Chapter 1

General introduction and outline of thesis

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Based in part on:


Introduction

The invention of hemodialysis, during the second world war by Willem Kolff, turned out to be a major breakthrough for the treatment of end stage renal disease, a condition up until that moment universally fatal\(^1\). However, initially this technique was considered not to be suitable for chronic application, mainly because of the repeated necessity of gaining access to the blood stream. This hurdle was subsequently overcome by the pioneering work of Belding Scribner creating the first arteriovenous access suitable for long-term dialysis\(^2\). Since then, hemodialysis has evolved to a widespread utilized technique, which has extended lives for many patients for many years, providing reasonable uremic control. However, large cohort analyses revealed a still unacceptably high mortality rate for patients on dialysis, as compared to the general population\(^5\). Observations like this led to attempts to further improve uremic control, as quantified by Kt/V\(_{\text{urea}}\). Unfortunately, the HEMO trial, failed to demonstrate improved outcome by either aiming at higher Kt/V or using high-flux dialysers in order to improve clearance of higher molecular weight uremic toxins\(^6\). Also, enthusiasm for frequent nocturnal hemodialysis has been tempered by the inability to prove superiority in terms of clinical outcome\(^7\).\(^8\).

The apparent limited ability of hemodialysis to improve outcome for patients suffering end-stage renal disease (ESRD) can have several explanations. First it is possible that the treatment itself adds pathology, for instance by procedure-related complications, or by the induction of micro-inflammation due to bio-incompatibility, adding to cardiovascular risk\(^10\). However, the evidence for the latter is weak, and not performing dialysis is not an option for obvious reasons. Also, the overwhelming causes of death in ESRD are not hemodialysis procedure-related (USRDS annual report 2010). Second, the retention of several uremia-related toxins are not removed by dialysis and may contribute to morbidity and mortality\(^11\).

A conceptual different explanation for the excess dismal outcome, despite sophisticated dialysis equipment and schedules, is the recognition that kidney-disease related comorbidity may have other causes, apart from the retention of potentially toxic uremic substances. Proteinuria\(^12\),\(^13\), disturbances of erythropoiesis\(^14\), blood pressure and volume regulation, oxidant stress and inflammation\(^15\)-\(^17\), and several deranged metabolic pathways may all be involved, and thus potential targets for treatment, on top of uremic control. Among the most prominent pathologically changed systems in ESRD are bone metabolism,
and homeostasis of calcium, phosphate, parathyroid hormone (PTH) and vitamin D. Indeed, large observational studies in dialysis populations consistently demonstrated increased all-cause mortality, and cardiovascular mortality and morbidity, associated with indicators of this system. A suggestion for a causal relation between these biochemical markers and cardiovascular disease comes from the observation that sustained control of levels of calcium, phosphate and PTH is associated with better outcome, as compared to no, or poor control. The associations found between these laboratory values, bone disease and cardiovascular pathology have led to defining this as a distinct clinical syndrome, named chronic kidney disease-mineral and bone disease (CKD-MBD). All the above-mentioned consequences of CKD are present in more early stages of this disease as well. Therefore, it was not surprising that a huge cohort analysis indisputably identified CKD, as defined as a decline in estimated glomerular filtration rate (eGFR), as an independent risk factor for all-cause and cardiovascular mortality, after correcting for a wide range of possible confounders, including Framingham risk factors for cardiovascular disease. In fact, it became clear that for the majority of patients suffering CKD their risk for mortality exceeds the risk for progression of CKD to dialysis-dependent renal failure. This mortality-risk obviously is also age dependent, and the interacting effect of age on these two competing risks was recently clarified, as shown in figure 1. This figure demonstrates that the GFR above which the mortality risk outweighs the risk of

Figure 1: The competing risk of death and development of ESRD, depending on age. Reproduced with permission from O’Hare et al.

ESRD declines with age, and that that GFR is quite low for all age groups. The implication of these observations is that therapeutic interventions for CKD-patients should not only be
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focused on slowing deterioration of kidney function, but also on the prevention of cardiovascular disease.

