducted by DXA should routinely include examination of the distal forearm. Failing to do so would have resulted in missed diagnosis of osteoporosis in over one third of osteoporotic subjects within our study. Our findings broadly agree with those of Roe and colleagues who in a study of 134 prevalent male transplant recipients found a 72% prevalence of hyperparathyroidism, the level of which strongly correlated with areal bone mineral density at the radial site and with the proportion of men diagnosed as having osteoporosis rising from 30 to 41% with examination of the radial site.[167] The clinical significance of this is further underscored by the high frequency of distal upper and lower limb fracture seen in renal transplant recipients. We report the significant and independent association between osteoporosis and iPTH despite adjustment for multiple confounders which may adversely influence bone health. While we cannot in this cross-sectional study assess the extent that the presence of osteoporosis may have resulted from pre-transplant as distinct to post transplant levels of PTH, the strong correlation of iPTH seen with biochemical markers of bone turnover, with their

short half-life, supports the hypothesis that hyperparathyroidism in at least some of these subjects is exerting an ongoing active and possibly maladaptive influence on bone turnover. However, the limited available data from post-transplant bone biopsies have reported disparate results, with two such studies finding no correlation between bone turnover and PTH.[245, 254]

We found a substantially higher prevalence of hypocalcaemia by measuring ionized calcium rather than total calcium based measurements. Evenepoel and colleagues recently reported similar poor agreement between ionized and total calcium although in their study total calcium overestimated ionized calcium in only 5% of subjects and underestimated it in 35%.[255]

We report the significant association of relatively mild acidosis with hyperparathyroidism and hypercalcaemia as has previously been shown by Yakupoglu et al.[256] The broader effects of even mild acidosis on bone health and renal survival of transplant recipients is worthy of additional prospective study.

While vascular calcification was not associated with Pulse Wave Velocity in this cross-sectional data there was a trend for both to be associated with past history of atherosclerotic cardiovascular events. The lack of association of Pulse Wave Velocity with vascular calcification may relate to imprecision in the assessment of vascular calcification using lateral radiographs of the lumbar spine, our limited sample size and to incidence-prevalence bias resulting from

the cross sectional nature of the analysis, whereby those subjects with greater degrees of vascular damage may have been more likely to have died and thereby not be available for study enrolment. Follow-up of these outcomes over time may help clarify these issues. Additional limitations of our study is that it is based in a single Irish institution and is susceptible to local practice patterns and possibly genetic predispositions occurring within Ireland, nevertheless the observed relationships between different CKD-MBD parameters should be reasonably generalizable. We do not have FGF-23 measurements which may contribute to the presence of hypophosphatemia, although available data suggests that this is most influential in the first year post transplant,[257, 258] and therefore would be relevant in only 25% of our study population.

In conclusion, while the severity of CKD-MBD varies with the post transplantation level of renal function, it is regardless highly prevalent even in those with excellent allograft function. Both autonomous iPTH secretion and vitamin D deficiency are likely to contribute to its perpetuation. Post transplant hyperparathyroidism may independently contribute to the development of osteoporosis and subsequent fracture risk. Finally the presence of persistent vascular and bone pathology may represent an essential mechanism in driving post-transplant morbidity and mortality. These data support the need to limit the initial development of these abnormalities at earlier stages of kidney disease through appropriate preventative measures

and call for research into the optimal management and secondary prevention of CKD-MBD in renal transplant recipients.

Chapter 5

The Association of Hyperparathyroidism and Bone Mineral Density with Vascular Calcification after Renal Transplantation

Introduction

Vascular calcification independently predicts cardiovascular disease, which accounts for up to 50% of all-cause mortality in renal transplant recipients.[145] Mortality rates, while substantially lower than that of the dialysis population[144], remain markedly elevated after renal transplantation.[146] Risk factors for cardiovascular disease after renal transplantation include the traditional risk factors common to the general population, such as; older age, pre-existing Diabetes Mellitus, hypercholesterolemia, hypertension and Left Ventricular Hypertrophy. Post renal transplant status confers additional cardiovascular risk factors namely the often suboptimal levels of renal function provided by the allograft, de-novo hyperglycaemia and hyperlipidemia due to immunosuppressive agents and residual disturbances in mineral metabolism.[147] Parameters of mineral metabolism typically improve over the first year post renal transplantation, although in a substantial proportion of patients PTH concentrations remain elevated, albeit at lower levels, and are frequently associated with hypercalcaemia.[148, 150, 151] It is typically assumed that with normalisation of bone mineral parameters and improvements in PTH levels, vascular calcification stabilizes or improves. However, few studies have examined the natural history of vascular calcification post renal transplantation and results have been conflicting. The prevalence of vascular calcification post transplantation, determined by Electron Beam Computed Tomography (EBCT) has been reported to be between 65% and 92%, which

was similar to comparative dialysis populations.[152, 153] Moe et al., in a prospective study of 23 renal transplant recipients found no evidence of progression of vascular calcification scores, determined by spiral CT over 15-20 months of follow-up. Several other small studies have shown improvements in vascular function and vascular stiffness following transplantation. More recently, Marechal et al. demonstrated significant increases in spiral CT determined vascular calcification scores in 281 renal transplant recipients over 3.5 years of follow up.[158]

In contrast to vascular calcification, the natural history of disorders of bone metabolism and fracture risk are well described post renal transplantation. Fracture risk is elevated in Chronic Kidney Disease and End Stage Kidney Disease and the risk of fracture further increases post transplantation. The cumulative incidence of any fracture post transplantation is reported to be as high as 60%[159] and is accompanied by an increased risk in all-cause mortality compared to the general population.[163] Risk factors for fracture post transplantation include dialysis vintage prior to transplant, severity of pre-transplantation hyperparathyroidism, duration since transplant, female gender, diabetes mellitus, older age, decreased Bone Mineral Density, prior history of fracture and immunosuppressive therapies (corticosteroids and calcineurin inhibitors).[165]

Bone metabolism and vascular calcification are both complex, multifactorial processes. Vascular calcification is a highly regulated process which closely resembles bone mineralization. Several epidemiological studies in the general population have demonstrated a relationship between decreased Bone Mineral Density and increased measures of vascular calcification.[136, 137, 259] This relationship has also been observed in the CKD population[260] and in patients treated with dialysis.[261] However, while individual components of CKD-MBD, such as residual mineral disturbances, fracture risk and vascular calcification, have been examined in the post transplant population, whether the complex inverse relationship between bone health and vascular calcification seen in CKD and dialysis patients persists in the post transplant era remains poorly described, but may influence the long term health of the successful transplant recipient. In this chapter we present cross-sectional data examining the presence, strength, independence and significance of (1) iPTH and (2) Bone Mineral Density with vascular calcification in a cohort of successful renal transplant recipients.

Methods

We examine the association of Bone Mineral Density and osteoporosis with measures of vascular calcification in 90 subjects prospectively enrolled in the Association of Bone and Cardiovascular Health after Renal Transplantation (ABC-HEART) study, by the author as described in detail in Chapter 4. The current report uses baseline data at study entry on 64 patients who underwent CT assessment of aortic vascular calcification and bone mineral density. In brief, to be eligible subjects had to be between 0.5 and 12 years post transplant and to have a current transplant eGFR>30ml/min/1.73m^2 and to be in their usual state of health. All study procedures were performed on an outpatient basis. Informed written consent was obtained from all participants and the study protocol was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Information on demographics, clinical details and past medical history were obtained by patient self-report and abstracted from the medical record. Biochemical measures of renal function and of bone metabolism were obtained as described in detail in Chapter 4. In addition, previously stored samples were analysed in batch for serum osteoprotegerin and Fetuin A. Osteoprotegerin was measured using an ELISA assay (Biomedica, Austria), and expressed in pmol/L. Fetuin A was measured using Human Fetuin A ELISA (Epitope Diagnostics, Inc, USA) and expressed in g/L.

Assessment of Bone Mineral Density

Areal bone mineral density was measured at the lumbar spine, the femoral neck and total femur of both hips and the non-dominant forearm, using a Lunar IDXA scanner (General Electric) and expressed as a T-score. Osteoporosis was defined as a lowest T-score of -2.5 or less and osteopenia as a T-score of -1.5-2.49.[16]

Assessment of Vascular Calcification

Consenting patients also underwent a non-contrast CT scan using a 64 slice General Electric Medical Systems Lightspeed VCT XTE CT scanner. A helical series from the L1 to L4 vertebral bodies was performed in all patients. An acquisition slice thickness of 0.625 mm was utilised. Calcification was scored using the semi-quantitative Aortic Calcification Index (ACI). The Aortic calcification index (ACI) is a semi-quantitative score where the cross section of each of 10 slices of the abdominal aorta is divided into 12 radial sectors. The number of sectors showing calcification in each slice is counted, and the total number of calcified sectors in the 10 slices is summed. All detectable calcifications ≥100 Hounsfield units are included, and the sum is then divided by 120 and multiplied by 100 to be expressed as a percentage.[262]

Aortic vascular calcification was quantified from plain lateral lumbar spine radiographs using the Framingham method, scoring on a scale of 1-3 the presence of calcification along the anterior and posterior margin of the expected aortic contour adjacent to the 4 lumbar vertebrae, resulting in a composite score of between 0-24.[129]

Carotid Femoral Pulse Wave Velocity (PWV) was measured using a dedicated Pulse Trace 400 PWV system (Viasys Healthcare) by a single investigator (SK) following the method described by London.[131] Participants were studied in the supine position after approximately 5 minutes of rest. Brachial systolic and diastolic blood pressure was recorded on 3 separate occasions at 5 minute intervals during the examination using an oscillometric device. Doppler ultrasound with simultaneous ECG recording was used to assess the pulse wave between the carotid and femoral sites. Pulse wave transit distances were recorded from the suprasternal notch to the femoral recording site. The velocity of the pulse wave between the carotid and femoral site was recorded during 10 – 15 ventricular contractions and the average PWV was calculated by the system software and expressed as meters per second.

Statistical Analysis

Clinically implausible and outlying data were checked against the original clinical record. Normality was examined using box-plots and Shapiro-Wilks

test and distribution described using mean (sd) or median (intra quartile range (IQR)) as appropriate. Non parametric analytic methods were exclusively used including Spearman rank correlation and Mann-Whitney U test. We examined the independence of the association of iPTH and osteoporosis (DXA T-score less than -2.5) with measures of vascular calcification, (PWV, lateral lumbar radiograph and Aortic Calcification Index) using multivariate linear regression, adjusting sequentially for age, smoking history, mean arterial blood pressure, renal function and history of Diabetes Mellitus. Analysis was conducted using SPSS (Chicago, Illinois) V16, with a 2-sided Type one error rate of 0.05.

Results

Data on 64 subjects who underwent CT scan were included for analysis. The baseline clinical characteristics of the included study population are shown in Table 5.1 and were similar to the baseline characteristics of the 26 subjects who did not undergo CT scanning. Mean age (sd) of the study participants was 47.3 (12.9) years, 61% were male and mean (sd) MDRD eGFR was 54.1 (17.6) ml/min/1.73m^2. Median (IQR) duration of dialysis and transplantation was 2.2 (1.4, 3.3) and 3.7 (0.9, 8.1) years respectively. Mean (sd) osteoprotegerin was 4.09 pmol/L (1.37) (Reference Range 0.6 – 6.9pmol/L). Mean (sd) Fetuin-A was 0.51 g/L (0.19), (Reference Range 0.35 – 0.95g/L). Using the CT determined Aortic Calcification Index (ACI), 62.5% of the study population had evidence of vascular calcification. Presence of vascular calcification was associated with

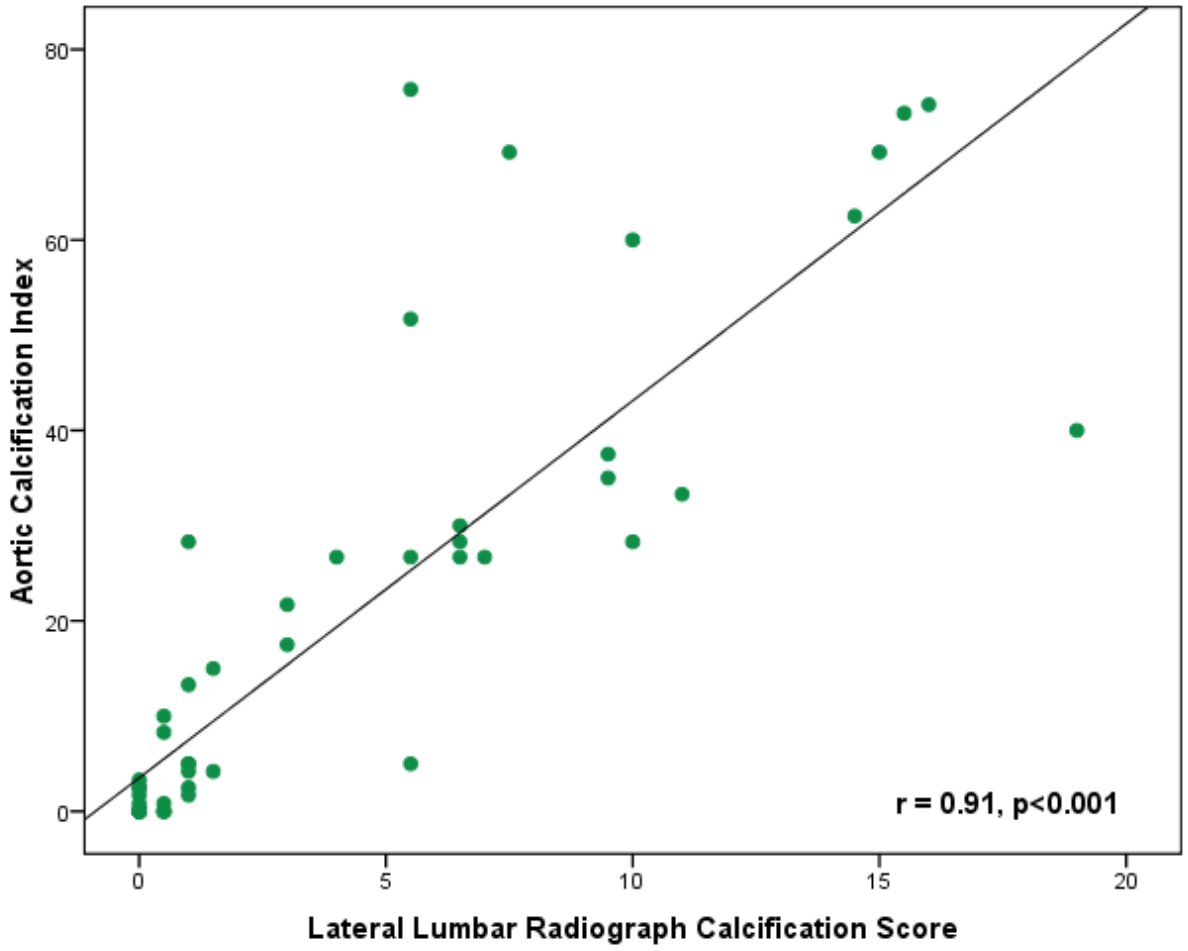
older age, lower level of renal function, longer dialysis vintage prior to transplantation, higher mean arterial blood pressure, prior cardiovascular event and higher total serum cholesterol, but not iPTH level. (Table 5.1)

Table 5.1: Characteristics of Study Population by Aortic Calcification Index Calcification Status

