Nocturnal hemodialysis and kidney transplantation:

From vascular calcification to beyond

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van vaatverkalking tot daar voorbij
(met een samenvatting in het Nederlands)

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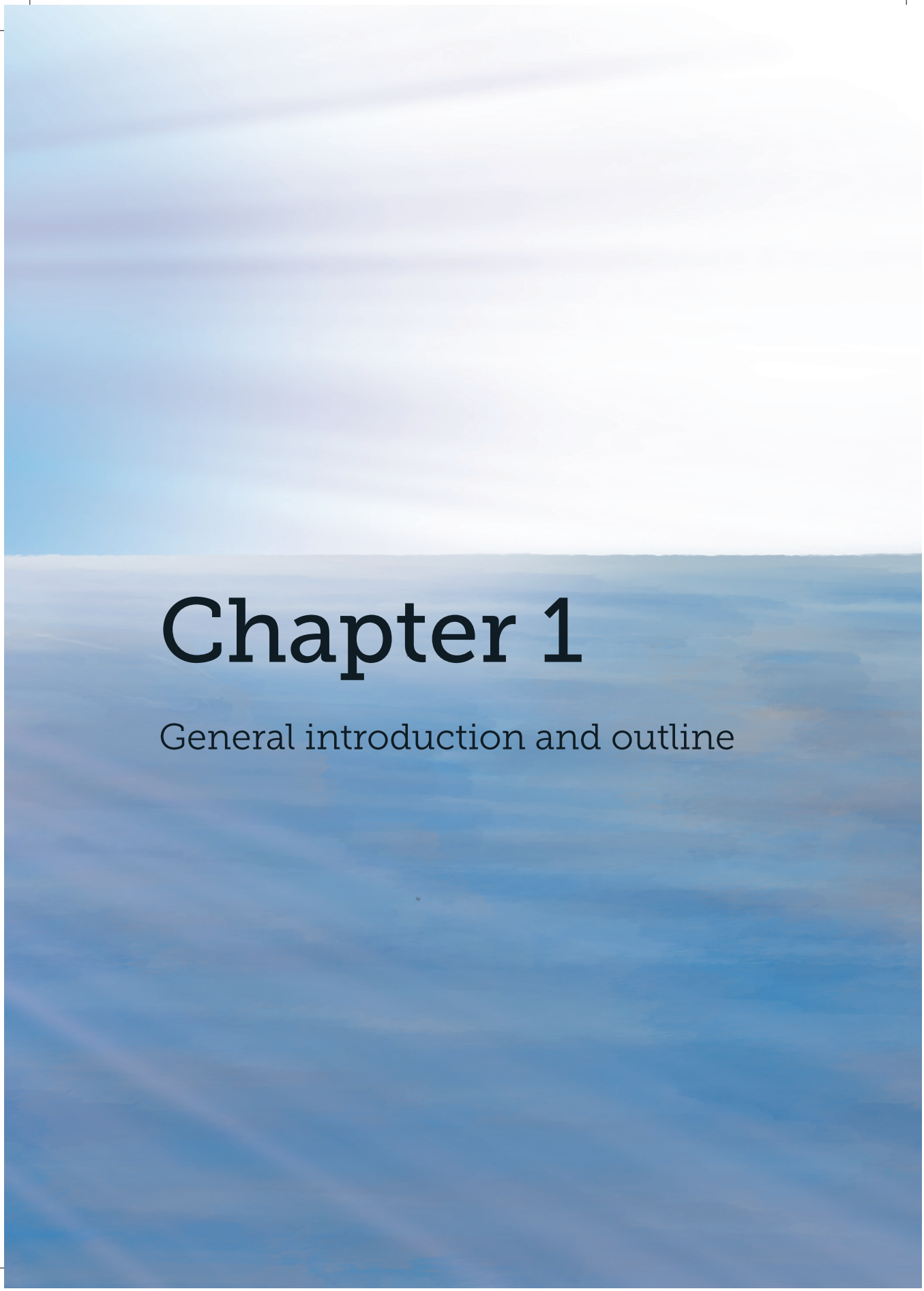

Chapter 1

General introduction and outline

General introduction

Over 17,000 patients in the Netherlands have end-stage kidney disease and require therapy to substitute their native kidney function[1]. This renal replacement therapy can either be done by transplantation of a donor kidney or by dialysis. Broadly, there are two types of dialysis: peritoneal dialysis, which can be performed at home and uses the peritoneum as a membrane to filter out fluid and waste products from the blood; and hemodialysis, which involves filtering the blood with a machine. Hemodialysis is usually delivered 3x 4 hours per week in a dialysis center, but may also be delivered more frequently during longer sessions at home, such as in nocturnal hemodialysis.

Unfortunately, mortality and morbidity among patients with end-stage kidney disease are extremely high, with 5-year survival rates of 88-94% among kidney transplant recipients and of as low as 42% among patients on dialysis[1]. The leading cause of death is cardiovascular disease, accounting for over 50% of deaths[2, 3]. An important role here is supposedly played by the disturbed mineral metabolism in end-stage kidney disease[4]. At the same time, the disturbed mineral metabolism in patients with end-stage kidney disease affects the bone. Patients with end-stage kidney disease have high fracture rates, with for example hip fracture rates four times higher compared to the general population[5]. Nevertheless, several aspects and consequences of the disturbed mineral metabolism in end-stage kidney disease are poorly understood. Furthermore, the disturbed mineral metabolism may be influenced by different renal replacement therapies. Therefore, this thesis will study how end-stage kidney disease and different renal replacement therapies impact the vessels, the bone, quality of life and mortality.

End-stage kidney disease and the vessels: vascular calcification

The high cardiovascular mortality in end-stage kidney disease is strongly associated with vascular calcification[6, 7]. Since the 2000s, studies with computed tomography (CT) have shown that vascular calcification occurs frequently and progresses rapidly in patients with end-stage kidney disease, even at a young age[3]. Vascular calcification in end-stage kidney disease is often of a different nature than in the general population. In the general population, vascular calcification is mostly found in the intimal layer of the vessel wall[8]. This type of calcification develops in atherosclerotic plaques, which may cause vessel stenosis and tissue ischemia (e.g. myocardial infarction)[9]. Indeed, coronary artery calcification in the general population is strongly associated with risk of coronary artery disease[10] and mortality[11].

In end-stage kidney disease, vascular calcification not only occurs in the intimal, but also in the medial layer of the vessel wall (Figure 1). This type of calcification is already present in the early stages of chronic kidney disease[12]. Medial calcification may result from disturbances in mineral metabolism, notably hyperphosphatemia[13], which frequently occurs in patients on dialysis. Contrary to what was previously thought, phosphate and calcium do not precipitate passively; rather, medial calcification is an actively regulated process, involving transdifferentiation of vascular smooth muscle cells to osteoblast-like phenotypes, and inhibition of calcification by multiple regulatory molecules[14].

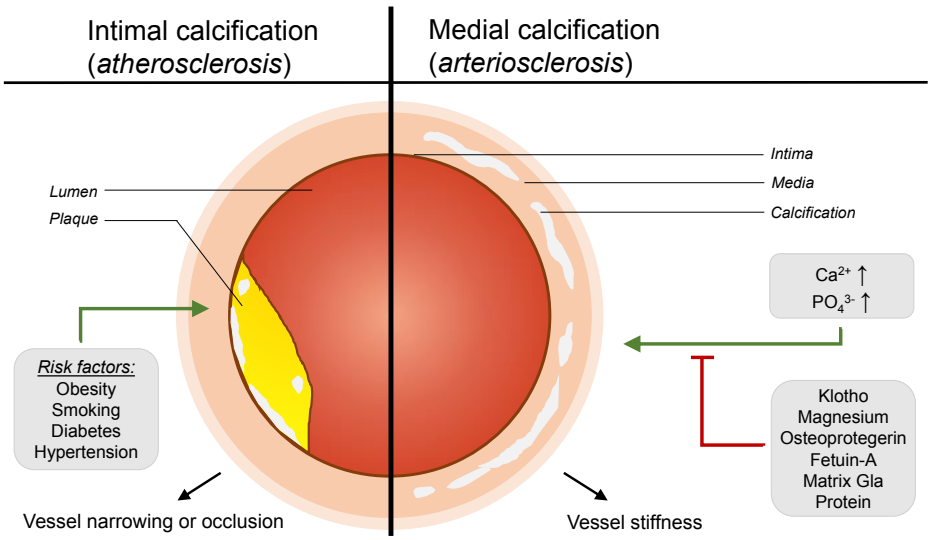

Figure 1. In end-stage kidney disease, vascular calcification (grey) may occur in the intimal (left panel) and medial layer of the vessel wall (right panel). These types of calcification have different causes and consequences. For example, medial calcification is driven by high calcium (Ca^{2+}) and phosphate (PO_4^{3-}) levels and is regulated by several molecules indicated in the lower right corner. On the other hand, intimal calcification has different risk factors, outlined in the lower left corner.

A study that assessed medial calcification on X-ray based on visual appearance indicated that medial calcification is independently associated with mortality in end-stage kidney disease[7]. Nevertheless, it is incompletely understood by which mechanisms. Medial calcification does not cause vessel occlusion but instead leads to vascular stiffness and increased pulse pressure. It has been hypothesized that this may result in left ventricular hypertrophy and heart failure[15]. Current imaging such as CT cannot reliably distinguish between intimal and medial calcification, which has impeded clinical studies into consequences of medial calcification.

The high mortality rate associated with vascular calcification in end-stage kidney disease has caused a growing research interest in factors that may mitigate vascular calcification. Much research has focused on phosphate, an important driver of vascular calcification[16]. Phosphate levels are considerably lower when clearance on dialysis is increased due to frequent (≥4 times per week) and longer treatment (≥8 hours per session, extended hours, e.g. nocturnal hemodialysis)[17]. Furthermore, phosphate levels are even lower and in many cases are no longer elevated when kidney function is restored by a kidney transplant[18]. Nevertheless, it is not known whether increased clearance such as in nocturnal hemodialysis results in less development of vascular calcification compared to conventional hemodialysis (3 times 4 hours per week), or whether restoration of kidney function by a kidney transplant leads to even less development of vascular calcification. In addition, we do not know whether conventional hemodialysis and peritoneal dialysis have a different effect on vascular calcification.

Vascular calcification is inhibited by various regulatory molecules, including Matrix Gla protein. Matrix Gla protein needs to be activated by vitamin K in order to inhibit vascular calcification[14]. However, patients with end-stage kidney disease are often deficient in vitamin K[19]. On top of that, in vitro studies have shown that phosphate binders, i.e. drugs given to prevent high phosphate levels, also bind vitamin K[20, 21], thus potentially limiting vitamin K absorption. Nevertheless, it is not known whether phosphate binder use is associated with worse vitamin K status in patients with end-stage kidney disease. Furthermore, it is not known whether a kidney transplantation is associated with better vitamin K status than conventional hemodialysis.

End-stage kidney disease and the bone: renal osteodystrophy

The disturbed mineral metabolism in end-stage kidney disease also affects bone metabolism, especially in patients on dialysis. A key role here is played by parathyroid hormone. This hormone is produced by the parathyroid glands and stimulates bone remodeling by indirectly stimulating osteoclast cells, cells which break down bone[22]. In end-stage kidney disease, high phosphate levels increase parathyroid hormone secretion, leading to secondary hyperparathyroidism (Figure 2). This results in excessive breakdown of bone tissue and increases fracture risk[23]. However, parathyroid hormone may also be oversuppressed in patients on dialysis. This can be due to relatively high doses of vitamin D analogs[24], calcium-containing phosphate binders, or high calcium concentrations of dialysate[25], which patients often receive in order to prevent secondary hyperparathyroidism. Oversuppressed parathyroid hormone or relatively too low parathyroid hormone due to skeletal resistance to its actions result in low or absent bone formation, i.e. adynamic bone disease. In this state,

physiological bone remodelling and repair do not take place[26]. Bone biopsy studies have shown that most patients on dialysis have some bone disease due to end-stage kidney disease[27-30], collectively termed renal osteodystrophy.

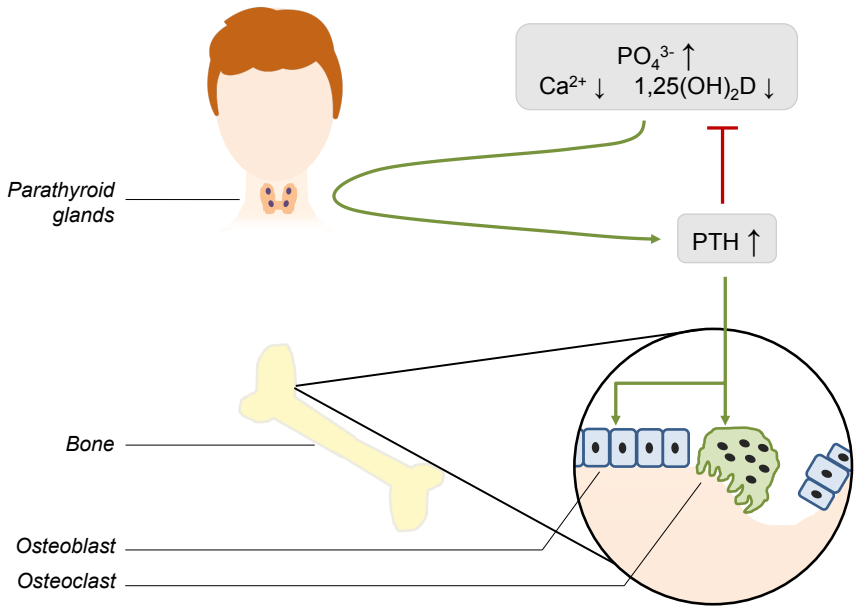

Figure 2. The physiology of parathyroid hormone in bone remodelling. The parathyroid glands secrete parathyroid hormone (PTH) in response to low calcium (Ca^{2+}) levels. Also, high phosphate (PO_4^{3-}) levels and low active vitamin D ($1,25(OH)_2D$) levels stimulate parathyroid hormone secretion. In turn, parathyroid hormone induces bone remodelling by stimulating osteoblasts and osteoclasts, raises calcium levels, raises renal phosphate excretion, and stimulates renal vitamin D activation. In patients on dialysis, high phosphate levels and low active vitamin D levels may induce secondary hyperparathyroidism, causing excessive bone resorption. On the other hand, excessive calcium loading may suppress parathyroid hormone, leading to absent bone remodelling.

Renal osteodystrophy increases the risk of fracture in end-stage kidney disease. The risk of hip fractures is four times higher in patients on dialysis compared to the general population[5]. However, while hip fractures are mostly clinically apparent, vertebral fractures often present atypically[31]. Vertebral fractures are the most common type of fragility fracture[32] but are easily missed on radiographs[33]. Hence, they often remain undiagnosed. Nevertheless, even undiagnosed vertebral fractures negatively impact physical functioning[34], quality of life[35], and mortality risk[36]. The prevalence of vertebral fractures is poorly documented in cohorts and dialysis registries[37] and is

therefore unclear in patients with end-stage kidney disease. Furthermore, it is unclear whether the risk of vertebral fracture can be assessed with parathyroid hormone levels or bone mineral density of the vertebrae.

Quality of life and survival in end-stage kidney disease

Patients with end-stage kidney disease have a poor quality of life. In one study, patients with end-stage kidney disease rated their quality of life even lower than patients with congestive heart failure, chronic lung disease, or cancer[38]. It is not completely understood by which mechanisms end-stage kidney disease impairs quality of life, but several studies have outlined the high symptom burden in end-stage kidney disease, which includes fatigue, pain, muscle cramps, difficulty with sleep, and sexual dysfunction[39]. Furthermore, dialysis therapy is time-consuming, requires rigorous dietary and fluid restrictions, and comes with one of the highest pill burdens in any chronic disease state[40]. This requires extensive lifestyle modifications.

Some renal replacement therapies may allow more freedom in lifestyle than others. Kidney transplantation takes away the need for many of the restrictions accompanying dialysis therapy, and has been shown to substantially improve quality of life and survival[41]. Nocturnal hemodialysis significantly reduces pill burden[42], improves quality of life[43], and may improve survival due to substantially longer hemodialysis sessions[44-47]. Yet, previous studies into the effect of nocturnal hemodialysis on pill burden have been short-term, which disregards that patients may change their lifestyle and diet when they have been on nocturnal hemodialysis for some time. Also, it is unknown how quality of life after kidney transplantation compares to nocturnal hemodialysis. Finally, previous studies into the effect of longer hemodialysis sessions (≥6 hours/session) on survival have been limited to the United States. In general, American patients on hemodialysis more often have diabetes, have shorter dialysis treatment times with higher blood flow rates, and less often use an arteriovenous fistula than European patients[48, 49]. Thus far, no study has looked into the association of longer hemodialysis sessions (≥6 hours/session) with survival in European patients.

Outline of this thesis

The keystone of this thesis is the NOCTx study (Arterial Calcifications in Nocturnal Hemodialysis and Renal Transplantation Versus Conventional Hemodialysis, ClinicalTrials.gov Identifier NCT00950573). NOCTx is a prospective study that measured progression of calcification of the coronary arteries, the vascular bed where vascular calcification is most commonly measured. Its hypothesis was that progression of coronary artery calcification would be less as phosphate levels were lower as a consequence of the type of renal replacement therapy, with less progression hypothesized in nocturnal hemodialysis and

the lowest in kidney transplantation. The NOCTx study therefore investigated patients treated with nocturnal hemodialysis, conventional hemodialysis, peritoneal dialysis, and kidney transplant recipients. Between 2010 and 2016, 181 patients, recruited from eight centers in the Netherlands, underwent cardiac CT scanning and blood sampling, and filled out quality of life questionnaires until up to 3 years follow-up at University Medical Center Utrecht, Utrecht, the Netherlands. Lateral chest radiographs performed as part of routine care were collected.

The main results of NOCTx involve vascular calcification and are discussed in **Part I** of this thesis. First, we give an overview of the current evidence on the effects of different dialysis modalities and kidney transplantation on progression of coronary artery calcification, by systematically reviewing the current literature (*Chapter 2*). Next, we describe the main results of NOCTx: progression of coronary artery calcification compared between nocturnal hemodialysis, conventional hemodialysis, and kidney transplantation (*Chapter 3*); and between conventional hemodialysis and peritoneal dialysis (*Chapter 4*). Finally, we describe the associations of kidney transplantation and phosphate binder use with vitamin K status, a vitamin needed for calcification inhibition, in participants of NOCTx (*Chapter 5*).

In **Part II**, we discuss results of NOCTx regarding the bone. In *Chapter 6*, we report the prevalence and incidence of vertebral fractures as measured on lateral chest radiographs in a subset of participants of NOCTx. In addition, this chapter describes the roles of parathyroid hormone and vertebral bone mineral density.

Finally, **Part III** covers the results of NOCTx and other studies regarding quality of life and survival. First, we report pill burden and several intermediary outcomes in the long term of patients that switched to nocturnal hemodialysis compared to matched patients treated with conventional hemodialysis/hemodiafiltration from the Convective Transport Study (*Chapter 7*). Second, we compare quality of life between participants in the NOCTx study that switched to nocturnal hemodialysis and participants that received a kidney transplant (*Chapter 8*). Finally, we describe the association of longer hemodialysis session duration with survival in a large cohort of European patients on hemodialysis, using data from the European Renal Association – European Dialysis and Transplantation Association Registry (*Chapter 9*).

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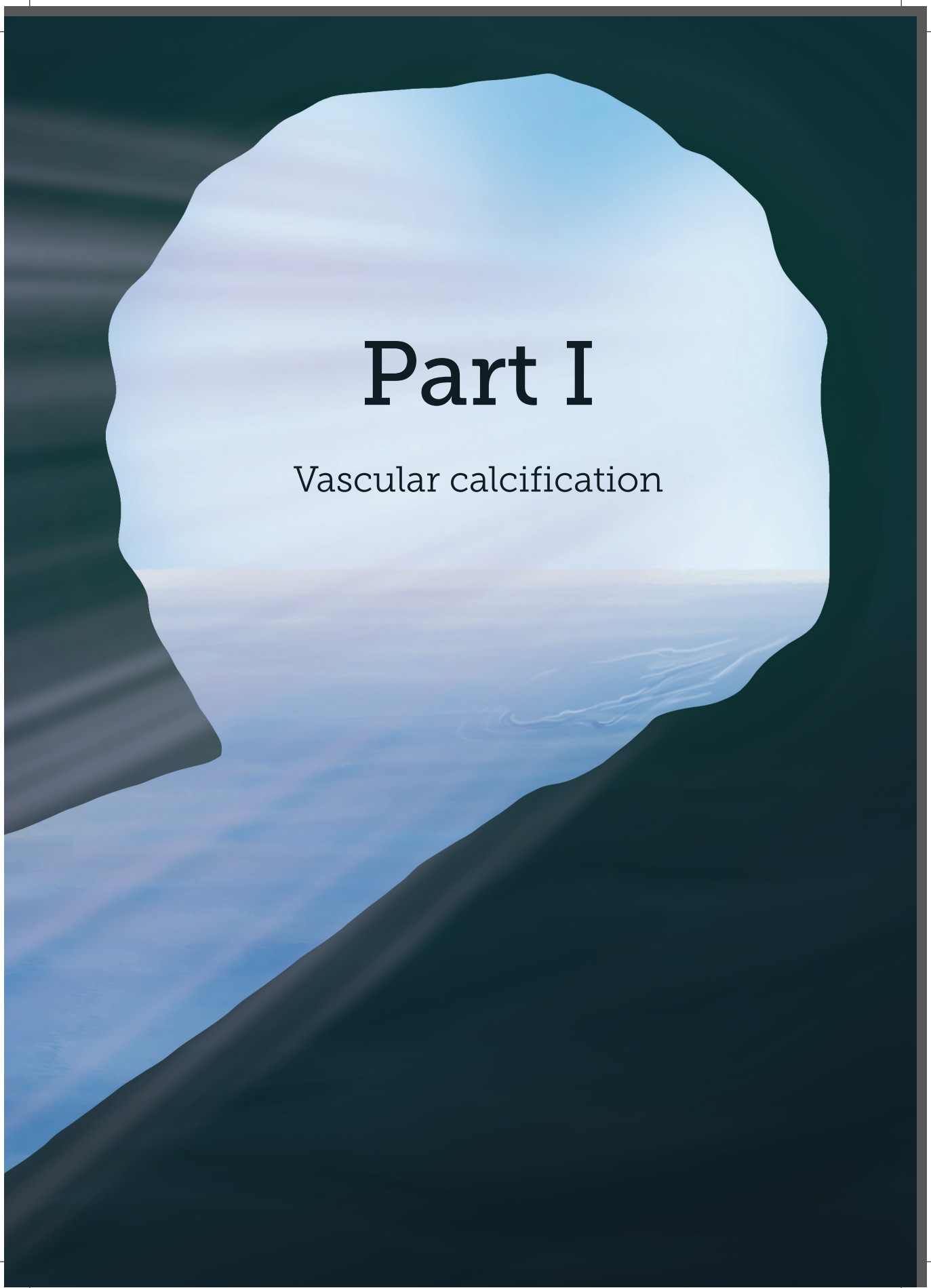

Part I

Vascular calcification

Chapter 2

Is progression of coronary artery calcification influenced by modality of renal replacement therapy? A systematic review

T.T. Jansz, M.C. Verhaar, G.M. London, B.C. van Jaarsveld.
Clin Kidney J. 2018 Jun;11(3):353-361.

Abstract

Background

Progression of coronary artery calcification is an important marker for cardiovascular morbidity in end-stage renal disease patients. We aimed to review the evidence on coronary artery calcification progression in different renal replacement therapies.

Methods

We used the MEDLINE (PubMed), EMBASE and TRIP databases (1999–2016). Additionally, bibliographies were searched by hand and citation tracking of key publications was performed. Prospective studies were included that examined coronary artery calcification with ≥2 multi-slice computed tomography scans ≥6 months apart, in patients 18–75 years old receiving any renal replacement therapy, including kidney transplantation. Reporting of separate scores for different modalities was required. Two researchers extracted data independently with pilot-tested forms and assessed risk of bias using a validated tool.

Results

We identified 29 eligible studies that assessed coronary artery calcification progression in end-stage renal disease patients. 19 studies evaluated hemodialysis, 8 kidney transplantation. Evidence on progression in peritoneal dialysis (3 studies) and nocturnal hemodialysis (1 study) was limited. Meta-analysis was not possible due to diverse reporting methods of coronary artery calcification scores and definitions of progression. Median coronary artery calcification scores were considerably higher in hemodialysis cohorts at baseline, presumably due to a generally higher age and dialysis vintage. Median coronary artery calcification progressed universally. Visual inspection suggested the least progression in kidney transplant recipients.

Conclusions

There is insufficient evidence to compare the influence of renal replacement therapies on coronary artery calcification progression. We advocate the adoption of a standardized reporting method of coronary artery calcification progression.

Introduction

Cardiovascular disease is the leading cause of death in patients with end-stage renal disease (ESRD), accounting for over 50% of deaths[1]. Often, this cardiovascular disease burden is linked to the extensive vascular calcifications observed in ESRD patients. Contrasting with the general population, in which vascular calcifications are confined to atherosclerotic plaques in the intima, vascular calcifications also occur in the tunica media of the arterial wall in ESRD patients[2].

Calcifications of the coronary arteries (CAC) are highly prevalent in ESRD patients, and are associated with clinically overt cardiovascular disease[3]. Although CAC has been established as a predictor of mortality[4], there is an ongoing debate on the implications of CAC progression in ESRD. It has been argued that vascular calcification is merely a healing process, and as such does not play a causal role in cardiovascular disease in ESRD[5]. However, even though there is dearth of evidence to conclusively confirm that CAC progression corresponds with clinical endpoints in ESRD, meta-analytical data on for instance phosphate binders suggest that attenuation of CAC progression is reflected by a reduction in mortality[6-9].

As for the mechanisms by which vascular calcifications may be linked to mortality, calcifications in the ESRD population presumably carry additional risks besides myocardial ischemia through associated vascular stiffness[10, 11], progressive left ventricular hypertrophy, myocardial fibrosis[12], and conductive abnormalities[10]. Although the complex pathogenetic mechanisms have not been fully elucidated, it is tenable that CAC and CAC progression are a portentous sign in patients with in ESRD.

Kidney transplantation is considered the treatment of choice for ESRD, but recipients still suffer from a high cardiovascular risk[1], and CAC is highly prevalent in kidney transplant recipients[13]. Thus far, it has not been delineated whether certain renal replacement therapies (e.g. hemodialysis, kidney transplantation, peritoneal dialysis or intensive forms of hemodialysis such as nocturnal hemodialysis) have different effects on the progression of CAC. Therefore, to examine the comparative influence of different renal replacement therapies on CAC progression, we systematically reviewed prospective studies that assessed CAC in patients treated with hemodialysis, peritoneal dialysis, nocturnal hemodialysis or kidney transplantation.

Methods

We conducted a systematic review according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[14] and a prespecified protocol (CRD42016053649). In short, we included all English-, French-, German-, Dutch- and Spanish-language studies that performed ≥2 repeated CAC score measurements with a scanning interval of ≥6 months in patients receiving any form of renal replacement therapy, including kidney transplantation. Prospective studies (randomized clinical trials, observational cohorts) studying adult patients 18–75 years old with multi-slice computed tomography (MSCT) were included. Studies were excluded if CAC scores for different renal replacement therapy modalities were not provided separately.

We searched the MEDLINE, TRIP, and EMBASE databases for studies published from 1999 to 01 January 2017, as MSCT was introduced in 1999. The following terms were used as MeSH terms (shown in italics) and free text terms: (*chronic kidney failure*, *renal replacement therapy*, *renal dialysis*, *hemodiafiltration*, *peritoneal dialysis*, *home hemodialysis*, *kidney transplantation*, dialysis modality, coronary artery calcification, progression, advance, change, increase, decrease). Last search was run on 31 March, 2017. A complete draft of the search strategy is available as supplemental material. We also hand-searched bibliographies of relevant publications, and used ISI Web of Science for citation tracking of relevant publications.