As in ESRD, levels of calcium, phosphate, PTH and 25-hydroxycholecalciferol (25vitD), are frequently abnormal in more early stages of CKD\textsuperscript{29}. Evidence that these abnormalities may contribute to dismal clinical outcome mainly comes from studies in the general population.

Low levels of 25vitD have been associated with peripheral arterial disease, myocardial infarction, cardiovascular and all-cause mortality in the general population\textsuperscript{30-33}. Elevated levels of phosphate, and even levels in the high-normal range, also are associated with increased cardiovascular disease in the absence of overt kidney disease\textsuperscript{34,35}. Therefore, almost all guideline on CKD-MBD advocate treating all laboratory abnormalities of CKD-MBD in both ESRD and CKD. The recent KIDIGO guideline on diagnosis and treatment of CKD-MBD acknowledges the frequently poor level of evidence, i.e. mainly observational studies, and formulates several “guidelines” as suggestions\textsuperscript{36}. Although plausible biological mechanisms exist by which CKD-MBD may lead to cardiovascular disease, as will be discussed briefly, caution is warranted, especially when treatments itself could be harmful. Treatment of hyperhomocysteinemia, hypercholesterolemia, and anemia in CKD, based on sound pathophysiological principles, failed to demonstrate improved outcome\textsuperscript{37-40}. Recent trials even question the role of too aggressive reduction or prevention of proteinuria, though undisputed for renal protection\textsuperscript{41}, as cardioprotective manoeuvre\textsuperscript{42,43}. An example more closely related to CKD-MBD is the observation that the use of the calcimimetic cinacalcet in CKD stages III and IV, led to a worrisome decline in calcium-levels and increased phosphate level\textsuperscript{44}. In contrast to these well-documented side effects, its purpose, reducing PTH in predialysis, is not a firmly established treatment goal\textsuperscript{45}. The wide gap that exists between detailed knowledge from observational data and biological mechanisms on the one hand, and the lack of data establishing causal relationships and treatment effects on the other, warrants caution, additional studies, and a scrupulous interpretation of available data.

**Vitamin D**

The nomenclature used for several metabolites of vitamin D can be confusing. Since chemical structure, plasma concentration, physiological properties and behaviour in disease differ between compounds it is important that the same name is used for the same molecule\textsuperscript{46}. Native vitamin D is the product from ultraviolet B light, formed in the skin,
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mainly from the sun\textsuperscript{47}, and its chemical name is cholecalciferol. After passage through the
liver it is hydroxylated to 25-hydroxycholecalciferol, named calcidiol, and abbreviated as
25\textit{vit}D. This is the main circulating compound, bound to vitamin D binding protein (DBP) and
available to all tissues. In the kidney a second hydroxylation step takes place, forming 1,25-
dihydroxycholecalciferol, named calcitriol, and abbreviated as 1,25\textit{vit}D\textsuperscript{48}. Its plasma levels
are a thousand fold lower compared to 25\textit{vit}D, and it binds to the same DBP. Almost all
biological activity comes from 1,25\textit{vit}D\textsuperscript{49}.
Declining levels of 1,25\textit{vit}D developed early in the course of progressive renal disease\textsuperscript{29}, and
have been thought to be mainly the consequence of declining renal capacity to hydroxylate
its parent compound. In severe nephrotic syndrome, 25\textit{vit}D losses, bound to DBP can be high\textsuperscript{49,50}. This deficiency contributes to the development of secondary hyperparathyroidism,
hypocalcemia and renal osteodystrophy\textsuperscript{51}. For this reason, supplementation of an active
form of vitamin D, have been applied for decades to treat or ameliorate these complications.
Results from several large observational studies changed the scope entirely\textsuperscript{52-56}. These
studies, collectively covering over 100.000 patients, consistently demonstrated a survival
benefit for dialysis patients taking any form of active vitamin D. Unfortunately, a prospective
trial has never been performed. These results pointed to effects of active vitamin D that go
beyond its traditional effects on bone and calcium homeostasis\textsuperscript{57}. These so-called pleiotropic
effects may play a role in myocardial hypertrophy\textsuperscript{58}, arterial wall function\textsuperscript{59}, and regression
of proteinuria\textsuperscript{60,61}, possibly mediated through the inhibitory effects of active vitamin D on
renin expression\textsuperscript{62}. The pleiotropic effects may be even more widespread, giving the
distribution of the vitamin D receptor\textsuperscript{57}, and may also positively affect immunity\textsuperscript{63}, insulin
sensitivity\textsuperscript{64}. On top of that 25\textit{vit}D deficiency is associated with the metabolic syndrome in
the general population\textsuperscript{65}. All these effects may be involved in the apparent survival benefit
for hemodialysis patients on an active form of vitamin D therapy. However, despite all these
data, definitive proof of benefit for the use active vitamin D is still lacking\textsuperscript{45,66,67}.
Deficiency of 25\textit{vit}D also is frequently encountered in chronic kidney disease\textsuperscript{68,69}, and is also
related to all cause mortality and heart disease, independent from levels of 1,25\textit{vit}D\textsuperscript{33,70}.
This possibly points to the ability of non-renal tissues to convert 25\textit{vit}D to its active form
1,25\textit{vit}D by local expression of 1\textalpha;-hydroxylase\textsuperscript{71}. This locally produced 1,25\textit{vit}D can act in an
autocrine fashion, regulating a wide range of genes\textsuperscript{72}. Since this local production is
dependent on levels of its substrate, being 25vitD from the circulation, adequate levels of the latter are mandatory. For CKD patients a level of 30ng/ml is recommended\textsuperscript{46,69}.