Characteristic	**Total N=64**	**No Calcification N = 24**	**Calcification N =40**	**p- value**
Age (years) (sd)	47.3 (12.9)	35.8 (8.5)	52.3 (9.6)	0.001
Male (%)	39 (61)	18 (75)	21 (52.5)	0.074
eGFR (ml/min/1.73m^2) (sd)	54.1 (17.6)	57.8 (14.8)	48.3 (14.9)	0.002
CKD Stage, N (%)				
Stage 1T-2T	22 (34.4)	14 (58.3)	8 (20)	
Stage 3AT	20 (31.2)	6 (25)	14 (35)	0.006
Stage 3BT	22 (34.4)	4 (16.7)	18 (45)	
Cause ESKD, N (%)				
GN	22 (34.4)	7 (29.2)	15 (37.5)	
PKD/Alports	13 (20.3)	3 (12.5)	10 (25)	0.466
DM	6 (9.4)	2 (8.3)	4 (10)	
Other	23 (35.9)	12 (50)	11 (27.5)	
Dialysis Modality, N (%)				
Haemodialysis	39 (60.9)	15 (62.5)	24 (60)	0.954
Peritoneal Dialysis	23 (35.9)	8 (33.3)	15 (37.5)	
Pre-emptive	2 (3.2)	1 (4.2)	1 (2.5)	
Median Duration of Dialysis, (years) (IQR)	2.2 (1.4, 3.3)	1.6 (0.8, 2.0)	3.0 (1.7, 3.8)	0.034
Median Duration of Transplant, (years), (IQR)	3.7 (0.9, 8.1)	3.2 (0.9, 8.8)	4.1 (0.9, 7.1)	0.398
Mean Arterial Blood Pressure, mmHg	102.7 (9.7)	100.0 (8.8)	104.7 (8.9)	0.012
Ever Smoked, n (%)	28 (43.8)	8 (33.3)	20 (50)	0.193
Diabetes, n (%)	10 (15.6)	3 (12.5)	7 (17.5)	0.594
Cardiovascular Event, n	6	0	6	0.046
Tacrolimus, n (%)	56 (87.5)	21 (87.5)	35 (87.5)	1.0
Mycophenolate mofetil, n (%)	52 (81.2)	17 (70.8)	35 (87.5)	0.098
Current Steroid Use, n (%)	28 (43.8)	10 (41.7)	18 (45)	0.795
Previous Parathyroidectomy, n	8	3	5	1.0
Current Vitamin D Use, n	5	1	4	0.642
Median intact PTH, ng/ml (IQR)	91.5 (73, 168)	106.5 (83.5, 175)	86.5 (57, 128)	0.846
Mean 25 OH Vitamin D, nmol/L (sd)	44.9 (18.1)	47.7 (20.1)	45.2 (19.8)	0.430
Mean Total Calcium, mmol/L (sd)	2.57 (0.17)	2.52 (0.16)	2.63 (0.17)	0.062
Mean Haemoglobin, g/dl (sd)	13.3 (1.8)	13.3 (1.9)	13.2 (1.9)	0.972
Mean LDL Cholesterol, mmol/L (sd)	2.7 (0.9)	2.3 (0.8)	2.9 (0.9)	0.052
Mean HDL Cholesterol, mmol/L (sd)	1.35 (0.4)	1.27 (0.35)	1.44 (0.46)	0.211
Mean Total Cholesterol, mmol/L (sd)	4.7 (1.1)	4.2 (0.9)	5.0 (1.0)	0.006
Mean Osteoprotegrin, pmol/L (sd)	4.09 (1.37)	4.2 (1.8)	4.2 (1.3)	0.776
Mean Fetuin A, g/L (sd)	0.51 (0.19)	0.51 (0.23)	0.47 (0.15)	0.441

Using Spearman rank correlation the Aortic Calcification Score correlated significantly with the Lateral Lumbar Radiograph Calcification score ($r = 0.91$, $p < 0.001$) (Figure 5.1). The correlation of Aortic Calcification Score with Pulse Wave Velocity was weaker but similar in direction, $r = 0.29$, $p = 0.02$.

Figure 5.1: Scatter-plot of Correlation of Aortic Calcification Index with Lateral Lumbar Radiograph Calcification Score



On univariate linear regression, PWV was significantly associated with prior history of cardiovascular event (ß (95% Confidence Interval), 5.4 (2.4, 8.4)) and history of diabetes mellitus (ß (95% Confidence Interval), 3.1 (0.54, 5.56). Lateral Lumbar Radiograph Calcification Score and Aortic Calcification Index were both univariately associated with age, prior cardiovascular event, mean arterial blood pressure, osteoprotegerin, and lower DXA measures of Bone Mineral Density but not iPTH. (Table 5.2)

Table 5.2: Linear Regression Univariate Associations of Measures of Vascular Calcification with demographic, clinical and laboratory variables.

	ß (95% Confidence Interval)		
Parameter	**Pulse Wave Velocity**	**Lateral Lumbar Radiograph Calcification Score**	**Aortic Calcification Index**
Age (decades)	0.469 (-0.268, 1.206)	**1.81 (1.04, 2.6) p<0.001**	**10.49 (6.91, 14.08) p <0.001**
ESKD Vintage (years)	0.076 (-0.159, 0.311)	0.076 (-0.198, 0.35)	0.636 (-0.77, 2.04)
History of Prior CV event	**5.4 (2.4, 8.4) p=0.001**	**4.67 (1.22, 8.05) p = 0.008**	**30.86 (12.76, 48.96) p = 0.001**
History of Smoking	0.55 (-1.33, 2.43)	1.75 (-0.42, 3.92)	10.09 (-1.21, 21.39)
History of Diabetes Mellitus	**3.1 (0.54, 5.56) p = 0.018**	0.658 (-2.46, 3.78)	-0.796 (-16.63, 15.04)
Mean Arterial Blood Pressure (mmHg)	0.057 (-0.032, 0.145)	**0.111 (0.005, 0.217) p = 0.04**	**0.928 (0.371, 1.48) p =0.001**
25 OH Vitamin D (nmol/L)	0.003 (-0.053, 0.058)	0.001 (-0.066, 0.068)	-0.077 (-0.394, 0.241)
FetuinA (g/L	4.19 (-0.91, 9.28)	0.663 (-6.05, 7.37)	2.57 (-27.75, 32.89)
Osteoprotegerin (pmol/L)	0.226 (-0.56, 1.01)	**1.05 (0.131, 1.96) p = 0.026**	**4.68 (0.015, 9.34) p = 0.049**
eGFR (ml/min/1.73m^2)	0.034 (-0.02, 0.09)	-0.05 (-0.118, 0.019)	-0.298 (-0.62, 0.022)
Phosphate (mmol/L)	3.36 (-1.12, 7.8)	3.89 (-1.26, 9.04)	13.71 (-12.02, 39.45)
Total Calcium (mmol/L)	-5.72 (-11.63, 0.186)	4.3 (-2.47, 11.07)	7.29 (-27.54, 42.13)
Total Cholesterol (mmol/L)	-0.5 (-0.95, 0.85)	0.324 (-0.699, 1.35)	-0.91 (-6.29, 4.47)
iPTH (ng/ml)	-0.004 (-0.17, 0.008)	0.006 (-0.008, 0.02)	0.041 (-0.028, 0.110)
Prior Parathyroidectomy	-1.62 (-4.71, 1.48)	0.794 (-2.78, 4.37)	1.49 (-15.89, 18.87)
Cinacalcet Use	-0.84 (-3.7, 2.0)	**4.43 (1.48, 7.38) p =0.004**	16.04 (-0.864, 32.94)
Lowest T-score	0.615 (-0.2, 1.43)	**-1.43 (-2.3, -0.56) p = 0.002**	**-6.25 (-11.2, -1.29) p = 0.014**
Osteoporosis (Lowest T-score <-2.5)	-1.078 (-3.13, 1.158)	**3.79 (1.43, 6.16) p= 0.002**	**20.48 (8.46, 32.5) p = 0.001**

On multivariate linear regression analysis osteoporosis (defined as a DXA T-score less than -2.5) was significantly associated with Lateral Lumbar Radiograph Calcification Score, even with simultaneous adjustment for age, gender, mean arterial blood pressure, smoking history, and intact PTH (adjusted ß (95% confidence Interval), 3.27 (0.88, 5.66), p =0.008). (Table 5.3, Model 5)

Similarly, on multivariate linear regression analysis, osteoporosis was independently associated with Aortic Calcification Index, even following adjustment for the above variables, (adjusted ß (95% Confidence Interval), 12.45 (1.16, 23.75), p = 0.031). (Table 5.3, Model 5) With further adjustment for osteoprotegerin in addition to the variables in Model 5, the relationship between osteoporosis and Aortic Calcification Index was substantially attenuated an no longer statistically significant (adjusted ß (95% Confidence Interval), 7.17 (-5.3, 19.6), p = 0.25). Similarly, with adjustment for Fetuin A, in addition to the variables in Model 5 the relationship between osteoporosis and Aortic Calcification Index was no longer significant, (adjusted ß (95% Confidence Interval), 8.77 (-2.7, 20.3), p = 0.13).

Table 5.3: Linear Regression Crude and Adjusted Association of Osteoporosis with measures of Vascular Calcification, ß (95% Confidence Interval)

	Pulse Wave Velocity	**Lateral Lumbar Radiograph Calcification Score**	**CT Aortic Calcification Index**
Model 1	-1.08 (-3.3, 1.16) p = 0.340	**3.79** **(1.43, 6.16)** **p = 0.002**	**20.48** **(8.47, 32.5)** **p =0.001**
Model 2	-1.35 (-3.64, 0.935) p = 0.243	**2.81** **(0.68, 4.94)** **p = 0.011**	**12.63** **(2.71, 22.55)** **p = 0.014**
Model 3	-1.18 (-3.33, 0.97) p = 0.278	**2.76** **(0.62, 4.91)** **p = 0.012**	**11.74** **(1.5, 21.9)** **p= 0.025**
Model 4	-1.26 (-3.44, 0.93) p = 0.256	**2.58** **(0.41, 4.75)** **p = 0.02**	**10.4** **(0.26, 20.57)** **p= 0.045**
Model 5	-0.93 (-3.33, 1.47) p =0.443	**3.27** **(0.88, 5.66)** **p = 0.008**	**12.45** **(1.16, 23.75)** **p = 0.031**

Model 1: Crude Association of Osteoporosis (Lowest DXA T-score <-2.5) and measure of Vascular Calcification.
Model 2: Adjusted for Age (decades) and Gender
Model 3: Adjusted for Age (decades), Gender and Mean Arterial Blood Pressure
Model 4: Adjusted for Age (decades), Gender, Mean Arterial Blood Pressure and history of smoking
Model 5: Adjusted for Age (decades), Gender, Mean Arterial Blood Pressure, history of smoking and intact PTH (ng/ml)

Intact PTH showed no significant association with Pulse Wave Velocity or with either of the calcification scores on univariate or any of the above mentioned multivariate models.

Discussion

We report that in a cohort of successful renal transplant recipients, the presence of osteoporosis but not the prevalent intact PTH is associated with measures of vascular calcification. This relationship was independent of potential confounders; age, gender mean arterial blood pressure, smoking history and PTH level. We found that vascular calcification is highly prevalent post-transplant, 62.5% of our study population had evidence of abdominal aortic calcification, determined by CT. While lower than the prevalence of 80-95% in the CKD and haemodialysis population,[260, 263, 264] our findings are similar to those of by Rosas et al. who reported evidence of vascular calcification to be present in 65% of 79 incident renal transplant recipients, determined by CT measures of Coronary Artery Calcification.[152] The lower prevalence of vascular calcification seen in renal transplant recipients compared to those with CKD and ESKD still on dialysis may reflect selection or survival bias in those patients evaluated and successfully listed for renal transplantation, for whom severe vascular disease would be a relative contra-indication.

Vascular calcification is an independent predictor of cardiovascular disease and in patients with End Stage Kidney Disease, the presence of vascular calcification is predictive of both all cause and cardiovascular mortality.[128, 130] While originally thought to be an unregulated process due to passive mineral deposition in the vascular wall, evidence from human and animal studies have demonstrated that vascular calcification is an active, cell-mediated process

which closely resembles bone mineralisation. However, while bone mineralisation is a physiological process, vascular calcification is a pathological process with important clinical consequences, both in the general population and in patients with renal disease.

Several epidemiological studies in the general population have reported associations between vascular calcification and decreases in bone mineral density. In the Framingham Heart Study, Kiel et al. demonstrated that there was a significant association between percentage change in Bone Mineral Density (measured by metacarpal relative cortical area (MCA)) and progression of abdominal aortic calcification (measured by lateral lumbar radiograph) in women though not men over a 25 year follow up period.[136] Similarly, in a cross-sectional study of 2348 post-menopausal women, Schluz et al. found aortic calcification scores were inversely related to bone mineral density (both parameters determined by CT). This study also reported that the Odds Ratios (95% Confidence Interval) for vertebral and hip fractures in those women with calcification were 4.8 (3.6 – 6.5) and 2.9 (1.8 – 4.8) respectively, compared to those without evidence of calcification. In a subgroup analysis of 228 women with available longitudinal data, this study also demonstrated a significant graded association between progression of vascular calcification and bone loss, where women with the greatest increase in vascular calcification had 4 times greater yearly bone loss than women of similar age in the lowest vascular calcification quartile.[137] In a larger prospective study of 624 men and

women over the age of 50 years, Naves at al. showed that progression of aortic calcification (determined by lateral lumbar radiograph) was associated with the rate of decline in Bone Mineral Density (determined by DXA) over 4 years of follow-up. This relationship remained significant following adjustment for age, gender, smoking history and diabetic status.[138]

This relationship between vascular calcification and bone metabolism has also been demonstrated in patients with CKD and End Stage Kidney Disease treated with dialysis. In a cross-sectional study of 48 patients with NKF Stage 3 CKD (mean eGFR 35ml/min/1.73m^2), Toussaint et al. demonstrated an inverse association between superficial femoral artery vascular calcification score (measured by CT) and femoral T-scores (measured by DXA).[260] London et al reported, in a cross-sectional study of 58 haemodialysis patients, that the extent of vascular calcification, assessed by ultrasonography and plain radiography at 4 separate anatomical sites was associated with low bone turnover and adynamic bone disease on bone histomorphometry.[261]

Few studies have examined whether the complex relationship between bone metabolism and vascular calcification persists in the post-transplant setting. In the present study, we report that measures of vascular calcification, determined both by lateral lumbar radiographs and by CT, were associated with presence of osteoporosis, defined as a lowest DXA T-score less than -2.5. These relationships remained significant even following adjustment for age,

gender, mean arterial blood pressure, history of smoking, and PTH. Although cross-sectional, these data broadly concur with the earlier observation by Schulz at al.[137] and Naves et al.[138] In the general population, the associations between increased vascular calcification and decreased bone mineral density occur in the absence of disorders of mineral metabolism and are often considered a degenerative consequence of aging. In CKD, the prevalence of vascular calcification increases with progressively decreasing renal function, even in young patients and is greater than in the general population. The associations between vascular calcification and bone health observed in the CKD population occur in the context of abnormal bone mineral homeostasis, Vitamin D and PTH dysregulation and disruptions in the complex inter-play between promoters and inhibitors of vascular calcification. In the current study we did not observe an association between measures of vascular calcification and PTH, Vitamin D or serum calcium and phosphate levels which may indicate that these factors are not of clinical importance in a well functioning renal allograft or that the presence of vascular calcification was dominated by the historical –during dialysis- rather than ambient derangements in mineral metabolism. Alternatively given out modest sample size it is possible that the lack of association may represent a type II statistical error.