One investigator (TJ) screened for eligibility based on titles and abstracts. We retrieved the full text of any potentially relevant study. Two investigators (TJ and BJ) reviewed full texts, independently assessed eligibility in a standardized manner, unblinded for author and journal. Each investigator extracted data using a pilot tested form. Data on patient characteristics (including age, sex, dialysis vintage, history of cardiovascular disease, diabetes mellitus, phosphate, calcium, PTH, CRP, creatinine, blood pressure, and BMI), sample size (with second CAC score), type of renal replacement therapy, CAC scores (preferentially in Agatston units[15]) and follow-up duration were extracted. Whenever more than one study provided data from the same cohort population, we included the study with the most complete data. When data were reported in strata, data were pooled when possible, or extracted in separate cohorts. Whenever CAC scores were available at more than one follow-up moment within the same cohort, we used the most complete data on the longest follow-up duration. Studies were excluded when neither CAC scores at follow-up nor other workable measures of CAC progression were provided. Disagreements were resolved by consensus. 28 study authors were contacted for further information, 9 responded and 7 provided data that had not been presented in the original publication.

Risk of bias was assessed with an adaption of the Quality in Prognostic Studies tool (QUIPS)[16]. The QUIPS tool evaluates six domains: study participation, study attrition, prognostic factor management, outcome measurement, study confounding and statistical analysis and reporting. A summary judgment of low, moderate or high risk of bias is made based on criteria scored for the domain concerned. For example, we considered risk of bias in study participation high when a highly selected patient group was enrolled as a result of in-/exclusion criteria, or when patient selection was not described at all. We considered risk of attrition bias high when >30% of patients whose CAC was measured at baseline did not undergo follow-up CT. We considered risk of bias in statistical reporting high when CAC scores were reported as means ± SD. Further criteria used in risk of bias assessment are available as supplemental material. Risk of bias assessment was performed in duplicate (TJ and BJ), with disagreements resolved by consensus.

To describe CAC progression, we report median and (preferably interquartile) range of CAC score at baseline and at follow-up with follow-up duration. Because of the fundamentally skewed nature of CAC scores, and consequent heterogeneity in statistical reporting and analysis of CAC progression, it was not possible or appropriate to perform meta-analysis.

Results

Study characteristics

Our search yielded 445 individual citations after discarding duplicates. 391 of these were discarded after reviewing abstracts made clear these citations did not meet eligibility criteria. An additional 4 citations were discarded because full texts were not available, and abstracts provided too little data. Full texts of the 50 remaining citations were examined in detail, 29 of which met inclusion and exclusion criteria and were included in the systematic review. Citation tracking and hand-searching bibliographies of included publications did not bring forth citations that were unidentified by previous searches. Figure 1 displays the screening and study selection process.

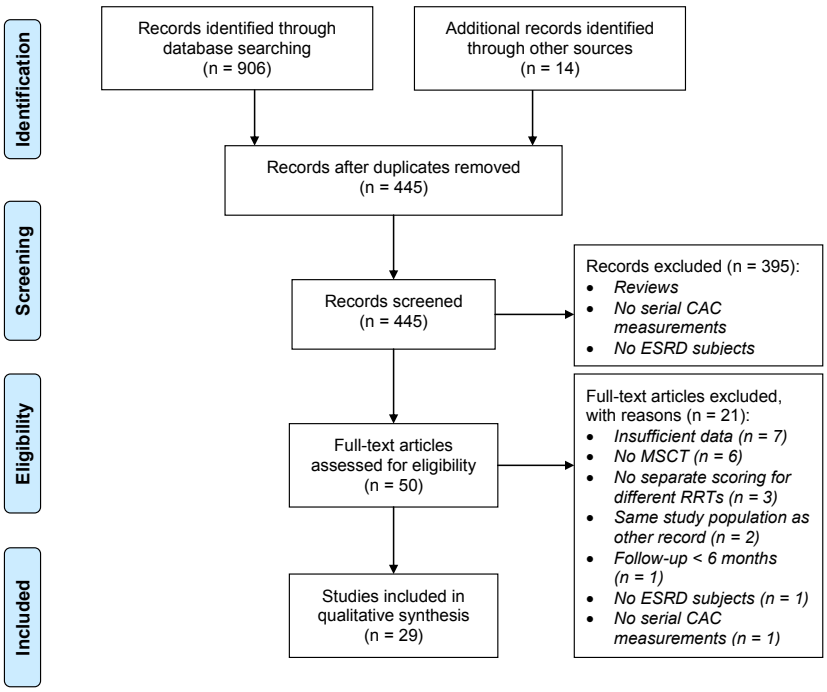

Figure 1. Flowchart of the study screening and selection process.

Of the 29 included studies (32 unique cohorts), most focused on hemodialysis patients[10, 17-35] (twenty studies; 1499 patients) or kidney transplant recipients[30, 33, 36-41] (eight studies; 649 patients), two of which investigated both hemodialysis patients and kidney transplant recipients[30, 33]. Three studies (92 patients) investigated peritoneal dialysis patients[29, 42, 43], one of which also investigated hemodialysis patients[29]. One study (38

patients) investigated nocturnal hemodialysis patients[44]. Six studies were randomized controlled trials[17, 22, 23, 32, 35, 36] (five evaluating pharmacological interventions, one evaluating dialysate calcium), two studies were non-randomized controlled trials[27, 34] (one of which incorporated a retrospective control group[34]) and one was a pilot study[25] (all three evaluating pharmacological interventions). The 20 other studies were observational cohort studies. Sample sizes ranged between 7 and 235, and follow-up durations ranged from 6 months to 52.8 months. Study characteristics are shown in Table 1.

Risk of bias assessment

Results of risk of bias assessment of included studies are summarized in Figure 2. Many studies did not report the patient recruitment process. Also, inherent to research in the dialysis setting, attrition rates were substantial in many studies. Frequently, subjects that did not complete follow-up were different from subjects that did complete follow-up[17, 24, 32, 33, 36, 38, 41, 42], or were not described sufficiently[18, 21, 23, 25-30, 34, 36-40, 43]. In three publications, renal replacement therapy was described as "dialysis", not otherwise specified. Authors of two of these publications confirmed that these cohorts concerned hemodialysis patients only[10, 30]. As the authors of the third publication did not respond, we assumed the third publication concerned hemodialysis patients exclusively as well, but consequently adjudged this study a high risk of bias on the prognostic factor domain[20]. Four studies on kidney transplant recipients did not report transplant function[30, 33, 39, 40]. All but two studies reported relatively homogeneous follow-up durations[18, 31], and two studies reported CAC scores normalized for a one-year interval assuming a linear increase in CAC[26, 44]. All studies reported CT scanning and CAC measurement procedures. CAC scores were reported in various ways, although most studies reported medians with (interquartile) ranges. Seven studies reported CAC scores as means only[23, 24, 28, 40] , and upon request authors of three of these provided median CAC scores[10, 30, 44].

CAC progression in different renal replacement therapies

As can be seen from Figure 3, median CAC scores were high in hemodialysis cohorts, ranging between 52 and 1409 at baseline, and progressed to a range of 120 to 1462 at follow-up. One large study on hemodialysis patients did not report CAC scores at follow-up, but reported a median increase of 94 and 149 in two strata (treatment with cinacalcet and low-dose vitamin D or flexible doses of vitamin D, respectively) over a one-year time span[35]. Remarkably, median CAC scores regressed (1409 to 1333) in one cohort receiving bisphosphonate treatment[34]. Of note, two studies on hemodialysis included only patients with baseline CAC scores ≥30[35] or ≥300[27].

Table 1. Characteristics of 29 included studies.

HAEMODIALYSIS				
Author, year	N° of pts with 2nd CAC	Follow-up (months)	CAC score at baseline (Agatston units)	CAC score at follow-up (Agatston units)
Ok, 2016[17]	224	24	97 (IQR 0 – 521)	166 (IQR 5- 800)
Barros, 2016[18]	37	23.9 ±4.7	267 (IQR 15 – 1206)	477 (IQR 33 – 1524)
Wang, 2015[19]	42	48	259 (IQR 48 – 1350)	545 (IQR 110 – 1761)
Malluche, 2015[20]	122	12	353 (IQR 20 – 1186)	552 (IQR 57 – 1608)
Ozkok, 2013[21]	74	12	52 (IQR 1 – 767)	120 (IQR 1 – 796)
Ohtake, 2013[22]	52	6	1020 (range 12 – 8462)	1246 (range 24 – 7887)
Di Iorio, 2011[10]	132	12	290 (IQR 10 – 986)	322 (IQR 20 – 1019)
Kakuta, 2011[23]	163	12	*875 ±1262*	*1014 ±1409*
Kurnatowska, 2011[24]	33	30	*1037 ±1571*	*1571 ±1705*
Raggi, 2011[35]	235	12	695 (p10/p90 98 – 1959) [n = 115] 590 (p10/p90 71 – 2583) [n = 120]	-
Cejka, 2010[25]	7	6	361 (range 0 – 5197)	543 (range 17 – 5254)
Coen, 2010[26]	81	12‡	481 (1783)¶	528 (12406)¶‡
Adirekkiat, 2010[27]	32	8.4 ±1.4	1008 (IQR 537 – 1723)	1075 (IQR 606 – 1955)
Caro, 2010[28]	33	12	*1913 ±?*	*2235 ±?*
Lee, 2010a[29]	18	12	110 (433)¶	175 (543)¶
Mazzaferro, 2009a[30]	30	24 ±3	239 (IQR 8 – 1109)	318 (IQR 128 – 1648)
Barreto, 2008[32]	71	12	123 (IQR 0 – 823)	175 (IQR 0 – 994)
Jung, 2006[31]	40	21.6 ±4.5	191 (range 0 – 2403)°	253 (range 0 – 2745)°
Moe, 2004a[33]	17	13.2 ±1.0	22 (range 0 – 391)°	154 (range 0 – 555)°
Nitta, 2004[34]	56	12	1409 (range 168 – 8768)° [n = 35] 1303 (range 231 – 3133)° [n = 21]	1333 (range 213 – 7348)° [n = 35] 1462 (range 220 – 3450)° [n = 21]
RENAL TRANSPLANTATION				
Author, year	N° of pts with 2nd CAC	Follow-up (months)	CAC score at baseline (Agatston units)	CAC score at follow-up (Agatston units)
Yazbek, 2016[36]	100	12	0 (IQR 0 – 71)	0 (IQR 0 – 94)
Seyahi, 2012[37]	150	33.7 ±4.7	0 (IQR 0 – 15)	3 (IQR 0 – 46)
Maréchal, 2012[38]	197	52.8 ±3.4	110 (IQR 1 – 582)	202 (IQR 8 – 936)
Bargnoux, 2009[39]	76	12	54 (range 0 – 4897)	84 (range 0 – 4192)
Abedi, 2009[40]	31	6	*40 ±63*	*24 ±40*
Mazzaferro, 2009b[30]	41	25 ±4	5 (IQR 0 – 300)	12 (IQR 0 – 668)
Oschatz, 2006[41]	31	12	250 (range 0 – 3152)	366 (range 0 – 4460)
Moe, 2004b[33]	23	15 ±1.9	19 (range 0 – 1764)°	44 (range 0 – 2801)°

	Age (years)	Male (%)	Diabetes (%)	Cardio-vascular disease (%)	Dialysis vintage (months)	Phosphate (mmol/l)
	60 ±14	56	26	22	51 ±42	1.41 ±0.32
	60 ±15	46	30	32*	45 (IQR 21 – 80)	1.85 ±0.55
	64 ±14	50	27	38	63 ±11	1.53 ±0.39
	53 ±13	61	43	23*	40 (IQR 3 – 292)	1.84 ±0.52
	52 ±14	49	-	-	54 (IQR 23 – 96)	1.78 ±0.49
	68 ±6	60	43	26	124 ±48	1.74 ±0.48
	65 ±17	64	24	34	< 4	1.58 ±0.48
	58 ±12	54	21	7	112 ±88	1.84 ±0.22
	54 ±9	-	-	0	51 ±11	1.84 ±0.50
	62 ±13	58	43	28*	38 (p10/p90 9 – 105)	1.87 ±0.58
	61 ±5	43	-	-	48 ±10	1.78 ±0.24
	59 ±11	67	10	-	45 (100)¶	1.74 ±0.45
	60 ±13	60	54	38*	46 ±31	1.60 ±0.39
	68 ±11	73	30	42*	43 ±37	-
	57 (17)¶	61	22	-	48 (range 12 – 300)	1.94 ±0.10
	51 ±14	67	13	13	68 ±65	1.78 ±0.61
	47 ±13	68	14	-	37 ±25	2.30 ±0.61
	56 ±12	65	43	33	27 (range 1 – 111)	1.64 ±0.25
	51 ±8	-	12	-	80 ±77	1.87 ±0.36
	62 ±8	80	17	-	88 ±69	1.80 ±0.27

	Age (yrs)	Male (%)	Diabetes (%)	Cardio-vascular disease (%)	Dialysis vintage (months)	Phosphate (mmol/l)
	41 ±10	56	7	0	28 (IQR 14 – 66) [n = 51] 18 (IQR 9 – 38) [n = 49]	0.87 ±0.25
	39 ±11	67	5	0	16 (range 0 – 114)	1.04 ±0.16
	52 ±12	57	13	25	24 ±24	1.00 ±0.30
	51 (range 22 – 66)	62	7	27	35 (range 1 – 267)	1.45 (range 0.77 – 2.79)
	38 ±14	55	-	-	20 ±16	-
	48 ±13	61	10	10	58± 52	1.07 ±0.29
	52 ±12	78	15	29*	38 ±33	1.04 ±0.21
	-	-	-	-	-	-

Table 1. Continued.

PERITONEAL DIALYSIS				
Author, year	N° of pts with 2nd CAC	Follow-up (months)	CAC score at baseline (Agatston units)	CAC score at follow-up (Agatston units)
Lee, 2010b[29]	15	12	3 (824)¶	76 (1386)¶
Ammirati, 2007[42]	30	12	8 (IQR 0 – 136)	20 (IQR 0 – 263)
Stompór, 2004[43]	47	12	23 (range 0 – 5503)	84 (range 0 – 5001)
NOCTURNAL HAEMODIALYSIS				
Author, year	N° of pts with 2nd CAC	Follow-up (months)	CAC score at baseline (Agatston units)	CAC score at follow-up (Agatston units)
Yuen, 2006[44]	38	12‡	0 (IQR 0 – 221)	3 (IQR 0 – 336)‡

Data are reported as means ±standard deviation, median (IQR) or median (range). Mean CAC scores are printed in italics to indicate the defective interpretability of this summary measure.

*Coronary artery disease was reported instead of cardiovascular disease.

‡1-year standardized CAC score was reported

¶Distance between 25th and 75th percentiles

°Volume scores (mm^3) instead of Agatston units

In kidney transplantation cohorts, median CAC scores were lower, ranging between 0 and 250 at baseline, and progressed slightly to a range of 0 to 366 at follow-up. However, patients in kidney transplantation cohorts were also younger (mean age per cohort ranging between 38 and 52 years) than patients in hemodialysis cohorts (mean age per cohort ranging between 47 and 68 years), and had a shorter dialysis vintage (mean and median vintages per cohort ranging between 20–58 and between 16–35 months respectively) than patients in hemodialysis cohorts (mean and median vintages per cohort ranging between 3 –124 and between 27–54 respectively, disregarding one study on incident dialysis patients[10]). In addition, two studies on kidney transplantation exclusively included patients with no history of coronary artery disease[36, 37]. The two studies that compared CAC progression between hemodialysis and kidney transplantation indeed reported greater CAC progression in the former group[30, 33]. Yet, hemodialysis patients and kidney transplant recipients were significantly different with regard to baseline CAC scores and other characteristics in these two studies.

	Age (yrs)	Male (%)	Diabetes (%)	Cardio-vascular disease (%)	Dialysis vintage (months)	Phosphate (mmol/l)
	53 (6)[¶]	40	33	-	24 (range 5 – 96)	1.94 ±0.32
	52 (range 20 – 70)	45	35	29*	24 (range 3 – 120)	1.55 (range 0.87 – 2.87)
	53 ±13	53	-	-	18 (range 1 – 96)	1.74 ±0.52

	Age (yrs)	Male (%)	Diabetes (%)	Cardio-vascular disease (%)	Dialysis vintage (months)	Phosphate (mmol/l)
	43 ±2	55	18	-	45 ±10	1.56 ±0.08

In peritoneal dialysis cohorts, median CAC scores were also lower compared to hemodialysis, ranging between 3 and 23 at baseline and progressing to a range of 20–84 at follow-up, while median ages (range 52–53 years) and median dialysis vintages (range 18–24 months) were also low. At the same time, the one study that compared CAC progression between hemodialysis and peritoneal dialysis did not find significant differences in CAC progression[29].

Median CAC scores in the sole study on nocturnal hemodialysis increased from 0 at baseline to 3 at follow-up. Here, mean age was 43 years and mean dialysis vintage 45 months.

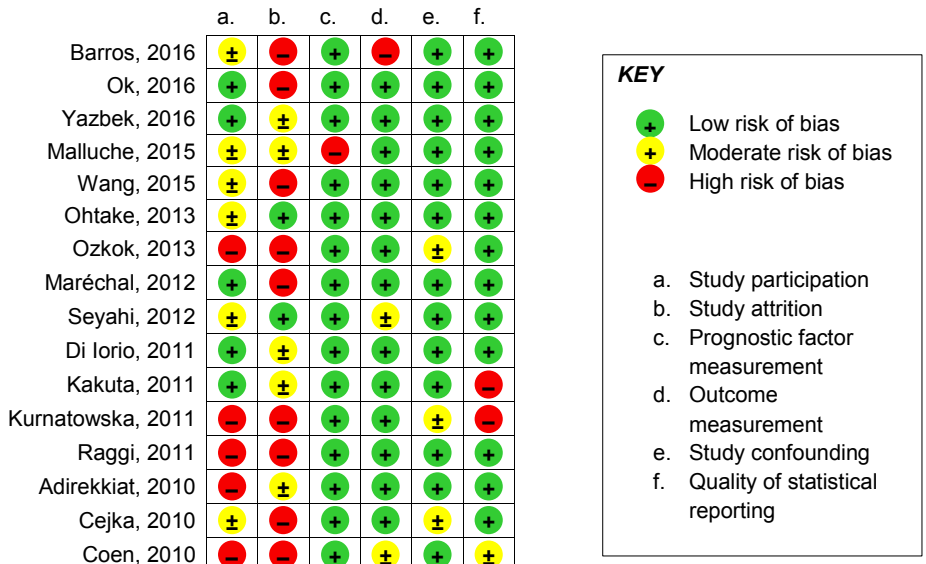

Figure 2. Risk of bias in the 29 included studies.

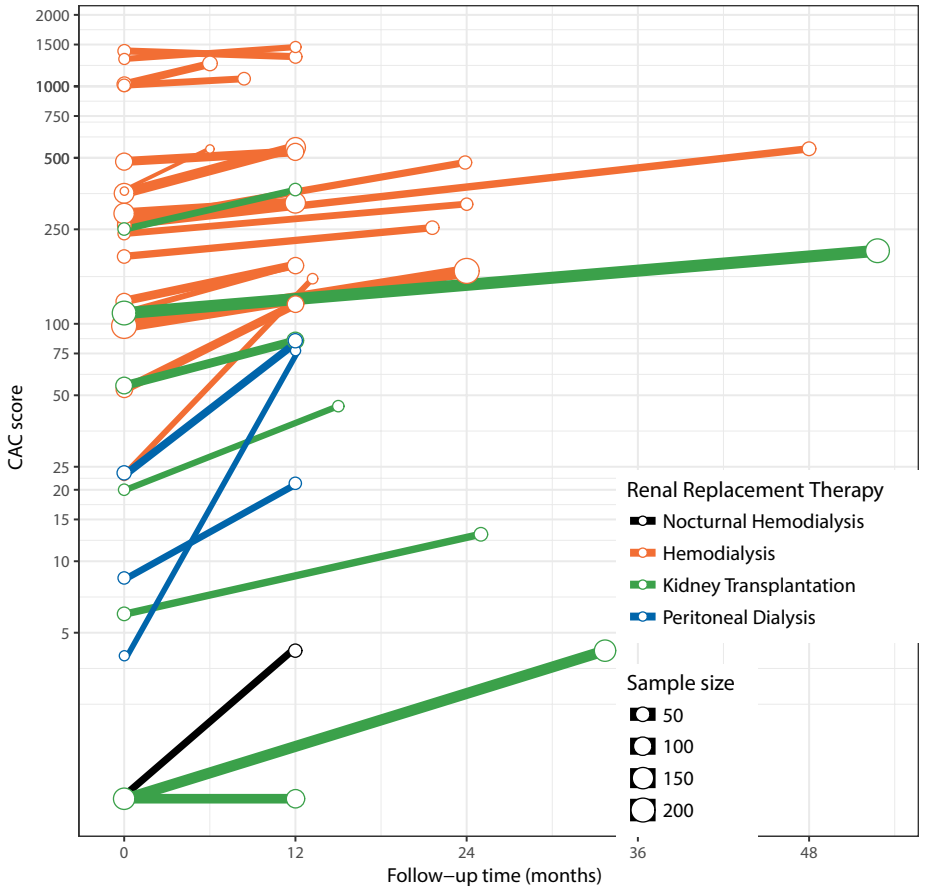

Figure 3. Median CAC score [logarithmic scale] at baseline and at follow-up in 24 studies on 18 hemodialysis cohorts (orange lines), 7 kidney transplant cohorts (green lines), 3 peritoneal dialysis cohorts (blue lines) and one nocturnal hemodialysis cohort (black line).CAC scores are in Agatston units (with the exception of volume scores in mm^3 in 3 studies[31, 33, 34]).Note: as a trade-off for the logarithmic scale, slopes are not readily comparable when baseline CAC scores are disparate.

Discussion

In this paper, we systematically reviewed the current literature on progression of CAC in different renal replacement therapies. CAC progression is observed in every study on patients with ESRD. Overall, the evidence is insufficient to determine the comparative influence of different renal replacement therapies on the progression of CAC. Although progression appears to be slower in kidney transplantation compared to hemodialysis, a proper comparison is hampered by important differences between these patient groups, i.e. lower age, shorter dialysis vintage and considerably lower baseline CAC scores in kidney transplantation cohorts. Moreover, meta-analysis was unworkable due to differences in statistical reporting of CAC scores and progression. Based on the limited available studies, it is unclear whether CAC progression is different between peritoneal dialysis, nocturnal hemodialysis and hemodialysis.

A major limitation of this systematic review is that reporting methods of CAC scores and CAC progression are far from concordant across studies. CAC scores are highly skewed, while scores of zero are also frequent, limiting the usefulness of common transformations such as log-transformation. Furthermore, a lack of consensus on the definition of CAC progression has led to various reporting methods and definitions of CAC progression, e.g. (normalized) absolute or percentage differences[17, 18, 22, 24, 25, 28, 29, 33-36, 38, 40, 41, 43], at times with varying cut-off values to define progression[10, 19, 21, 23, 37, 38, 42]; difference in square-root transformed CAC[17, 20]; odds ratios of progression to higher quantiles of CAC scores[26]; the method described by Hokanson et al.[45] (change in √volume score ≥2.5 mm^3)[27, 31, 37, 39] or the method described by Sevrukov et al.[46] (Agatston score change ≥4.93 x √baseline CAC score, or Agatston score at follow up >11.6 when baseline CAC = 0)[30, 37]. Previous systematic reviews and meta-analyses comparing pharmacological interventions on CAC progression have used mean (percentage) annualized progression rates[47], or mean differences[9, 48], both of which yield biased results. Currently, direct comparisons of CAC scores and CAC progression across renal replacement therapies by meta-analysis are infeasible without individual participant data.

Another limitation of this review is the high risk of attrition bias in many included studies. Risk of attrition bias was high in 16 studies, and moderate in another 8. This possibly led to an underestimation of CAC progression, since – as far as described – patients without a second CAC assessment were generally older and had higher CAC scores at baseline[24, 32, 33, 36, 38, 41, 42].

In many studies, the scanning interval was around 6 to 12 months. As incident hemodialysis patients with low or nil CAC develop minimal to no progression for up to 30 months[49], it is questionable if 6 to 12 months follow-up time is enough to effectively detect CAC progression. Therefore, we recommend adequate scanning intervals (>30 months) in future studies.

Considerable CAC progression in peritoneal dialysis was observed in three studies; then again, patients in peritoneal dialysis cohorts had a lower age, shorter dialysis vintage and considerably lower baseline CAC scores than patients in hemodialysis cohorts. As phosphate levels, associated with vascular calcification, are notoriously low in nocturnal hemodialysis, one would expect slow CAC progression in nocturnal hemodialysis. Indeed, the only one publication on CAC progression in nocturnal hemodialysis found moderate CAC progression. All the same, the evidence to determine the comparative influence of peritoneal dialysis or nocturnal hemodialysis on CAC progression remains insufficient.

From the summary of study characteristics (Table 1) and Figure 3, it is apparent that CAC progressed remarkably more in some cohorts. Generally, mean / median dialysis vintages[21, 29, 33, 41] and mean phosphate levels[21, 29] were high in these cohorts. On the other hand, little to nil progression was observed in two kidney transplantation cohorts, with low mean ages, low median dialysis vintages and low mean phosphate levels, and a zero median CAC score at baseline[36, 37]. Likely, dialysis vintage, age and phosphate levels are risk factors for the progression of CAC, which is endorsed by some of the included studies[20, 21, 33, 41-43].

Further research on the comparative influence of different renal replacement therapies on CAC progression is needed. A suitable design for future studies would be to longitudinally measure CAC in cohorts of different renal replacement therapies that are similar in characteristics such as age, sex and dialysis vintage. Furthermore, we advocate the adoption of a standardized manner of reporting CAC scores and CAC progression. For instance, both median (interquartile range) and quantiles should be reported for CAC scores, and both median (annualized) progression rates and odds ratios of progression to distinct categories should be reported for CAC progression.

In conclusion, CAC progresses in ESRD patients undergoing any form of renal replacement therapy. As CAC progression is a strong predictor of mortality, and likely has a causal role, it is of utmost importance to identify interventions that can slow down CAC progression. Given the central role of phosphate in the development and progression of CAC, high-quality research is needed that compares the effects of treatments that can control phosphate levels, such as kidney transplantation but also intensive forms of hemodialysis such as frequent or nocturnal hemodialysis.