**Parathyroid hormone**

Levels of PTH increase as CKD progresses, leading to a high prevalence of secondary hyperparathyroidism (SHPT) in patients reaching ESRD\textsuperscript{73}. Hypocalcemia, vitamin D deficiency and hyperphosphatemia all are physiological stimulants for PTH secretion\textsuperscript{73}. On top of that, during CKD the expression of the VDR, the calcium-sensing receptor and klotho on parathyroid glands declines, thereby abolishing the inhibitory effects of 1,25vitD, calcium and fibroblast growth factor 23 (FGF23) on PTH production and secretion\textsuperscript{74-78}. All these factors lead to hyperplasia of the parathyroid glands, which, once established, is hardly reversible\textsuperscript{79}. Originally, PTH was considered to be an indicator of renal bone disease, but especially with advanced CKD, PTH performs poor as a predictor of bone histology\textsuperscript{80}. When considered along with levels of alkaline phosphatase, these markers show better predictive value for bone histology\textsuperscript{81}. In ESRD, PTH turned out to be associated with mortality, and this association, after multivariable adjustment, showed a U-shaped curve for studies using both cross-sectional values and time-varying values\textsuperscript{19,20,82,83}. Unfortunately, no consistency exists across these studies about the optimum level of PTH in ESRD, and for that reason, the KDIGO guideline could only suggest to prevent PTH levels from moving outside extreme ranges\textsuperscript{96}. Apart from the epidemiological association that exists between levels of PTH in SHPT and mortality, two additional lines of evidence suggest a role for PTH in the causal pathway to dismal clinical events, including all-cause mortality. First, when considering the role of PTH on outcome, in patients with primary hyperparathyroidism, lacking potential uremic confounders increased mortality exists, mainly because of cardiovascular complications\textsuperscript{84} and left ventricular hypertrophy\textsuperscript{85}. Second, as the PTH receptor is present on cardiomyocytes and vascular smooth muscle cells (VSMC)\textsuperscript{86}, and its activation leads to changes in function of these cells, such as disturbed calcium and energy handling, a possible biological mechanism exists.

Treatment options for SHPT are preventing hyperphosphatemia and hypocalcemia, correction of 25vitD deficiency\textsuperscript{87,88}, use of active vitamin D compounds\textsuperscript{89-92}, initiating the calcimimetic cinacalcet (only for ESRD)\textsuperscript{93-95}, combinations of cinacalcet with active vitamin D compounds\textsuperscript{96} (also used in the IMPACT trial presented at ASN 2011), or surgery\textsuperscript{97}. Whether
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or not surgical or pharmacological improvement in PTH levels will translate into improved clinical outcome is at present unknown, and is addressed in the ongoing EVOLVE trial. A post-hoc analysis did suggest improved mortality when PTH is controlled using cinacalcet.