However, we did observe a univariate association between osteoprotegerin levels and measures of vascular calcification. Osteoprotegerin is a glycoprotein

member of the tumour necrosis factor (TNF) receptor family. Osteoprotegerin is an important modulator of bone remodelling and by inhibiting activation of RANK it inhibits osteoclast formation, differentiation, activation and survival, preventing bone resorption. It is expressed by endothelial cells, VSMC's and osteoblasts.[112] Osteoprotegerin levels are significantly higher in patients with CKD and ESKD than in those without CKD and osteoprotegerin levels are higher at lower levels of renal function.[115] Observational studies in haemodialysis patients have shown a positive association between osteoprotegerin levels and severity and progression of vascular calcification[117, 118] and osteoprotegerin levels were predictive of future cardiovascular events.[119, 120] In a large cohort of renal transplant recipients osteoprotegerin has recently been independently associated with renal events, cardiovascular events and mortality.[121] The role of osteoprotegerin in the pathogenesis of vascular calcification in these studies is unclear but it has been suggested that osteoprotegerin levels increase as a defensive response to rapidly progressive mineral deposition in the vessel wall.[122] With the addition of osteoprotegerin to our multivariate linear regression model, the observed relationship between osteoporosis and vascular calcification was attenuated and no longer significant. A similar effect was seen with the addition of Fetuin A to the statistical model. Fetuin A is an extracellular protein which is an inhibitor of calcium-phosphate precipitation. It is a negative acute phase protein, with reduced circulating levels during inflammation. Fetuin A acts both systemically

and locally to potently inhibit vascular calcification. The effect of osteoprotegerin and Fetuin A, 2 potent calcification inhibitors, on our results may represent incident-prevalence bias in this cross sectional study –whereby the elevated osteoprotegerin and Fetuin A levels may result as a consequence of rather than as a cause of the vascular calcification.

The presence and severity of vascular calcification strongly predicts cardiovascular morbidity and mortality in patients with CKD in whom Electron Beam CT (EBCT) or Multi Slice CT (MSCT) is considered the gold standard for the detection and quantification of vascular calcification. However, these CT based techniques are expensive and not as readily available as plain radiography. The KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) suggest that a lateral abdominal radiograph can be used to detect the presence or absence of vascular calcification as a reasonable alternative to CT based imaging.[39] We performed lateral lumbar radiographs in our study and applied the semi-quantitative scoring system for vascular calcification, originally described by Kauppila.[129] We found a strong correlation between this scoring system and the CT derived Aortic Calcification Index, Spearman Rank Correlation, $r = 0.91$, $p < 0.001$. Similarly, in multivariate linear regression models, the adjusted relationship between osteoporosis and vascular calcification, using both these outcome variables showed comparable results, suggesting that semi-quantitative determination of vascular

calcification by lateral lumbar radiograph is a valid alternative to CT based measures.

In conclusion, osteoporosis but not intact PTH was significantly and independently associated with CT and radiographic measures of aortic calcification in this cross-sectional study of renal transplant recipients. Whether the relationship between these two important conditions persists over time warrants further prospective evaluation as vascular calcification and fracture remain extremely deleterious to the long-term health of the successful renal transplant recipient.

Chapter 6

Association of Hyperparathyroidism and Health Related Quality of Life Post Renal Transplantation

Introduction

Renal transplantation is the treatment of choice for advanced Chronic Kidney Disease.[265] The risk of death for a successful renal transplant recipient is less than half of that for dialysis patients.[266] Patient and allograft survival rates following renal transplantation have improved dramatically over the past 30 years; one year allograft survival rates currently exceed 88% for deceased donor allografts and 95% for living donor allografts. One year patient survival exceeds 94% for deceased donor allografts and 98% for living donor allografts.[267] With this improvement in allograft and recipient survival rates, recent attention has increasingly focused on minimizing the long-term complications related to immunosuppression and cardiovascular risk and optimizing the Health Related Quality of Life and functional health status of transplant recipients.

Secondary hyperparathyroidism (SHPT) is a common complication of Chronic Kidney Disease (CKD) and End Stage Kidney Disease (ESKD). Hyperparathyroidism typically develops early in the course of CKD and is initially compensatory, serving to help maintain a normal serum calcium and phosphate level. However, over time, prolonged secondary hyperparathyroidism may result in nodular hyperplasia and autonomous parathyroid hormone secretion, termed tertiary hyperparathyroidism. Such tertiary hyperparathyroidism is distinct to the secondary condition and resembles primary hyperparathyroidism in that in the setting of a functioning

kidney it may lead to actual hypercalcaemia and phosphate wasting. Successful renal transplantation restores endogenous renal function and in the absence of tertiary hyperparathyroidism serum calcium and phosphate values typically normalize over weeks to months. However, whether hyperplastic parathyroid tissue ever spontaneously involutes is controversial[176, 268] and renal transplantation frequently does not lead to normalization of parathyroid hormone levels despite adequate renal function.[150, 238]

Primary hyperparathyroidism has classically been associated with symptoms of bone pain, nephrolithiasis and neuropsychiatric disturbance. In recent years, with increased routine screening of serum calcium levels, the presentation of primary hyperparathyroidism has changed. Most patients with primary hyperparathyroidism exhibit vague non-specific manifestations of the disease such as mood swings, irritability, fatigue and increased absenteeism from work.[170] These non-classical manifestations of primary hyperparathyroidism are associated with decreased measures of Health Related Quality of Life which demonstrate an improvement following appropriate treatment.[171-173]

Both primary and secondary hyperparathyroidism have been associated with abnormal bone turnover, decreased bone mineral density and increased cardiovascular risk but the relationship between these outcomes and elevated parathyroid hormone post-transplantation is unclear. Moreover, the effect of residual hyperparathyroidism on the quality of life and over-all well-being of

the renal transplant recipient has not been examined. We conducted the following study to examine the relationship of post transplant hyperparathyroidism with parathyroid associated symptoms and Health Related Quality of Life in patients with good allograft function.

Methods

We examine the association of health related Quality of Life with hyperparathyroidism in 90 subjects enrolled in the Association of Bone and Cardiovascular Health after Renal Transplantation (ABC-HEART) study, as described in detail in chapter 4. The current report uses baseline data at study entry on 90 patients who completed Parathyroid Assessment of Symptoms and Short Form 12 Quality of Life questionnaires at study entry. In brief, to be eligible subjects had to be between 0.5 and 12 years post transplant and to have a current transplant eGFR>30ml/min/1.73m^2 and to be in their usual state of health. All study procedures were performed on an outpatient basis. Informed written consent was obtained from all participants and the study protocol was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

Information on demographics, clinical details and past medical history were obtained by patient self-report and abstracted from the medical record. Serum creatinine was measured using the modified Jaffe reaction on the

Olympus 5400 analyser. The method was changed to an IDMS traceable form in October 2009 and was used in the last 10 subjects recruited into the study. eGFR (ml/min/1.73m^2) was calculated using the 4-variable MDRD prediction equation[2] and categorized as Transplant CKD Stage 1T-2T for eGFR >60, Stage 3AT for 45-59 and Stage 3B for 30-44ml/min/1.73m^2. Parathyroid hormone (PTH) was measured by several different methods. Intact PTH, which uses a second generation PTH assay, was initially measured in all subjects by electrochemical luminescence (ECLIA) immunoassay on the Roche E170 modular analyzer. Second generation PTH assays, which are the most widely used in clinical practice in Europe, use 2 separate antibodies. One is directed against the C-terminal fragment of the PTH molecule and the second is directed against the N-terminal fragment. These assays were initially thought to quantify only the full-length, biologically active 1-84 PTH molecule. It is now known that they also measure other large PTH fragments, predominantly 7-84 PTH, the biological activity of which is uncertain. Additional samples were taken at the same blood draw, centrifuged, aliquoted and frozen. In a subgroup of 70 patients these samples were subsequently analyzed in batch, under the supervision of Dr Paula O' Shea, Department of Clinical Biochemistry, University College Hospital, Galway), using a third generation "Whole" PTH assay (Scantibodies™) which only measures the biologically active 1-84 PTH as well as a separate measurement of the "Total" PTH, which measures both Whole (1-84) PTH and the 7-84 N-truncated PTH fragment. The

Scantibodies™ third generation PTH assays also use a two-antibody technique but in these assays one antibody is radio-labelled and directed only against the first six N-terminal amino acids of 1-84 PTH, while the second antibody binds to the C-terminal region of 1-84 PTH. These assays are more sensitive and specific for the measurement of biologically active 1-84 PTH. Typically, mean measured PTH levels are 30-60% lower using the third generation PTH assay as compared to a second generation assay[77] and current guidelines for therapeutic targets in patients with renal disease were developed using second generation assays.

Serum 25-hydroxyvitamin D (25(OH) D) was measured by a competitive radioimmunoassay (IDS). Inter assay coefficients of variations were 6.2% and 7.7% at concentrations of 28.8 nmol/l and 105.4 nmol/l, respectively. The intra-assay coefficients of variations were 3.0% and 2.7% at concentrations of 28.9 nmol/l and 73.9 nmol/l, respectively. Optimal values were considered to be over 50 nmol/l.[73] Serum calcium was measured by the Arsenazo 111 method on the Olympus 5400 analyzer.

Subjects completed the standard 4-week recall Short Form 12. (SF-12v2™ Health Survey © 2002 Quality Metric Incorporated). This is a multipurpose short form survey with 12 questions selected from the Short Form 36 Health survey. It is a generic measure of health status and does not target a specific age or disease group. It is used to assess the physical, functional, emotional

and social dimensions of Health Related Quality of Life. It was developed to provide a shorter but valid alternative to the Short Form 36. The Short Form 12 covers 8 health domains, with one or two questions per domain. The domains covered are Physical Functioning, Role-Physical, Bodily Pain, General Health, Vitality, Social Functioning, Role-Emotional and Mental Health. These dimensions are scored on a scale with a low of 0 and a high of 100. General population norms are used to facilitate direct comparisons across different health domains. For example, using the 0-100 scoring, the Physical Function scale General population norm is between 80 and 90, while the Vitality general population norm is approximately 60 on the 100 point scale. A software algorithm computes these standardized domains as well as a composite Physical Function Component and a Mental Health Component score using a standardized mean score of 50 and a standard deviation of 10. All of the health domains contribute to the scoring of both the Physical and Mental Component Summary scores, although to a varying extent. The Physical Function, Role Physical, Bodily Pain and General Health domains contribute more to the Physical Component Score, while the Vitality, Social Functioning, Role Emotional and Mental Health domains contribute more to the Mental Component Score. Higher scores represent higher functioning, a clinically relevant reduction in Health related Quality of Life is defined as a group mean that is below 47.[269]

Patients also completed the Parathyroid Assessment of Symptoms (PAS) questionnaire; this tool documents the patients symptoms using a Visual Analog Scale ranging from 0 (no experience of the symptom) to 100 (experiencing the most extreme aspect of the symptom) for 13 disease specific items. Symptoms evaluated are bone pain, fatigue, mood swings, depression, abdominal pain, weakness, irritability, joint pain, forgetfulness, difficulty getting out of a chair or car, headaches, itchy skin and being thirsty. This disease specific tool has been validated by a number of studies in patients with symptomatic and asymptomatic primary hyperparathyroidism and has been utilized in a study of patients with both secondary and tertiary hyperparathyroidism due to ESKD. [53, 171, 270]

Statistical Analysis

Clinically implausible and outlying data were checked against the original clinical record. Normality was examined using box-plots and Shapiro-Wilks test and distribution described using mean (sd) or median (intra quartile range (IQR)) as appropriate. Non parametric analytic methods were exclusively used including Spearman rank correlation. Clinical characteristics and laboratory results in those above and below the sample median for iPTH were compared using the Mann-Whitney U test or Chi square test as appropriate. Short Form 12 scores were initially compared to the general population norms, stratified

by age and gender. Subsequently, univariate relationships of clinical characteristics and laboratory data for subjects with Physical Component Score and Mental Component Score above or below the sample median value for the respective distribution were quantified using linear regression. We examined the presence, strength, significance and independence of the association of iPTH with SF 12 domain and composite scores using logistic regression, adjusting sequentially for age, gender, eGFR, haemoglobin, serum calcium and co-morbidities (prior cardiovascular event and diabetes mellitus). The relationship of Parathyroid Assessment of Symptoms score and PTH was examined using linear regression modelling. Routine model diagnostics were performed on all final models and included assessment of points of high leverage and/or influence and for logistic regression models measurement of overall 'Goodness of Fit' using the Hosmer and Lemeshow test. All analyses were conducted using SPSS (Chicago, Illinois), V16, with a 2 sided type one error rate of 0.05. In keeping with methodological approach usually taken in Quality of Life studies we do not adjust the type I error rate for multiple comparisons across the different Quality of Life domains but instead report the nominal p value in a non selective fashion for all of the domains examined.

Results

Data on 90 subjects were included for analysis. The clinical characteristics of the study sample are shown in Table 6.1 and were broadly representative of our prevalent transplant population. The median (IQR) iPTH was 100ng/ml, (73-148), with 45 subjects above and 45 below this value. Significantly more patients with CKD Stage 3BT had an iPTH level above the median of 100ng/ml (44.4% vs 24.4%, p = 0.045. The documented cause of ESKD was also significantly different between patients with iPTH above or below 100ng/ml, p chi square = 0.045, with more chronic glomerulonephritis and less Diabetes, Polycystic Kidney Disease and Alports syndrome in the higher iPTH group. Subjects with iPTH >100ng/ml also had spent significantly longer on dialysis prior to transplantation (2.7 vs 1.7 years, p = 0.0012), but had no significant difference in transplant vintage.

Seventy percent of subjects had sub-optimal 25 OH Vitamin D levels (<50nmol/L). Subjects with elevated iPTH, (>100ng/ml, Roche) had significantly lower mean 25 OH Vitamin D levels, (37.4 Vs 50.6 nmol/L, p<0.001).