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Supplementary material

Criteria for risk of bias assessment

- Study participation
  - Flowchart or in-text description of number of patients screened for eligibility and patients that were eventually enrolled: *LOW*
  - No reported in-/exclusion criteria, nor description of location/setting or time frame of recruitment; or highly selected patient groups owing to in-/exclusion criteria: *HIGH*
  - Else: *MODERATE*
- Study attrition
  - Kidney transplantation:
    - Attrition not described: *HIGH*
    - <10% attrition: *LOW*
    - 10-30% attrition: MODERATE
    - >30% attrition: *HIGH*
    - Depending on whether patients lost to follow-up were described and there were important differences between patients lost to follow-up and patients that completed follow-up, the reviewers can decide to adjudge a higher or lower risk of bias.
  - Dialysis:
    - Attrition not described: *HIGH*
    - <20% attrition: *LOW*
    - >20% attrition: MODERATE
    - >30% attrition: *HIGH*
    - Depending on whether patients lost to follow-up were described and there were important differences between patients lost to follow-up and patients that completed follow-up, the reviewers can decide to adjudge a higher or lower risk of bias.
- Prognostic factor measurement
  - Dialysis: presumably hemodialysis, but not explicitly mentioned: *HIGH*
  - Kidney transplantation: no eGFR or creatinine levels reported: *MODERATE*
  - Else: *LOW*
- Outcome measurement
  - Heterogeneous follow-up durations (standard deviation of follow-up duration > 20% of mean follow-up duration): *HIGH*
  - Heterogeneous follow-up durations, but accounted for with normalized (annualized) scores: *MODERATE*
  - Else: *LOW*

- Confounders
  - Most important confounders reported (at least 5 out of age, sex, diabetes mellitus, dialysis vintage, prevalence of cardiovascular disease or coronary artery disease and phosphate levels): *LOW*
  - 3 or 4 of most important confounders reported: *MODERATE*
  - 2 or less of most important confounders reported: *HIGH*
- Quality of statistical reporting
  - CAC scores reported as median + (interquartile) range: *LOW*
  - CAC scores reported as median + distance between 25th and 75th percentiles: *MODERATE*
  - CAC scores reported as means ± standard deviation: *HIGH*

Embase search strategy

1. 'end stage renal disease'/de OR 'chronic kidney failure':ab,ti OR 'end stage kidney disease':ab,ti OR 'end-stage renal disease':ab,ti OR 'chronic renal failure':ab,ti OR 'end-stage kidney disease':ab,ti OR 'ESRD':ab,ti
2. 'renal replacement therapy'/de OR 'renal replacement therap*':ab,ti OR 'kidney replacement therap*':ab,ti
3. 'hemodialysis'/de OR 'h*modialys*':ab,ti
4. 'hemodiafiltration'/de OR 'h*modiafiltration':ab,ti OR 'HDF':ab,ti
5. 'peritoneal dialysis'/de OR 'peritoneal dialys*':ab,ti OR 'CAPD':ab,ti OR 'CCPD':ab,ti OR 'APD':ab,ti OR 'PD':ab,ti
6. 'home dialysis'/de OR 'home *dialysis':ab,ti OR 'nocturnal h*modialysis':ab,ti OR 'frequent h*modialysis':ab,ti OR 'intensive h*modialysis':ab,ti
7. 'kidney transplantation'/de OR 'kidney transplant*':ab,ti OR 'renal transplant*':ab,ti OR 'kidney graft':ab,ti OR 'renal graft':ab,ti
8. 'dialysis modalit*':ab,ti
9. 'coronary artery calcification'/de OR ('coronary' NEAR/3 'calcification'):ab,ti
10. 'progress*':ab,ti OR 'advanc*':ab,ti OR 'chang*':ab,ti OR 'increas*':ab,ti OR 'decreas*':ab,ti
11. [1999-2016]/py
12. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8
13. 9 AND 10
14. 11 AND 12 AND 13

Pubmed search strategy

1. "Kidney Failure, Chronic"[Mesh] OR End-Stage Kidney Disease [tiab] OR End Stage Kidney Disease[tiab] OR Chronic Kidney Failure [tiab] OR End-Stage Renal Disease[tiab] OR End-Stage Renal Failure[tiab] OR Chronic Renal Failure[tiab] OR ESRD[tiab]
2. "Renal Replacement Therapy"[Mesh] OR Renal Replacement Therap*[tiab] OR Kidney Replacement Therap*[tiab]
3. "Renal Dialysis"[Mesh] OR Renal Dialys*[tiab] OR Hemodialys*[tiab] OR Haemodialys*[tiab] OR Extracorporeal Dialys*[tiab] OR HD[tiab]
4. "Hemodiafiltration"[Mesh] OR Hemodiafiltration[tiab] OR Haemodiafiltration[tiab] OR HDF[tiab] OR Acetate-Free Biofiltration[tiab]
5. "Peritoneal Dialysis"[Mesh] OR Peritoneal Dialys*[tiab] OR PD[tiab] OR CAPD[tiab] OR CCPD[tiab] OR APD[tiab]
6. "Hemodialysis, Home"[Mesh] OR Home Hemodialysis[tiab] OR Home Haemodialysis[tiab] OR Home HD[tiab] OR Nocturnal Hemodialysis[tiab] OR Nocturnal Haemodialysis[tiab] OR Nocturnal HD[tiab] OR Frequent Hemodialysis[tiab] OR Frequent Haemodialysis[tiab] OR Frequent HD[tiab] OR Intensive Hemodialysis[tiab] OR Intensive Haemodialysis[tiab] OR Intensive HD[tiab]
7. "Kidney Transplantation"[Mesh] OR Kidney Transplant*[tiab] OR Renal Transplant*[tiab] OR Kidney Graft*[tiab] OR Renal Graft*[tiab]
8. Dialysis modality[tiab] OR Dialysis modalities[tiab]
9. Coronary Artery Calcification*[tiab] OR Coronary Calcification*[tiab]
10. Progress*[tiab] OR Advanc*[tiab] OR chang*[tiab] OR increas*[tiab] OR decreas*[tiab]
11. "1999/01/01"[PDat] : "2017/01/01"[PDat]
12. 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8
13. 9 AND 10
14. 11 AND 12 AND 13

TRIP search strategy

(kidney failure OR end stage renal disease OR renal failure OR end stage kidney disease OR renal replacement therapy OR kidney replacement therapy OR hemodialysis OR haemodialysis OR peritoneal dialysis OR HD OR PD OR CCPD OR APD OR CAPD OR haemodiafiltration OR hemodiafiltration OR HDF OR nocturnal OR home hemodialysis OR home haemodialysis OR frequent hemodialysis OR frequent haemodialysis OR intensive hemodialysis OR intensive haemodialysis OR kidney transplant OR renal transplant OR dialysis modality) AND ((coronary artery calcifcation OR coronary calcification) AND (progression OR advancement OR change OR increase OR decrease)

Chapter 3

Coronary artery calcification in hemodialysis and peritoneal dialysis

T.T. Jansz, F.E. van Reekum, A. Özyilmaz, P.A. de Jong, F.T.J. Boereboom, T. Hoekstra, M.C. Verhaar, B.C. van Jaarsveld.
Am J Nephrol. 2018;48(5):369-377

Abstract

Background

Vascular calcification is seen in most patients on dialysis and is strongly associated with cardiovascular mortality. Vascular calcification is promoted by phosphate, which generally reaches higher levels in hemodialysis than in peritoneal dialysis. However, whether vascular calcification develops less in peritoneal dialysis than in hemodialysis is currently unknown. Therefore, we compared coronary artery calcification (CAC), its progression, and calcification biomarkers between patients on hemodialysis and peritoneal dialysis.

Methods

We measured CAC in 134 patients who had been treated exclusively with hemodialysis (*n=94*) or peritoneal dialysis (*n=40*) and were transplantation candidates. In 57 of them (34 on hemodialysis and 23 on peritoneal dialysis), we also measured CAC progression annually up to 3 years and the inactive species of matrix Gla protein (dp-ucMGP), fetuin-A, osteoprotegerin. We compared CAC cross-sectionally with Tobit regression. CAC progression was compared in two ways: with linear mixed models as the difference in square root transformed volume score per year (ΔCAC SQRV) and with Tobit mixed models. We adjusted for potential confounders.

Results

In the cross-sectional cohort, CAC volume scores were 92 mm^3 in hemodialysis and 492 mm^3 in peritoneal dialysis (adjusted difference 436 mm^3; 95% CI -47, 919; P=0.08). In the longitudinal cohort, peritoneal dialysis was associated with significantly more CAC progression defined as ΔCAC SQRV (adjusted difference 1.20; 95% CI 0.09, 2.31; P=0.03), but not with Tobit mixed models (adjusted difference in CAC score increase per year 106 mm^3; 95% CI -140, 352; P=0.40). Peritoneal dialysis was associated with higher osteoprotegerin (adjusted P=0.02), but not with dp-ucMGP or fetuin-A.

Conclusions

Peritoneal dialysis is not associated with less CAC or CAC progression than hemodialysis, and perhaps with even more progression. This indicates that vascular calcification does not develop less in peritoneal dialysis than in hemodialysis.

Introduction

Cardiovascular disease is the leading cause of death among patients with end-stage renal disease[1, 2]. This high cardiovascular mortality is strongly associated with vascular calcification[3, 4], which occurs frequently[2] and progresses almost universally in end-stage renal disease[5]. Vascular calcification can be measured at various sites, such as the coronary arteries, and is promoted by phosphate, which is frequently elevated in end-stage renal disease[6, 7].

Remarkably, it is unknown whether vascular calcification is affected by dialysis modality, of which the two major types are hemodialysis and peritoneal dialysis. In theory, peritoneal dialysis might induce less vascular calcification than hemodialysis, because patients on peritoneal dialysis generally have lower serum phosphate[8] probably owing to their continuous clearance. However, there have never been randomized studies on this subject, as randomization to dialysis modality is generally refused by patients[9]. Moreover, patients on peritoneal dialysis are typically younger and healthier due to the prerequisites of treatment at home[10], which has hampered previous observational research that did not attempt to statistically adjust for this[11-14].

To overcome this, we compared patients treated with hemodialysis or peritoneal dialysis who were all eligible for transplantation and thus relatively comparable in age and comorbidities. First, we compared coronary artery calcification (CAC) cross-sectionally between prevalent patients who had been treated exclusively with hemodialysis or peritoneal dialysis. Second, we compared CAC progression up to 3 years among those who underwent follow-up measurements. Additionally, we studied calcification biomarkers in relation to CAC progression, and compared these between patients on hemodialysis and peritoneal dialysis.

Methods

Study population

We analyzed a cross-sectional sample of patients that had been treated exclusively with conventional hemodialysis or peritoneal dialysis and participated in the NOCTx study. NOCTx (NCT00950573) is a prospective non-randomized study that included patients on chronic conventional hemodialysis or peritoneal dialysis with a minimum dialysis vintage of 2 months, patients that switched to nocturnal hemodialysis, and patients who received a kidney transplant. Thus, all patients had been treated with hemodialysis or peritoneal dialysis at inclusion. Patients were eligible when aged between 18 and 75 years and were candidates for transplantation when on dialysis. NOCTx excluded patients with a life expectancy <3 months, non-adherence to dialysis regimens, drug abuse, and pregnancy.

Between December 2009 and February 2016, 329 patients were screened for eligibility in 8 Dutch dialysis centers. NOCTx included 181 of these patients, of whom 135 were being treated with hemodialysis and 46 with peritoneal dialysis at inclusion. We excluded patients who were treated with hemodialysis >16 hours per week (*n=14*), as we theorized that more intensive dialysis regimens might mitigate calcification. Furthermore, we excluded patients who had a history of treatment with the other modality of over 3 months (*n=33*), leaving a sample of 134 patients.

Longitudinal cohort

We analyzed a longitudinal sample of patients from the NOCTx study who continued treatment with conventional hemodialysis or peritoneal dialysis after inclusion and completed at least one follow-up visit (*n=57*). In NOCTx, CAC was measured at inclusion, and after 1, 2 and 3 years. Also, blood was collected in 4.5 mL potassium-ethylenediaminetetraacetic acid (EDTA) vacutainers (on a non-dialysis day in case of hemodialysis), immediately centrifuged and stored in aliquots at -80°C without thawing at inclusion. Patients left the study if their renal replacement therapy was changed.

Treatment characteristics

Patients were treated according to the Kidney Disease: Improving Global Outcomes guidelines by the attending nephrologists[15]. Hemodialysis was delivered 3 times a week 4 hours with a default 1.50 mmol/L dialysate calcium concentration, and peritoneal dialysis as automatic or continuous ambulant peritoneal dialysis with a default 1.25 mmol/L dialysate calcium concentration.

CAC measurements

We determined CAC scores on non-enhanced prospectively triggered cardiac multi-slice computed tomography (iCT 256, Philips Healthcare, Best, the Netherlands). Acquisition parameters were as follows: 120 kV, 40-50 mAs, rotation time 270 ms, and 128 x 0.625 mm collimation. Metoprolol was administered intravenously if heart rate was above 60/min to improve imaging quality. We used a calcium threshold of ≥130 Hounsfield units. A single observer (TJ) read all scans chronologically per patient in order to exclude segments with severe motion artefacts or stents at a given scan from an entire set. Using commercially available software (Heartbeat CS, Philips Healthcare, The Netherlands), we calculated calcium volume scores. Reproducibility of coronary artery calcification measurements has been shown to be excellent, with an intraclass correlation coefficient of >0.95[16].

Calcification biomarker measurements

Plasma levels of desphospho-uncarboxylated matrix Gla protein (dp-ucMGP) were determined as described before[17]. The within-run and total variations of this assay were 0.8–6.2% and 3.0–8.2%, respectively. The assay measuring range was between 300 and 12,000 pmol/L and was linear up to 11,651 pmol/L[18]. The dp-ucMGP assays were performed in a single run by the laboratory of Coagulation Profile, department of Biochemistry, Maastricht, the Netherlands. Plasma fetuin-A and osteoprotegerin levels were measured with a Bio-Plex system (Bio-Rad) multiplex assay by the laboratory of the University Medical Center Utrecht, Utrecht, the Netherlands. All assays were executed in a single run.

Other study variables

Study personnel recorded demographic and clinical parameters at inclusion (pre-dialysis blood pressure and post-dialysis weight averaged from routine measurements during 3 hemodialysis sessions or 2 outpatient visits in case of peritoneal dialysis). Laboratory parameters (total calcium, albumin, phosphate, C-reactive protein, and parathyroid hormone) were obtained at inclusion by averaging routine measurements from 3 months, performed with standard laboratory techniques at the local treatment facilities. We classified residual urine production as present (≥100 mL/24h) or absent. We defined dialysis vintage as the time between the first day of dialysis and the day of scanning, minus the time with a functioning kidney transplant.

Statistical analyses

We reported normally distributed variables as mean (± standard deviation), non-normally distributed variables as median (interquartile range, IQR), and categorical data as number (percentage). We compared normally distributed variables with Student's t-tests, non-normally distributed variables with Mann-Whitney-U tests, and categorical data with Chi-squared tests.

We compared CAC volume scores cross-sectionally with Tobit regression. Tobit regression can be used to analyze variables with floor and/or ceiling effects[19]. This may be the case with CAC scores, when calcification can be present below the detection limit while the CAC score is 0. With Tobit regression, we assume our outcome variable is actually a normally distributed variable that has been truncated (here CAC score truncated at zero). By modeling this latent underlying variable, values of zero do not need to be excluded from the analyses and do not severely skew the results[19, 20].

To compare CAC progression, we used two different approaches, since a valid standard method to analyze CAC progression is lacking. First, we analyzed CAC progression with linear mixed models as change per year in square root transformed volume scores (ΔCAC SQRV). This approach, also known as Hokanson's method, accounts for interscan variability[21] and has been used by others[22]. We adjusted these analyses for CAC SQRV at inclusion. Second, we used Tobit mixed models to analyze CAC progression. We adjusted for factors known to induce calcification[23]: age (years), sex (male/female), presence of diabetes mellitus (yes/no), dialysis vintage (months), presence of residual urine production ≥100 mL/24h (yes/no), and vitamin K antagonist use (yes/no).

We used linear regression to compare biomarker levels between dialysis modalities. Dp-ucMGP levels were log-transformed, as these were right-skewed. We adjusted for potential confounders as described above. To determine the relationship between biomarkers and ΔCAC SQRV between inclusion and 1 year, we calculated Pearson's correlation coefficients.

We report regression coefficients with 95% confidence intervals (95% CI). We considered P-values ≤ 0.05 (two-tailed) statistically significant and used R 3.4.1 (R Foundation Statistical Computing) for all analyses.

Results

Cross-sectional cohort

The cross-sectional cohort included 134 patients who had been treated exclusively with hemodialysis (*n=94*) or peritoneal dialysis (*n=40*). The mean age of this cohort was 54 ±12 years, 94 (70%) were male, median dialysis vintage was 17 (IQR 10–34) months, and 24 (18%) had diabetes mellitus. The patients on hemodialysis had a median 6-month longer dialysis vintage, were somewhat heavier, had higher blood pressures, and had lower calcium and higher albumin levels than the patients on peritoneal dialysis (Table 1). Phosphate levels were not significantly higher in the patients on hemodialysis.

CAC volume scores were 92 (IQR 1–663) in the patients on hemodialysis and 492 (IQR 92–1139) in the patients on peritoneal dialysis. The distribution of the CAC volume scores is illustrated by a smoothed version of a histogram (Kernel density plot) in Figure S1. In Tobit regression, the CAC volume scores were not significantly (P=0.15) higher in patients on peritoneal dialysis compared to patients on hemodialysis (difference 342 mm^3; 95% CI -125, 808). When adjusted for age, sex, diabetes mellitus, dialysis vintage, residual urine production, and vitamin K antagonist use, peritoneal dialysis was also not significantly (P=0.08) associated with more CAC than hemodialysis (difference 436 mm^3; 95% CI -47, 919).

Longitudinal cohort

The longitudinal cohort included 57 patients treated with hemodialysis (*n=34*) or peritoneal dialysis (*n=23*) who completed at least one follow-up visit. The mean age of this cohort was 52 ±13 years, 37 (65%) were male, median dialysis vintage was 17 (IQR 8–47) months, and 7 (12%) had diabetes mellitus. The patients on hemodialysis were somewhat heavier, had higher systolic blood pressures, tended to have longer dialysis vintages, tended to use more vitamin K antagonists, and had lower calcium and higher albumin levels than the patients on peritoneal dialysis (Table 1), whereas their other characteristics were comparable. Notably, CAC volume scores at inclusion were not significantly different between patients on hemodialysis (median 163, IQR 5–745) and patients on peritoneal dialysis (median 76, IQR 2–696, P=0.68), nor was the proportion of patients with zero calcification (*n=8,* 24% versus *n=6*, 26%, respectively, P=0.99). There were also no significant differences between the longitudinal cohort (*n=57*) and those who underwent treatment with hemodialysis or peritoneal dialysis after inclusion but did not undergo follow-up CAC measurements (Table S1).

Table 1. Characteristics of the 134 patients included in the cross-sectional CAC analysis and of the 57 patients included in the CAC progression analyses, stratified by dialysis modality.

	Cross-sectional cohort	
	Hemodialysis (n = 94)	*Peritoneal dialysis* (n = 40)
Demographics and medical history		
Age (years)	56 ±11	51 ±15
Male (%)	62 (66%)	32 (80%)
Body mass index (kg/m²)	26.2 ±4.4	24.7 ±3.3
Systolic blood pressure (mmHg)	143 ±20	135 ±13
Diastolic blood pressure (mmHg)	79 ±12	85 ±10
Diabetes mellitus (%)	21 (22%)	3 (8%)
Cardiovascular disease (%)	23 (25%)	10 (25%)
Current smoker (%)	13 (14%)	5 (13%)
History of kidney disease		
Dialysis vintage (months)	19 (11–35)	13 (7–31)
Cause of end-stage renal disease (%)		
• Cystic kidney disease	18 (19%)	6 (15%)
• Interstitial nephritis	5 (5%)	1 (3%)
• Glomerulonephritis	24 (26%)	7 (18%)
• Vascular disease	21 (22%)	11 (28%)
• Diabetic nephropathy	10 (11%)	2 (5%)
• Other	8 (9%)	7 (18%)
• Unknown	8 (9%)	6 (15%)
Dialysis therapy and kidney function		
Dialysis therapy		
• Weekly hemodialysis sessions	3.1 ±0.5	-
• Weekly hemodialysis hours	11.4 ±2.0	-
• Daily peritoneal dialysis dwells	-	4.4 ±0.6
• Daily peritoneal dialysis volume (L)	-	9.8 ±2.4
Kidney function		
• Residual urine production ≥100mL/24h (%)	55 (59%)	29 (73%)
*Medication use**		
Vitamin K antagonist (%)	12 (15%)	1 (3%)
Vitamin D analogue (%)	61 (75%)	32 (89%)
Calcium-containing phosphate binder (%)	28 (35%)	14 (39%)
Cinacalcet (%)	16 (20%)	7 (19%)

		Longitudinal cohort		
	P for difference	*Hemodialysis* (n = 34)	*Peritoneal dialysis* (n = 23)	*P for difference*
	0.06	54 ±12	49 ±14	0.17
	0.16	20 (59%)	17 (74%)	0.37
	0.05	26.7 ±4.9	24.5 ±3.4	0.04
	0.03	142 ±20	132 ±18	0.05
	<0.01	79 ±11	82 ±14	0.29
	0.07	6 (18%)	1 (4%)	0.28
	0.99	7 (21%)	5 (22%)	0.99
	0.99	4 (12%)	1 (4%)	0.62
	0.05	26 (10–56)	12 (5–47)	0.06
	0.42			0.49
		4 (12%)	5 (22%)	
		2 (6%)	1 (4%)	
		9 (26%)	5 (22%)	
		8 (24%)	5 (22%)	
		5 (15%)	1 (4%)	
		4 (12%)	6 (26%)	
		2 (6%)	0	
		2.9 ±0.3	-	
		11.0 ±2.0	-	
		-	4.3 ±0.6	
		-	9.0 ±2.2	
	0.18	20 (59%)	14 (61%)	0.99
	0.11	7 (21%)	0	0.06
	0.15	29 (85%)	19 (83%)	0.99
	0.81	18 (53%)	10 (44%)	0.67
	0.99	8 (24%)	3 (13%)	0.52

Table 1. Continued.

	Cross-sectional cohort	
	Hemodialysis (n = 94)	*Peritoneal dialysis* (n = 40)
Laboratory parameters		
Calcium (mmol/L)	2.3 ±0.1	2.4 ±0.1
Albumin (g/L)	41.4 ±3.2	38.3 ±3.6
Phosphate (mmol/L)	1.6 ±0.4	1.6 ±0.4
C-reactive protein (mg/L)	3 (2–6)	3 (1–13)
Parathyroid hormone (pmol/L)	22 (15–41)	22 (14–37)

Data are presented as mean ±standard deviation, median (interquartile range) or number (percentage). *In the cross-sectional cohort, data on medication use were available in 81 patients on hemodialysis and 36 patients on peritoneal dialysis; in the longitudinal cohort data on medication use were available in all patients.

The maximum follow-up duration was 1 year for 25 patients, 2 years for 18 patients, and 3 years for 14 patients. CAC progressed in most patients, but only in 2 of 8 patients on hemodialysis without CAC at inclusion, and in 2 of 6 patients on peritoneal dialysis without CAC at inclusion (Figure 1). We analyzed CAC progression as ΔCAC SQRV with linear mixed models and with Tobit mixed models.

CAC progressed with 1.72 ΔCAC SQRV per year in patients on hemodialysis (95% CI 0.81, 2.64) and with 2.73 ΔCAC SQRV per year in patients on peritoneal dialysis (95% CI 1.58, 3.88) (Figure 2). As can be seen in Table 2, peritoneal dialysis was not significantly (P=0.18) associated with higher ΔCAC SQRV than hemodialysis in unadjusted analyses (difference in ΔCAC SQRV per year 1.01; 95% CI -0.47, 2.47). When adjusted for CAC SQRV at inclusion, age, sex, diabetes mellitus, dialysis vintage, vitamin K antagonist use, and presence of residual urine production, peritoneal dialysis was significantly (P=0.03) associated with 1.20 ΔCAC SQRV per year higher CAC progression compared to hemodialysis (95% CI 0.09, 2.31).

In Tobit mixed models, CAC progressed with 99 mm^3 per year in hemodialysis (95% CI -42, 240) and with 288 mm^3 per year in peritoneal dialysis (95% CI 57, 519). As can be seen in Table 2, peritoneal dialysis was not significantly associated with higher CAC progression than hemodialysis in both crude and adjusted analyses with Tobit mixed models (crude difference 189 mm^3 per year; 95% CI -81, 459; P=0.17; and fully adjusted difference 106 mm^3 per year; 95% CI -140, 352; P=0.40).

	Longitudinal cohort		
P for difference	*Hemodialysis* (n = 34)	*Peritoneal dialysis* (n = 23)	*P for difference*
0.01	2.3 ±0.1	2.4 ±0.1	0.01
<0.001	40.8 ±3.0	38.5 ±3.5	0.02
0.69	1.6 ±0.3	1.6 ±0.3	0.50
0.93	2 (2–7)	2 (1–18)	0.91
0.53	30 (17–48)	22 (15–41)	0.53

Calcification biomarkers

At inclusion, we measured calcification biomarkers in the longitudinal cohort (*n=57*). Dp-ucMGP levels were median 1689 (IQR 1304–3470) pmol/L in hemodialysis, and 1548 (IQR 900–1822) pmol/L in peritoneal dialysis. Fetuin-A levels were mean 0.20 ±0.06 g/L in hemodialysis and 0.21 ±0.08 g/L in peritoneal dialysis. Osteoprotegerin were mean 3.2 ±1.4 μg/L in hemodialysis and 3.3 ±1.2 μg/L in peritoneal dialysis. In univariate analyses, peritoneal dialysis was not associated with differences in dp-ucMGP, fetuin-A, or osteoprotegerin levels (Table S2). When adjusted for age, sex, diabetes mellitus, dialysis vintage, vitamin K antagonist use, and presence of residual urine production, peritoneal dialysis was associated with 0.83 μg/L higher osteoprotegerin levels than hemodialysis (95% CI 0.13, 1.52; P=0.02), but not with differences in dp-ucMGP or fetuin-A. Osteoprotegerin correlated with ΔCAC SQRV (Pearson's correlation coefficient 0.32, P=0.05), while dp-ucMGP and fetuin-A did not correlate with ΔCAC SQRV (Pearson's correlation coefficients 0.23, P=0.13; and -0.01, P=0.93).

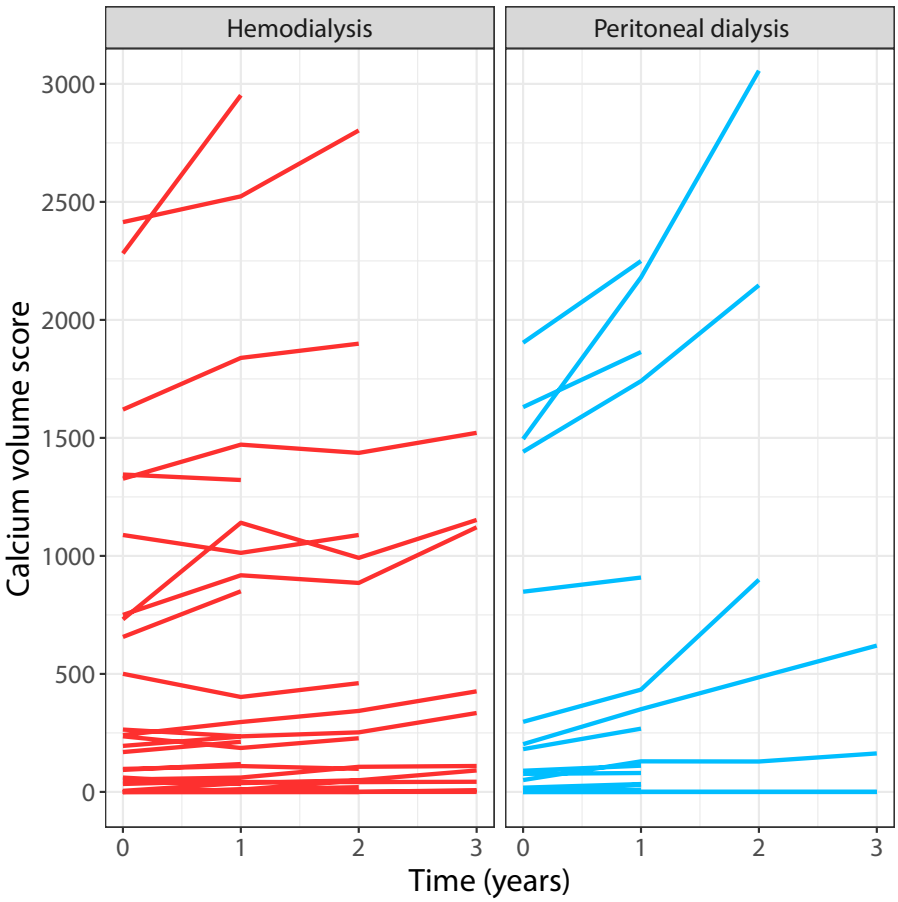

Figure 1. Coronary artery calcification progression in 57 patients on dialysis stratified by dialysis modality, depicted as individual trajectories of calcium volume scores. Individual trajectories of change in calcium volume score in patients on hemodialysis (left panel) and patients on peritoneal dialysis (right panel). Trajectories of two patients on hemodialysis and one on peritoneal dialysis with scores >5000 are not shown in this figure. Number of patients shown at 0, 1, 2, and 3 years: hemodialysis 32, 32, 21, and 10; peritoneal dialysis: 22, 22, 7, and 4. Note that lines may overlap around 0.