**Phosphate and calcium**

Phosphate homeostasis is almost entirely regulated by renal excretion. Renal excretion in turn, is regulated at the tubular level, where variable amounts of ultrafiltered phosphate from the glomeruli is reabsorbed. The amount of ultrafiltered phosphate at the glomerular level is rather fixed by the GFR. The tubular reabsorption is mainly accomplished in the proximal convoluted segment via NaPi2 sodium-phosphate co-transporters, present in the luminal membrane of tubular cells. The abundance of the phosphate channels is regulated, and thus phosphate homeostasis is accomplished. The expression of NaPi2a at the luminal side of the proximal tubule is under hormonal control of PTH, FGF23 and possibly klotho, but the exact mechanism of this has not been clarified.

![Graph](image.png)

**Figure 2:** The association between levels of serum phosphate and relative risk of death in dialysis-patients. Reproduced with permission from Block.

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Given the central role of the kidney in phosphate homeostasis, hyperphosphatemia develops in all patients with progressive CKD, typically starting with GFR below 45-30 ml/min\(^2^9\). At higher GFR single nephron phosphate excretion increases, under the influence of early rises in FGF23\(^1^0^7\). Elevated phosphate is the single most important inducer of uremic arterial calcification\(^1^0^8,1^0^9\). Detailed knowledge of this process has been elucidated, showing phosphate-induced transdifferentiation of vascular smooth muscle cells (VSMC) to osteoblastic cell types and the induction of apoptosis of VSMC that along with excreted vesicles form nuclei for matrix calcification. Hyperphosphatemia also leads to a locally changed profile of mediators that facilitate calcification, like upregulated osteopontin, alkaline phosphatase, and downregulated inhibitors like MGP\(^1^1^0\). This process likely is more enhanced by decreased levels of fetuin A\(^1^1^1\), and inactive forms of MGP\(^1^1^2,1^1^3\). Of all biochemical markers of CKD-MBD the association with mortality and cardiovascular morbidity is by far the strongest for phosphate throughout the entire spectrum of stages of CKD\(^2^0,3^4,3^5,4^5,8^2,1^1^4\), as shown in figure 2 for patients on dialysis. For this reason, guidelines suggest to aim for phosphate levels in the normal range for all stages of CKD, even though this appears unattainable for the majority of dialysis patients\(^2^1,3^6\). Restriction of dietary phosphate intake is an important measure, both in early stage CKD and in ESRD\(^1^1^5,1^1^6\). Calcium containing binders, sevelamer, lanthanumcarbonate and aluminium-based binders all have been proven to be efficacious in lowering phosphate levels\(^1^1^7\). The only study with clinical endpoints comparing calcium-based phosphate binder therapy with sevelamer was negative on its primary endpoint (all-cause mortality after 2 years), but suggested advantages for the sevelamer arm for patients older than 65 years\(^1^1^8\). In this study overall mortality was lower than expected, the dropout rate was high and an on-treatment analysis was performed, all making results difficult to interpret. A smaller study did find a survival advantage for sevelamer, but the study was not designed for that endpoint\(^1^1^9\). This study did show attenuated arterial calcification, confirming previous findings\(^1^1^9,1^2^0\). Other studies, controlling for changes in lipid profile found no differences between phosphate binder type on calcification score\(^1^2^1,1^2^2\). Extended dialysis duration does provide good control of phosphate, but this has not been translated in proven improvements in clinical outcome\(^7\). Compared to phosphate the role of calcium is less clear, although being associated with dismall outcome as well\(^1^9,8^2,1^2^3\). A sustained hypercalcemia in CKD is a less frequent finding compared to hyperphosphatemia or elevated levels of PTH\(^2^1\). However, its role may be
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underestimated because transient and unnoticed periods of hypercalcemia may be important inducers of vascular pathology\textsuperscript{124,125}. To make things even more complex, apart from systemic transient hypercalcemia, also local hypercalcemia in the arterial wall, as a consequence of VSMC apoptosis or locally formed calcium-containing nanocrystals, can initiate and exacerbate pathological changes\textsuperscript{126,127}. From a clinical perspective this observation is of importance, because these locally increased levels of calcium are not likely to be influenced by adaptations of dosing of either calcium-containing phosphate binders or vitamin D. In conjunction with phosphate, calcium has been implicated in the process of VSMC transdifferentiation\textsuperscript{110}. On the other hand, signaling of the calcium-sensing receptor (CaSR), present on VSMC, was shown to halt the process of calcification, possibly via effects on matrix Gla protein (MGP)\textsuperscript{128}. Apart from its effects on PTH, this may be a beneficial effect of calcimimetics and of clinical importance.