We observed a small but significant difference in mean total calcium and haemoglobin levels between the two groups, The percentage of patients with a total serum calcium level above the upper limit of normal (>2.6mmol/l) was 37.8%. Mean (sd) phosphate was 1.03 (0.2) mmol/L. Only 1 patient was hyperphosphatemic (which was marginal at 1.51mmol/L), whereas 11 patients

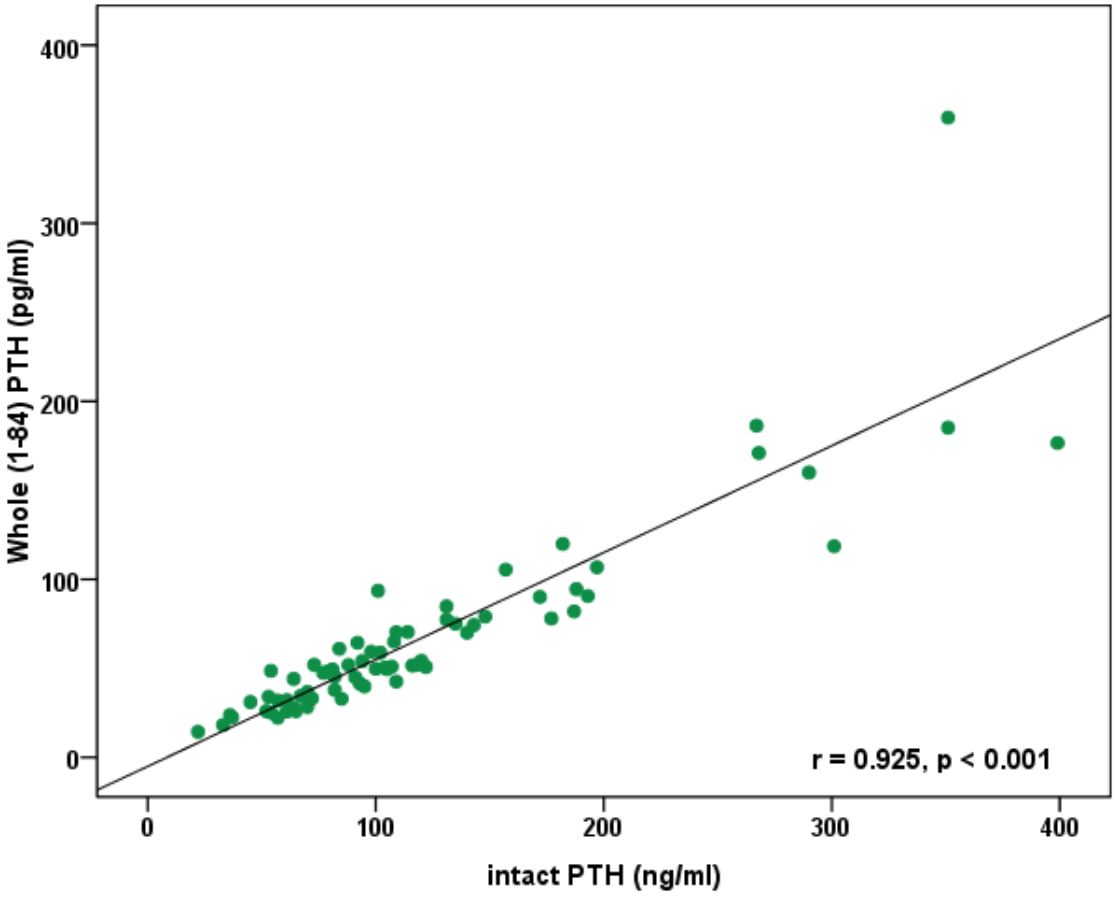
(12.2%) were hypophosphatemic (<0.8mmol/L) of whom 9 had an iPTH level >100ng/ml.

Table 6.1: Patient Characteristics by iPTH level

Characteristic	**Enrolled Study Population**			**p-value**
	Total N=90	**Intact PTH <100ng/ml N = 45**	**Intact PTH >100ng/ml N = 45**	
Age (years) (sd)	46.4 (12.6)	46.5 (13.3)	46.3 (12.1)	0.954
Male (%)	54 (60)	30 (66.7)	24 (53.3)	0.197
eGFR (ml/min/1.73m^2) (sd)	53.6 (16.6)	55.9 (16.0)	51.3 (17.1)	0.188
CKD Stage, N (%) Stage 1T-2T Stage 3AT Stage 3BT	 31 (34.3) 28 (32.9) 31 (32.9)	 15 (33.3) 19 (42.3) 11 (24.4)	 16 (35.3) 9 (20.0) 20(44.4)	 0.045
Cause ESKD, N (%) GN PKD/Alports DM Other	 28 (31.0) 21 (23.3) 8 (9.0) 33 (36.7)	 12 (26.7) 14 (31.1) 7 (15.5) 12 (26.7)	 16 (35.6) 7 (15.6) 1 (1.1) 21 (46.7)	 0.044
Dialysis Modality, N (%) Haemodialysis Peritoneal Dialysis Pre-emptive	 40 (57.1) 25 (35.7) 5 (7.2)	 23 (67.6) 9 (26.5) 2 (5.9)	 17 (47.2) 16 (44.4) 3 (8.3)	 0.196
Median Duration of Dialysis (IQR)	2.5 (1.4 – 3.2)	1.7 (0.74 – 3.2)	2.7 (1.9 – 3.6)	0.012
Median Duration of Transplant (IQR)	2.5 (1.1 – 6.4)	3.4 (1.7 – 6.6)	2.0 (0.7 - 5.6)	0.084
Tacrolimus, N (%)	59 (84.3)	38 (84.4)	38 (84.4)	1.0
Mycophenolate mofetil, N (%)	56 (80.0)	33 (73.3)	39 (86.7)	0.114
Current Steroid Use, N (%)	28 (40.0)	20 (57.1)	15 (42.9)	0.280
Previous Parathyroidectomy, N	9	4	5	1.0
Current Vitamin D Use, N	6	4	2	0.677
Median intact PTH, (Roche assay) ng/ml (IQR)	100 (70 – 143)	73 (57 -85)	148 (116 – 193)	 <0.001
Intact (Total) PTH (Scantibodies Assay) pg/ml (IQR)	65.9 (49.4 – 111.8)	48.6 (38.8 – 62.7)	111.8 (80.4 – 158.1)	0.4
Whole (1-84) PTH (Scantibodies Assay) pg/ml (IQR)	51.6 (36.8 – 78.0)	34.5 (26.9 – 47.9)	78 (56.5 – 106.2)	0.547
Mean 25 OH Vitamin D, nmol/L (sd)	42.7 (15.8)	50.6 (18.2)	37.4 (14.5)	<0.001
Mean Total Calcium, mmol/L (sd)	2.57 (0.16)	2.5 (0.17)	2.6 (0.18)	0.015
Mean Haemoglobin, g/dl (sd)	13.3 (1.6)	13.6 (1.6)	12.9 (1.6)	0.043
Median SF 12 Physical Component Score (IQR)	52.6 (44.8 – 58)	56.1 (44.8 – 58.4)	52.2 (44.8 – 57.1)	0.008
Median SF 12 Mental Health Component Score (IQR)	54.9 (50.8 – 57.9)	54.6 (49.9 – 57.4)	57.3 (52.2 – 59.2)	0.001

In 70 patients with the necessary data, median (IQR) Scantibodies™ Whole (1-84) PTH and Total Intact PTH were 51.6 (36.8-78) and 65.9 (49.4 -111.8) pg/ml respectively. Spearman Rank correlation of iPTH (Roche) and Scantibodies™ Whole (1-84) PTH was r =0.924, p<0.001, (Figure 6.1).

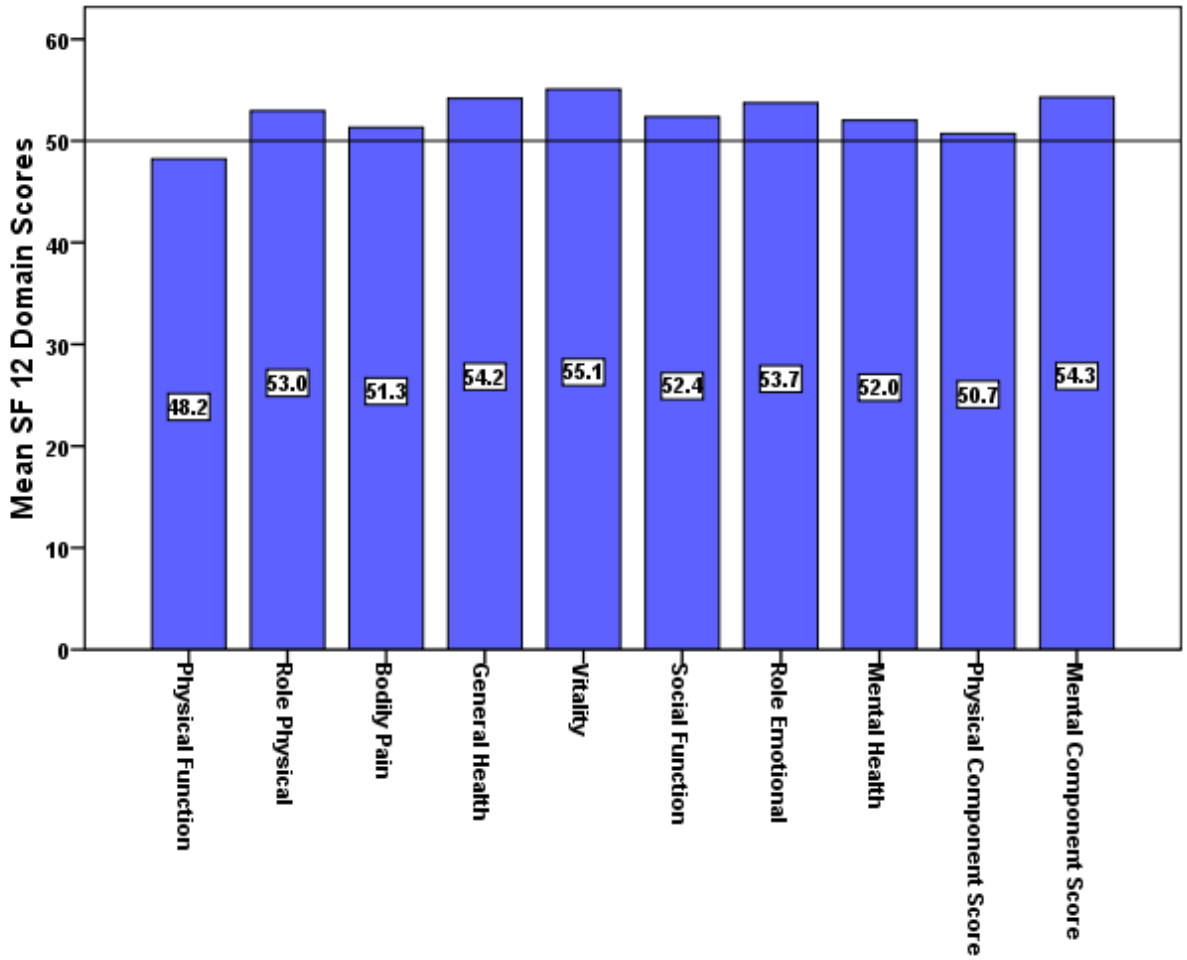
Figure 6.1: Scatter-plot of Correlation of iPTH (ng/ml) with Whole (1-84) PTH (pg/ml)



Comparison of Short Form 12 Scores with General Population

Median (IQR) Physical Component Short Form 12 Score (standardized to a general population mean score of 50) was 53.6 (44.8 – 57.6). Median (IQR) Mental Component Short Form 12 score was 55.6 (51.0 – 59.1); -General population median score, 50. Short Form 12 mean individual domain scores for the total study population are shown in Figure 6.2.

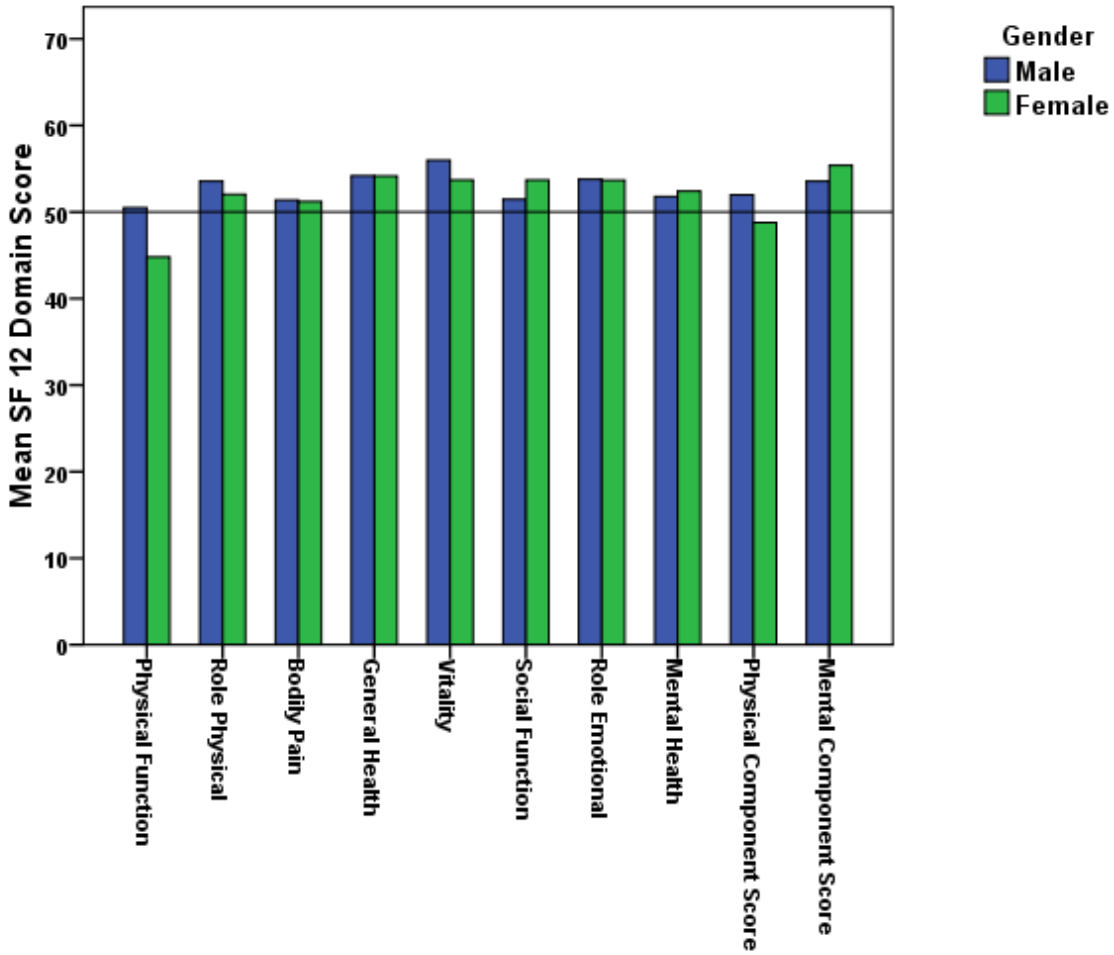
Figure 6.2: SF 12 domain and composite scores for Study Population (n= 90)



Physical Function scores were slightly lower than that of the general population. Other dimensions were similar to general population norms, with Mental Health dimensions (Mental Component Score, Vitality, Social Functioning, Role Emotional and Mental Health) scoring slightly higher than the general population average.) Overall 38.5% of subjects had a Physical Component Score below 50; the general population average and 23% had a Mental Component Score below 50.

When stratified by gender (Figure 6.3) scores in all dimensions for male transplant recipients were similar to the general population. Female transplant recipients had lower Physical Function scores than the general population average, (44.8 Vs 50, $p = 0.003$) however scores for Mental Component Score (55.4 Vs 50, $p<0.001$), Social Functioning (53.7 Vs 50 $p<0.001$ and Role Emotional (53.7 Vs 50, $p=0.001$) were significantly higher.

Figure 6.3: SF 12 Domain and Composite Scores for Study Population (n =90) stratified by Gender



Comparison of Short Form 12 Scores within the sample population

Lower MDRD eGFR (ml/min/1.73m^2) were significantly associated with SF 12 scores below the sample median for the General Health, Vitality, and Physical Component domains, mean eGFR approximately 48 Vs 56 ml/min/1.73m^2, all $p<0.05$. Distribution of demographic, clinical and laboratory variables for subjects with SF 12 scores below and above the sample median for the

Physical and Mental Component Scores are summarized in Tables 6.2 and 6.3 respectively.