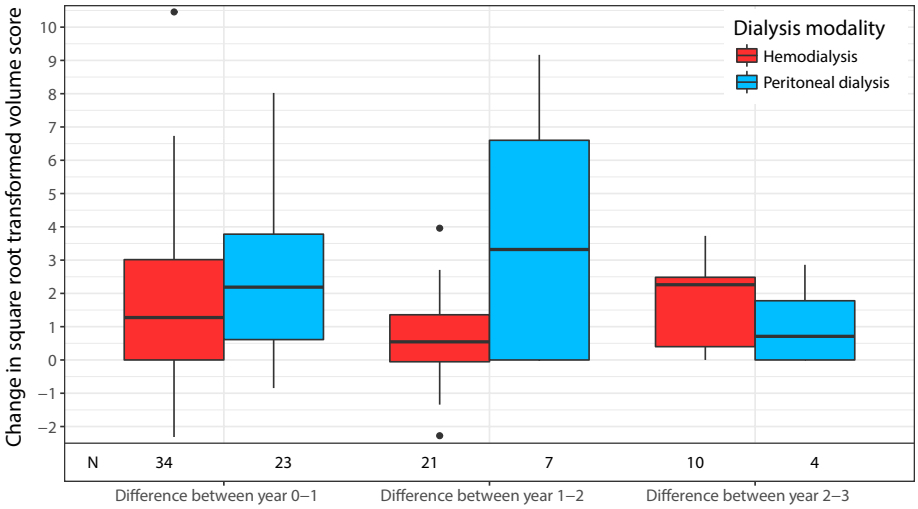

Figure 2. Coronary artery calcification progression per year in 57 patients on dialysis stratified by dialysis modality, depicted as boxplots of change in square root transformed volume score. Change in square root transformed volume scores (Y-axis) stratified by dialysis modality (X-axis) as boxplots. Note that square root transformations cannot be back-transformed. Number of patients per group per period (N) denoted below the boxplots. Crude P for difference in change in square root transformed volume score per year: 0.18; adjusted P for difference in change in square root transformed volume score per year: 0.03.

Table 2. Effect estimates of CAC progression for peritoneal dialysis (*n=23*) compared to hemodialysis (*n=34*) analyzed with linear mixed models as ΔCAC SQRV and with Tobit mixed models, with different multivariate adjustments.

	Unadjusted	CAC SQRV at inclusion*	Adjustment for age and sex†	Full adjustment‡
ΔCAC SQRV	1.01 (-0.47, 2.47)	1.22 (0.16, 2.29)	1.25 (0.19, 2.33)	1.20 (0.09, 2.31)
Tobit regression	189 (-81, 459)	-	77 (-184, 338)	106 (-140, 352)

95% confidence intervals between brackets.

*Tobit mixed models could not be adjusted for CAC SQRV at inclusion.

†The linear mixed models of ΔCAC SQRV were additionally adjusted for CAC SQRV at inclusion.

‡Full adjustment included age, sex, diabetes mellitus, dialysis vintage, presence of residual urine production, and vitamin K antagonist use. The linear mixed models of ΔCAC SQRV were additionally adjusted for CAC SQRV at inclusion.

Discussion

Our study investigated whether vascular calcification develops less in peritoneal dialysis than in hemodialysis, cross-sectionally and longitudinally. In a large cross-sectional cohort, we found that patients treated with peritoneal dialysis do not have less CAC than patients treated with hemodialysis. In the longitudinal cohort, we found that patients on peritoneal dialysis do not have less CAC progression than patients on hemodialysis. Altogether, this indicates that vascular calcification does not develop less in peritoneal dialysis than in hemodialysis.

Few studies have compared vascular calcification between hemodialysis and peritoneal dialysis, that is three cross-sectional studies and one longitudinal study. An American cross-sectional study found more frequent coronary artery calcification in pediatric patients on hemodialysis *(9/21 patients)* than on peritoneal dialysis *(2/17 patients)*[11]. An Albanian cross-sectional study found more frequent cardiac valve calcification in adult patients on hemodialysis *(24/34 patients)* than on peritoneal dialysis *(10/30 patients)*[12]. A Korean cross-sectional study did not find a significant difference in CAC score between patients on hemodialysis *(n=31,* median score 30*)* and peritoneal dialysis *(n=15,* median score 16*)*[13]. Finally, a Taiwanese study did not find significant differences in CAC score or one-year CAC progression between patients on hemodialysis *(n=18,* median score increased from 110 to 175*)* and patients on peritoneal dialysis (*n=15*, median score increased from 3 to 76)[14, 24]. However, all of these studies had imbalances in age, dialysis vintage, or comorbidities between groups, which were not adjusted for statistically. In addition, the longitudinal study analyzed CAC progression as percentage change, which yields biased results because of the skewed distribution of CAC scores with excessive zeros[5].

The problematic distribution of CAC scores precludes many strategies commonly used to analyze changes. This is also why a standard method to analyze CAC progression is lacking. We therefore chose to analyze CAC progression with two approaches that are valid from a statistical viewpoint: as ΔCAC SQRV with linear mixed models and with Tobit mixed models. Our data show a significant effect with the former approach that appears larger than that with the latter approach. In fact, if we maintained the definition of CAC progression ≥2.5 ΔCAC SQRV by Hokanson et al.[21], there would be 6 out of 34 patients on hemodialysis with progression and 9 out of 23 patients on peritoneal dialysis with progression, implying a number needed to harm of 4.7 patients to make one more patient progress in CAC on peritoneal dialysis than on hemodialysis. However, this larger effect may be due to modest right-skewness of ΔCAC SQRV, despite the square root transformation. On the other hand, this larger

effect may be because Tobit mixed models could not be adjusted for CAC score at inclusion. Nevertheless, the estimates from both analyses are in the same direction, which support our conclusion that peritoneal dialysis is not associated with *less* CAC progression than hemodialysis.

There could be some explanations for our unexpected finding that peritoneal dialysis is not associated with less CAC or CAC progression. On the one hand, patients on peritoneal dialysis may have had a greater time-averaged exposure to phosphate than patients on hemodialysis. After all, time-averaged phosphate exposure in hemodialysis is lower than suggested by pre-dialysis phosphate levels because of the sawtooth pattern in hemodialysis[25]. On the other hand, there could have been differences in calcium balance. Patients on hemodialysis might have had a positive calcium balance with the default dialysate calcium concentration of 1.50 mmol/L based on kinetic modeling studies[26], but it is unknown whether patients on peritoneal dialysis also had a positive calcium balance with a default dialysate calcium concentration of 1.25 mmol/L, as this also depends on ultrafiltration[27]. This would require detailed calcium balance studies.

Our findings regarding the three calcification biomarkers do not allow firm conclusions and need further exploration. First, we found that osteoprotegerin was associated with progression of CAC, in accordance with previous studies[28, 29]. Nevertheless, osteoprotegerin should theoretically protect against vascular calcification by preventing the receptor activator of nuclear factor-κB ligand (RANKL) from binding to the RANK receptor[30]. Whether our finding indicates a compensatory response requires additional study. Second, we did not find a significant association between CAC progression and dp-ucMGP. Dp-ucMGP is an inverse marker of calcification inhibition potential, as its active form inhibits vascular calcification after carboxylation by vitamin K[31]. It is possible that larger samples are needed to study the relationship between CAC progression and dp-ucMGP. Third, we did not find any relationship between fetuin-A and CAC progression. Fetuin-A is a hepatic protein that forms soluble complexes with calcium and phosphate (calciprotein particles, CPPs) and thus prevents calcification[32]. The reason we did not find a relationship with CAC progression lies probably in these CPPs: an ordinary fetuin-A measurement includes the CPP-bound fetuin-A, which is the fetuin-A fraction that has already been used up. Future studies should measure the non-CPP-bound fraction of fetuin-A after an extra centrifugation step[33], or should measure CPPs directly[34].

Our results should be interpreted within certain limitations. The size of our longitudinal cohort was small and patients on peritoneal dialysis had a limited follow-up duration. Larger studies are necessary to investigate whether peritoneal

dialysis may be associated with more CAC than hemodialysis and to investigate the relationship between calcification biomarkers and CAC progression. Also, our study was non-randomized, although randomization to dialysis modalities has proven infeasible in earlier studies[9].

Our study also has several strengths. This study is the largest so far to compare vascular calcification between hemodialysis and peritoneal dialysis, combining a large cross-sectional cohort with follow-up data on progression of CAC. Also, we accounted for the skewness and zero-inflation of CAC scores by using two statistically valid approaches that enabled essential adjustment for potential confounders. On top of that, the patients on hemodialysis and peritoneal dialysis in our study were relatively comparable, as this study only included patients eligible for transplantation.

In conclusion, peritoneal dialysis is not associated with less CAC nor less CAC progression than hemodialysis. This indicates that vascular calcification does not develop less in peritoneal dialysis. Further studies should investigate whether vascular calcification develops even more in peritoneal dialysis.

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Supplementary material

Table S1. Characteristics of the patients that underwent treatment with hemodialysis or peritoneal dialysis after inclusion but did not undergo follow-up CAC measurements (*n=35*), compared to the longitudinal cohort (*n=57*).

	No follow-up CAC measurement (n = 35)	Longitudinal cohort (n = 57)	*P for difference with longitudinal cohort*
Demographics and medical history			
Age (years)	52 ±14	52 ±13	0.86
Male sex (%)	25 (71%)	37 (65%)	0.68
Body mass index (kg/m²)	26.2 ±4.3	25.8 ±4.5	0.68
Systolic blood pressure (mmHg)	137 ±19	138 ±20	0.87
Diastolic blood pressure (mmHg)	79 ±12	80 ±12	0.74
Diabetes mellitus (%)	9 (26%)	7 (12%)	0.17
Cardiovascular disease (%)	8 (23%)	12 (21%)	0.99
Active smoker (%)	8 (23%)	5 (9%)	0.11
History of kidney disease			
Dialysis vintage (months)	23 (13–46)	17 (8–49)	0.30
Cause of end-stage renal disease (%)			0.17
• Cystic kidney disease	1 (3%)	9 (16%)	
• Interstitial nephritis	3 (9%)	3 (5%)	
• Glomerulonephritis	12 (34%)	14 (25%)	
• Vascular disease	8 (23%)	13 (23%)	
• Diabetic nephropathy	3 (9%)	6 (11%)	
• Other	3 (9%)	10 (18%)	
• Unknown	5 (14%)	2 (4%)	
Dialysis therapy and kidney function			
Dialysis therapy (%)			0.36
• Hemodialysis	25 (71%)	34 (60%)	
• Peritoneal dialysis	10 (29%)	23 (40%)	
Kidney function			
• Residual urine production ≥100mL/24h (%)	19 (54%)	34 (60%)	0.90
Baseline CAC score			
Volume score (mm³)	217 (1–1183)	97 (3–750)	0.44
Agatston score	264 (0–1383)	103 (1–816)	0.45
Zero calcification (%)	9 (26%)	14 (25%)	0.99

Table S1. Continued.

	No follow-up CAC measurement (n = 35)	Longitudinal cohort (n = 57)	*P for difference with longitudinal cohort*
Reasons for not undergoing follow-up CAC measurements			
• Kidney transplantation	14 (40%)		
• Loss to follow-up	7 (20%)		
• Change of dialysis modality	7 (20%)		
• Withdrawal of informed consent	4 (11%)		
• Death	3 (9%)		

Data are presented as mean ±standard deviation, median (interquartile range) or number (percentage).

Table S2. Regression coefficients of linear regression of dialysis modality and natural log-transformed dp-ucMGP, fetuin-A, and osteoprotegerin, in 34 patients on hemodialysis and 23 on peritoneal dialysis. Reference is hemodialysis.

	Hemodialysis (n = 34)	Peritoneal dialysis (n = 23)	
		Crude	*Adjusted**
Log (dp-ucMGP)	*1.0 (reference)*	-0.39 (-0.86, 0.09)	-0.15 (-0.66, 0.35)
Fetuin-A (g/L)	*1.0 (reference)*	0.01 (-0.03, 0.05)	0.00 (-0.04, 0.05)
Osteoprotegerin (µg/L)	*1.0 (reference)*	0.08 (-0.70, 0.87)	0.83 (0.13, 1.52)

Abbreviations: dp-ucMGP: desphosho-uncarboxylated matrix Gla protein. 95% confidence intervals are shown between brackets.

*Adjusted for age (years), sex (male/female), presence of diabetes mellitus (yes/no), dialysis vintage (months), presence of residual urine production ≥100 mL/24h (yes/no), and vitamin K antagonist use (yes/no). Dp-ucMGP levels were right-skewed and were therefore natural log-transformed.

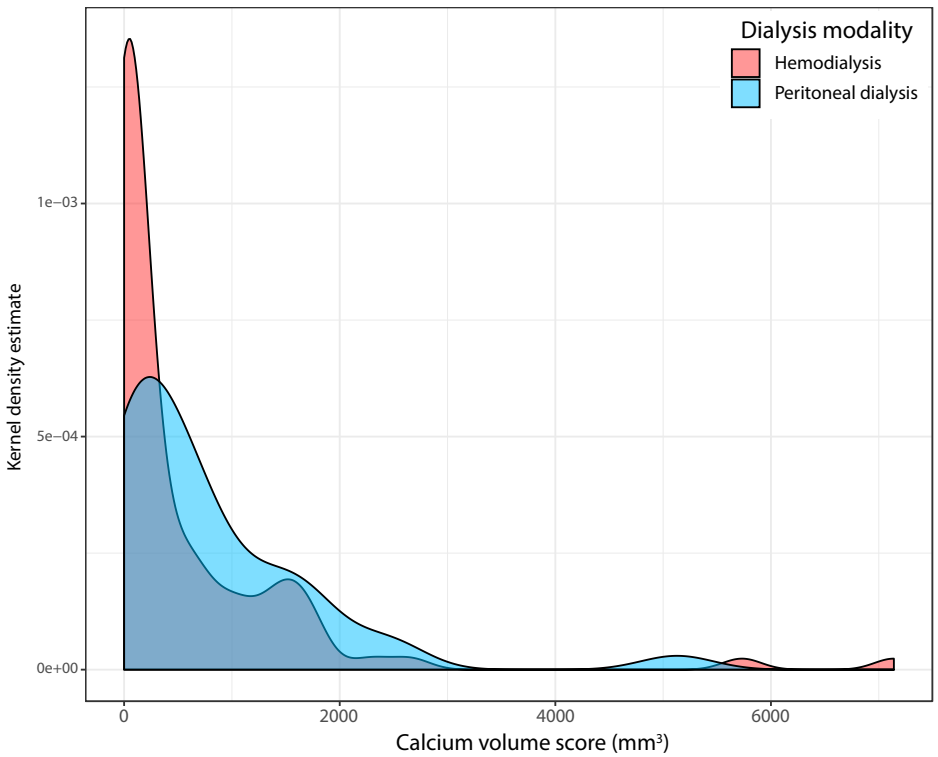

Figure S1. Distribution of calcium volume scores in 134 patients on dialysis at inclusion, stratified by dialysis modality, displayed as a smoothed histogram (kernel density estimates).

Figure legend: red shaded area: hemodialysis; blue shaded area: peritoneal dialysis. Note that the area under the curve of the kernel density estimates amounts to 1 per group.

Chapter 4

Progression of coronary artery calcification in conventional hemodialysis, peritoneal dialysis, nocturnal hemodialysis, and kidney transplantation

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Manuscript in preparation.

Abstract

Background

Cardiovascular disease is the leading cause of death in end-stage renal disease (ESRD) and is strongly associated with vascular calcification. Vascular calcification is promoted by high phosphate levels, but phosphate levels are lower when patients initiate nocturnal hemodialysis or receive a kidney transplant. However, it is unknown whether nocturnal hemodialysis or kidney transplantation thus mitigate vascular calcification. We therefore studied progression of coronary artery calcification (CAC) in patients treated with nocturnal hemodialysis, a kidney transplant, conventional hemodialysis, and peritoneal dialysis.

Methods

We measured CAC annually up to 3 years in 135 patients with ESRD that were transplantation candidates: 32 that continued conventional hemodialysis, 21 that continued peritoneal dialysis, 34 that initiated nocturnal hemodialysis (≥4x 8 hours/week), and 48 that received a kidney transplant. We compared CAC progression between groups as the difference in square root transformed volume scores per year (ΔCAC SQRV) using linear mixed models. Reference category was conventional hemodialysis.

Results

The mean age of the study population was 52 ±13 years, 90 (67%) were male, and median dialysis duration was 25 (IQR 11–52) months. Median CAC score at inclusion was 144 (IQR 4–653), which did not differ significantly between groups (P=0.91). Compared to conventional hemodialysis, CAC progression was non-significantly different for nocturnal hemodialysis 0.04 (95% CI -0.61 to 0.70) and kidney transplantation -0.22 (95% CI -0.84 to 0.39) in models adjusted for age, sex, diabetes mellitus, dialysis duration, vitamin K antagonist use, and CAC score at inclusion.

Conclusions

Nocturnal hemodialysis and kidney transplantation are not associated with significantly less CAC progression compared to conventional hemodialysis during up to 3 years follow-up. These findings raise important questions about which type of calcification is measured with CAC in end-stage kidney disease and what CAC progression means with regard to cardiovascular risk in end-stage kidney disease.

Introduction

Cardiovascular disease is the leading cause of death among patients with end-stage renal disease[1, 2]. This high cardiovascular mortality is strongly associated with vascular calcification[3, 4], which occurs frequently and progresses rapidly in end-stage renal disease[2, 5]. Vascular calcification can be measured at various sites, such as the coronary arteries, and is promoted by phosphate, which is frequently elevated in end-stage renal disease[6, 7].

Phosphate levels are considerably lower in patients who dialyze longer and more frequently, such as in frequent nocturnal hemodialysis[8, 9]. By improving phosphate control, nocturnal hemodialysis could mitigate progression of vascular calcification. Similarly, it is thought that kidney transplantation could halt progression of vascular calcification. However, thus far, the effect of nocturnal hemodialysis on progression of vascular calcification has not been investigated. Furthermore, only two previous studies compared progression of vascular calcification between kidney transplant recipients and patients on hemodialysis[10, 11]. However, only one of these compared kidney transplant recipients to transplantation-eligible patients on hemodialysis[11], and both did not account for calcification at inclusion, which is strongly associated with progression[12].

We therefore conducted the NOCTx study, the first study to comprehensively compare progression of vascular calcification between different renal replacement therapies. This prospective study evaluated progression of vascular calcification by measuring coronary artery calcification (CAC) at inclusion and annually during 3 years in 4 groups of patients with end-stage renal disease: transplantation-eligible patients that were treated with conventional hemodialysis or peritoneal dialysis, transplantation-eligible patients that switched from conventional to nocturnal hemodialysis, and patients on dialysis that received a kidney transplant.

Methods

Study design and population

NOCTx (NCT00950573) is a prospective study designed to compare CAC progression between different renal replacement therapies. NOCTx included patients that continued chronic conventional hemodialysis or peritoneal dialysis after at least 2 months on dialysis, patients that switched from conventional hemodialysis to nocturnal hemodialysis (≥4x 8 hours per week), and patients on dialysis who received a kidney transplant. Patients were eligible when aged between 18 and 75 years and were candidates for transplantation when on dialysis. NOCTx excluded patients with a life expectancy <3 months, pre-emptive transplantation, non-adherence to dialysis regimens, drug abuse, and pregnancy. All participants gave written informed consent. NOCTx has been approved by the Medical Ethics Committee of the University Medical Center Utrecht and was conducted according to the Declaration of Helsinki.

Between December 2009 and February 2016, 329 patients were screened for eligibility in 8 Dutch dialysis centers. NOCTx included 181 of these patients, who underwent study exams at University Medical Center Utrecht at inclusion and after 1, 2, and 3 years. Patients left the study if they switched renal replacement therapy, except for 8 patients on dialysis that received a kidney transplant within 6 months after inclusion and continued participation in the kidney transplantation group.

Treatment characteristics

Patients were treated according to the Kidney Disease: Improving Global Outcomes (KDIGO) 2009 guidelines by the attending nephrologists[13]. Conventional hemodialysis (3x 4 hours per week) and nocturnal hemodialysis (4-6x 8 hours per week) were delivered with a default dialysate calcium concentration of 1.50 mmol/L. Peritoneal dialysis was delivered as automatic or continuous ambulant peritoneal dialysis with a default 1.25 mmol/L dialysate calcium concentration. Kidney transplant recipients received standard immunosuppressant regimens consisting of a calcineurin inhibitor (tacrolimus), mycophenolate mofetil, and prednisolone in tapering doses.

CAC measurements

CAC scores were determined at each study exam using non-enhanced prospectively triggered cardiac multi-slice computed tomography (iCT 256 or IQon, Philips Medical Systems, Best, the Netherlands). Acquisition parameters were as follows: 120 kV, 40-50 mAs, rotation time 270 ms, and 128 x 0.625 mm collimation (iCT 256) / 64 x 0.625 mm collimation (IQon). To improve imaging quality, metoprolol was given intravenously if heart rate was above 60/min. We used a calcium threshold of ≥130

Hounsfield units. A single reader (TJ) read all scans blinded for treatment group and chronologically per patient, in order to exclude coronary segments with severe motion artefacts or stents at a given scan from an entire set. We calculated calcium volume and Agatston scores. These scores are highly correlated (Spearman's ρ=0.99). Reproducibility of coronary artery calcification measurements has been shown to be excellent (intraclass correlation coefficient >0.95)[14].

Other variables

At each study exam, study personnel collected data on laboratory parameters (total calcium, albumin, phosphate, and parathyroid hormone) by averaging values of routine measurements of the 3 months preceding the study exam. Residual urine production was classified as present (≥100 mL/24h) or absent. We assessed history of kidney disease, current dialysis schedule, and presence of comorbidities by chart review, and evaluated medication use by medication inventory.

We estimated glomerular filtration rate with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 equation for kidney transplant recipients. We defined dialysis duration as the time between the first day of dialysis and inclusion, minus the time with a functioning kidney transplant. We defined cardiovascular events as any myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, aortic aneurysm repair, stroke, new intermittent claudication, peripheral artery angioplasty or bypass grafting.

Statistical analyses

We reported normally distributed variables as mean (± standard deviation), non-normally distributed variables as median (interquartile range, IQR), and categorical data as number (percentage).

We evaluated the associations of treatment group with CAC progression by defining CAC progression as change per year in square root transformed volume scores (ΔCAC SQRV). This approach, also known as Hokanson's method, accounts for interscan variability[15] and has been used by others[16, 17]. We adjusted these analyses for CAC SQRV at inclusion and used mixed-effects to account for repeated measurements. We adjusted for factors related to calcification[18]: age (years), sex, presence of diabetes mellitus, dialysis duration (months), and vitamin K antagonist use. Conventional hemodialysis was the reference group.

We reported regression coefficients with 95% confidence intervals (95% CI). We considered p-values of ≤ 0.05 (two-tailed) statistically significant and used R 3.4.1 (R Foundation Statistical Computing) for all analyses.

Sensitivity analyses

To test the robustness of the associations, we repeated the analyses of treatment group with CAC progression using Agatston scores instead of volume scores.

Results

Study population

A total of 181 patients were included in the NOCTx study (Figure 1). We excluded patients that did not attend any follow-up exam (*n=46*), leaving an analytical sample of 135 patients (Figure 1). The mean age of the study population (*n=135)* was 52 ±13 years, 90 (67%) were male, dialysis duration (including historical dialysis duration of kidney transplant recipients) was median 25 (IQR 11–52) months, and 15 (11%) had diabetes mellitus (Table 1). There were 32 patients treated with conventional hemodialysis, 21 treated with peritoneal dialysis, 34 treated with nocturnal hemodialysis, and 48 kidney transplant recipients. Patients on nocturnal hemodialysis were enrolled after a training period of about 3 months. Kidney transplant patients were included about 3 months after transplantation, since many (25/48) received a deceased-donor transplant. Compared to patients on conventional hemodialysis, patients on nocturnal hemodialysis less often used vitamin D analogs (42% versus 84%) or calcium-containing phosphate binders (15% versus 50%). Also, patients on nocturnal hemodialysis had lower phosphate levels than patients on conventional hemodialysis (mean 1.2 versus 1.5 mmol/L), as did kidney transplant recipients (mean 0.9 mmol/L). These differences in phosphate levels subsisted during follow-up (Figure S1).

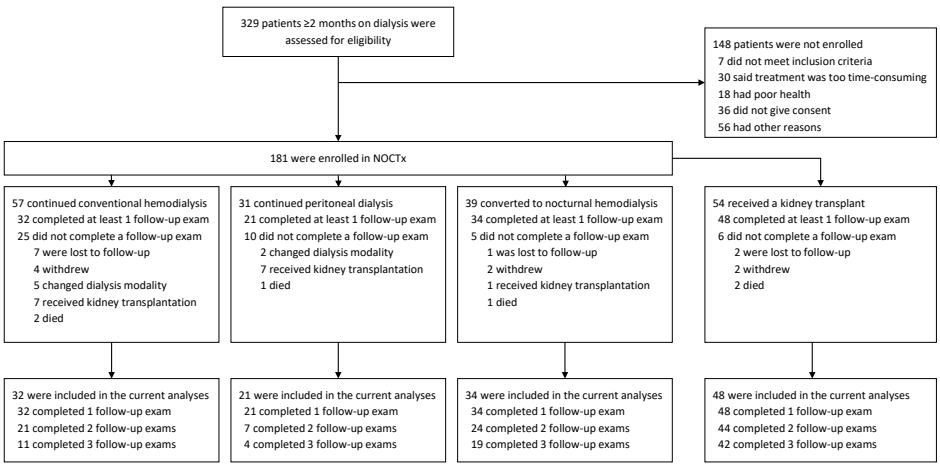

Figure 1. Study flowchart.

Table 1. Characteristics of 135 patients with end-stage renal disease stratified by renal replacement therapy.

	Conventional hemodialysis (n = 32)	Peritoneal dialysis (n = 21)	Nocturnal hemodialysis (n = 34)	Kidney transplantation (n = 48)
Demographics and medical history				
Age (years)	53 ±12	48 ±14	52 ±13	52 ±14
Male sex (%)	19 (59%)	15 (71%)	20 (59%)	36 (75%)
Diabetes mellitus (%)	6 (19%)	1 (5%)	5 (15%)	3 (6%)
Cardiovascular disease (%)	7 (22%)	5 (24%)	9 (27%)	6 (13%)
Current smoker (%)	4 (13%)	1 (5%)	6 (18%)	6 (13%)
History of kidney disease				
Dialysis duration (months)	27 (11–58)	12 (5–45)	29 (16–56)	28 (12–51)
Cause of end-stage renal disease (%)				
• Cystic kidney disease	4 (13%)	4 (19%)	6 (18%)	14 (29%)
• Interstitial nephritis	2 (6%)	1 (5%)	0	1 (2%)
• Glomerulonephritis	8 (25%)	5 (24%)	11 (32%)	9 (19%)
• Vascular disease	8 (25%)	5 (24%)	4 (12%)	10 (21%)
• Diabetic nephropathy	5 (16%)	1 (5%)	2 (6%)	2 (4%)
• Other	3 (9%)	5 (24%)	6 (18%)	5 (10%)
• Unknown	2 (6%)	0	5 (15%)	7 (15%)
Dialysis therapy and kidney function				
Dialysis therapy				
• Weekly dialysis sessions	2.9 ±0.4	-	5.2 ±0.8	-
• Weekly dialysis hours	11.0 ±2.0	-	41.2 ±6.4	-
Kidney function				
• Residual urine production ≥100mL/24h (%)	19 (59%)	15 (71%)	11 (32%)	-
• eGFR (mL/min)	-	-	-	57 ±20
Medication use				
Vitamin K antagonists (%)	7 (22%)	0	5 (15%)	4 (8%)
Vitamin D analogs (%)	27 (84%)	17 (81%)	14 (42%)	5 (10%)
Calcium-containing phosphate binder (%)	16 (50%)	9 (43%)	5 (15%)	-
Cinacalcet (%)	7 (22%)	3 (14%)	9 (27%)	2 (4%)

Table 1. Continued.