**Novel markers of CKD-MBD**

The discrepancy between the linear relation of the added mortality risk due to progressive renal failure\textsuperscript{24} and the rather late in the course of CKD developing plasma changes of calcium, phosphate and PTH\textsuperscript{29}, suggests that some hidden processes may be responsible for this early increased risk. Possible candidates are not necessarily directly related to the syndrome of CKD-MBD, and inflammation and early increased oxidant stress may play an important role\textsuperscript{129}. A recent review by the Cochrane group on the efficacy of inhibitors of the renin-angiotensin system in early non-diabetic CKD found no evidence for cardiovascular protection of this intervention\textsuperscript{130}. There are possible candidates that are related to CKD-MBD that can be involved in this early increased risk. As discussed above, an early decline in GFR requires tubular adaptations to maintain phosphate homeostasis, and this is accomplished by the actions of fibroblast growth factor (FGF)-23\textsuperscript{105}. This bone-derived hormone in turn has been associated with increased mortality, not only in hemodialysis patients\textsuperscript{131}, but also in more early stages of CKD\textsuperscript{107,132-134}. In all these cohort, FGF23 was independently positively associated with clinical endpoints, including all-cause and cardiovascular mortality and progression of CKD. Multivariable adjustments did not attenuate the predictive value of FGF23 as an important risk factor. An argument against FGF23 being a direct culprit for the increased cardiovascular risk is the recent observation that in the general population it is associated with a wide range of established cardiovascular risk factors, like age, body mass
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index, smoking and hypertension\textsuperscript{135}. Therefore, it cannot be excluded, that FGF23 modifies
the detrimental effects of these risk profiles instead of being a risk factor on its own.

Another important argument against FGF23 as a causative agent is the lack of a presumed
mechanism by which it may exert its presumed detrimental effect. In two human study it
was suggested that FGF23 associates with endothelial function, but these study did not
prove a direct effect\textsuperscript{136,137}. A recent study in mice however, did show beyond doubt that

FGF23 directly induces left ventricular hypertrophy\textsuperscript{138}. Another remarkable finding from that

study was that these effects were entirely klotho-independent, while up until this study

klotho was considered a prerequisite for FGF23 signal transduction via FGF receptors\textsuperscript{139}. If

this finding can be reproduced in human, then FGF23 indeed could be an important target

for therapy.

A second possible highly important protein involved in CKD-MBD is klotho. Originally,

mutation of the gene encoding klotho led to a premature aging phenotype in mice\textsuperscript{140}, and

since then, numerous effects that may explain this aging process have been revealed. As

mentioned, klotho is necessary for FGF23 signaling for all described human effects of FGF23

so far\textsuperscript{141}. Klotho exists as a membrane-bound protein, but also as a humeral factor in both
circulation and urine. In urine it interferes with calcium and phosphate handling\textsuperscript{106,142}. In the
circulation, klotho protects against calcification\textsuperscript{143}, is of importance for endothelial cell
calcium handling\textsuperscript{144}, and plays a role in protection against oxidant stress\textsuperscript{145}.