Table 6.2: Distribution demographic, clinical and laboratory variables for subjects with SF 12 Physical Component Score below Vs above the sample median value of 53.6

	Total N=90	**PCS <53.6 N = 45**	**PCS >53.6 N= 45**	**p**
Male, n (%)	54 (60)	24 (53.3)	30 (66.7)	0.20
Mean (sd)Age, yrs	46.4 (12.6)	52.3 (11.6)	40.6 (10.9)	<0.001
Median (IQR)ESKD Vintage (yrs)	5.5 (3.4, 9.2)	5.4 (3.6, 9.2)	5.6 (3.2, 9.9)	0.96
History of Diabetes, n	14	8	6	0.53
Mean (sd)MDRD eGFR (ml/min/1.73m^2)	53.6 (16.6)	49.9 (15.8)	57.3 (16.7)	0.03
Mean (sd)Total Calcium (mmol/l)	2.57 (0.16)	2.57 (0.17)	2.58 (0.14)	0.71
Median (IQR) Intact PTH (ng/ml)	99 (73, 148)	105 (82, 182)	95 (65, 128)	0.03
Median (IQR) Whole PTH (pg/ml)	51.3 (34.5, 78)	54.2 (39.7, 100)	49.6 (32.9, 65)	0.03
Median (IQR) Intact SB PTH (pg/ml)	65.7 (48.6, 111.8)	73.9 (50.3, 147.4)	64.5 (46.9, 88.3)	0.06
Mean (sd) 25-OH Vitamin D (nmol/l)	43.9 (17.6)	42.8 (16.3)	45.1 (19.1)	0.57
Mean (sd) Haemoglobin (g/dl)	13.3 (1.6)	13.2 (1.6)	13.4 (1.6)	0.44

Abbreviations: sd, standard deviation, IQR, Intra-Quartile Range, ESKD, End Stage Kidney Disease, MDRD eGFR, Modification of Diet in Renal Disease estimated Glomerular Filtration Rate, PTH, Parathyroid Hormone, SB, Scantibodies™.

Table 6.3: Distribution of demographic, clinical and laboratory variables for subjects with SF 12 Mental Component Score below Vs above the sample median value of 55.6

	Total N=90	**MCS <55.6 N = 45**	**MCS >55.6 N= 45**	**p**
Male, n (%)	54 (60)	29 (64.4)	25 (55.6)	0.39
Mean (sd)Age, yrs	46.4 (12.6)	45.9 (11.4)	47.0 (13.9)	0.67
Median (IQR) ESKD Vintage (yrs)	5.5 (3.4, 9.2)	6.6 (3.6, 9.8)	5.1 (2.9, 9.2)	0.59
History of Diabetes, n	14	8	6	0.53
Mean (sd)MDRD eGFR (ml/min/1.73m^2)	53.6 (16.6)	53.3 (17.0)	53.9 (16.3)	0.87
Mean (sd)Total Calcium (mmol/l)	2.57 (0.16)	2.56 (0.13)	2.59 (0.18)	0.42
Median (IQR)Intact PTH (ng/ml)	99 (73, 148)	84 (61, 122)	104 (88, 168)	0.05
Median (IQR)Whole PTH (pg/ml)	51.3 (34.5, 78)	48.6 (33.2, 65.7)	64.4 (47.9, 92.1)	0.35
Median (IQR)Intact SB PTH (pg/ml)	65.7 (48.6, 111.8)	65.5 (47, 88.1)	80.1 (52.3, 130.9)	0.56
Mean (sd)25-OH Vitamin D (nmol/l)	43.9 (17.6)	45.9 (17.6)	41.9 (17.7)	0.29
Mean(sd) Haemogloblin (g/dl)	13.3 (1.6)	13.2 (1.6)	13.4 (1.7)	0.75

Abbreviations: sd, standard deviation, IQR, Intra-Quartile Range, ESKD, End Stage Kidney Disease, MDRD eGFR, Modification of Diet in Renal Disease estimated Glomerular Filtration Rate, PTH, Parathyroid Hormone, SB, Scantibodies™.

Univariate Linear Regression Associations of SF 12 Domain and Composite Scores

Summaries of univariate associations using linear regression for each of the SF 12 domains with demographic, clinical and laboratory characteristics of the study population are shown in Tables 6.4 and 6.5.

Table 6.4: Univariate Linear Regression of the Associations of SF-12 Physical Domains with demographic, clinical and laboratory variables, ß (95% Confidence Interval)

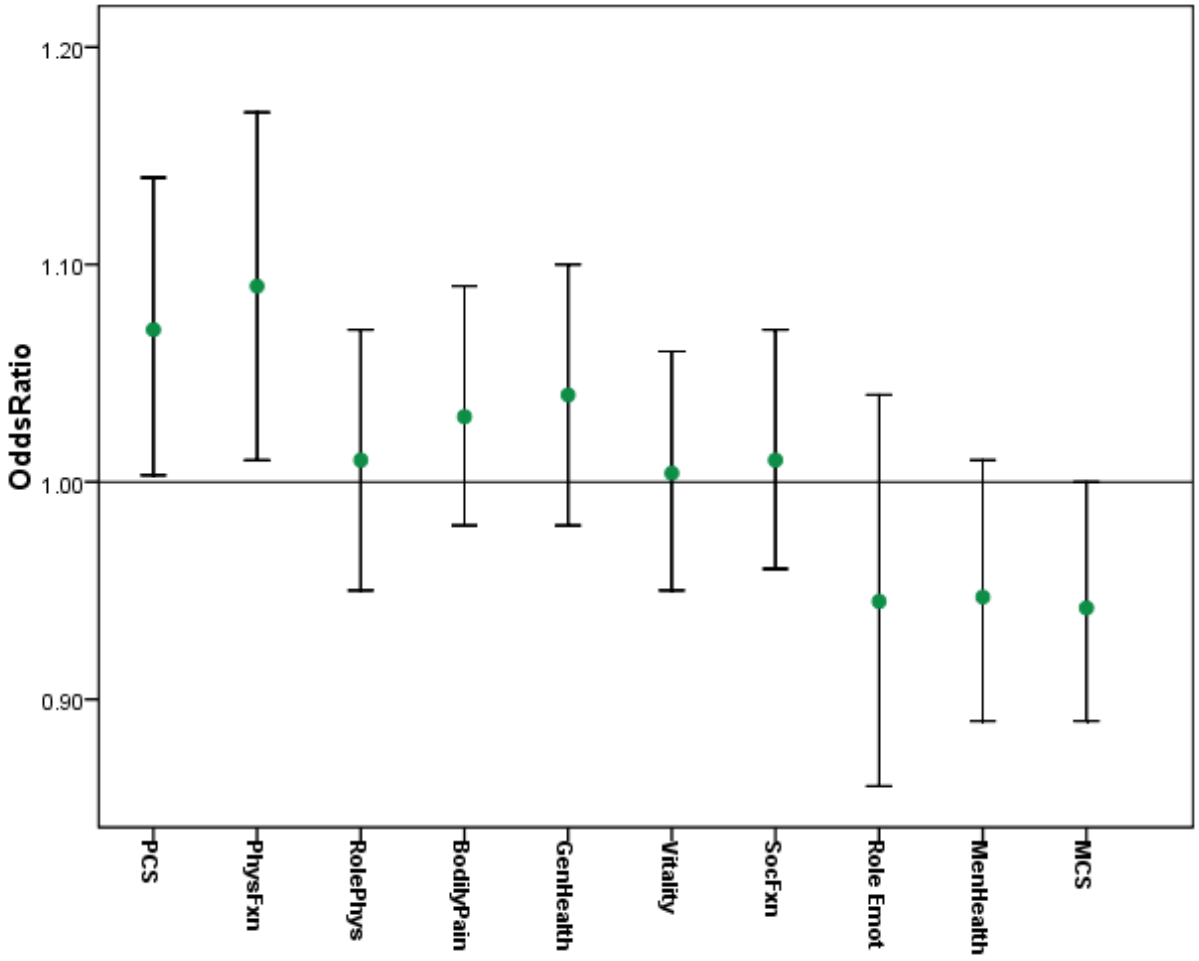
	Physical Component Score	Physical Functioning	Physical Role	Bodily Pain	General Health
iPTH (per 10ng/ml)	**1.07** **1.003, 1.138** **p=0.04**	**1.09** **1.01, 1.17** **p= 0.02**	1.006 0.95, 1.07	1.03 0.98, 1.09	1.04 0.98, 1.1
Age (years)	**1.09** **1.05, 1.15** **p <0.001**	**1.09** **1.05, 1.14** **P< 0.001**	**1.06** **1.02, 1.1** **p = 0.007**	**1.08** **1.04, 1.13** **p < 0.001**	**1.057** **1.02, 1.10** **p = 0.005**
Gender (Male)	1.75 0.75, 4.1	**3.23** **1.28, 8.14** **p = 0.01**	1.14 0.45, 2.89	0.82 0.34, 1.9	0.822 0.34, 1.96
ESKD Vintage (years)	1.003 0.90, 1.11	0.976 0.88, 1.09	1.04 0.93, 1.17	0.99 0.88, 1.1	1.005 0.901, 1.12
Previous CV event	**7.92** **0.93, 67.3** **p = 0.06**	5.33 0.63, 45.3	2.68 0.62, 11.7	**14.0** **1.64, 119.7** **p = 0.02**	**5.38** **1.02, 28.39** **p = 0.05**
Diabetes Mellitus	1.44 0.46, 4.57	0.667 0.21, 2.09	**2.95** **0.92, 9.5** **p = 0.07**	1.78 0.56, 5.6	0.366 0.09, 1.42
MDRD eGFR (ml/min/1.73m^2)	**0.97** **0.95, 0.99** **p = 0.04**	0.981 0.96, 1.01	0.987 0.96, 1.02	0.985 0.96, 1.01	**0.963** **0.94, 0.99** **p = 0.01**
Total Calcium >2.6mmol/L	1.0 0.43, 2.34	0.820 0.35, 1.94	1.04 0.41, 2.66	0.638 0.26, 1.56	0.957 0.40, 2.29
Phosphate <0.8mmol/L	1.89 0.51, 6.97	2.02 0.50, 8.17	2.302 0.64, 8.34	1.36 0.38, 4.85	1.361 0.38, 4.85
Vitamin D <50nmol/L	0.794 0.29, 2.09	0.876 0.32, 2.4	0.739 0.25, 2.14	0.821 0.30, 2.24	0.677 0.25, 1.83
Haemoglobin (g/dl)	0.903 0.70, 1.17	0.804 0.61, 1.05	0.951 0.72, 1.26	1.02 0.78, 1.33	1.014 0.78, 1.32

Table 6.5: Univariate Linear Regression Associations of SF-12 Mental/Emotional Domains with demographic, clinical and laboratory variables, ß (95% Confidence Interval)

	MCS	Vitality	Social Function	Role Emotional	Mental Health
iPTH (per 10ng/ml)	0.942 0.89, 1.00	1.004 0.95, 1.06	1.012 0.96, 1.07	0.945 0.86, 1.04	0.947 0.89, 1.01
Age (years)	0.993 0.96, 1.03	**1.072** **1.03, 1.12** **p =0.001**	1.007 0.97, 1.04	0.963 0.92, 1.01	1.021 0.99, 1.06
Gender (Male)	0.69 0.29, 1.61	1.70 0.72, 3.99	0.485 0.19, 1.23	1.00 0.32, 3.1	0.822 0.34, 1.96
ESKD Vintage	1.03 0.93, 1.15	1.04 0.93, 1.16	0.974 0.87, 1.09	0.98 0.84, 1.14	1.009 0.91, 1.13
Previous CV event	1.03 0.24, 4.38	4.84 0.92, 25.5	2.12 0.49, 9.13	1.92 0.35, 10.64	2.99 0.67, 13.42
Diabetes Mellitus	1.027 0.33, 3.21	0.707 0.22, 2.31	1.11 0.34, 3.67	**3.61** **1.004, 12.99** **p = 0.49**	1.19 0.37, 3.78
MDRD eGFR (ml/min/1.73m^2)	0.998 0.97, 1.02	**0.958** **0.93, 0.99** **p = 0.005**	0.988 0.96, 1.02	**1.037** **1.004, 1.072** **p = 0.03**	1.007 0.98, 1.03
Total Calcium >2.6mmol/L	0.828 0.35, 1.94	0.933 0.39, 2.21	1.307 0.54, 3.18	0.355 0.09, 1.36	0.957 0.40, 2.29
Phosphate <0.8mmol/L	0.330 0.08, 1.34	0.471 0.12, 1.91	1.699 0.47, 6.09	1.128 0.22, 5.84	0.551 0.14, 2.24
Vitamin D <50nmol/L	0.525 0.19, 1.44	0.559 0.21, 1.51	0.872 0.31, 2.42	0.533 0.16, 1.82	1.07 0.39, 2.95
Haemoglobin (g/dl)	0.958 0.74, 1.24	**0.726** **0.54, 0.97** **p = 0.03**	0.882 0.67, 1.16	1.125 0.80, 1.58	1.01 0.78, 1.32

Intact PTH (per 10ng/ml increment) was significantly associated with Physical Component Score and Physical Functioning but not with any of the other SF 12 domain scores. (Figure 6.4).

Figure 6.4: Univariate Linear Regression association of SF 12 Domains (all domains) with intact PTH (per 10ng/ml increment)



Age was associated with Physical Component Score, Physical Functioning, Physical Role, General Health and Vitality on univariate analysis, but not with Mental Component Score, Social Function, Emotional Role or Mental Health domains. Gender was univariately associated with Physical Function but not with the other SF 12 domains.

Multivariate Logistic Regression Associations of SF 12 Domain and Composite Scores

On multivariate binary logistic regression analysis, Physical Function score below the sample median of 53.6 was significantly associated with intact PTH (per 10ng/ml increment), even with simultaneous adjustment for gender, eGFR, age, haemoglobin and elevated total serum calcium, (adjusted Odds Ratio (95% CI), 1.115, (1.009, 1.233, p = 0.03). With additional adjustment for co-morbidities (prior cardiovascular event and history of Diabetes), this association was somewhat attenuated, adjusted Odds Ratio (95% CI) 1.107 (0.999, 1.227, p =0.05). (Table 6.6, Figure 6.5).

Table 6.6: Crude and adjusted Odds Ratio (95% Confidence Interval) for Association of iPTH (per 10ng/ml) with Physical Domains of SF 12

	Model 1 Crude OR	Model 2 Adjusted OR	Model 3 Adjusted OR	Model 4 Adjusted OR
Physical Component Score	**1.069** **1.003, 1.14** **p = 0.04**	1.063 0.98, 1.15	1.067 0.99, 1.16	1.063 0.98, 1.15
Physical Function	**1.089** **1.01, 1.17** **p = 0.02**	**1.099** **1.00, 1.21** **p = 0.05**	**1.115** **1.01, 1.23** **p = 0.03**	**1.107** **0.999, 1.23** **p= 0.05**
Role Physical	1.006 0.95, 1.07	0.994 0.93, 1.06	0.990 0.93, 1.06	1.001 0.94, 1.07
Bodily Pain	1.034 0.98, 1.09	1.031 0.97, 1.1	1.042 0.97, 1.12	1.036 0.96, 1.12
General Health	1.040 0.98, 1.10	1.021 0.96, 1.09	1.026 0.96, 1.10	1.016 0.94, 1.09

Model 1: Unadjusted

Model 2: Adjusted for Gender, MDRD eGFR (ml/min/1.73m^2) and Age (years)

Model 3: Adjusted for Gender, MDRD eGFR (ml/min/1.73m^2), Age (years), Haemoglobin (g/dl), Total Calcium >2.60 mmol/L.

Model 4: Adjusted for Gender, MDRD eGFR (ml/min/1.73m^2), Age (years), Haemoglobin (g/dl), Total Calcium >2.60 mmol/L, History of prior CV event, History of Diabetes Mellitus.

On univariate logistic regression iPTH was not significantly associated with Mental Component Score or with any of the domains; on multivariate analysis higher iPTH was significantly associated with a better Mental Component Score. (Table 6.7, Figure 6.5) Some of this unexpected association may relate to uncontrolled confounding with subjects having a more severe clinical course on dialysis having higher post transplant PTH levels and having a greater perceived improvement in their quality of life with successful renal transplantation.