	Conventional hemodialysis (n = 32)	Peritoneal dialysis (n = 21)	Nocturnal hemodialysis (n = 34)	Kidney transplantation (n = 48)
Physical and laboratory parameters				
Body mass index (kg/m²)	26.8 ±5.0	24.0 ±3.1	25.8 ±5.4	24.4 ±3.5
Systolic blood pressure (mmHg)	142 ±19	133 ±18	138 ±19	128 ±14
Diastolic blood pressure (mmHg)	79 ±11	82 ±14	77 ±11	77 ±9
Calcium (mmol/L)	2.3 ±0.1	2.4 ±0.1	2.3 ±0.2	2.4 ±0.1
Albumin (g/L)	40.8 ±3.1	38.3 ±3.6	41.7 ±3.6	39.9 ±3.4
Phosphate (mmol/L)	1.6 ±0.3	1.6 ±0.3	1.2 ±0.3	0.9 ±0.5
Parathyroid hormone (pmol/L)	30 (17–48)	22 (15–46)	5 (3–14)	11 (7–21)

Data are presented as mean ±standard deviation, median (interquartile range) or number (percentage). Data of patients on nocturnal hemodialysis and kidney transplant recipients were measured at inclusion, i.e. about 3 months after initiating this treatment.
Abbreviations: eGFR: estimated glomerular filtration rate, calculated with the Chronic Kidney Disease-Epidemiology Collaboration equation 2009.

Patients that were excluded from the current analyses as they did not complete any follow-up exam (*n=46*) were on average 51 ±14 years old, 31 (67%) were male, dialysis duration was median 28 (IQR 14–58) months, and 11 (24%) had diabetes mellitus. Their median CAC score was 213 (IQR 0–1185).

During follow-up, 2 patients on conventional hemodialysis died (6%), as did 3 patients on peritoneal dialysis (14%), 0 patients on nocturnal hemodialysis, and 2 kidney transplant recipients (4%). Cardiovascular events occurred in 2 patients on conventional hemodialysis (6%), in 3 patients on peritoneal dialysis (14%), in 5 patients on nocturnal hemodialysis (15%), and in 3 kidney transplant recipients (6%). Eleven patients on conventional hemodialysis received a kidney transplant (34%), as did 7 patients on nocturnal hemodialysis (21%), and 9 patients on peritoneal dialysis (43%).

Associations of renal replacement therapy with CAC progression

At inclusion, CAC scores were median 182 (IQR 3–835) in patients on conventional hemodialysis, 74 (IQR 0–848) in patients on peritoneal dialysis, 176 (IQR 16–501) in patients on nocturnal hemodialysis, and 110 (IQR 10–523) in kidney transplant recipients (P=0.91 for difference). Eight patients on conventional hemodialysis (25%) and 6 patients on peritoneal dialysis (29%) had no calcification, compared to 7 on nocturnal hemodialysis (21%) and 9 kidney transplant recipients (19%). During 3 years of follow-up, CAC progressed in most patients (Table 2 and Figure 2).

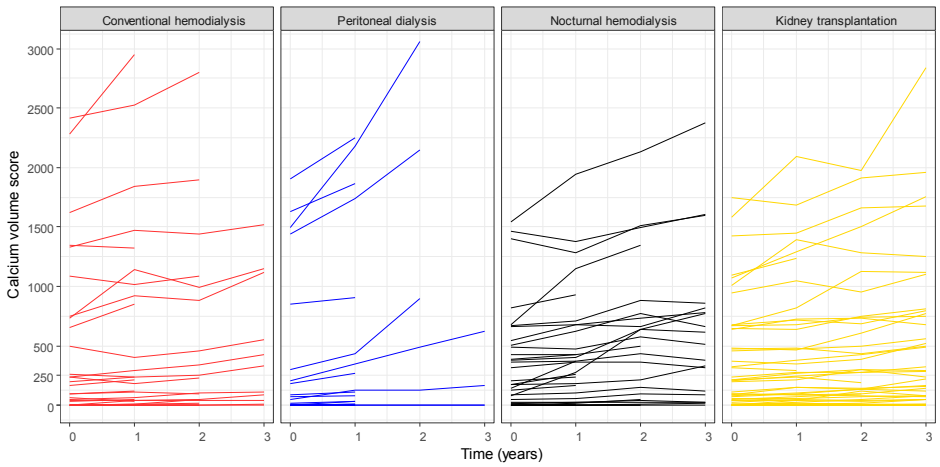

Figure 2. Progression of coronary artery calcification in 135 patients with end-stage renal disease stratified by renal replacement therapy. Two patients on conventional hemodialysis and one patient on peritoneal dialysis are off-scale, with CAC scores at inclusion/after 1 year of 5129/6793, 5731/7424, and 7140/13655.

Table 2. Coronary calcium scores at annual follow-up exams in 135 patients with end-stage renal disease.

	*N**	Inclusion	*N*	Year 1	*N*	Year 2	*N*	Year 3
Conventional hemodialysis	*32*	182 (3–835)	*32*	199 (27–1045)	*21*	106 (20–885)	*11*	334 (67–835)
Peritoneal dialysis	*21*	76 (0–848)	*21*	111 (6–908)	*7*	486 (64–5123)	*4*	82 (0–277)
Nocturnal hemodialysis	*34*	176 (16–501)	*34*	267 (20–583)	*24*	464 (46–743)	*19*	511 (107–799)
Kidney transplantation	*48*	103 (10–523)	*48*	154 (35–559)	*44*	144 (37–648)	*42*	194 (48–731)

Coronary calcium scores in mm^3 are presented as median (IQR).

*Patients without any follow-up exams were not included in the current analyses.

In patients on conventional hemodialysis, ΔCAC SQRV was 1.34 per year (95% CI 0.83 to 1.85) and in patients on peritoneal dialysis 2.62 (95% CI 1.86 to 3.39), while it was 1.29 per year in patients on nocturnal hemodialysis (95% CI 0.83 to 1.76) and 0.90 per year in kidney transplant recipients (95% CI 0.54 to 1.26). Patients on nocturnal hemodialysis and kidney transplant recipients did not have significantly less CAC progression compared to patients on conventional hemodialysis, both in unadjusted and adjusted analyses (Table 3). CAC progression was also not significantly less in kidney transplant recipients when compared to patients on conventional and nocturnal hemodialysis combined (adjusted difference in ΔCAC SQRV -0.30, 95% CI -0.80 to 0.22). However, CAC progression was significantly lower in kidney transplant recipients compared to patients on peritoneal dialysis (adjusted difference in ΔCAC SQRV -1.71, 95% CI -2.49 to -0.92). The above associations were similar when we used Agatston scores instead of volume scores (Table S1 and Table S2).

Table 3. Longitudinal changes in calcium scores between annual follow-up exams in 135 patients with end-stage renal disease.

	N	Mean change per year	Unadjusted difference
Conventional hemodialysis	32		
ΔCAC SQRV		1.34 (0.83 to 1.85)	0.0 *(reference)*
Peritoneal dialysis	21		
ΔCAC SQRV		2.62 (1.86 to 3.39)	1.28 (0.33 to 2.23)
Nocturnal hemodialysis	34		
ΔCAC SQRV		1.29 (0.83 to 1.76)	-0.05 (-0.73 to 0.64)
Kidney transplantation	48		
ΔCAC SQRV		0.90 (0.54 to 1.26)	-0.44 (-1.06 to 0.18)

95% confidence intervals between brackets.

*Model 1 = Adjusted for CAC SQRV at inclusion.

†Model 2 = Model 1 + age and sex.

‡Model 3 = Model 2 + diabetes mellitus, dialysis duration, and vitamin K antagonist use.

	Model 1*	Model 2†	Model 3‡
	0.0 *(reference)*	0.0 *(reference)*	0.0 *(reference)*
	1.31 (0.46 to 2.15)	1.41 (0.59 to 2.24)	1.43 (0.58 to 2.29)
	0.03 (-0.63 to 0.68)	0.02 (-0.61 to 0.66)	0.04 (-0.61 to 0.70)
	-0.32 (-0.91 to 0.27)	-0.27 (-0.85 to 0.31)	-0.22 (-0.84 to 0.39)

Discussion

Our study shows that nocturnal hemodialysis is not associated with less CAC progression compared to conventional hemodialysis during up to 3 years of follow-up. To our knowledge, this is the first study to report this. Furthermore, our study shows that kidney transplantation is not associated with significantly less CAC progression compared to conventional hemodialysis, although kidney transplantation is associated with less CAC progression compared to peritoneal dialysis.

Contrary to expected, we did not find less CAC progression in nocturnal hemodialysis or kidney transplantation compared to conventional hemodialysis, despite substantial serum phosphate reductions. An interpretation could be that vascular calcification progresses regardless of the type of renal replacement therapy. This contrasts with the prevailing paradigm that kidney transplant recipients have lower cardiovascular morbidity and mortality in part due to mitigating effects of kidney transplantation on vascular calcification. Rather, based on our current findings, one might also say that kidney transplant recipients have lower cardiovascular morbidity and mortality *despite* progressive vascular calcification. This suggests a discrepancy between progression of vascular calcification and hard endpoints.

Previously, others have also pointed out important discrepancies between the effects of several drugs on vascular calcification and mortality[19]. For example, several trials have demonstrated that drugs such as cinacalcet or sevelamer may slow down progression of coronary artery calcification[20, 21] but lack benefit on mortality[21, 22]. These discrepancies suggest that vascular calcification may only be a secondary phenomenon to vascular damage and may not be harmful in itself.

On the other hand, our data do not exclude the possibility that progression of vascular calcification is less after kidney transplantation compared to dialysis. We did find a significant difference in CAC progression for kidney transplant recipients compared to patients on peritoneal dialysis, and a non-significant difference for kidney transplant recipients compared to patients on conventional hemodialysis in the same direction. One previous study compared CAC progression between 41 kidney transplant recipients and 30 transplantation-eligible patients on hemodialysis during 2 years, and reported less frequent CAC progression among kidney transplant recipients[11]. Another earlier study also reported less frequent CAC progression in 23 kidney transplant recipients compared to 17 patients on hemodialysis during variable follow-up durations, although these patients were not matched on transplantation eligibility[10]. In light of these previous findings, it is possible that progression of vascular calcification is less after kidney transplantation,

but that we could not detect a significant difference due to sample size. Furthermore, it is unclear to what extent CAC progression concerns progression of medial calcification, which is where an effect of kidney transplantation could be expected. Coronary artery calcification has been suggested to be predominantly intimal[18]. This is based on one post-mortem study in 23 patients on dialysis with known coronary artery disease, which showed that coronary artery calcification was mostly located in intimal plaques, although it also occurred in the media[23]. Moreover, intimal calcification has been suggested to reinforce atherosclerotic plaque[19], which further complicates the interpretation of CAC progression with regard to its potential cardiovascular consequences.

Although our data may not exclude an effect of kidney transplantation on progression of vascular calcification, they clearly do not suggest less CAC progression in nocturnal hemodialysis compared to conventional hemodialysis. The effect estimates for nocturnal hemodialysis were close to zero and do not indicate an undetected difference. Only one previous study reported CAC progression in patients on nocturnal hemodialysis but had no control group[24]. An explanation for similar CAC progression in nocturnal hemodialysis and conventional hemodialysis may be that longer and more frequent hemodialysis could also increase clearance of certain water-soluble calcification inhibitors. These include pyrophosphate[25, 26] and magnesium[27, 28], which have been shown to be lost during hemodialysis. Whether nocturnal hemodialysis indeed results in lower serum levels of pyrophosphate or magnesium needs further study.

Our study has some important strengths. To our best knowledge, this is the first study to comprehensively compare CAC progression between different dialysis modalities and kidney transplantation, with a up to 3 years of follow-up, sufficiently long to detect CAC progression[29]. Also, all CT scans were read by a single reader blinded to treatment group, eliminating inter-observer variability. A unique strength of our study is that patients on dialysis and kidney transplant recipients were well comparable, as all patients on dialysis were transplantation-eligible, and all kidney transplant recipients had been on dialysis before transplantation. This study should however be viewed within the context of some limitations. Our study did not have a large sample size, although this is similar to many studies in patients on dialysis due to high transplantation and mortality rates[5]. Moreover, the close to zero effect estimates for nocturnal hemodialysis on CAC progression do not suggest an undetected effect of nocturnal hemodialysis. Another limitation is that the conventional hemodialysis group had a shorter follow-up, mainly due to high transplantation rates. Further, we could not measure certain calcification inhibitors or serum calcification propensity. This could have provided additional information.

In conclusion, nocturnal hemodialysis and kidney transplantation are not associated with significantly less CAC progression compared to conventional hemodialysis during up to 3 years follow-up. These findings raise important questions about which type of calcification is measured with CAC in end-stage kidney disease and what CAC progression means with regard to cardiovascular risk in end-stage kidney disease.

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Supplementary material

Table S1. Agatston scores at annual follow-up exams in 135 patients with end-stage renal disease.

	*N**	Inclusion	*N*	Year 1	*N*	Year 2	*N*	Year 3
Conventional hemodialysis	*32*	206 (1–897)	*32*	234 (20–1041)	*21*	103 (7–890)	*11*	426 (81–852)
Peritoneal dialysis	*21*	89 (0–1088)	*21*	129 (2–1028)	*7*	465 (64–1909)	*4*	71 (0–242)
Nocturnal hemodialysis	*34*	165 (18–586)	*34*	269 (12–751)	*24*	494 (51–780)	*19*	602 (131–880)
Kidney transplantation	*48*	112 (6–606)	*48*	138 (20–612)	*44*	131 (28–727)	*42*	178 (39–824)

Agatston scores are presented as median (IQR).

*Patients without any follow-up exams were not included in the current analyses.

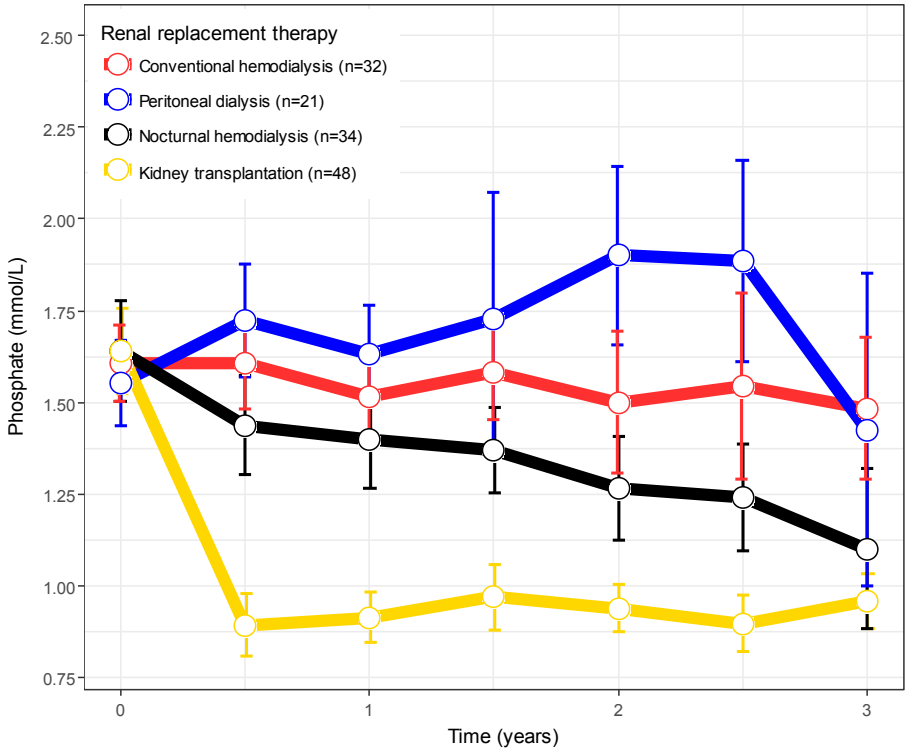

Figure S1. Phosphate levels during follow-up in 135 patients with end-stage renal disease, stratified by renal replacement therapy.

Table S2. Longitudinal changes in Agatston scores between annual follow-up exams in 135 patients with end-stage renal disease.

	N	Mean change per year	Unadjusted difference
Conventional hemodialysis	32		
ΔCAC SQRA		1.48 (0.86 to 2.10)	0.0 *(reference)*
Peritoneal dialysis	21		
ΔCAC SQRA		2.80 (1.96 to 3.64)	1.28 (0.283 to 2.36)
Nocturnal hemodialysis	34		
ΔCAC SQRA		1.31 (0.73 to 1.88)	-0.17 (-1.02 to 0.68)
Kidney transplantation**	48		
ΔCAC SQRA		0.93 (0.48 to 1.39)	-0.55 (-1.32 to 0.22)

95% confidence intervals between brackets.

*Model 1 = Adjusted for CAC SQRA at inclusion.

†Model 2 = Model 1 + age and sex.

‡Model 3 = Model 2 + diabetes mellitus, dialysis duration, and vitamin K antagonist use.

**adjusted difference in ΔCAC SQRA between kidney transplant recipients and patients on peritoneal dialysis: -1.80, 95% CI -2.66 to -0.94)

	Model 1*	Model 2†	Model 3‡
	0.0 *(reference)*	0.0 *(reference)*	0.0 *(reference)*
	1.43 (0.50 to 2.38)	1.53 (0.61 to 2.45)	1.46 (0.48 to 2.44)
	-0.02 (-0.77 to 0.73)	-0.03 (-0.77 to 0.70)	-0.10 (-0.86 to 0.66)
	-0.32 (-1.01 to 0.36)	-0.30 (-0.97 to 0.37)	-0.34 (-1.05 to 0.37)

Chapter 5

The role of kidney transplantation and phosphate binder use in vitamin K status

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Abstract

Background

Cardiovascular disease is the leading cause of death in end-stage renal disease and is strongly associated with vascular calcification. Both kidney transplantation and phosphate binders may lower the risk of vascular calcification. Vascular calcification is actively inhibited by vitamin-K-dependent matrix γ-carboxyglutamic acid protein (MGP). Whether kidney transplantation or phosphate binders affect vitamin K status is unknown. Therefore, we studied the influence of kidney transplantation and phosphate binder use on vitamin K status.

Methods

We measured plasma desphospho-uncarboxylated MGP (dp-ucMGP), a marker reflecting low vitamin K status, in a cross-sectional study of patients on hemodialysis *(n=82)*, peritoneal dialysis *(n=31)* or who recently received a kidney transplantation *(n=36)*. By medication inventory, we assessed phosphate binder use. With linear regression, we assessed the influence of kidney transplantation and phosphate binder use on natural-log-transformed dp-ucMGP, adjusting for potential confounders.

Results

Mean age of patients was 52±13 years; 102 (68%) were male. Dp-ucMGP levels were significantly lower in kidney transplant recipients (median 689 pmol/L) compared to patients on dialysis (median 1537 pmol/L, p<0.001). Eighty-nine patients on dialysis used phosphate binders. Using any phosphate binder was not associated with dp-ucMGP levels (median 1637 pmol/L, p=0.09) compared to no phosphate binders (median 1142 pmol/L). Twenty-six patients used sevelamer monotherapy, which was associated with higher dp-ucMGP levels (median 1740 pmol/L, p=0.04) after adjusting for age, sex and vitamin K antagonist use.

Conclusions

Recent kidney transplantation is associated with lower dp-ucMGP levels suggesting improved vitamin K status after transplantation. Sevelamer monotherapy is associated with higher dp-ucMGP levels suggesting worsening of vitamin K status. Both findings warrant more attention to vitamin K status in patients on dialysis, as vitamin K is necessary for protection against vascular calcification.

Introduction

Cardiovascular disease accounts for over 50% of deaths in end-stage renal disease (ESRD)[1], and is advanced by vascular calcification, often encountered in ESRD[2-4]. Vascular calcification is inhibited by matrix γ-carboxyglutamic acid protein (MGP)[5], which needs vitamin K for carboxylation to its active form. High levels of inactive MGP, desphospho-uncarboxylated MGP (dp-ucMGP), indicate vitamin K deficiency[6] and are associated with vascular calcification in chronic kidney disease[7, 8].

Kidney transplantation is the preferred treatment for ESRD, and rapidly normalizes serum phosphate[9]. Phosphate contributes importantly to vascular calcification[10]. However, kidney transplantation does not normalize cardiovascular disease risk, even though it prolongs life expectancy[11-13]. Interestingly, kidney transplant recipients are often vitamin K deficient[14], which is associated with increased mortality[15].

Patients on dialysis are routinely prescribed phosphate binders to lower serum phosphate levels. Despite their widespread use, definite proof for beneficial outcomes is lacking[16]. Remarkably, phosphate binder use did not improve and even worsened vascular calcification compared to placebo in a randomized trial in patients with chronic kidney disease, despite lowering of slightly elevated baseline phosphate levels[17]. Therefore, it is conceivable that phosphate binders exert adverse effects that are yet unknown[17].

Recent *in-vitro* studies indicate that various phosphate binders may not only bind phosphate, but also fat-soluble vitamins such as vitamin K[18-20]. Phosphate binders might thus worsen vitamin K status and hence promote vascular calcification.

It is unclear whether kidney transplantation improves low vitamin K status in patients on dialysis[21]. Moreover, it is unknown whether phosphate binder use affects vitamin K status in patients on dialysis. Therefore, we measured dp-ucMGP in a cohort of patients with ESRD, to assess whether kidney transplantation is associated with better vitamin K status, i.e. lower dp-ucMGP levels, as compared to hemodialysis and peritoneal dialysis. Finally, we investigated whether use of phosphate binders was associated with worse vitamin K status, i.e. higher dp-ucMGP levels in patients on dialysis.

Methods

Study population

We analyzed a cross-sectional cohort from the ongoing NOCTx study (NCT00950573), a prospective cohort study that included prevalent patients on hemodialysis and peritoneal dialysis with a minimum dialysis vintage of 2 months, and patients who received a kidney transplant 2–3 months before inclusion. Patients were eligible when aged between 18 and 75 years, and were candidates for transplantation when on dialysis. All study participants gave written informed consent. NOCTx excluded patients with a life expectancy <3 months, pre-emptive transplantation, non-adherence to dialysis regimens, drug abuse, and pregnancy. NOCTx has been approved by the Medical Ethics Committee of the University Medical Centre Utrecht and is conducted according to the Declaration of Helsinki. None of the transplant donors were from a vulnerable population and all donors or next of kin provided written informed consent that was freely given.

Between December 2009 and February 2016, NOCTx included 181 patients who were referred for study participation to the University Medical Centre of Utrecht, the Netherlands, by 8 Dutch dialysis centers. Patients were treated according to guidelines by the attending nephrologists. For kidney transplant recipients, standard immunosuppressant regimens consisted of a calcineurin inhibitor (tacrolimus), mycophenolate mofetil, and prednisone in tapering doses.

Blood samples were collected in 4.5 mL potassium-ethylenediaminetetraacetic acid vacutainers (on a non-dialysis day in case of hemodialysis), immediately centrifuged and stored in aliquots at -80°C without thawing. For the present analyses, we excluded patients whose blood samples were not available *(n=28)* or with missing data for medication prescriptions *(n=4)*, leaving a final sample of 149 patients, 113 of which were on dialysis.

Dp-ucMGP measurements

We determined vitamin K status by measuring dp-ucMGP. Dp-ucMGP is a sensitive marker of vitamin K status, as opposed to circulating vitamin K_1 and K_2, which may fluctuate substantially as a result of dietary intake[21] and degrade when exposed to light[22]. Plasma dp-ucMGP levels were determined using the commercially available IVD CE-marked chemiluminescent InaKtif MGP assay on the IDS-iSYS system (IDS, Boldon, United Kingdom). Patient sample and internal calibrators were incubated with magnetic particles coated with murine monoclonal antibodies dpMGP, acridinium-labelled murine monoclonal antibodies ucMGP, and an assay buffer. The magnetic particles were captured using a magnet and washed to remove any unbound analyte. Trigger reagents were added; the resulting light emitted by the acridinium label was directly proportional to the level

of dp-ucMGP in the sample. The within-run and total variations of this assay were 0.8–6.2% and 3.0–8.2%, respectively. The assay measuring range was between 300 and 12,000 pmol/L and was linear up to 11,651 pmol/L[8]. All assays were performed in a single run by the laboratory of Coagulation Profile, department of Biochemistry, Maastricht, the Netherlands.

Phosphate binder use

Study personnel recorded phosphate binder use with lists of prescribed medication at time of blood sample collection. Similarly, vitamin K antagonist (VKA) and vitamin D analog use were recorded. We categorized patients according to phosphate binder use: no binders or any binder, subcategorized as exclusively non-calcium containing binders or exclusively calcium containing binders. Additionally, we subcategorized patients according to monotherapy with a single binder (sevelamer hydrochloride or carbonate, lanthanum carbonate, calcium carbonate, calcium acetate/magnesium carbonate, or calcium acetate).

Other study variables

At time of sampling, study personnel recorded demographic and clinical parameters (pre-dialysis blood pressure and post-dialysis weight averaged from routine measurements during 3 hemodialysis sessions or 2 outpatient visits). Total calcium, albumin, phosphate, parathyroid hormone, C-reactive protein and total cholesterol were routinely measured at local treatment facilities (pre-dialysis for patients on hemodialysis). Smoking status and height were self-reported. Study personnel evaluated residual urine production with a 24h-urine collection. We assessed history of kidney disease, current dialysis schedule and presence of comorbidities by chart review. We defined diabetes mellitus as the necessity for oral diabetes medication or insulin therapy, and cardiovascular disease as any history of angina, myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, aortic aneurysm ≥5 cm, stroke, intermittent claudication, peripheral artery angioplasty or bypass grafting. We defined dialysis vintage as the time since the first day of dialysis, minus the time with a functioning kidney transplant. For kidney transplant recipients, we estimated glomerular filtration rate with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 2009 equation.

Statistical analyses

We present results as mean (± standard deviation) for normally distributed variables, as median (interquartile range, IQR) for non-normally distributed variables, or as number (percentage) for categorical data. We tabulated baseline characteristics and medication use by renal replacement therapy. We compared dialysis vintage and dp-ucMGP between groups with Mann-Whitney-U tests.

The distribution of dp-ucMGP levels was right-skewed and we therefore natural-log-transformed dp-ucMGP levels. With boxplots and linear regression we examined the associations of both renal replacement therapy and phosphate binder use with log-transformed dp-ucMGP. We compared log-transformed dp-ucMGP between kidney transplantation and dialysis (hemodialysis and peritoneal dialysis jointly), and between hemodialysis and peritoneal dialysis. We compared log-transformed dp-ucMGP between phosphate binder categories in patients on dialysis only, as none of the kidney transplant recipients used phosphate binders. We compared patients using any phosphate binder, exclusively non-calcium containing phosphate binders, exclusively calcium containing phosphate binders and sevelamer monotherapy to non-phosphate binder users. We adjusted stepwise for potential confounders age (years), sex (male/female), VKA use (yes/no), intensive hemodialysis regimens (≥18h/week or <18h/week) and residual urine production (≥100mL/24h or absent). Additionally, we analyzed non-VKA users only. In the final models of both renal replacement therapy and phosphate binder use, we adjusted for age (years), sex (male/female) and VKA use (yes/no).