Conclusion

Taking together, in the field of CKD-MBD randomized trials on meaningful clinical endpoint

are sorely lacking. Most clinical trials have focused on intermediate endpoints. In addition,

there is a lack of data that describe how knowledge from these clinical trials have been

integrated into everyday clinical practice. Apart from that, it remains doubtful if “optimal

management” of the traditional markers of CKD-MBD, i.e. calcium, phosphate and PTH

levels, and vitamin D metabolism, will completely alleviate the added risk that is associated

with disturbances in these biochemical markers of CKD-MBD. For this reason, ongoing in-

depth research on mechanisms, tools to modulate these mechanism and clinical trials

targeting the old and new players in the field of mineral and bone metabolism with clinical

endpoints are both necessary and thrilling challenges for the next years.
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Outline of the thesis

The studies in this thesis aim to optimize current treatment of CKD-MBD in part one, and to delineate future directions in part two. As the evidence is growing that CKD-MBD is tightly connected to especially cardiovascular disease, by far the most prevalent complication of kidney disease, the ultimate goal is to target these devastating consequences.

Part I: Optimizing current treatments

In chapter 1 we critically appraise the methodology used in several studies that suggested mortality benefit form usage of active vitamin D compounds in patients on dialysis. As mentioned, all these studies were historical cohort analysis, and not prospective randomised trials. Translation of these studies into everyday clinical practice is difficult because its design is prone for bias. On the other hand, distrust for complex statistical methods could lead to therapeutic nihilism, and missed opportunity for patient care. We aimed to assist physicians in developing balanced appreciation of these studies.

Cinacalcet treatment for secondary hyperparathyroidism has proven efficacy in improving levels of PTH in several phase III clinical trials. However, there can be huge differences between the well-described population in clinical trials, treated following strict protocols, and everyday clinical practice. For this reason the pan-European ECHO-study was conducted. In chapter 3, we describe the results for the Dutch subgroup of this study. As differences in real-life practice between countries can provide important information about the potential of cinacalcet, we analysed the differences between participating countries in the ECHO-study in chapter 4. Most studies using cinacalcet in ESRD, found that apart from its well-established effects on PTH-levels, a decline of phosphate could be observed as well. Giving the fact that phosphate associates with dismal outcome, and has a sound biological basis underlining its relevance, this observation could be of clinical importance. However, several factors influence phosphate levels as well. Therefore, in chapter 5, we analysed the phosphate-lowering potential of cinacalcet, using the European data-base of the ECHO study.
Part II: Delineating future treatment direction

In the last few years two factors, fibroblast growth factor 23 (FGF23) and klotho, have emerged as important regulators of calcium, phosphate, PTH, and vitamin D metabolism. In chapter 6 we summarize current knowledge on function, regulation and potential clinical impact of these two new players in the field of CKD-MBD. However, research and even clinical application of newly discovered circulating substances often precedes solid determination of the validity of assays used. Therefore we tested several available assays used for establishing FGF23 levels in chapter 7. FGF23 currently is believed to be the main regulator of phosphate balance, giving its influence on tubular phosphate handling and vitamin D metabolism. However, conflicting data exist, on how FGF23 levels respond to dietary changes in phosphate loading in healthy subjects. Also, several studies used isolated phosphate loading to study these effects instead of meals containing high-phosphate content. The latter probably reflects reality better. Finally, it is unknown if presumed changes in FGF23 in healthy subjects indeed leads to a decline in levels of vitamin D. In chapter 8 we describe a detailed study of FGF23 and vitamin D changes in healthy volunteers, following diets with set amounts of calcium and phosphate.

Just as the abovementioned “classical” markers of CKD-MBD, the assumption that FGF23 associates with clinical endpoints is mainly based on observational data. Therefore, in addition to prospective trials, investigating how FGF23 coincides with established cardiovascular risk markers is mandatory for proper appraising individual weight of these risk factors and to generate hypothesis on how these factors may interact. To that end we analysed the MASTERPLAN cohort using prospectively collected baseline data and stored samples in chapter 9. Anticipating on proof of vitamin D, FGF-23 and klotho being causally related to clinical endpoints, including progression of CKD, we studied the relation between these three markers and other biological systems, with emphasis on the renin-angiotensin system in chapter 10. In this analysis we aimed to identify novel treatment targets for established interventions, and novel interventions for established treatment goals considering these systems.

Finally in chapter 11, results are summarized, and suggestions on how to proceed are made.
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