Table 6.7: Crude and adjusted Odds Ratio (95% Confidence Interval) for Association of iPTH (per 10ng/ml) with Mental/Emotional Domains of SF 12

	Model 1 Crude OR	**Model 2 Adjusted OR**	**Model 3 Adjusted OR**	**Model 4 Adjusted OR**
Mental Component Score	0.942 0.89, 1.00	0.938 0.88, 1.00	**0.934** **0.873, 1.00** **p = 0.05**	**0.918** **0.851, 0.990** **p = 0.03**
Vitality	1.004 0.95, 1.06	0.965 0.91, 1.03	0.959 0.90, 1.03	0.953 0.88, 1.03
Social Function	1.012 0.96, 1.07	1.011 0.95, 1.08	1.002 0.94, 1.07	0.985 0.92, 1.06
Role Emotional	0.945 0.86, 1.04	0.956 0.86, 1.07	0.972 0.87, 1.09	0.997 0.88, 1.11
Mental Health	0.947 0.89, 1.01	0.948 0.89, 1.01	0.942 0.88, 1.01	0.936 0.87, 1.01

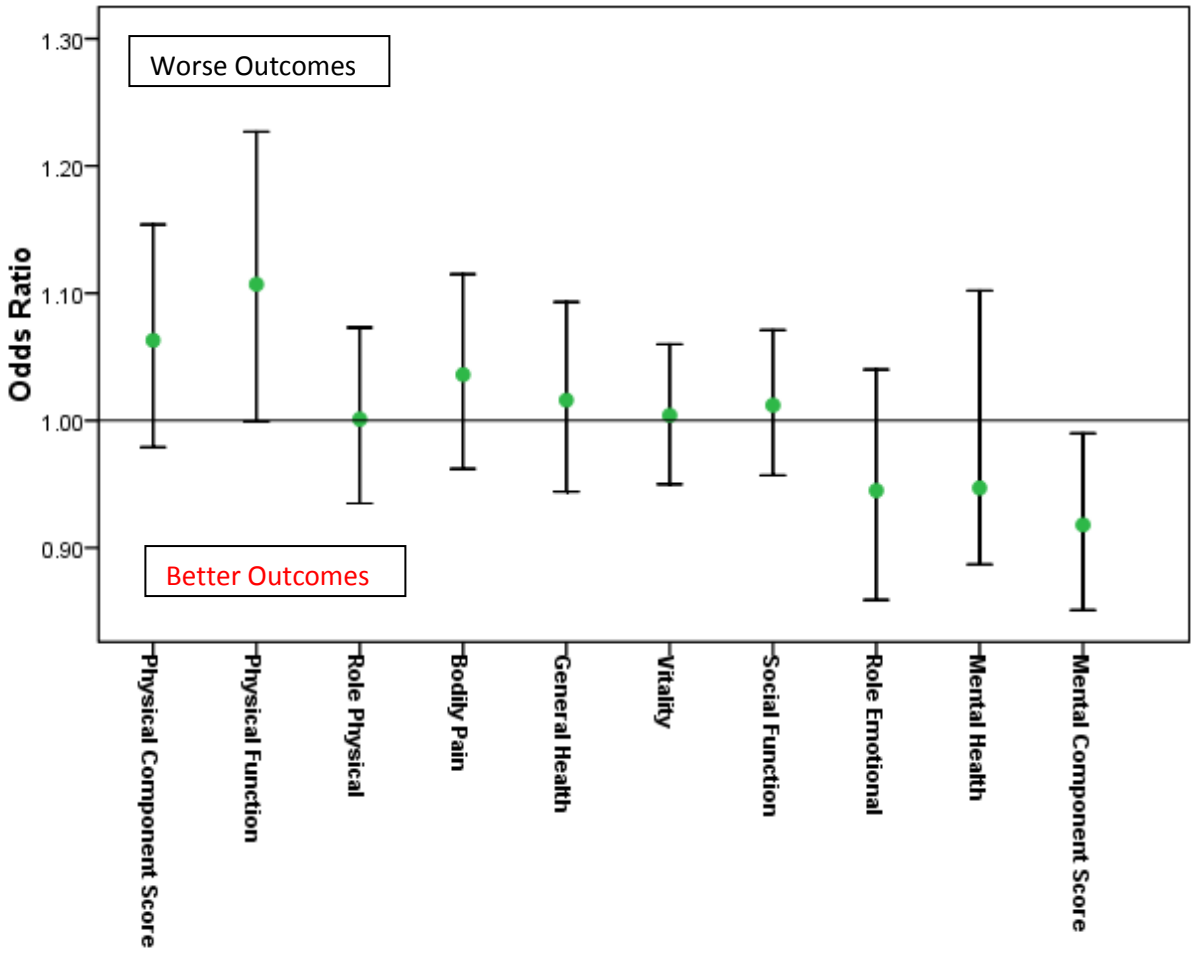
Model 1: Unadjusted

Model 2: Adjusted for Gender, MDRD eGFR (ml/min) and Age (years)

Model 3: Adjusted for Gender, MDRD eGFR (ml/min), Age, Haemoglobin (g/dl), Total Calcium >2.60 mmol/L.

Model 4: Adjusted for Gender, MDRD eGFR (ml/min), Age, Haemoglobin (g/dl), Total Calcium >2.60 mmol/L, History of prior CV event, History of Diabetes Mellitus.

Figure 6.5: Multivariate associations of SF 12 Domains (all domains) with intact PTH (per 10ng/ml increment) adjusted for Gender, MDRD eGFR (ml/min), Age, Haemoglobin (g/dl), Total Calcium >2.60 mmol/L, History of prior CV event, History of Diabetes Mellitus.



Multivariate Logistic Regression Associations of SF 12 Domain and Composite Scores with Whole and Total Intact PTH.

We repeated the above analysis in the subgroup of 70 patients in whom we measured Whole 1-84 PTH results as outlined in Methods; examining its relationship with each of the SF 12 domain scores. The association of Whole 1-84 PTH with the Physical Functioning domain of SF 12 was stronger and remained significant in the fully adjusted model, adjusted OR (95% CI) 1.399, (1.043 – 1.877, p = 0.025) (Table 6.8).

Table 6.8: Crude and adjusted OR (95% CI) for Association of Scantibodies™ Whole 1- 84 PTH (per 10pg/ml) with SF 12 Domain Scores

	Crude	Adjusted*
Physical Component Score	**1.148** **1.001, 1.316** **p = 0.05**	1.088 0.916, 1.292
Physical Function	**1.358** **1.086, 1.699** **p = 0.007**	**1.399** **1.043, 1.877** **p = 0.03**
Role Physical	0.972 0.87, 1.09	0.942 0.83, 1.07
Bodily Pain	1.076 0.97, 1.19	1.070 0.92, 1.24
General Health	1.034 0.94, 1.13	0.959 0.83, 1.10
Mental Component Score	0.959 0.87, 1.05	0.932 0.83, 1.04
Vitality	1.013 0.93, 1.11	0.882 0.77, 1.02
Social Function	0.988 0.99, 1.09	0.946 0.83, 1.07
Role Emotional	0.941 0.80, 1.10	0.995 0.85, 1.16
Mental Health	0.937 0.83, 1.05	0.932 0.83, 1.05

* Adjusted for Gender, MDRD eGFR (ml/min), Age, Haemoglobin (g/dl), Total Calcium >2.60 mmol/L, History of prior CV event, History of Diabetes Mellitus.

Similarly, Scantibodies Intact PTH (per 10pg/ml increment) was significantly associated with SF 12 Physical Functioning Score, even following simultaneous adjustment for gender, eGFR, age, haemoglobin level, hypercalcaemia and co-morbidities (history of cardiovascular event and diabetes) (Table 6.9).

Table 6.9: Crude and adjusted OR (95% CI) for Association of Intact PTH (Scantibodies Assay) (per 10pg/ml) with SF 12 Domain Scores

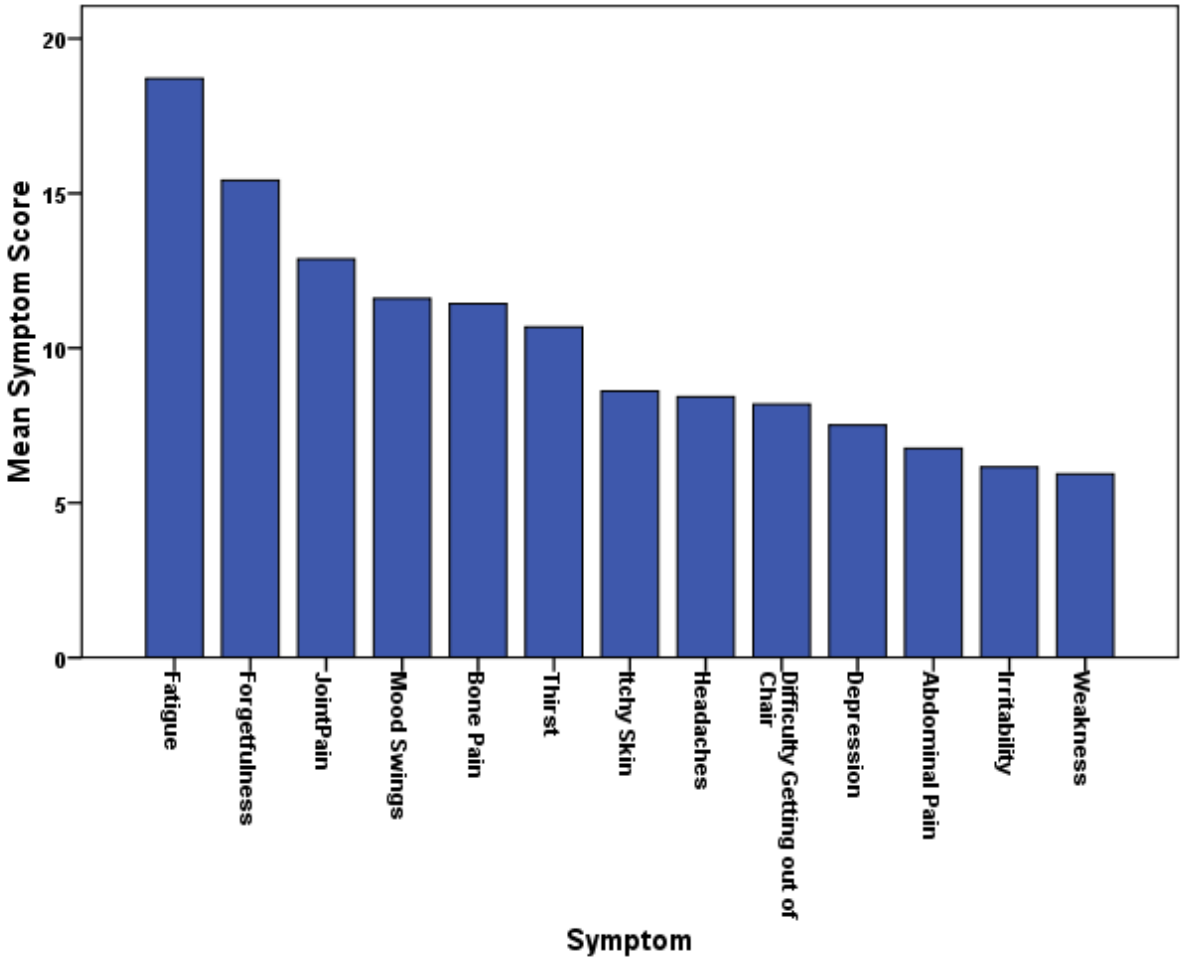
	Crude	Adjusted*
Physical Component Score	1.083 0.99, 1.189	1.040 0.94, 1.15
Physical Function	**1.225** **1.053, 1.424** **p = 0.008**	**1.244** **1.024, 1.512** **p = 0.03**
Role Physical	0.980 0.91, 1.06	0.959 0.88, 1.05
Bodily Pain	1.034 0.97, 1.10	1.025 0.93, 1.13
General Health	1.023 0.97, 1.08	0.976 0.89, 1.06
Mental Component Score	0.984 0.93, 1.04	0.975 0.91, 1.04
Vitality	1.012 0.96, 1.07	0.933 0.86, 1.01
Social Function	0.984 0.92, 1.05	0.960 0.88, 1.04
Role Emotional	0.947 0.84, 1.07	0.977 0.875 1.09
Mental Health	0.980 0.92, 1.05	0.973 0.91, 1.04

* Adjusted for Gender, MDRD eGFR (ml/min), Age, Haemoglobin (g/dl), Total Calcium >2.60 mmol/L, History of prior CV event, History of Diabetes Mellitus.

Parathyroid Assessment of Symptoms Score

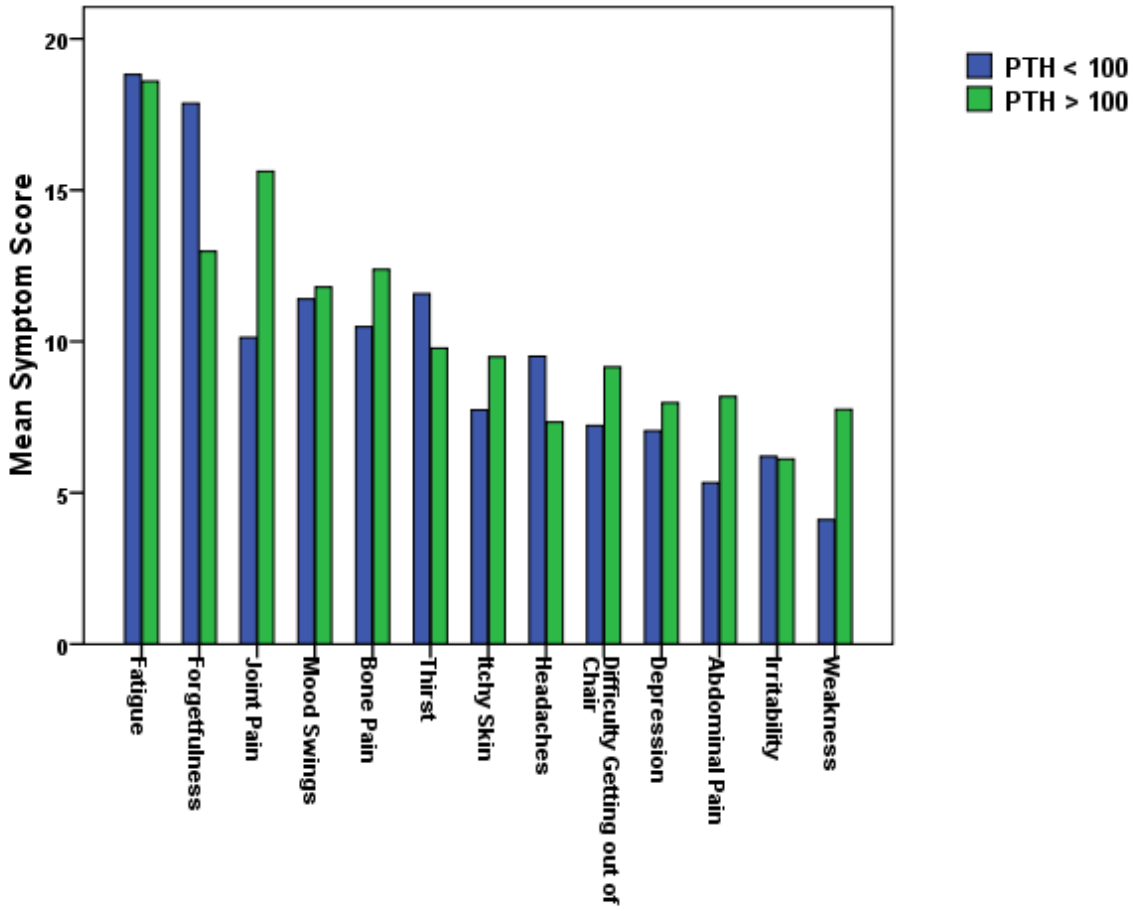
Median (IQR) total Parathyroid Assessment of Symptom Score was 100 (31 – 170). The most commonly reported symptoms were fatigue, forgetfulness, joint pain and mood swings. (Figure 6.6)

Figure 6.6: Responses to Parathyroid Assessment of Symptoms Questionnaire (n=90)



There was no significant difference is Parathyroid Assessment of Symptoms Score between patients who had an iPTH level above or below the median value of 100ng/ml, (137 vs 127ng/ml, p = 0.7). Similarly there was no significant difference in the individual disease specific symptom scores between patients who had iPTH levels above or below the median value of 100ng/ml. (Figure 6.7)

Figure 6.7: Comparison of individual disease specific symptom scores between patients with iPTH value above or below 100ng/ml.