We reported regression coefficients with 95% confidence intervals (95% CI). Regression coefficients should be interpreted multiplicatively, i.e. as a ratio, after exponentiation. We considered P-values of ≤ 0.05 (two-tailed) statistically significant and performed all analyses with R 3.3.3 (R Foundation Statistical Computing).

Results

Study population

The mean age of the study population *(n=149)* was 52 ±13 years, 102 patients (68%) were male. Dialysis vintage (including historical dialysis vintage of kidney transplant recipients) was 23 months (IQR 11–49 months), 21 patients (14%) had diabetes mellitus and 16 patients (11%) used VKAs (acenocoumarol or phenprocoumon; 4 kidney transplant recipients and 12 patients on hemodialysis). Eighty-two patients were treated with hemodialysis, 31 with peritoneal dialysis and 36 were kidney transplant recipients (Table 1).

Table 1. Characteristics of the 149 patients with end-stage renal disease stratified by renal replacement therapy.

	Hemodialysis (n = 82)	Peritoneal dialysis (n = 31)	Kidney transplantation (n = 36)
Demographics and medical history			
Age (y)	53.3 ±12.2	49.8 ±14.2	50.4 ±15.1
Male (%)	53 (65%)	21 (68%)	28 (78%)
BMI (kg/m²)	26.1 ±5.0	24.2 ±3.3	24.9 ±3.3
Systolic blood pressure (mmHg)	140 ±19	134 ±13	128 ±13
Diastolic blood pressure (mmHg)	77 ±11	84 ±12	77 ±7
Diabetes mellitus (%)	17 (21%)	1 (3%)	3 (8%)
Prior cardiovascular disease (%)	19 (23%)	6 (19%)	5 (14%)
Active smoker (%)	13 (16%)	5 (16%)	4 (11%)
History of kidney disease			
Dialysis vintage (months)	28 (13 – 59)	12 (6 – 22)	31 (12 – 55)
Cause of ESRD (%)			
• Cystic kidney disease	9 (11%)	3 (10%)	12 (33%)
• Interstitial nephritis	3 (4%)	1 (3%)	1 (3%)
• Glomerulonephritis	27 (33%)	7 (23%)	6 (17%)
• Vascular disease	15 (18%)	7 (23%)	7 (19%)
• Diabetic nephropathy	9 (11%)	0 (0%)	3 (8%)
• Other	13 (16%)	7 (23%)	3 (8%)
• Unknown	6 (7%)	6 (19%)	4 (11%)
Dialysis therapy and kidney function			
Dialysis therapy			
• Weekly HD sessions	3.5 ±0.9	-	-
• Weekly HD hours	16.9 ±11.1	-	-
• Daily PD dwells	-	4.4 ±0.6	-

Table 1. Continued.

	Hemodialysis (n = 82)	Peritoneal dialysis (n = 31)	Kidney transplantation (n = 36)
• Daily PD volume (L)	-	9.3 ±2.1	-
Kidney function			
• Residual urine production ≥100mL/24h (%)	38 (46%)	21 (68%)	-
• eGFR (mL/min)	-	-	53 ±20
Laboratory parameters			
Calcium (mmol/L)	2.3 ±0.2	2.3 ±0.1	2.4 ±0.1
Albumin (g/L)	41.5 ±3.2	38.7 ±3.1	40.3 ±3.2
Phosphate (mmol/L)	1.7 ±0.4	1.6 ±0.4	0.8 ±0.3
Parathyroid hormone (pmol/L)	20 (11 – 43)	21 (14 – 42)	13 (10 – 27)
C-reactive protein (mg/L)	3.0 (2.0 – 5.0)	1.5 (1.0 – 6.8)	3.0 (1.5 – 5.0)
Total cholesterol (mmol/L)	4.4 ±1.1	5.1 ±1.6	4.8 ±1.2
dp-ucMGP (pmol/L)	1605 (993 – 2390)	1195 (921 – 1807)	689 (489 – 1078)

Data are presented as mean ±standard deviation, median (interquartile range) or number (percentage). Abbreviations: BMI: body mass index; ESRD: end-stage renal disease; eGFR: estimated glomerular filtration rate, calculated with the Chronic Kidney Disease-Epidemiology Collaboration equation 2009; dp-ucMGP: desphospho-uncarboxylated matrix Gla-protein.

The mean age of patients not included in the evaluation sample *(n=32* without samples or medication lists) was 52±13 years, 19 (59%) were male, dialysis vintage was 32 months (IQR 15–69 months, P=0.15 versus study population) and 5 patients (16%) had diabetes mellitus. Nineteen of these patients were treated with hemodialysis, 7 with peritoneal dialysis and 6 were kidney transplant recipients.

Phosphate binder use

Twenty-four of the 113 patients on dialysis (21%) did not use phosphate binders, while 89 (79%) used any phosphate binder. Fifty-three of these patients used exclusively non-calcium containing phosphate binders, whereas 10 used exclusively calcium containing phosphate binders (Table 2). Thirty-eight of the patients on dialysis (34%) used a single phosphate binder, 45 patients (40%) two types of phosphate binders, and 6 patients (5%) three types of phosphate binders. None of the kidney transplant recipient used phosphate binders, apart from 500 mg calcium carbonate once daily, commonly prescribed as supplement post-transplantation *(n=22)*. We therefore excluded kidney transplant recipients from the phosphate binder analyses.

Table 2. Medication prescriptions in 149 patients with end-stage renal disease, stratified by renal replacement therapy.

	Hemo-dialysis (n = 82)	Peritoneal dialysis (n = 31)	Kidney transplantation (n = 36)
Vitamin K antagonists (%)	12 (15%)	0 (0%)	4 (11%)
Vitamin D analogs (%)	57 (70%)	25 (81%)	4 (11%)
Any phosphate binder (%)	60 (73%)	29 (94%)	0 (0%)
• Exclusively non-calcium containing phosphate binders (%)	36 (44%)	17 (55%)	0 (0%)
○ Sevelamer monotherapy (%)	20 (24%)	6 (19%)	0 (0%)
• Exclusively calcium containing phosphate binders (%)	6 (7%)	4 (13%)	0 (0%)

Data are presented as numbers (percentage). Vitamin K antagonists prescribed were acenocoumarol and phenprocoumon, and vitamin D analogs prescribed were alfacalcidol.

Overall, sevelamer was the most frequently prescribed phosphate binder (73 patients, 65%), followed by lanthanum carbonate (37 patients, 33%), calcium carbonate (29 patients, 26%), calcium acetate/magnesium carbonate (4 patients, 4%) and calcium acetate (3 patients, 3%). Sevelamer was prescribed as monotherapy in 26 patients (23%). Monotherapy with lanthanum carbonate, calcium carbonate, calcium acetate/magnesium carbonate and calcium acetate occurred too infrequently (in 2, 8, 1 and 1 patients, respectively) and was therefore not studied separately.

Dp-ucMGP levels

Median plasma levels of dp-ucMGP in all 149 patients with ESRD were 1302 (IQR 739–1838) pmol/L. Dp-ucMGP levels were highest in VKA users (median 4718, IQR 3028–6672 versus 1146, IQR 704–1716 pmol/L in non-users, P<0.001). Dp-ucMGP levels were right-skewed and therefore natural-log-transformed for regression analyses.

Associations between kidney transplantation and dp-ucMGP

In kidney transplant recipients, median dp-ucMGP levels were 689 (IQR 489–1078) pmol/L. In patients on hemodialysis, median dp-ucMGP levels were 1605 (IQR 993–2390) pmol/L, and in patients on peritoneal dialysis 1195 (IQR 921–1807) pmol/L (Figure 1). In crude regression analyses, dp-ucMGP levels were lower in kidney transplant recipients as compared to patients treated with hemo- and peritoneal dialysis (regression coefficient -0.64, 95% confidence interval [CI] -0.91; -0.36). This association remained numerically similar when adjusted for age, sex and VKA use (regression coefficient -0.61, 95% CI -0.84; -0.37). Hemodialysis was associated with higher dp-ucMGP levels compared to peritoneal dialysis in crude regression analyses (regression coefficient 0.26, 95% CI -0.05; 0.56), but

not when adjusted for age, sex and VKA use (regression coefficient 0.05, 95% CI -0.31; 0.21). The above associations remained numerically similar when analyzed in non-VKA users only (Table S1).

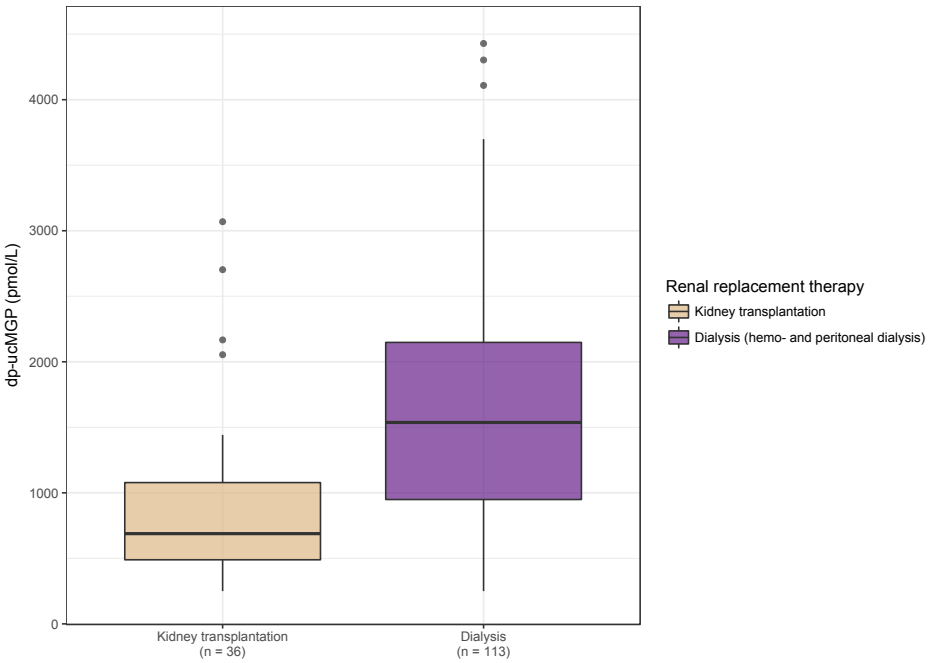

Figure 1. Dp-ucMGP levels stratified by renal replacement therapy, as boxplots.

Figure legend: Kidney transplant recipients *(n=36)*: sand-color boxplot, and both patients on hemo- and peritoneal dialysis *(n=113)*: purple boxplot. Median values of dp-ucMGP: 689 and 1537 pmol/L respectively.

Associations between phosphate binder use and dp-ucMGP

In patients on dialysis without phosphate binders *(n=24)*, median dp-ucMGP levels were 1142 (IQR 841–1642) pmol/L, while in those on any phosphate binder *(n=89)* median dp-ucMGP levels were 1637 (IQR 994–2307) pmol/L. This difference was non-significant, and remained numerically similar when adjusted for age, sex and VKA use (Table 3). In patients on dialysis on exclusively non-calcium containing phosphate binders *(n=53)* and exclusively calcium containing phosphate binders *(n=10)*, median dp-ucMGP levels were 1615 (IQR 1068–2061) and 2330 (IQR 1014–3476) pmol/L, respectively. Compared to patients on dialysis without phosphate binders, these dp-ucMGP levels were not significantly different, and the differences remained numerically similar in the adjusted model. In patients on dialysis on sevelamer monotherapy *(n=26)*, median dp-ucMGP levels were 1740 (IQR 1363–2267) pmol/L. These dp-ucMGP levels were significantly higher compared

to patients on dialysis without phosphate binders in the adjusted model (Figure 2). The above associations remained numerically similar when analyzed in non-VKA users only (Table S2).

Table 3. Regression coefficients of linear regression analysis of phosphate binder prescription and log-transformed dp-ucMGP levels in 113 patients with end-stage renal disease on dialysis.

	N	Crude regression coefficient 95% CI	Adjusted regression coefficient* 95% CI
No phosphate binders	24	0.0 *(reference)*	0.0 *(reference)*
Any phosphate binder	89	0.26 (-0.08; 0.60)	0.25 (-0.04; 0.53)
• Exclusively non-calcium containing phosphate binder	53	0.23 (-0.10; 0.56)	0.28 (-0.02; 0.59)
○ Sevelamer monotherapy	26	0.34 (-0.04; 0.72)	0.35 (0.02; 0.68)
• Exclusively calcium containing phosphate binder	10	0.42 (-0.12; 0.95)	0.28 (-0.20; 0.76)

Plasma dp-ucMGP was skewed to the right and therefore log-transformed. Hence, regression coefficients should be interpreted as a ratio after exponentiation. CI: confidence interval

*Adjusted for age (years), sex (male/female) and vitamin K antagonist use (yes/no).

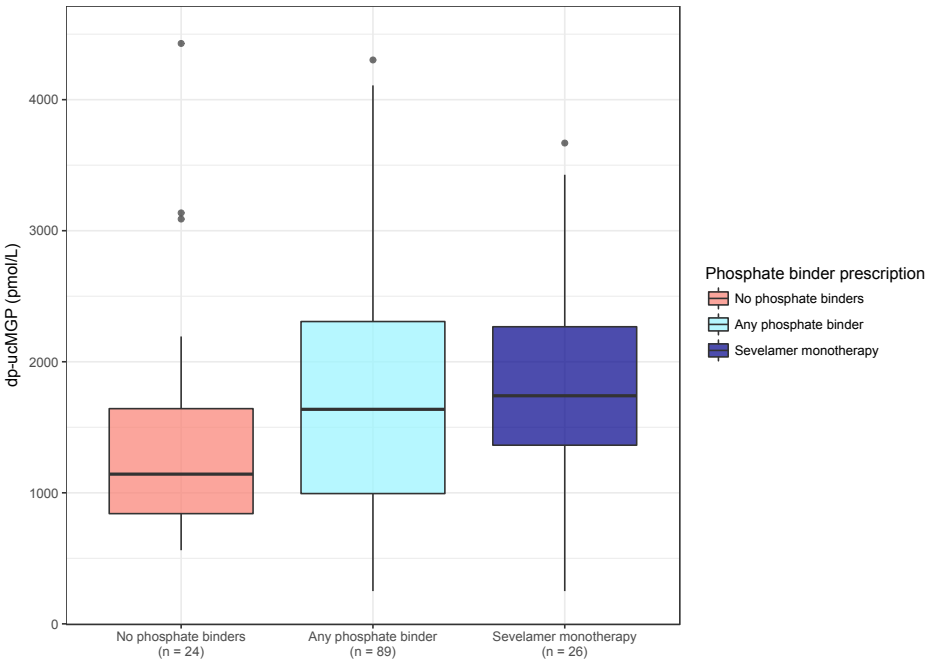

Figure 2. Dp-ucMGP levels stratified by phosphate binder use in patients on dialysis, as boxplots.

Figure legend: Patients using no phosphate binders *(n=24)*: pink boxplot, patients using any phosphate binder *(n=89)*: light blue boxplot, and a subgroup of patients using sevelamer monotherapy *(n=26)*: dark blue boxplot. Median values of dp-ucMGP: 1142; 1637; and 1740 pmol/L, respectively.

Discussion

Our study shows that kidney transplant recipients have substantially lower dp-ucMGP levels compared to patients on any form of dialysis, indicating better vitamin K status after restoration of kidney function. Phosphate binder use in general is not associated with dp-ucMGP levels. However, sevelamer monotherapy is associated with significantly higher dp-ucMGP levels compared to no phosphate binders, suggesting a negative effect of sevelamer on vitamin K status.

To our knowledge, vitamin K status and dp-ucMGP levels have only been studied in kidney transplant recipients or patients on dialysis in isolation. Previous studies have examined stable kidney transplant recipients (median 6 years after transplantation) with a similar age to our population (mean and median age 51[15] and 56 years[14]), and found similar dp-ucMGP levels to our measurements 2-3 months after transplantation[14, 15]. A previous study of healthy individuals (mean age 53 years) has reported slightly lower dp-ucMGP levels (447 ±188 pmol/L) as compared to kidney transplant recipients in our study (median 689 pmol/L)[23]. In our study, kidney transplant recipients had about twice as low dp-ucMGP levels compared to patients on dialysis (median 689 versus 1537 pmol/L). Likely, dp-ucMGP levels in kidney transplant recipients have been higher before transplantation, because they had all been on dialysis. Altogether, this suggests that vitamin K status improves rapidly after kidney transplantation.

There are several possible explanations for the lower dp-ucMGP levels in kidney transplant recipients. Uremia impairs carboxylation of MGP by reducing γ-carboxylase[24]; thus, relief of uremia by kidney transplantation might restore carboxylation capacity of MGP as reflected by lower dp-ucMGP levels. Furthermore, dp-ucMGP levels could be lower because of improved vitamin K intake after kidney transplantation; vitamin K intake is poor in patients on dialysis, partly due to the dietary restrictions[21]. Nonetheless, vitamin K deficiency was still common after transplantation, reflected by dp-ucMGP >500 pmol/L[23] in 20 out of 32 kidney transplant recipients that did not use VKAs.

We found lower dp-ucMGP levels in patients on hemodialysis compared to previous literature. These include cross-sectional studies that examined older patients on hemodialysis (median age 74[8] and 65 years[21]), as well as a randomized trial on vitamin K_2 supplementation (mean age 64 years[25]). Interestingly, in our study dp-ucMGP levels were higher among patients on hemodialysis than on peritoneal dialysis, which could be explained by patients on hemodialysis using VKAs more frequently. Frequent VKA use may predispose patients on hemodialysis to vitamin K deficiency. Possibly, this difference will be attenuated when patients on dialysis will use direct oral anticoagulants instead of VKAs in the future.

Recent *in vitro* studies have investigated the impact of phosphate binders on vitamin K, demonstrating that most classes of binders can bind fat-soluble vitamins including vitamin K[18, 19]. This implies that phosphate binders may aggravate vitamin K deficiency, hampering activation of MGP and limiting protection against vascular calcification. Currently, little is known about the impact of phosphate binders on vitamin K status in humans. Counterintuitively, an observational study in patients on hemodialysis has reported lower total ucMGP levels with calcium carbonate use[26]. However, total ucMGP reflects vitamin K status poorly[23], unlike dp-ucMGP, which was not measured. To our knowledge, no human study has investigated the impact of phosphate binders on vitamin K status, as measured by dp-ucMGP.

Our study shows an association between sevelamer monotherapy and higher dp-ucMGP levels after adjusting for age, sex and VKA use, but remarkably no association between phosphate binder use in general and dp-ucMGP levels, although we did observe a trend towards higher dp-ucMGP levels. This discrepancy could be explained by confounding by better dietary intake, including vitamin K intake, by patients using multiple phosphate binders. These patients had higher phosphate levels (1.74±0.30 mmol/L) compared to patients using a single phosphate binder (1.58±0.39 mmol/L), which might suggest better dietary intake. On the other hand, the supposed vitamin K-lowering effect of phosphate binders could have been masked by medication non-adherence in patients using multiple phosphate binders.

Our findings may be clinically relevant: the better vitamin K status after kidney transplantation may in part explain the lower risk of cardiovascular disease after transplantation[13], given that high dp-ucMGP is associated with vascular calcification and all-cause mortality in chronic kidney disease[27]. This therefore warrants more attention to vitamin K status in patients on dialysis by improving dietary vitamin K intake, or even by vitamin K supplementation. Furthermore, it is a safety signal that sevelamer may negatively affect vitamin K status; the same may apply to other phosphate binders when tested in larger studies. Possibly, this supposed effect of phosphate binder use caused the remarkable progression of vascular calcification seen in the study by Block et al[17]. This placebo-controlled randomized trial found worsening of vascular calcification in patients allocated to phosphate binders compared to placebo, despite lowering of serum phosphate[17]. When confirmed, this justifies even more attention to vitamin K status of patients on dialysis, especially of those on phosphate binders.

Our study has some limitations. First, there may have been indication bias for no phosphate binder use. Using no phosphate binders may have been related to malnutrition, and hence a lower production of various proteins, including MGP and thereby dp-ucMGP.

Conversely, malnutrition could also have increased dp-ucMGP levels by poor vitamin K intake. Importantly, both albumin levels and BMI did not suggest malnutrition in non-phosphate binder users (albumin 42.4 ±3.2 g/L and BMI 26.2 ±4.4 kg/m^2 versus 40.3 ±3.4 g/L and 25.4 ±4.8 in kg/m^2 in phosphate binder users). A second limitation is that the aim of the main study was about dialysis modality and vascular calcification, and hence dietary intake and medication adherence were not measured. On the one hand, dosages of phosphate binders were therefore not taken into account. On the other hand, medication non-adherence could have protected patients from iatrogenic vitamin K deficiency by phosphate binders, masking the supposed adverse effect. Third, monotherapy with phosphate binders other than sevelamer occurred too infrequently to be analyzed separately. Finally, we did not measure vitamin D status, which might also be affected by phosphate binder use[28], or other MGP species, such as total ucMGP and total MGP. Nevertheless, dp-ucMGP remains the most accurate marker of vitamin K status of all MGP species[23, 29].

Our study also has several strengths. First, dp-ucMGP levels were measured in a single run with a validated, standardized technique. Second, patients in our study originated from 8 large dialysis centers, representing an urban as well as a rural population. We therefore believe our results can be generalized to other ESRD populations. Third, all patients on dialysis were transplantation candidates, while all kidney transplant recipient had been transplanted after a period on dialysis. Thus, patients on dialysis and kidney transplant recipients were comparable.

In conclusion, recent kidney transplantation is associated with lower dp-ucMGP levels, indicating rapid improvement of vitamin K status following restoration of kidney function. In patients on dialysis, sevelamer monotherapy is associated with higher dp-ucMGP levels compared to no phosphate binders, suggesting worsening of vitamin K status by this phosphate binder. This calls for attention to vitamin K status in patients on dialysis, as vitamin K is necessary for protection against vascular calcification.

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Supplementary material

Table S1. Regression coefficients of linear regression analysis of renal replacement therapy and log-transformed dp-ucMGP levels in 149 patients with end-stage renal disease, stratified by vitamin K antagonist use.

	All patients	
	N	Crude regression coefficient
Hemo- and peritoneal dialysis	113	0.0 *(reference)*
Kidney transplantation	36	-0.64 (-0.91; -0.36)
	All patients	
	N	Crude regression coefficient
Peritoneal dialysis	31	0.0 *(reference)*
Hemodialysis	82	-0.26 (-0.57; 0.05)

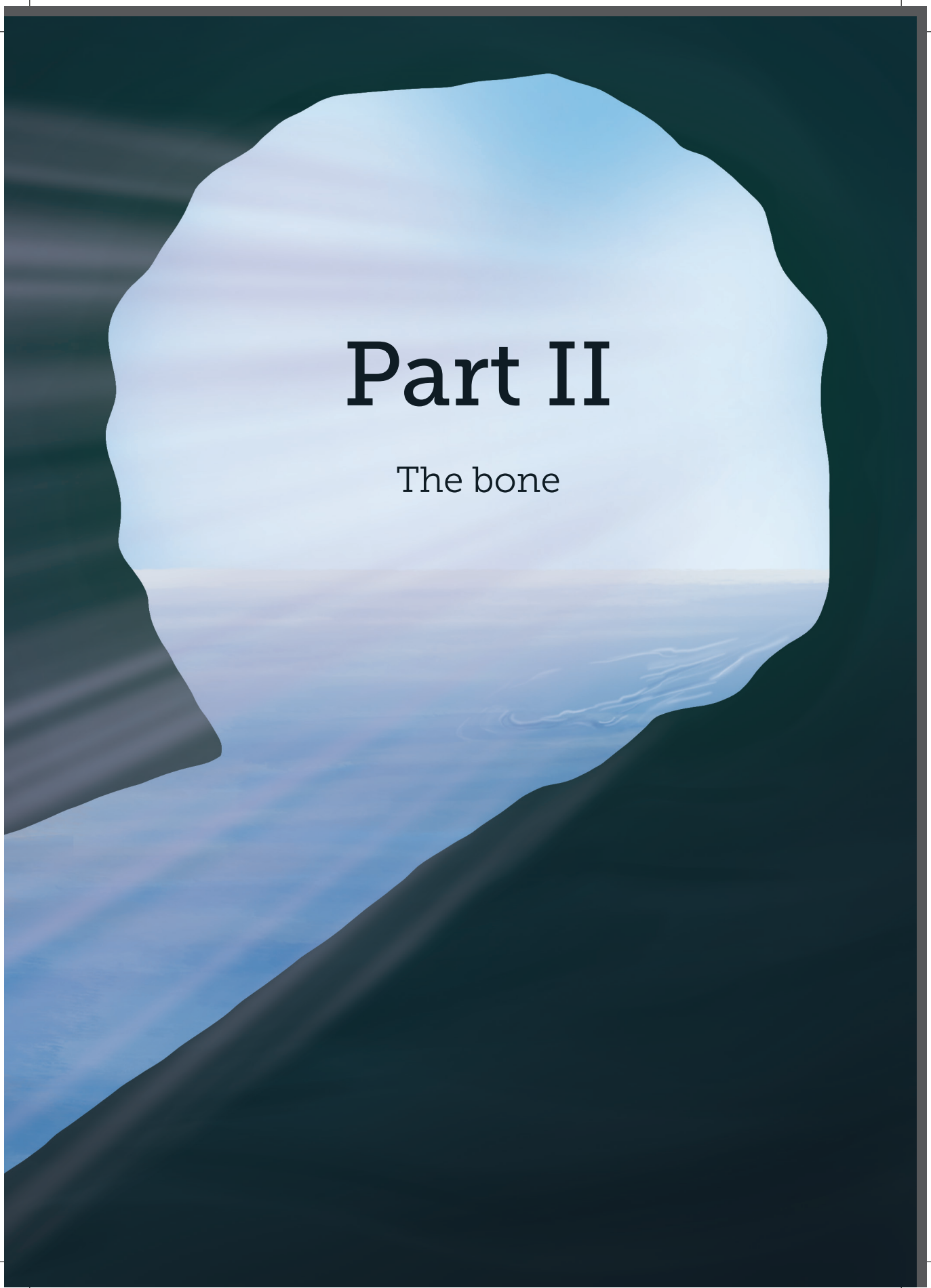
Table S2. Regression coefficients of linear regression analysis of phosphate binder use and log-transformed dp-ucMGP levels in 113 patients on dialysis, stratified by vitamin K antagonist use.

	All patients	
	N	Crude regression coefficient
No phosphate binders	24	0.0 *(reference)*
Any phosphate binder	89	0.26 (-0.08; 0.60)
• Exclusively non-calcium containing phosphate binder	53	0.23 (-0.10; 0.56)
○ Sevelamer monotherapy	26	0.34 (-0.04; 0.72)
• Exclusively calcium containing phosphate binder	10	0.42 (-0.12; 0.95)

Non-vitamin K antagonist users		Vitamin K antagonist users	
N	Crude regression coefficient	N	Crude regression coefficient
101	0.0 *(reference)*	12	0.0 *(reference)*
32	-0.64 (-0.89; -0.39)	4	-0.66 (-1.30; -0.02)

Non-vitamin K antagonist users		Vitamin K antagonist users	
N	Crude regression coefficient	N	Crude regression coefficient
31	0.0 *(reference)*	0	*n/a*
70	-0.07 (-0.35; 0.21)	12	*n/a*

Non-vitamin K antagonist users		Vitamin K antagonist users	
N	Crude regression coefficient	N	Crude regression coefficient
22	0.0 *(reference)*	2	0.0 *(reference)*
79	0.21 (-0.10; 0.53)	10	0.32 (-0.37; 1.01)
50	0.27 (-0.05; 0.60)	3	0.07 (-0.57; 0.70)
23	0.34 (-0.03; 0.70)	3	0.07 (-0.57; 0.70)
8	0.29 (-0.21; 0.79)	2	0.21 (-2.92; 3.34)



Part II

The bone

Chapter 6

The prevalence and incidence of vertebral fractures in end-stage renal disease and the role of parathyroid hormone

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Abstract

Introduction

Vertebral fractures are often overlooked, but even undiagnosed vertebral fractures negatively impact physical functioning, quality of life, and mortality. The risk of vertebral fractures in end-stage renal disease (ESRD) patients is unclear, and parathyroid hormone (PTH) might play a role in the development of vertebral fractures. We therefore determined vertebral fracture prevalence and incidence in ESRD patients and assessed associations of vertebral trabecular bone mineral density (BMD) and PTH with vertebral fracture.

Methods

In 146 transplantation-eligible patients on dialysis, we determined vertebral fractures on lateral chest radiographs, which image the thoracic and upper lumbar spine. We determined incident vertebral fractures in 70 patients with follow-up radiographs (23 received a kidney transplant) after median 1.8 years. Vertebral trabecular BMD was measured with computed tomography, and PTH measured with 2-site immunoassays, categorized in tertiles with the middle tertile as reference. We used Poisson regression to assess associations of vertebral trabecular BMD and PTH with vertebral fracture.

Results

Mean age of the study population was 52±13 years, and 98 (67%) were male. Median dialysis duration was 26 (IQR 13–55) months. Vertebral fractures were present in 50/146 patients (34%) and incident vertebral fractures occurred in 20/70 patients (29%). Vertebral trabecular BMD was not associated with vertebral fracture prevalence (relative risk 0.97, 95% CI 0.89 to 1.04). For the lowest PTH tertile (<11 pmol/L), the relative risk of vertebral fracture was greater although not significant (2.28, 95% CI 0.97 to 5.97) and was significantly greater for the highest PTH tertile (≥30 pmol/L; 2.82, 95% CI 1.22 to 7.27) after adjustment for potential confounders.

Conclusions

The prevalence and incidence of vertebral fractures is high even in relatively young and healthy ESRD patients. Vertebral trabecular BMD is not associated with vertebral fracture, and the association of PTH with vertebral fracture risk appears U-shaped.

Introduction

Patients with end-stage renal disease (ESRD) have a high risk of fractures. The risk of hip fractures is four times higher in patients on dialysis compared to the general population[1]. While hip fractures are mostly clinically apparent, many vertebral fractures are presented atypically[2]. Vertebral fractures are easily missed on radiographs[3]. Hence, they often remain undiagnosed, but even undiagnosed vertebral fractures negatively impact physical functioning[4], quality of life[5], and mortality risk[6]. Nevertheless, due to poor documentation in cohorts and dialysis registries, the risk of vertebral fracture in ESRD remains largely unknown[7].

Patients with ESRD have distinct risk factors for fracture beyond traditional risk factors due to chronic kidney disease-mineral and bone disorder[7]. The disturbed bone metabolism, also known as renal osteodystrophy, comprises a spectrum of loss of bone mass due to high bone turnover, and microstructural abnormalities due to suppressed bone turnover[8]. These specific types of renal osteodystrophy cannot be distinguished with conventional markers of bone fragility, such as bone mineral density (BMD)[9]. Clinicians commonly rely on parathyroid hormone (PTH) to estimate bone turnover in ESRD, however, the prognostic utility of BMD and PTH for fracture risk in ESRD is unclear[10]. A recent meta-analysis indicated that lower BMD at the lumbar spine was associated with prevalent fractures among patients on dialysis, but not in age-adjusted analyses[11]. This suggests that vertebral BMD may not independently predict fracture risk in ESRD. Furthermore, studies observed that both low and high PTH values were associated with hip and vertebral fracture risk among patients with ESRD, but with various cut-off values[12-15]. There is also an unclear relationship of PTH with BMD, with three studies of in total 289 patients on dialysis reporting negative associations of PTH with BMD at cortical sites (such as the distal radius), but inconsistent associations with BMD at trabecular sites (such as the spine)[16-18].

In this study, we determined vertebral fracture prevalence in patients on dialysis using lateral chest radiographs and determined vertebral fracture incidence in patients with follow-up radiographs, who remained on dialysis or received a kidney transplant. We examined the relationships of vertebral trabecular BMD and PTH with the risk of vertebral fracture, and additionally the relationship of PTH with vertebral trabecular BMD.

Methods

Study population

We used data from the NOCTx study, a prospective cohort that compared progression of coronary artery calcification between patients treated with different renal replacement therapies. Between December 2009 and February 2016, NOCTx recruited 181 patients from eight centers in the Netherlands, who were on chronic hemodialysis or peritoneal dialysis with a minimum dialysis duration of two months, who were on hemodialysis and switched to nocturnal hemodialysis, or who were on dialysis and received a kidney transplant. At inclusion, all dialysis patients were eligible for transplantation. NOCTx included patients between 18 and 75 years and excluded patients with a life expectancy <3 months or pre-emptive transplantation. All subjects gave written informed consent. NOCTx has been approved by the Medical Ethics Committee of the University Medical Center Utrecht and was conducted in accordance with the declaration of Helsinki.

Patients were treated according to the KDIGO guidelines by their attending nephrologist[10]. Conventional hemodialysis was delivered 3 x 4–5 hours per week in-center with default 3.0 mEq/L dialysate calcium. Peritoneal dialysis was delivered as continuous ambulant peritoneal dialysis (*n=16*) or automated peritoneal dialysis (*n=8*) with default 2.5 mEq/L dialysate calcium. Nocturnal hemodialysis was delivered ≥4 x 8 hours per week at home with default 3.0 mEq/L dialysate calcium. Kidney transplant recipients received standard immunosuppressant regimens consisting of tacrolimus, mycophenolate mofetil, and prednisone in tapering doses (cumulative dose in first three months typically 1.0–1.4 g, maintenance dose 5–7.5 mg/day from three months post-transplantation). Acute rejections were treated with 3 x 1 g of methylprednisolone intravenously.

We studied vertebral fracture prevalence in patients that underwent a lateral chest radiograph as part of routine care within six months of inclusion in NOCTx (*n=148*). For kidney transplant recipients, this was the screening radiograph performed the day of transplantation. We excluded two patients because of poor quality radiographs leaving a sample of 146 patients. We studied associations of PTH in all patients with no history of parathyroidectomy and non-missing PTH values (*n=131*). We assessed the incidence of vertebral fractures in patients that underwent a second lateral chest radiograph as part of routine care >1 year after the first radiograph during maximum three years follow-up (*n=70*).

BMD measurements

In NOCTx, all patients underwent non-enhanced cardiac multi-slice computed tomography (CT) at inclusion, using a single iCT 256 scanner (Philips Medical Systems, Best, the Netherlands), which is checked for image quality and stability at least once every 2 weeks as part of our quality assurance system. The acquisition parameters were as follows: 120 kV, 40-50 mAs, rotation time 270 ms, and 128 x 0.625 mm collimation; table height was set by the attending medical physicist in order to position the patient in the isocenter of the gantry. A single reader (TJ) determined volumetric trabecular BMD of three consecutive thoracic vertebrae in the T7-T10 range[19]. The reader placed a region of interest (ROI) at the center of the vertebra and changed its placement to exclude the cortical edge, bone islands, large veins, or calcified herniated disks, or excluded a vertebra entirely if it was fractured (*n=6*). We calculated BMD in mg/cm^3 by multiplying the mean CT value per patient (in Hounsfield units) with a calibration factor of 0.871 (calibration factor coefficient of variation 6.1%)[20]. With this iCT 256 scanner-specific calibration factor, vertebral trabecular BMD can be measured accurately without a phantom, correlating highly with phantom-based BMD values (r=0.987)[20].

In a random sample of 85 scan re-reads by the same reader, the 95% limits of agreement were -7.6 (95% CI -8.4 to -6.8) and 11.5 (95% CI 10.7 to 12.3) mg/cm^3, and intra-observer reliability ($ICC_{agreement}$) was 0.99. In previous studies, the interscan variation has been shown to be low (2.8%)[19].

Parathyroid hormone

Parathyroid hormone was measured as part of routine care at clinical laboratories, with intact 2-site immunoassays (Beckman-Coulter, Fullerton, USA; Abbott Diagnostics, Abbott Park, USA; or Roche Diagnostics, Indianapolis, USA). Reference intervals of these assays are 1.3–9.3 pmol/L; 1.6–7.2 pmol/L; and 1.6–6.9 pmol/L, respectively. PTH values were averaged over measurements of the 3 months preceding the first lateral chest radiograph (mostly two measurements (60%), range 1–4 measurements).

Vertebral fracture assessment

Using lateral chest radiographs, which show the thoracic and upper lumbar spine, two trained physicians (TJ and NG) independently identified vertebral fractures and graded them according to Genant's semi-quantitative method[21]. This method allows excluding other possible causes of vertebral deformity, such as Scheuermann's disease, remodeling of vertebral bodies due to degenerative disk disease, or scoliosis. Lateral chest radiographs are reliable for the diagnosis of vertebral fractures, with excellent agreement and reliability compared to lateral spine radiographs (95–98% and 0.88–0.91 respectively)[22]. Fractures were graded by type (wedge, biconcave or crush deformity) and severity: grade

1 (20–25% height loss), grade 2 (25–40% height loss), or grade 3 (>40% height loss). All radiographs were re-read in consensus readings and verified by an expert geriatrician (HW). On average, we evaluated 10.8±1.4 vertebrae per patient, which included the seventh thoracic vertebra in 145 (99%) patients and the first lumbar vertebra in 124 (85%) patients. The prevalence of vertebral fractures did not differ significantly between patients with ≤10 evaluable vertebrae (18/61 fractures) and patients with >10 evaluable vertebrae (32/85 fractures) (P=0.40).

We defined the incidence of a fracture as any new vertebral fracture not present on the first radiograph or deterioration of an existing fracture to a higher grade. We defined the follow-up time as the time between the first and second radiograph. We evaluated an average of 10.6±1.3 vertebrae per patient, which included the seventh thoracic vertebra in all patients and the first lumbar vertebra in 53 (76%) patients.

Other study variables

At inclusion, study personnel recorded demographics at the University Medical Center Utrecht. Body mass index was calculated by dividing weight (kg) by height (m^2), based on chart review. Smoking status was self-reported and categorized as current and non-current smoking. Biochemical parameters (total calcium, albumin, phosphate, C-reactive protein, and total alkaline phosphatase) were measured in clinical laboratories in the 8 recruiting centers, and values were averaged over measurements of three months preceding inclusion (commonly 2-3 measurements). Medication use and medical history were assessed by chart review. We defined dialysis duration as the time between the first day of dialysis and the inclusion date, minus the time with a functioning kidney transplant, expressed in months.

Statistical analyses

We reported normally distributed variables as mean (± standard deviation), non-normally distributed variables as median (interquartile range, IQR), and categorical data as number (percentage). We tabulated patient characteristics according to vertebral fracture prevalence. We categorized PTH as tertiles, as we expected a U-shaped relationship with vertebral fracture[7].

We evaluated associations of kidney transplantation versus dialysis with vertebral fracture incidence, of vertebral trabecular BMD with vertebral fracture prevalence, and lastly of PTH tertiles with both vertebral fracture prevalence and vertebral trabecular BMD. For vertebral fracture prevalence and incidence as outcome measure, we used Poisson regression due to the high event rate and reported relative risks (RR) with 95% confidence intervals (CI)[23]. For vertebral fracture prevalence as outcome measure, we

adjusted for potential confounders age (years), sex, dialysis duration (months), and diabetes (type I or type II/absent). For vertebral fracture incidence as outcome measure, we adjusted for age (years), sex, and follow-up time (years), and stratified by dialysis or kidney transplantation, as we suspected distinct fracture etiologies. For vertebral trabecular BMD as outcome measure, we used linear regression and adjusted for potential confounders as described above.

To evaluate the continuous associations of PTH with vertebral fracture prevalence and vertebral trabecular BMD, we constructed P-splines adjusted for age (years) and sex[24]. We considered P-values ≤0.05 (two-tailed) statistically significant and used R 3.4.1 (R Foundation Statistical Computing) for all analyses.

Sensitivity analyses

To test the robustness of the associations, we repeated the analyses of the association of PTH with vertebral fracture prevalence stratified by age (<50 or ≥50 years); sex; history of diabetes, cardiovascular disease, current smoking, or transplantation; treatment with hemodialysis or peritoneal dialysis; use of prednisone; calcium-containing phosphate binders; vitamin D analogues; and cinacalcet. Furthermore, we repeated the analyses of the association of vertebral trabecular BMD with vertebral fracture prevalence excluding grade 1 fractures.

Results

Study population

The mean age of the study population (*n=146)* was 52 ±13 years, 98 (67%) were male, median dialysis duration was 26 (IQR 13–55) months, and 18 (12%) had diabetes. Eighteen patients (12%) were on long-term corticosteroids (low-dose up to 10 mg/day), while none used bisphosphonates and 23 (18%) used cinacalcet.

Patients that could not be evaluated for vertebral fractures (*n=35*) due to missing radiographs were similar in patient characteristics: mean age 51 ±14 years, 23 (66%) were male, median dialysis duration was 31 (IQR 15–74) months, and eight (23%) had diabetes (Table S1).

Vertebral fracture prevalence and incidence

In total, 50 patients (34%) had 84 prevalent vertebral fractures. Patients with prevalent vertebral fractures were older, more often male, had longer dialysis durations, and more often used cinacalcet than patients without prevalent vertebral fractures (Table 1).

Table 1. Characteristics of the 146 patients with end-stage renal disease stratified by prevalence of a vertebral fracture on lateral chest radiograph.

	No vertebral fracture (*n* = 96)	Prevalent vertebral fracture (*n* = 50)
Demographics and medical history		
Age (years)	50 ±13	57 ±12
Male (%)	58 (60%)	40 (80%)
Body mass index (kg/m²)	25.6 ±4.6	26.0 ±4.4
Diabetes mellitus (%)	13 (14%)	5 (10%)
Cardiovascular disease (%)	22 (23%)	12 (24%)
Current smoker (%)	7 (7%)	8 (16%)
History of kidney disease		
Dialysis duration (months)	21 (11–48)	38 (19–70)
Previous transplantation (%)	20 (21%)	16 (32%)
Parathyroidectomy (%)	4 (4%)	5 (10%)
Cause of end-stage renal disease (%)		
• Cystic kidney disease	16 (17%)	11 (22%)
• Interstitial nephritis	2 (2%)	2 (4%)
• Glomerulonephritis	27 (28%)	11 (22%)
• Vascular disease	18 (19%)	14 (28%)
• Diabetic nephropathy	6 (6%)	3 (6%)

Table 1. Continued.

	No vertebral fracture (*n* = 96)	Prevalent vertebral fracture (*n* = 50)
• Other	13 (14%)	5 (10%)
• Unknown	14 (15%)	4 (8%)
Dialysis treatment (%)		
• Hemodialysis	68 (71%)	40 (80%)
• Peritoneal dialysis	28 (29%)	10 (20%)
*Medication use at inclusion**		
Corticosteroids (%)	10 (10%)	8 (16%)
Calcium-containing phosphate binders (%)	30 (36%)	11 (26%)
Vitamin D analogues (%)	64 (77%)	32 (74%)
Cinacalcet (%)	10 (12%)	13 (30%)
Laboratory parameters		
Calcium (mmol/L)	2.34 ±0.14	2.35 ±0.13
Phosphate (mmol/L)	1.36 ±0.54	1.32 ±0.48
Parathyroid hormone (pmol/L)	17 (10–34)	18 (9–49)
C-reactive protein (mg/L)	3 (2–6)	3 (2–9)
Total alkaline phosphatase (IU/L)	83 (69–114)	73 (59–96)
Bone mineral density and vertebral fracture		
Vertebral bone mineral density (mg/cm^3)	139 ±41	120 ±44
Number of vertebral fractures (%)		
• One	-	27 (54%)
• Two	-	14 (28%)
• Three	-	7 (14%)
• Four	-	2 (4%)
Highest vertebral fracture grade (%)		
• Grade 1	-	32 (64%)
• Grade 2	-	16 (32%)
• Grade 3	-	2 (4%)

Results are presented as mean ±standard deviation, median (interquartile range) or percentage.
*Data on medication use (except for corticosteroid use) were available in 126 patients.

We assessed incident vertebral fractures in a subset of 70 patients: 47 who continued treatment with dialysis and 23 who received a kidney transplant at inclusion. The kidney transplant recipients (*n=23*) had a mean estimated glomerular filtration rate of 54±19 mL/min/1.73m^2.

Patients that could not be evaluated for incident vertebral fractures (*n=76*) were similar in patient characteristics: mean age 51±13 years, 23 (70%) were male, median dialysis duration was 31 (IQR 16–64) months, and 9 (12%) had diabetes. Furthermore, these patients were similar regarding treatment modality (38 patients on hemodialysis, 13 on peritoneal dialysis, 25 kidney transplant recipients versus 35, 12, and 23 respectively) and vertebral fracture prevalence (27/76 patients, 36% versus 23/70, 33%).

After a median follow-up of 1.8 years (IQR 1.3–2.7) or 137 person-years, 20 patients (29%) developed 24 new vertebral fractures. Compared to patients without incident vertebral fracture, patients with incident vertebral fracture were older (mean 60 versus 52 years) and had more often a previous vertebral fracture (55% versus 24%) (Table 2). Patient characteristics of patients that received a kidney transplant (*n=23*) and patients that remained on dialysis (*n=47*) were comparable (Table S2), although the use of corticosteroids and bisphosphonates during follow-up was higher among kidney transplant recipients (100% versus 21% and 17% versus none). The risk of incident vertebral fracture did not differ significantly for kidney transplant recipients (6/23 with incident vertebral fracture) compared to patients on dialysis (14/47 with incident vertebral fracture) after adjustment for age, sex, and follow-up time (adjusted RR for kidney transplant recipients compared to patients on dialysis 0.94, 95% CI 0.32 to 2.43).

Table 2. Characteristics of the subset of 70 patients with end-stage renal disease who had follow-up radiographs, stratified by incidence of vertebral fracture.

	No incident vertebral fracture (n=50)	Incident vertebral fracture (n=20)
Demographics and medical history		
Age (years)	52 ±13	60 ±13
Male (%)	31 (62%)	14 (70%)
Diabetes mellitus (%)	7 (14%)	2 (10%)
Current smoker (%)	5 (10%)	0
History of kidney disease		
Dialysis duration (months)	18 (10–39)	25 (18–50)
Renal replacement therapy during follow-up (%)		
• Hemodialysis	24 (48%)	11 (55%)
• Peritoneal dialysis	9 (18%)	3 (15%)
• Kidney transplant	17 (34%)	6 (30%)
Medication use during follow-up		
Corticosteroids (%)*	26 (52%)	7 (35%)
Bisphosphonates (%)	3 (6%)	1 (5%)

Table 2. Continued.

	No incident vertebral fracture (n=50)	Incident vertebral fracture (n=20)
Calcium-containing phosphate binders (%)	24 (48%)	7 (35%)
Vitamin D analogues (%)	27 (54%)	12 (60%)
Cinacalcet (%)	4 (8%)	5 (25%)
Bone mineral density and vertebral fracture		
Vertebral bone mineral density (mg/cm^3)	132 ±39	119 ±36
Previous vertebral fracture (%)	12 (24%)	11 (55%)
Deterioration of existing fracture to higher grade (%)	-	1 (5%)
Number of new vertebral fractures (%)		
• One	-	16 (80%)
• Two	-	2 (10%)
• Three	-	1 (5%)
Highest incident vertebral fracture grade (%)		
• Grade 1	-	14 (70%)
• Grade 2	-	5 (25%)
• Grade 3	-	1 (5%)

Results are presented as mean ±standard deviation, median (interquartile range) or percentage.
*Acute rejections, treated with high doses of methylprednisolone, occurred in 3 patients who did not sustain a vertebral fracture, and in 2 patients who did sustain a vertebral fracture.

Associations of vertebral trabecular BMD with vertebral fracture

Mean vertebral trabecular BMD was 139±41 mg/cm^3 in patients without a prevalent vertebral fracture (*n=96*) and 120±44 mg/cm^3 in patients with a prevalent vertebral fracture (*n=50*). Vertebral trabecular BMD was associated with a lower risk of vertebral fracture prevalence in unadjusted models 0.93 (95% CI 0.86 to 0.99) but not when adjusted for potential confounders 0.97 (95% CI 0.89 to 1.04) (Table S3). These associations were numerically similar when grade 1 fractures (n=32) were excluded (unadjusted 0.90, 95% CI 0.79 to 1.01; and adjusted 0.95, 95% CI 0.83 to 1.06).

Associations of PTH with vertebral fracture and vertebral trabecular BMD

One hundred thirty-one patients had no history of parathyroidectomy and non-missing PTH values (Table S4). Vertebral fractures were the least prevalent in patients in the middle PTH tertile (16% versus 38% and 43%; P<0.05) (Table 3). Compared to the middle tertile, the adjusted RR of vertebral fracture prevalence was 2.28 for the lowest tertile (95% CI 0.97 to 5.97) and 2.82 for the highest tertile (95% CI 1.22 to 7.27). Correspondingly, the continuous association of PTH with prevalent vertebral fracture risk appeared U-shaped,

with the lowest risk around 29 pmol/L (Figure 1). Notably patients in the lowest tertile had significantly lower total alkaline phosphatase levels (median 69 versus 85 and 91 IU/L, P=0.02).

Table 3. Associations of parathyroid hormone with vertebral bone mineral density and vertebral fractures in a subset of 131 patients with end-stage renal disease.

	Parathyroid hormone		
	Lowest tertile (n=45)	Middle tertile (n=44)	Highest tertile (n=42)
PTH (pmol/L)	7 (5–10)	19 (16–23)	50 (36–66)
Alkaline phosphatase (IU/L)	69 (57–89)	85 (73–105)	91 (69–126)
Vertebral fracture prevalence	*17 (38%)*	*7 (16%)*	*18 (43%)*
Relative risk			
• Crude model	2.37 (1.02 to 6.14)	1.00 (ref)	2.69 (1.17 to 6.93)
• Model 1	2.16 (0.92 to 5.61)	1.00 (ref)	2.80 (1.22 to 7.20)
• Model 2	2.28 (0.97 to 5.97)	1.00 (ref)	2.82 (1.22 to 7.27)
Vertebral BMD (mg/cm^3)	*116±38*	*130±35*	*148±38*
Difference (mg/cm^3)			
• Crude model	-13.6 (-29.0 to 1.8)	0.00 (ref)	18.7 (3.0 to 140.5)
• Model 1	-9.4 (-22.5 to 3.7)	0.00 (ref)	14.2 (1.0 to 27.5)
• Model 2	-9.7 (-22.7 to 3.3)	0.00 (ref)	14.6 (1.4 to 27.8)

Values are mean ±standard deviation, median (interquartile range), percentage, relative risks estimated with Poisson regression (with 95% confidence intervals), or linear regression coefficients (with 95% confidence intervals).

Abbreviations: PTH: parathyroid hormone; BMD: bone mineral density.

Model 1: age (years) and sex.

Model 2: model 1 + dialysis duration (months) and diabetes (type I or type II/absent).

We repeated these analyses stratified by various patient characteristics (Table S5), which all showed similar trends. The U-shaped association of PTH with vertebral fracture risk appeared even stronger in vitamin D analogues users (*n=87*) (adjusted RR 3.68, 95% CI 1.12 to 16.52 for the lowest and 3.57, 95% CI 1.14 to 15.61 for the highest PTH tertile). Patient characteristics were similar between vitamin D analogue users and non-users (Table S6), except for higher phosphate (1.49 versus 1.19 mmol/L) and PTH values (23 versus 11 pmol/L) among vitamin D analogue users.

In the lowest PTH tertile, vertebral trabecular BMD tended to be lower with linear regression compared to the middle tertile (adjusted regression coefficient -9.7 mg/cm^3, 95% CI -22.7 to 3.3), while in the highest tertile vertebral trabecular BMD was significantly higher (14.6 mg/cm^3, 95% CI 1.4 to 27.8) (Table 3). For the continuous associations, lower PTH values tended to track with lower vertebral trabecular BMD values (Figure S1).

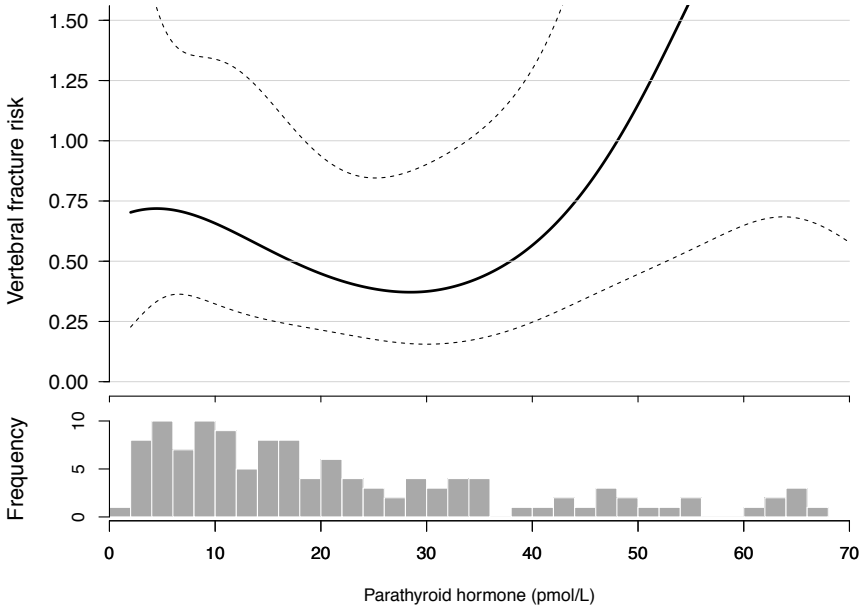

Figure 1. Continuous associations of parathyroid hormone with risk of prevalent vertebral fracture in 131 patients with end-stage renal disease, adjusted for age and sex. A histogram of parathyroid hormone is shown at the bottom.

Discussion

Our study indicates that vertebral fractures have a high prevalence (34%) and incidence (29% after median 1.8 years follow-up) in a relatively young population (mean age 52 years) of patients with ESRD. Also, our data show no relationship of CT-measured vertebral trabecular BMD with vertebral fracture and suggest a U-shaped relationship of PTH with vertebral fracture.

The vertebral fracture prevalence in our study is remarkably high compared to the general population. In a population-based study of European men and women aged 50-54 years, the prevalence ranged between 5% and 17% as determined with morphometric approaches[25], and between 2% and 11% in a population-based study of Dutch men and women aged 45-59 years as determined with algorithm-based qualitative methods and a morphometric approach[26]. In general, reproducibility is highest with algorithm-based qualitative methods[27], followed by Genant's semiquantitative technique used by us, which in turn has higher reproducibility than morphometric approaches (e.g. the McCloskey and Eastell methods and quantitative morphometry)[28]. Considering, vertebral fracture prevalence in our ESRD cohort is more than twice as high as in the general population, which could have been even higher if we had also evaluated the lower lumbar spine.

The prevalence of vertebral fractures is poorly documented in cohorts and dialysis registries[7] and has been reported in only few studies of patients on dialysis[29]. Four studies reported prevalences between 21% and 33% for patients generally older (mean ages between 54 and 69 years)[14, 30-32], whereas one study reported 9% vertebral fractures without mentioning age of the study population or method of vertebral fracture assessment[33] and another study reported 11% vertebral fractures among slightly younger incident kidney transplant recipients (49.7 versus 52.2 years), although it was unclear whether only the lumbar spine was evaluated[34]. In general, our estimates might be higher due to geographic variation in vertebral fracture prevalence between Northern and Southern Europe[25] or different referral patterns for dialysis[35]. Also, one of these studies used stricter criteria for wedge fractures (the most common type of vertebral fracture[26]) only adjudicating grade 2 and 3 fractures[14], and another study did not specify vertebral fracture adjudication method[34]. Yet another study found 55% vertebral fractures in older patients on hemodialysis (mean 64 years old) but used quantitative morphometry[36]. We employed Genant's semiquantitative technique to adjudicate fractures, which allows to exclude other possible causes of vertebral deformity[21] and has a higher reproducibility than morphometric approaches used in previous studies[28]. This supports the high vertebral fracture prevalence of 34% we currently report.