There was no correlation between Total Parathyroid Assessment of Symptoms Score with either intact PTH or Whole 1-84 PTH. Similarly, there was no difference in symptom scores for each of the 13 disease specific items on the parathyroid assessment of symptoms questionnaire between patients who had iPTH levels above the nominal general population reference range of 65ng/ml, or between patients who had iPTH values above or below the median value of 100ng/ml.

Discussion

To our knowledge, the current report is the first to demonstrate an independent relationship between elevated PTH levels post transplant and one element of Health Related Quality of Life. Contrary to our expectation we only found PTH levels to be associated with Physical Function Scores below the sample median, but not with any other domain or composite of Short Form 12. Renal transplantation is the treatment of choice for End Stage Kidney Disease. The objective of renal transplantation is to maximise longevity and health related quality of life, while minimising the complications of renal disease and cost of care. With increasing graft and patient survival rates, long-term Health Related Quality of Life has assumed greater importance as a relevant treatment-related outcome. Although published data is limited, studies evaluating Health Related Quality of Life after renal transplant have shown broadly similar results; Quality of Life substantially improves compared to

continuing on dialysis.[271-275] A recent meta-analysis has reported that the quality of life of renal transplant recipients has been improving over time, with significant improvements since the 1980's, likely reflecting improvements in renal transplant care and change in clinical practice and immunosuppressive protocols.[273]

The most widely employed tool to assess Health Related Quality of Life post renal transplant globally is the Short Form 36 questionnaire. Studies have reported that SF36 scores across the 8 domains are similar post-transplantation to that of the general population although there is some variability in results. Griva et al. reported that SF 36 scores in 102 transplant recipients with a mean transplant vintage of 8 years were similar to that of the general population, with the exception of physical functioning and general health perception scores which were slightly lower that population norms.[276] Similarly, Neipp et al. in a cross-sectional study of 139 long-term renal transplant recipients (transplant vintage > 15 years) found that SF 36 scores were similar to the general population in the domains role physical, social functioning, role emotional and mental health, while scores for physical functioning, physical pain, general health and vitality were reduced.[277] Rebello et al. demonstrated that Physical Functioning scores in transplant recipients were lower than the general population, but that role emotional and mental health scores were higher than the general population for female transplant patients.[275] To the best of our knowledge no previous study has examined the

effect of post transplant hyperparathyroidism with Health related Quality of Life. Our study broadly supports these earlier reports, we found that Physical Functioning scores were lower than general population norms, and were significantly and independently associated with PTH levels. Mental Component Scores significantly higher than the general population norm with PTH being significantly associated with higher (better) Mental Composite Scores on multivariate analysis. Given the observational nature of this study we cannot establish causality for any of the above associations, which may relate to residual uncontrolled confounding.

Several factors have been shown to be associated with reduced health related quality of life post renal transplantation. In a cross-sectional study of 281 renal transplant recipients Neri et al. showed that lower levels of renal function were associated with lower measures of Quality of Life, independent of age, gender, transplant vintage and co-morbidities (history of diabetes, hypertension or cardiovascular disease).[278] The immunosuppression protocol employed has been associated with improved Health Related Quality of Life measures, a number of studies have demonstrated better quality of life scores with the use of tacrolimus based protocols compared to cyclosporine.[279, 280] The first of these studies by Shield et al. also reported that patients who had experienced an acute rejection episode had lower quality of life scores.[279]

In Chapter 4 of this manuscript, we discuss that the pathophysiology of post transplant hyperparathyroidism may relate to a degree of secondary hyperparathyroidism in setting of diminished renal allograft function, associated vitamin D deficiency and diminished vitamin D receptor activation as occurs in the setting of native kidney CKD.[236] Alternatively, persistent autonomous PTH production from hyperplastic parathyroid tissue may resemble primary hyperparathyroidism which is associated with frank hypercalcaemia and hypophosphatemia.

Primary hyperparathyroidism is a biochemical diagnosis often made based on routine laboratory assessment of calcium levels in asymptomatic patients. Less than 20% of patients display the classic symptoms of bone pain, nephrolithiasis and neuropsychiatric disturbance. Whether the remaining 80% of patients are truly asymptomatic is controversial. Several studies have reported decreased measures of quality of life and elevated depression scores in patients with primary hyperparathyroidism which improves after surgical intervention. Weber at al. have recently shown that patients with primary hyperparathyroidism had significantly lower SF 36 Physical and Mental Component scores compared to a control group of patients with non-toxic goitre. SF 36 scores improved significantly over the first 12 months post parathyroidectomy.[174] In an earlier study the same authors demonstrated that patients with primary hyperparathyroidism had significantly lower SF 12 mental and physical component scores compared to the general population

and that mental component scores were significantly higher after parathyroidectomy.[173] In both these studies, pre-operative calcium levels were associated with higher depressive symptom scores. However, a study by Burney et al. demonstrated that patients with primary hyperparathyroidism have significant functional health status impairment independent of the level of serum calcium and that improvements in health related quality of life was seen in both the hypercalcaemic and normocalcaemic groups after surgical intervention.[270] In our study of post transplant patients with relatively well preserved renal function we demonstrate that lower levels of physical functioning were associated with higher PTH levels, independent of hypercalcaemia.

The original National Institutes of Health Consensus Conference Statement in 1991 stated that evidence of mineral bone loss, a decrease in renal function and classical symptoms were indications for surgical correction of primary hyperparathyroidism.[169] However, in light of this more recent data describing the more subtle physical and neurocognitive manifestations of the disease, which demonstrate improvement following surgical intervention, the original NIH recommendation has been revised. A consensus statement from the Proceedings of the Third International Workshop on Asymptomatic Primary Hyperparathyroidism states that "the balance of available evidence suggests that surgery is appropriate in the majority of patients with asymptomatic primary hyperparathyroidism".[175]

Pasieka et al. have developed a disease specific tool for hyperparathyroidism which has been validated by a number of studies in both symptomatic and asymptomatic patients.[171, 172] This disease specific tool has been utilised in a single study of patients with secondary and tertiary hyperparathyroidism due to ESKD including 10 renal transplant recipients, where tertiary hyperparathyroidism was defined as persistently elevated PTH above the normal population reference range six months post successful renal engraftment. This study compared the pre and post-operative disease specific tool scores and Quality of Life scores of patients with primary, secondary and tertiary hyperparathyroidism with those of a control group with non-toxic thyroid disease. Although the group with tertiary hyperparathyroidism (post transplant), was too small (n=10) for meaningful statistical analysis, they showed resolution of their symptoms similar to those in the primary hyperparathyroidism group.[53] We did not find a significant relationship between parathyroid assessment score and PTH levels in our study, this may relate in part to a type II statistical error given our modest sample size or to difficulty in dissecting out direct associations in a transplant population which typically has multiple contributing comorbidities which may all influence Health related Quality of Life. In the original studies validating the parathyroid assessment of symptoms questionnaire, median symptom scores were higher than in our cohort (350 vs 100) and while symptom scores in the original studies demonstrated a significant reduction following

parathyroidectomy, pre-operative symptom scores did not correlate with pre-operative PTH values.[172] Whether symptom scores in the post transplant population would improve following correction of secondary hyperparathyroidism, for example with Vitamin D supplementation, warrants further study.

The aetiology of persistent elevations of PTH is multifactorial, with autonomous PTH secretion and Vitamin D deficiency likely to contribute to its perpetuation. Regardless of its aetiology, elevated PTH post transplantation has been associated with decreased bone mineral density and increased fracture risk, with a predilection for cortical bone sites such as the distal radius. This pattern of bone loss is similar to that seen in primary hyperparathyroidism, where early bone loss is observed at the radius and is frequently asymptomatic.[281]

To our knowledge the current study is the first to demonstrate an independent relationship between elevated PTH levels and some element of Health Related Quality of Life namely physical functioning. Whether these symptom scores improve following correction of residual hyperparathyroidism post renal transplant is unknown and would require prospective interventional studies. While PTH levels typically decline post renal transplant, levels remain elevated above the normal range in a substantial proportion of patients despite adequate renal function. Given current uncertainty as to the optimal

treatment strategy for post-transplant hyperparathyroidism, results of the current analysis reinforce the potential benefits of controlling secondary hyperparathyroidism in native CKD and of strategies that prevent the development of persistent post transplant hyperparathyroidism.

Chapter 7

Conclusions

Chronic Kidney Disease (CKD), osteoporosis and mild hyponatremia are all prevalent chronic conditions, which may coexist and are often under-recognized. Mineral-Bone Disorder begins early in the natural history of CKD and results in complex abnormalities of bone which ultimately confers a well-established increased risk of fragility fractures in End Stage Kidney Disease. Hyponatremia is a novel, usually renal mediated metabolic perturbation, that most commonly occurs independently of the stage of renal dysfunction but which may also predispose to increased fracture risk. The extent to which either early stages of renal dysfunction or the presence of hyponatremia contribute to fracture occurrence in the general population, independently of osteoporosis, is unclear. Renal transplantation is the treatment of choice for ESKD and although it restores endogenous renal function it typically fails to normalize either the long term cardiovascular or fracture risk. One potential mechanism contributing to these elevated long-term risks and to diminished Health Related Quality of Life is persistent, post-transplant hyperparathyroidism.

In this study we examined the association of renal function and serum sodium with Bone Mineral Density and fracture occurrence in a retrospective cohort of 1930 female members of the general population who underwent routine DXA scan. We then prospectively recruited a cohort of 90 renal transplant recipients and examined the association of post transplant parathyroid hormone (PTH) level with measures of CKD Mineral Bone Disorder, including,

DXA Bone Mineral Density, Vascular Calcification (assessed using both abdominal radiograph and CT techniques, as well as indirectly by carotid-femoral pulse wave velocity) and Quality of Life (using the Short Form-12 and a PTH specific symptom score).

In Chapter 2, we examined the association of renal function with Bone Mineral Density and fracture occurrence in a retrospective cohort of 1702 female members of the general population who underwent routine DXA scan. Osteoporosis and CKD frequently co-exist, a relationship which we found to be confounded by age, as both conditions are increasingly more prevalent in older people. However, we report that moderate CKD (eGFR 30-59ml/min/1.73m^2), independently of DXA Bone Mineral Density and age was associated with the occurrence of self reported fracture with adjusted Odds Ratio (95% Confidence Interval), 1.37 (1.0, 1.89). This study confirms earlier reports of increased fracture prevalence in CKD; however we found that this association occurs independently of Bone Mineral Density and at moderate levels of previously undiagnosed CKD. Osteoporosis and CKD are both common chronic conditions associated with fracture risk. Risk stratification prediction models for fracture include Bone Mineral Density, but not renal function in the assessment of fracture risk. This may under estimate fracture risk in the general population, a major public health concern in an ageing population.

In Chapter 3, we examined the association of mild hyponatremia (<135mmol/L) with Bone Mineral Density and fracture occurrence. Serum sodium is a novel marker for bone health and has been associated with gait abnormalities, risk of falls and decreased Bone Mineral Density. We report that mild hyponatremia, at a level usually regarded as benign, was associated with the occurrence of self-reported non-vertebral fracture, adjusted Odds Ratio (95% Confidence Interval), 2.25 (1.24, 4.09), independently of Bone Mineral Density. In an editorial commentary on this work, Ayus et al.[230] suggest that serum sodium should be viewed as a novel marker of bone health and should be monitored and corrected when it develops, similar to the approach of measuring and correcting Vitamin D deficiency in osteoporotic patients. Subsequent studies have cited and confirmed our findings, Hoorn et al.[231] found that mild hyponatremia was associated with an increased risk of incident fractures, independently of age, gender and Bone Mineral Density, adjusted Odds Ratio (95% Confidence Interval), 1.39, (1.11, 1.73). Using the NHANES III data, Verbalis et al.[24] found a significant relationship between mild hyponatremia and risk of osteoporosis at the hip, Odds Ratio (95% Confidence Interval), 2.85 (1.03, 7.86). These studies emphasize the significant public health consequences of even mild levels of hyponatremia and the need to identify and modify long-term fracture risk.

In Chapter 4 we describe the myriad of mineral metabolism, bone turnover and vascular calcification abnormalities that are evident in a prospective cohort of 90 successful renal transplant recipients. Overall 91% of renal transplants with eGFR >30ml/min/m^2 had an elevated iPTH and/or suboptimal vitamin D level, 54% had aortic calcification and 42% osteoporosis, a prevalent vertebral collapse fracture or a post-transplant peripheral fragility fracture. These abnormalities appear to persist despite the restoration of adequate, and in some cases excellent, renal function. While it is likely that some of the long term morbidity seen in transplantation results from cumulative damage (e.g. vascular calcification or abnormal bone architecture that accrued during the period of advanced CKD or on dialysis, we nevertheless demonstrate an independent association of prevalent PTH levels with markers of bone turn over and with Bone Mineral Density, adjusted for other potential confounders, adjusted Odds Ratio (95% Confidence Interval), 1.15 (per 10ng/ml increment), (1.04, and 1.26). This raises the important possibility that in addition to previously acquired historical damage, persistent post transplant hyperparathyroidism may be actively exerting an ongoing deleterious effect on transplant outcomes at least with regard to bone mineral density and risk of fragility fracture risk. If confirmed in prospective studies this observation would support the need for interventions aimed at preventing or controlling excessive hyperparathyroidism in a post transplantation setting.

In Chapter 5, we report that the presence of osteoporosis, but not PTH, was independently associated with CT measures of vascular calcification in a cohort of 64 successful renal transplant recipients, adjusted ß (95% Confidence Interval), 12.45, (1.16, 23.75). In this cross-sectional study we cannot assess the progressive nature of this relationship but ongoing longitudinal investigations may further elucidate the nature and extent of this relationship and its implications on the long-term excess cardiovascular and fracture risk of renal transplant recipients.

In Chapter 6, we evaluate the association of PTH with Health Related Quality of Life in 90 subjects with good renal allograft function. Of the 8 health domains and 2 composite scores examined, post-transplant PTH (per 10ng/ml increment), was only significantly and independently associated with reduced Physical Functioning, (95% Confidence Interval), 1.12 (1.01, 1.23). The multitude of factors which influence the subjective physical and mental well-being of patients with chronic disease are diverse and not readily quantifiable. To our knowledge the current study is the first to demonstrate an independent relationship between elevated PTH levels and some element of Health Related Quality of Life namely physical functioning. Whether these symptom scores improve following correction of residual hyperparathyroidism post renal transplant is unknown and would require prospective interventional studies.

Given current uncertainty as to the optimal treatment strategy for post-transplant hyperparathyroidism, results of the above analyses at the very least reinforce the potential benefits of controlling secondary hyperparathyroidism in native CKD and of strategies that prevent the development of persistent post transplant hyperparathyroidism and furthermore support the conduct of additional prospective research to better delineate the influence of hyperparathyroidism on long term graft and patient outcomes.

In conclusion, Chronic Kidney Disease and hyponatremia are both common health problems that may contribute to fracture occurrence in the general population, a major on-going public health concern. PTH may be an important mediator of sub-optimal long-term outcomes post renal transplantation, influencing bone and vascular health and to a limited extent long term Health Related Quality of Life

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Appendices

Appendix 1: Patient Information Sheet and Consent Form Version 4

Clinical Research Ethics Committee Of The Cork Teaching Hospitals

CONSENT BY SUBJECT FOR PARTICIPATION IN RESEARCH PROTOCOL

Section A

Protocol Number: 06 Neph 02.2 Patient Name:_____

Title of Protocol: **Bone Mineral Density and Vascular Calcification in Chronic Kidney Disease.**

Doctors Directing Research:

Dr. Joseph Eustace	(Chief Investigator)	Phone:	021 4920199
Dr. Sinead Kinsella	(Research Co-ordinator)		021 4920199
Dr. Michael Molloy	(Bone Density Laboratory)		021 4922817
Dr. Joe Coyle	(Radiology SpR)		021 4922254
Prof Michael Maher	(Consultant Radiologist)		021 4922254
Dr. Denis O Mahony	(Brachial FMD)		021 4546400

You are being asked to participate in a research study. The doctors at University College Cork study the nature of disease and attempt to develop improved methods of diagnosis and treatment. In order to decide whether or not you want to be a part of this research study, you should understand enough about its risks and benefits to make an informed judgment. This process is known as informed consent. This consent form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

Section B

I. NATURE AND DURATION OF PROCEDURES:

You are being asked to join a study to look at the effects of high levels of parathyroid hormone and calcium on osteoporosis (bone strength) and

calcification, (hardening), of blood vessels after a kidney transplant. The study will take place in Cork University Hospital. There will be about 90 patients involved and it will last for 2 years.

High levels of phosphate, calcium and parathyroid hormone are very common in patients on dialysis. Previous research has shown that these high levels are associated with a higher risk of suffering a heart attack or stroke. It is also known that these high levels can affect the bone strength of people with kidney disease. The bones can become more fragile and fracture easily.

In most people the levels of calcium and parathyroid hormone go back to normal after a kidney transplant. However, in some people the levels stay above normal, even though the kidney transplant is working well. It is not clear whether these high levels continue to affect the blood vessels and the strength of the bones or if they make any difference to the long-term health of a person with a kidney transplant. The aim of this study is to find out if high levels of calcium and parathyroid hormone in the blood after a kidney transplant continue to affect the blood vessels and bones.

There are a number of tests that will be carried out as part of the study. Doctors and nurses working in Cork University Hospital will do these tests.

You will be asked to give a blood and urine sample. These samples will be repeated every 6 months over a 2-year period. Each time you have a blood sample taken, we will withdraw about 30mls (2 tablespoons) of blood. Blood will be taken by experienced staff.

You will then be asked to have a bone density scan done. This will look at the strength of the bones of your hips, lower back and forearms. This is a pain free procedure and does not require any special preparation on your part. The scan takes about 25 minutes in total. You will be asked to lie still on your back and left side. You will be able to breathe normally.

Next you will be asked to have an X ray of your back done on the same day as the bone density scan. This will be done in the Radiology (X Ray) Department of Cork University Hospital.

You will also be asked to have a CT scan done of your abdomen. This is a detailed type of X Ray test which will take about 10 minutes to complete. You will be asked to lie on your back on a table, which then moves through the scanning unit. You do not need to fast or have any contrast (dye) administered for this test. This examination is also done in the Radiology Department of Cork University Hospital.

You will have an ultrasound scan of an artery in your arm. This will not be an invasive test. But it will require that a blood pressure cuff be inflated above your elbow for 4 minutes before the scan is done. The amount that your blood vessel expands after the blood pressure cuff is taken off will then be measured by ultrasound scan.

The last test will measure how fast blood moves between 2 blood vessels in your body. This is not an invasive test. It involves placing pulse sensors on your skin over a blood vessel in your neck and at the top of your leg. You will be asked not to move for about 10 seconds while a computer measures how fast your blood is moving. You will be able to breathe normally. This test will take 5 to 10 minutes.

These tests will be done when you start the study and repeated 1 and 2 years later.

You will also be asked to fill in a questionnaire. This is to find out if you have symptoms related to high levels of calcium and parathyroid hormone in the blood and if these symptoms are affecting your day-to-day activities.

II. POTENTIAL RISKS AND BENEFITS:

When you have a blood sample taken you may experience some discomfort when the needle is being inserted. You may get a bruise at the site of entry of the needle.

During the bone density scan, you may experience some discomfort if you suffer from back pain, as you will be asked to lie on your back. The dose of radiation is minimal, similar to that experienced on a short haul flight. The same discomfort may be experienced when you have an X Ray of your back done. The dose of radiation is higher with an X Ray but still only similar to that experienced on a long haul flight. The dose of radiation in the CT scan is similar to that in the X Ray of your back.

You may feel the blood pressure cuff tight on your arm for the 4 minutes before the ultrasound scan, but this has not been shown to cause any harm to your arm. However it may cause some discomfort while it is inflated and after it is released. You might feel pins and needles in your arm for a few minutes. Ultrasound testing does not expose you to any harmful radiation and is a non-invasive test. Measuring how fast the blood moves between your blood vessels is a non-invasive test and is not harmful.

By carrying out this research, we hope to find out if high calcium and parathyroid hormone levels in the blood affect the blood vessels and bone strength of people with kidney transplants. We would also like to see if blood and urine tests are effective in detecting a change in bone strength and blood vessel calcification after a kidney transplant.

This research will help us identify people who might be at higher risk of heart attack, stroke and fragile bones because of their kidney disease. This will hopefully help us to prevent heart attack, strokes and fractures in these people.

III. POSSIBLE ALTERNATIVES:

You may choose not to take part in this study. This will not affect your future medical care. If you decide to join the study, you are free to withdraw from the study at any time, and again this will not affect your medical care.

Section C

AGREEMENT TO CONSENT

The research project and the treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available to me. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by government agencies and sponsors of the research.

I understand that the sponsors and investigators have such insurance as is required by law in the event of injury resulting from this research.

I, the undersigned, hereby consent to participate as a subject in the above described project conducted at the Cork Teaching Hospitals. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the doctor(s) listed above. If I have further queries concerning my rights in connection with the research, I can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Lancaster Hall, 6 Little Hanover Street, Cork.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

Doctor: _____ _____

Signature of Subject or Guardian

Witness:_____ Date: _____ Time:

Appendix 2: ABC-HEART Study Data Collection Form, Version 1

Appendix 2

ABC-HEART

The Association between Bone and Cardiovascular Health After Renal Transplantation

Department of Renal Medicine

Cork University Hospital

Baseline Visit. **Date:** ___ /___ /____

Surname: **First Name:**

DOB: ___ /____ /____

MRN: _____

Study Number: A B C ___ ___ ___

Eligibility Criteria:

1.	> 18 years	Yes	No	
2.	4 months – 12 years post transplant	Yes	No	
3.	eGFR > 30mls/min/1.73m^2	Yes	No	_____ml/min/1.73m^2

Informed Consent Signed Yes No

Renal History:

1.	Cause of ESKD			
2.	Date of Diagnosis of CKD	____ / ____ / ____		
3.	Start Date Dialysis	____ / ____ / ____		
4.	Modality	Haemodialysis		Peritoneal Dialysis
5.	Date Of Transplant	____ / ____ /____		
6.	Deceased Donor	Yes	No	LRD
7.	Parathyroidectomy	Yes	No	
	Date:	____ / ____ / ____		
	Implant:	Yes	No	

31-Jan-08

Sinead Kinsella

Appendix 2

Medication: List all including dose. **Specifically:**

1.	Calcium Supplements	Yes	No
2.	Vitamin D analogue	Yes	No
3.	Bisphosphonate	Yes	No
4.	Statin	Yes	No
5.	Cinacalcet	Yes	No

Height (cm):_____ Weight (kg):_____ BMI: _____

Post Menopausal Yes No
Age at Menopause: _____

Cardiovascular Risk Factors:

1.	Smoker	Current	Former	Never
2.	Family History (First Degree Relative <70 yrs)		Yes	No
3.	Hypercholesterolaemia		Yes	No
4.	Diabetes Mellitus		Yes	No
		Type 1	Type 2	
		PreTransplant	PostTransplant	

Cardiovascular History:

				Most Recent:	
1.	Current Angina	Yes	No		
2.	Myocardial Infarction	Yes	No	When	Where
3.	Angioplasty/Stenting	Yes	No	When	Where
4.	CABG	Yes	No	When	Where
5.	Stroke/TIA	Yes	No	When	Where
6.	PVD	Yes	No		
	Revascularisation	Yes	No	When	Where
	Amputation	Yes	No	When	Where

Neoplastic History:

Non Skin Malignancy	Yes	No
	Pre Transplant	Post Transplant
Skin Malignancy	Yes	No
	Pre transplant	Post Transplant

31-Jan-08

Sinead Kinsella

Appendix 2

Bone Health:

1.	Previous DEXA	Yes	No
2.	Osteoporosis	Yes	No
3.	Fracture	Yes	No
	Site		
	Approx Date	___ /___ /____	
	Where X Ray Done		
	Low Impact	Yes	No

Procedures:

Visit One:

DEXA Done	Yes	No	Due Date ___ / ___ / ___
Plain X Ray Done	Yes	No	Due Date

Visit Two: Date: ___ /___ /_____

1.	Patient Fasting	Yes	No
2.	Blood for Future Genetic Analysis	Yes	No

Blood samples:

1.	Bone Biomarkers	Yes	No
2.	Renal Function	Yes	No
3.	Calcium/Albumin/Phosphate	Yes	No
4.	PTH	Yes	No
5.	Bicarbonate	Yes	No
6.	CRP	Yes	No
7.	Troponin T	Yes	No
8.	Fasting Cholesterol/Lipid Profile	Yes	No

Urine samples:

1.	Bone Biomarkers	Yes	No
2.	Protein/Creatinine Ratio	Yes	No

Appendix 2

Pulse Pressure (mmHg):

Value One _____ / _____

Value Two _____ / _____

Value Three _____ / _____

PWV Performed Yes No

Result

Signed: **Date:** ___ /___ /____

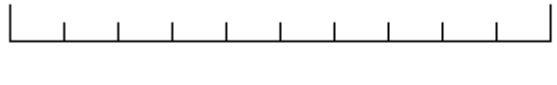
Appendix 3: Parathyroid Assessment of Symptoms Questionnaire, Version 1

Appendix 3 Surname: MRN:
First Name: Date: - - / - - / - - - -

Parathyroid Assessment of Symptoms.

Please answer the following questions by placing a vertical mark on the scale provided. 0 means not having the symptom and 100 is having the symptom in the worst state imaginable.

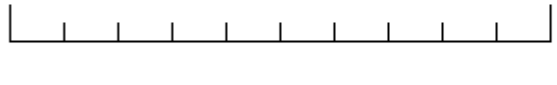
1. Do you have pain in your bones? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

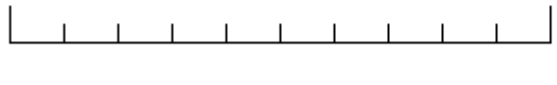
2. Do you feel tired easily? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

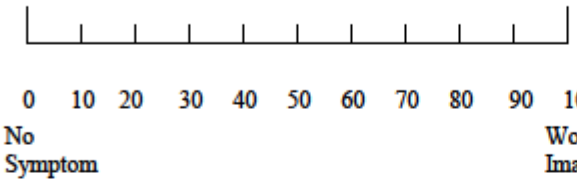
3. Do you have mood swings? Please place a mark on the line below to indicate how bad it is today.



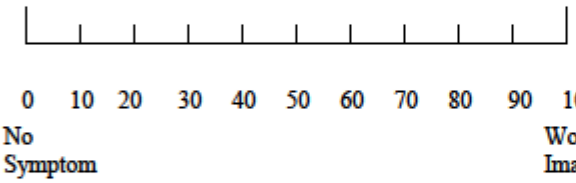
0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

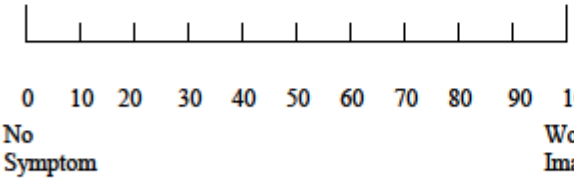
4. Do you feel "blue" or depressed? Please place a mark on the line below to indicate how bad it is today.



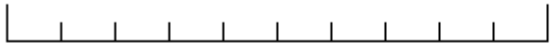
5. Do you have pain in your abdomen? Please place a mark on the line below to indicate how bad it is today.



6. Do you feel weak? Please place a mark on the line below to indicate how bad it is today.



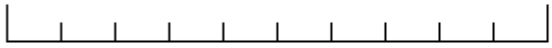
7. Do you feel irritable? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

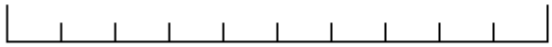
8. Do you have pain in your joints? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

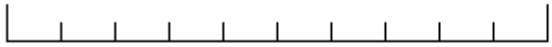
9. Are you forgetful? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

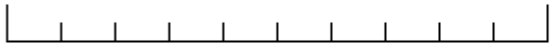
10. Do you have difficulty getting out of a chair or car? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

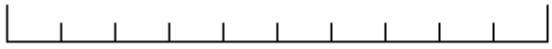
11. Do you have headaches? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

12. Do you have itchy skin? Please place a mark on the line below to indicate how bad it is today.

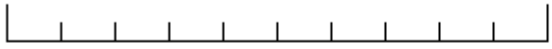


0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

Surname: MRN:
First Name: Date: - - / - - / - - - -

13. Do you feel thirsty? Please place a mark on the line below to indicate how bad it is today.



0 10 20 30 40 50 60 70 80 90 100

No Symptom — Worst Imaginable

Appendix 4: DXA scan request form and questionnaire

BONE DENSITY UNIT
CORK UNIVERSITY HOSPITAL
WILTON
CORK
PHONE: 021-4922549
FAX: 021-4922102

NB: IF REFERRAL IS NOT FILLED OUT IN FULL, APPOINTMENT WILL NOT BE MADE.

REFERRAL
Dual-Energy X-Ray Absorptiometry (DEXA)

(Block Capitals)

Surname: _____

First Names: _____

Address: _____
_____
_____
_____

Tel. No: _____

Date Of Birth: ____/_____/_____

Sex: M ¯ F ¯

Post Menopausal ¯
Approx Age: ____

L.M.P: ____/____/____

Family History of Osteoporosis:
Yes No

Clinical Information:
_____
_____
_____

Medication:

Steroid: Yes ¯ No ¯ Oral ¯ Inhaled ¯
Dose: ____ Duration: _____

Biphosphonates Yes No
Didronel Fosamax Actonel

Calcium / Vitamin D: Yes No
(Ideos ¯ Calcichew D3 ¯ Sandocal ¯ Other ¯)
Name: _____

HRT: Yes No Duration of HRT: _____
Evista: Yes ¯ No ¯
Contraception: Yes No Type: _____

Fracture History: _____

Hip Replacement: Yes No

Previous Scan: Yes ¯ No

Referring Doctor: _____

Surgery Address: _____
_____
_____

Medical Card No:
_____

Expiry Date: ____/____/____

MRN: _____