Our study is the first to report vertebral fracture incidence in patients with ESRD using radiographs to assess vertebral fractures. Two previous studies reported 4.8[12] and about 10[37] vertebral fractures per 1000 person-years in patients on dialysis, with vertebral fractures identified by *International Classification of Diseases, Ninth revision (ICD-9)* codes in claim files of the United States Renal Data system. However, this methodology is likely to underestimate vertebral fracture incidence because of clinical underdiagnosis[3]. We used standardized methods by 2 independent trained physicians to adjudicate fractures, which is much more sensitive than ICD-9 codes. Our data indicate a high vertebral fracture incidence (146 per 1000 person-years) among patients with ESRD. Although causes of vertebral fracture may differ for patients on dialysis and kidney transplant recipients, our data showed similar vertebral fracture rates for both groups, far exceeding rates reported in placebo groups of large osteoporosis drug trials[38]. This high incidence warrants a better understanding of the impact of mineral metabolism disturbances on bone strength in order to prevent these fractures.

In this first study to measure vertebral trabecular BMD with cardiac CT in patients with ESRD, we did not find associations of vertebral trabecular BMD with vertebral fracture, similar to a previous study using quantitative CT[32]. Cardiac CT vertebral trabecular BMD correlates well with quantitative CT lumbar spine BMD (r=0.91-0.93)[19], which is considered a useful and appropriate method for BMD testing[39, 40]. The discrepancy between vertebral trabecular BMD and fracture might be explained by physiological anabolic effects of PTH, which are most pronounced on trabecular bone[41]. Indeed, we found an association of higher PTH with higher vertebral trabecular BMD, whereas previous studies reported inconsistent associations[16-18]. Because of this, vertebral trabecular BMD may not be useful to assess fracture risk in ESRD. Moreover, our findings indicate that BMD testing is redundant in these patients. The clinical purpose of BMD is to identify high-risk patients for fracture, but with the currently reported vertebral fracture risk, patients on dialysis can all be regarded as high-risk patients for fracture and rather may benefit from radiographic screening for vertebral fractures.

Our data suggest a U-shaped association of PTH with vertebral fracture risk. We used continuous associations to indicate the precise direction of the associations per PTH pmol/L. This allowed us to estimate the shape of fracture risk for the whole range of PTH compared to predefined PTH categories or quantiles, indicating the lowest vertebral fracture risk around 29 pmol/L. Our finding complements previous studies reporting optimal PTH values for fracture risk around 32 pmol/L[12], above 21 pmol/L[13], above 7 pmol/L[14], between 16 and 32 pmol/L[15], and below 95 pmol/L[42]. In order to draw conclusions about the target PTH range in clinical practice, randomized controlled trials are needed to define optimal PTH values for fracture.

Our results should be interpreted within certain limitations. We used lateral chest radiographs and not spine radiographs, which may have complicated vertebral fracture diagnosis, although lateral chest radiographs have excellent agreement and reliability compared to spine radiographs[22]. Also, lateral chest radiographs did not allow for fracture assessment of the lower lumbar spine, as we could only evaluate the spine down to the first lumbar vertebra in most patients. Nevertheless, one could even expect a higher prevalence of vertebral fractures with additional lumbar spine radiography. Furthermore, the radiographs were performed as part of routine care, which may have introduced indication bias. However, only two radiographs (1%) were performed with the indication of back pain. Another limitation is that the majority of our study population was male, while it has been indicated that the inter-observer agreement is not good for the diagnosis of vertebral fracture in males[43]. Further limitations include that PTH was measured with multiple assays, that we did not additionally measure vertebral BMD using conventional dual-energy X-ray absorption (DXA), and that we did not have data on historical corticosteroid use or vitamin D status. Notwithstanding, most patients used vitamin D analogues, rendering vitamin D deficiency unlikely as cause of vertebral fractures. Finally, all patients in this study were eligible for transplantation and some were treated with home hemodialysis. This population may therefore be somewhat healthier than the average dialysis population. On the other hand, a high vertebral fracture risk in our population indicates a an even higher risk in the general dialysis population.

This study has several strengths. We adjudicated fractures on radiographs based on 2 independent trained physicians, instead of recall of fracture history or radiographic reports. We thus also detected less severe fractures, corroborating the high vertebral fracture prevalence. Also, the patients on dialysis and kidney transplant recipients were well comparable, as all dialysis patients were eligible for transplantation, and all kidney transplant recipients were on dialysis before transplantation.

In conclusion, even relatively young and healthy patients with ESRD are at high risk of vertebral fractures. CT-measured vertebral trabecular BMD is not associated with vertebral fracture and may therefore not be useful to assess fracture risk in ESRD. Rather, patients may benefit from radiographic screening for vertebral fractures. Nevertheless, our study did not measure vertebral BMD using DXA and assessed vertebral fractures using lateral chest radiographs and not spine radiographs. The association of PTH with vertebral fracture risk appears to be U-shaped, with the lowest risk around 29 pmol/L. The target PTH range for fracture needs investigation by randomized controlled trials.

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Supplementary material

Table S1. Characteristics of the patients with end-stage renal disease included in the current analyses (*n=146*) and those excluded because of missing lateral chest radiographs (*n=35*).

	Included (*n* = 146)	Excluded (*n* = 35)	P-value
Demographics and medical history			
Age (years)	52.2 ±13.2	51.2 ±14.2	0.72
Male (%)	98 (67%)	23 (66%)	0.99
Body mass index (kg/m²)	25.7 ±4.5	25.3 ±4.3	0.62
Diabetes mellitus (%)	18 (12%)	8 (23%)	0.18
Cardiovascular disease (%)	34 (23%)	6 (17%)	0.58
Current smoker (%)	15 (10%)	10 (29%)	0.01
History of kidney disease			
Dialysis vintage (months)	26 (13–55)	31 (15–74)	0.45
Previous transplantation (%)	36 (17%)	6 (17%)	0.47
Cause of end-stage renal disease (%)			0.11
• Cystic kidney disease	27 (18%)	2 (6%)	
• Interstitial nephritis	4 (3%)	3 (69%)	
• Glomerulonephritis	38 (26%)	10 (29%)	
• Vascular disease	32 (22%)	6 (17%)	
• Diabetic nephropathy	9 (6%)	5 (14%)	
• Other	18 (12%)	7 (20%)	
• Unknown	18 (12%)	2 (6%)	
Dialysis treatment (%)			0.86
• Hemodialysis	108 (74%)	27 (77%)	
• Peritoneal dialysis	38 (26%)	8 (23%)	

Table S2. Characteristics of the subset of 70 patients with end-stage renal disease who had follow-up radiographs, stratified by kidney transplantation or continued dialysis treatment.

	Continued dialysis treatment (n=47)	Received kidney transplant (n=23)
Demographics and medical history		
Age (years)	53 ±13	55 ±14
Male (%)	31 (66%)	14 (61%)
Diabetes mellitus (%)	7 (15%)	2 (9%)
Current smoker (%)	4 (9%)	1 (4%)
History of kidney disease		
Dialysis duration (months)	20 (10–46)	30 (12–47)
Renal replacement therapy before inclusion (%)		
• Hemodialysis	35 (74%)	16 (70%)
• Peritoneal dialysis	12 (26%)	7 (30%)
Medication use during follow-up		
Corticosteroids (%)*	10 (21%)	23 (100%)
Bisphosphonates (%)	0	4 (17%)
Calcium-containing phosphate binders (%)**	18 (38%)	4 (36%)
Vitamin D analogues (%)	37 (79%)	9 (82%)
Cinacalcet (%)	7 (15%)	1 (9%)
Bone mineral density and vertebral fracture		
Vertebral bone mineral density (mg/cm^3)	129 ±37	126 ±43
Previous vertebral fracture (%)	17 (36%)	6 (26%)
Incident vertebral fracture (%)	14 (30%)	6 (26%)

Results are presented as mean ±standard deviation, median (interquartile range) or percentage.
*Acute rejections, treated with high doses of methylprednisolone, occurred in 5 kidney transplant recipients.
**Including calcium carbonate prescribed post-transplantation.

Table S3. Associations of vertebral bone mineral density with vertebral fracture prevalence in 146 patients on dialysis and with vertebral fracture incidence in 70 patients with end-stage renal disease.

Relative risk of vertebral fracture prevalence	N	*Per 10 mg/cm³ higher vertebral BMD*
• Crude model	146	0.93 (0.86 to 0.99)
• Model 1	146	0.98 (0.90 to 1.06)
• Model 2	146	0.97 (0.89 to 1.04)
Relative risk of vertebral fracture incidence	**N**	*Per 10 mg/cm³ higher vertebral BMD*
• Crude model	70	0.92 (0.81 to 1.04)
• Model 1	70	1.00 (0.85 to 1.15)
○ Patients on dialysis only	47	0.94 (0.75 to 1.14)
○ Kidney transplant recipients only	23	1.10 (0.87 to 1.48)

Values are relative risks estimated with Poisson regression (with 95% confidence intervals).
Abbreviations: BMD: bone mineral density.
Model 1: age (years) and sex.
Model 2: model 1 + dialysis duration (months) and diabetes (type I or type II/absent).
For vertebral fracture incidence, all models were adjusted for follow-up time (years).

Table S4. Patient characteristics stratified by parathyroid hormone tertiles (*n=131*).

	Lowest tertile (*n* = 45)	Middle tertile (*n* = 44)	Highest tertile (*n* = 42)	P-value
Demographics and medical history				
Age (years)	55 ±12	52 ±11	50 ±15	0.11
Male (%)	29 (64%)	30 (68%)	26 (62%)	0.83
Body mass index (kg/m²)	25.1 ±4.4	26.1 ±4.3	25.8 ±4.8	0.52
Diabetes mellitus (%)	6 (13%)	5 (11%)	6 (14%)	0.92
Cardiovascular disease (%)	10 (22%)	10 (23%)	9 (21%)	0.99
Current smoker (%)	8 (18%)	1 (2%)	4 (10%)	0.05
History of kidney disease				
Dialysis duration (months)	21 (12–40)	21 (11–42)	29 (15–55)	0.30
Previous transplantation (%)	9 (20%)	5 (11%)	14 (33%)	0.04
Cause of end-stage renal disease (%)				0.57
• Cystic kidney disease	10 (22%)	8 (18%)	7 (17%)	
• Interstitial nephritis	0	0	1 (2%)	
• Glomerulonephritis	11 (24%)	15 (34%)	7 (17%)	
• Vascular disease	13 (29%)	7 (16%)	9 (21%)	
• Diabetic nephropathy	1 (2%)	3 (7%)	5 (12%)	
• Other	5 (11%)	6 (14%)	7 (17%)	
• Unknown	5 (11%)	5 (11%)	6 (14%)	

Table S4. Continued.

	Lowest tertile (*n* = 45)	Middle tertile (*n* = 44)	Highest tertile (*n* = 42)	P-value
Dialysis treatment (%)				
• Hemodialysis	37 (82%)	28 (64%)	29 (69%)	0.13
• Peritoneal dialysis	8 (18%)	16 (36%)	13 (31%)	
*Medication use at inclusion**				
Corticosteroids (%)	7 (16%)	4 (9%)	6 (14%)	0.59
Calcium-containing phosphate binders (%)	12 (33%)	12 (30%)	12 (32%)	0.95
Vitamin D analogues (%)	23 (64%)	31 (78%)	30 (87%)	0.07
Cinacalcet (%)	5 (14%)	3 (8%)	11 (29%)	0.03
Laboratory parameters				
Calcium (mmol/L)	2.38 ±0.11	2.32 ±0.13	2.35 ±0.16	0.18
Phosphate (mmol/L)	1.14 ±0.42	1.40 ±0.55	1.50 ±0.53	<0.01
Parathyroid hormone (pmol/L)	7 (5–10)	19 (16–23)	50 (36–66)	<0.01
C-reactive protein (mg/L)	3 (2–7)	4 (2–8)	2 (1–4)	0.36
Total alkaline phosphatase (IU/L)	69 (57–89)	85 (73–105)	91 (69–126)	0.02
Bone mineral density and vertebral fracture				
Vertebral bone mineral density (mg/cm^3)	116 ±38	130 ±35	148 ±38	<0.01
Prevalent vertebral fracture (%)	17 (38%)	7 (16%)	18 (43%)	0.02

Results are presented as mean ±standard deviation, median (interquartile range) or percentage.
P-values were calculated with one-way analyses of variance for compared normally distributed variables, with Kruskal-Wallis tests for non-normally distributed variables, and with Chi-squared tests for categorical data.
*Data on medication use (except for corticosteroid use) were available in 114 patients.
Conversion factors for units: calcium mg/dL to mmol/L, x0.2495; phosphate mg/dL to mmol/L, x0.3229.

Table S5. Associations of parathyroid hormone with vertebral fractures stratified by various patient characteristics in 131 patients with end-stage renal disease.

		Age <50 years	
		PTH	
	Lowest tertile (n=12)	Middle tertile (n=16)	Highest tertile (n=22)
PTH (pmol/L)	5 (4–7)	17 (15–21)	64 (53–75)
Vertebral BMD	140 ±41	149 ±28	170 ±31
Vertebral fracture prevalence	25%	13%	32%
Relative risk of vertebral fracture prevalence			
• Unadjusted	2.00 (0.33 to 15.18)	1.00 (ref)	2.55 (0.62 to 17.08)
• Model 1	2.11 (0.35 to 16.16)	1.00 (ref)	2.77 (0.67 to 18.60)
• Model 2	3.80 (0.53 to 36.85)	1.00 (ref)	3.38 (0.76 to 24.76)
		Male sex	
		PTH	
	Lowest tertile (n=29)	Middle tertile (n=30)	Highest tertile (n=26)
PTH (pmol/L)	6 (4–10)	19 (16–22)	45 (35–66)
Vertebral BMD	110 ±34	127 ±34	136 ±37
Vertebral fracture prevalence	45%	20%	50%
Relative risk of vertebral fracture prevalence			
• Unadjusted	2.24 (0.89 to 6.38)	1.00 (ref)	2.50 (0.99 to 7.12)
• Model 1	1.98 (0.77 to 5.69)	1.00 (ref)	2.38 (0.94 to 6.80)
• Model 2	2.08 (0.80 to 6.08)	1.00 (ref)	2.51 (0.98 to 7.21)
		No history of diabetes	
		PTH	
	Lowest tertile (n=39)	Middle tertile (n=39)	Highest tertile (n=36)
PTH (pmol/L)	7 (5–9)	20 (16–23)	50 (41–66)
Vertebral BMD	115 ±36	130 ±37	149 ±40
Vertebral fracture prevalence	39%	18%	42%
Relative risk of vertebral fracture prevalence			
• Unadjusted	2.14 (0.90 to 5.61)	1.00 (ref)	2.32 (0.98 to 6.08)
• Model 1	1.97 (0.83 to 5.20)	1.00 (ref)	2.46 (1.04 to 6.45)
• Model 2	2.10 (0.87 to 5.58)	1.00 (ref)	2.45 (1.03 to 6.44)

	Age ≥50 years	
	PTH	
Lowest tertile (n=33)	Middle tertile (n=28)	Highest tertile (n=20)
8 (5–10)	20 (16–25)	37 (34–47)
107 ±34	118 ±34	124 ±30
42%	18%	55%
2.38 (0.91 to 7.35)	1.00 (ref)	3.08 (1.12 to 9.78)
2.08 (0.79 to 6.50)	1.00 (ref)	2.43 (0.87 to 7.84)
2.07 (0.78 to 6.44)	1.00 (ref)	2.44 (0.87 to 7.86)
	Female sex	
	PTH	
Lowest tertile (n=16)	Middle tertile (n=14)	Highest tertile (n=16)
7 (5–10)	19 (16–23)	62 (51–73)
126 ±44	136 ±37	169 ±32
25%	7%	31%
3.50 (0.52 to 68.47)	1.00 (ref)	4.38 (0.71 to 83.82)
3.41 (0.50 to 66.74)	1.00 (ref)	5.79 (0.91 to 111.72)
3.92 (0.57 to 77.49)	1.00 (ref)	5.14 (0.77 to 100.72)
	History of diabetes	
	PTH	
Lowest tertile (n=6)	Middle tertile (n=5)	Highest tertile (n=6)
7 (5–10)	15 (15–17)	49 (33–79)
119 ±57	125 ±34	143 ±26
33%	0%	50%
-	1.00 (ref)	-
-	1.00 (ref)	-
-	1.00 (ref)	-

Table S5. Continued

	No history of cardiovascular disease		
	PTH		
	Lowest tertile (n=35)	Middle tertile (n=34)	Highest tertile (n=33)
PTH (pmol/L)	6 (4–9)	18 (15–22)	56 (42–72)
Vertebral BMD	121 ±40	136 ±36	158 ±35
Vertebral fracture prevalence	37%	18%	39%
Relative risk of vertebral fracture prevalence			
• Unadjusted	2.10 (0.83 to 5.99)	1.00 (ref)	2.23 (0.88 to 6.35)
• Model 1	1.76 (0.68 to 5.09)	1.00 (ref)	2.33 (0.92 to 6.64)
• Model 2	1.88 (0.72 to 5.53)	1.00 (ref)	2.28 (0.89 to 6.55)
	No current smoker		
	PTH		
	Lowest tertile (n=37)	Middle tertile (n=43)	Highest tertile (n=38)
PTH (pmol/L)	6 (4–9)	19 (16–23)	50 (40–71)
Vertebral BMD	119 ±40	130 ±35	150 ±37
Vertebral fracture prevalence	38%	14%	42%
Relative risk of vertebral fracture prevalence			
• Unadjusted	2.71 (1.09 to 7.66)	1.00 (ref)	3.02 (1.24 to 8.41)
• Model 1	2.27 (0.90 to 6.48)	1.00 (ref)	3.18 (1.31 to 8.85)
• Model 2	2.34 (0.92 to 6.71)	1.00 (ref)	3.25 (1.33 to 9.09)
	No history of transplantation		
	PTH		
	Lowest tertile (n=36)	Middle tertile (n=39)	Highest tertile (n=28)
PTH (pmol/L)	6 (4–9)	18 (16–22)	45 (34–62)
Vertebral BMD	119 ±41	128 ±35	142 ±38
Vertebral fracture prevalence	28%	15%	50%
Relative risk of vertebral fracture prevalence			
• Unadjusted	1.81 (0.67 to 5.31)	1.00 (ref)	3.25 (1.30 to 9.18)
• Model 1	1.42 (0.52 to 4.22)	1.00 (ref)	2.67 (1.06 to 7.62)
• Model 2	1.44 (0.53 to 4.27)	1.00 (ref)	2.67 (1.06 to 7.62)

	History of cardiovascular disease	
	PTH	
Lowest tertile (n=10)	Middle tertile (n=10)	Highest tertile (n=9)
9 (6–10)	23 (18–26)	43 (35–49)
98 ±25	107 ±19	112 ±25
40%	10%	56%
-	1.00 (ref)	-
-	1.00 (ref)	-
-	1.00 (ref)	-
	Current smoker	
	PTH	
Lowest tertile (n=8)	Middle tertile (n=1)	Highest tertile (n=4)
8 (5–10)	16	46 (35–56)
104 ±30	128	128 ±47
38%	100%	50%
-	1.00 (ref)	-
-	1.00 (ref)	-
-	1.00 (ref)	-
	History transplantation	
	PTH	
Lowest tertile (n=6)	Middle tertile (n=5)	Highest tertile (n=6)
7 (5–10)	15 (15–17)	49 (33–79)
119 ±57	125 ±34	143 ±26
33%	0%	50%
-	1.00 (ref)	-
-	1.00 (ref)	-
-	1.00 (ref)	-

Table S5. Continued

	Hemodialysis		
	PTH		
	Lowest tertile (n=37)	Middle tertile (n=28)	Highest tertile (n=29)
PTH (pmol/L)	7 (5–10)	18 (16–22)	52 (39–66)
Vertebral BMD	118 ±39	126 ±33	151 ±34
Vertebral fracture prevalence	35%	18%	48%
Relative risk of vertebral fracture prevalence			
• Unadjusted	1.97 (0.74 to 6.13)	1.00 (ref)	2.70 (1.03 to 8.37)
• Model 1	1.80 (0.68 to 5.61)	1.00 (ref)	2.98 (1.14 to 9.22)
• Model 2	2.01 (0.74 to 6.39)	1.00 (ref)	3.10 (1.18 to 9.64)
	No prednisone use		
	PTH		
	Lowest tertile (n=36)	Middle tertile (n=40)	Highest tertile (n=36)
PTH (pmol/L)	6 (4–10)	19 (16–23)	49 (35–66)
Vertebral BMD	121 ±40	130 ±36	147 ±39
Vertebral fracture prevalence	28%	18%	47%
Relative risk of vertebral fracture prevalence			
• Unadjusted	1.59 (0.61 to 4.37)	1.00 (ref)	2.70 (1.16 to 6.98)
• Model 1	1.41 (0.54 to 3.91)	1.00 (ref)	2.67 (1.15 to 6.92)
• Model 2	1.49 (0.56 to 4.14)	1.00 (ref)	2.55 (1.10 to 6.63)
	No calcium-containing phosphate binder use		
	PTH		
	Lowest tertile (n=24)	Middle tertile (n=28)	Highest tertile (n=26)
PTH (pmol/L)	8 (5–10)	19 (15–24)	55 (43–71)
Vertebral BMD	117 ±43	135 ±36	141 ±35
Vertebral fracture prevalence	42%	14%	50%
Relative risk of vertebral fracture prevalence			
• Unadjusted	2.92 (0.98 to 10.63)	1.00 (ref)	3.50 (1.24 to 12.43)
• Model 1	3.09 (1.01 to 11.45)	1.00 (ref)	3.46 (1.22 to 12.30)
• Model 2	2.98 (0.97 to 11.09)	1.00 (ref)	3.52 (1.24 to 12.54)

	Peritoneal dialysis	
	PTH	
Lowest tertile (n=8)	Middle tertile (n=16)	Highest tertile (n=13)
5 (4–9)	22 (16–26)	47 (35–66)
108 ±36	136 ±39	142 ±46
50%	13%	31%
-	1.00 (ref)	-
-	1.00 (ref)	-
-	1.00 (ref)	-
	Prednisone use	
	PTH	
Lowest tertile (n=7)	Middle tertile (n=4)	Highest tertile (n=6)
9 (7–10)	18 (14–22)	64 (51–95)
93 ±27	127 ±30	154 ±36
100%	0%	17%
-	1.00 (ref)	-
-	1.00 (ref)	-
-	1.00 (ref)	-
	Calcium-containing phosphate binder use	
	PTH	
Lowest tertile (n=12)	Middle tertile (n=12)	Highest tertile (n=12)
6 (5–8)	19 (16–22)	50 (41–68)
121 ±33	111 ±27	164 ±43
33%	17%	25%
2.00 (0.39 to 14.43)	1.00 (ref)	1.50 (0.25 to 11.39)
2.41 (0.44 to 18.50)	1.00 (ref)	1.52 (0.24 to 12.04)
-	1.00 (ref)	-

Table S5. Continued

	No vitamin D analogue use		
	PTH		
	Lowest tertile (n=13)	Middle tertile (n=9)	Highest tertile (n=5)
PTH (pmol/L)	6 (5–9)	18 (16–20)	66 (63–94)
Vertebral BMD	129 ±52	104 ±31	153 ±12
Vertebral fracture prevalence	31%	33%	60%
Relative risk of vertebral fracture prevalence			
• Unadjusted	-	1.00 (ref)	-
• Model 1	-	1.00 (ref)	-
• Model 2	-	1.00 (ref)	-
	No cinacalcet use		
	PTH		
	Lowest tertile (n=31)	Middle tertile (n=37)	Highest tertile (n=27)
PTH (pmol/L)	7 (5–10)	19 (16–23)	49 (35–74)
Vertebral BMD	122 ±41	128 ±37	149 ±44
Vertebral fracture prevalence	32%	16%	37%
Relative risk of vertebral fracture prevalence			
• Unadjusted	1.99 (0.74 to 5.85)	1.00 (ref)	2.28 (0.85 to 6.71)
• Model 1	1.96 (0.72 to 5.77)	1.00 (ref)	2.29 (0.85 to 6.75)
• Model 2	2.25 (0.82 to 6.83)	1.00 (ref)	2.65 (0.97 to 8.00)

Values are mean ±standard deviation, median (interquartile range), percentage, risk ratios estimated with Poisson regression (with 95% confidence intervals). Regression was not performed when any tertile within one stratus consisted of <10 subjects.

Abbreviations: PTH: parathyroid hormone; BMD: bone mineral density.

Model 1: age (years) and sex.

Model 2: model 1 + dialysis duration (months) and diabetes (type I or type II/absent).

	Vitamin D analogue use	
	PTH	
Lowest tertile (n=23)	Middle tertile (n=31)	Highest tertile (n=33)
7 (5–10)	21 (15–25)	48 (39–66)
113 ±30	134 ±34	148 ±41
44%	10%	39%
4.49 (1.37 to 20.04)	1.00 (ref)	4.07 (1.31 to 17.76)
4.21 (1.28 to 18.86)	1.00 (ref)	3.90 (1.25 to 17.05)
3.68 (1.12 to 16.52)	1.00 (ref)	3.57 (1.14 to 15.61)
	Cinacalcet use	
	PTH	
Lowest tertile (n=5)	Middle tertile (n=3)	Highest tertile (n=11)
9 (6–10)	24 (20–27)	56 (47–105)
95 ±15	120 ±10	148 ±23
80%	0%	55%
-	1.00 (ref)	-
-	1.00 (ref)	-
-	1.00 (ref)	-

Table S6. Patient characteristics stratified by vitamin D analogue users and non-users (*n=114*)*.

	Vitamin D analogue users (*n* = 87)	Non-users (*n* = 27)
Demographics and medical history		
Age (years)	52 ±12	54 ±11
Male (%)	55 (63%)	18 (67%)
Body mass index (kg/m²)	25.6 ±4.5	26.3 ±5.1
Diabetes mellitus (%)	10 (11%)	5 (19%)
Cardiovascular disease (%)	19 (22%)	8 (30%)
Current smoker (%)	8 (9%)	5 (19%)
History of kidney disease		
Dialysis duration (months)	25 (13–48)	30 (11–44)
Previous transplantation (%)	21 (24%)	5 (19%)
Cause of end-stage renal disease (%)		
• Cystic kidney disease	10 (11%)	8 (30%)
• Interstitial nephritis	1	0
• Glomerulonephritis	24 (28%)	6 (22%)
• Vascular disease	20 (23%)	6 (22%)
• Diabetic nephropathy	6 (7%)	2 (7%)
• Other	13 (15%)	3 (11%)
• Unknown	13 (15%)	2 (7%)
Dialysis treatment (%)		
• Hemodialysis	59 (68%)	23 (85%)
• Peritoneal dialysis	28 (32%)	4 (15%)
Laboratory parameters		
Calcium (mmol/L)	2.36 ±0.14	2.31 ±0.15
Phosphate (mmol/L)	1.49 ±0.52	1.19 ±0.32
Parathyroid hormone (pmol/L)	23 (11–44)	12 (6–20)
C-reactive protein (mg/L)	3 (2–6)	4 (2–8)
Bone mineral density and vertebral fracture		
Vertebral bone mineral density (mg/cm³)	134 ±38	125 ±43
Prevalent vertebral fracture (%)	26 (30%)	10 (37%)

Results are presented as mean ±standard deviation, median (interquartile range) or percentage.

*Data on medication use were available in 114 patients.

Conversion factors for units: calcium mg/dL to mmol/L, x0.2495; phosphate mg/dL to mmol/L, x0.3229.

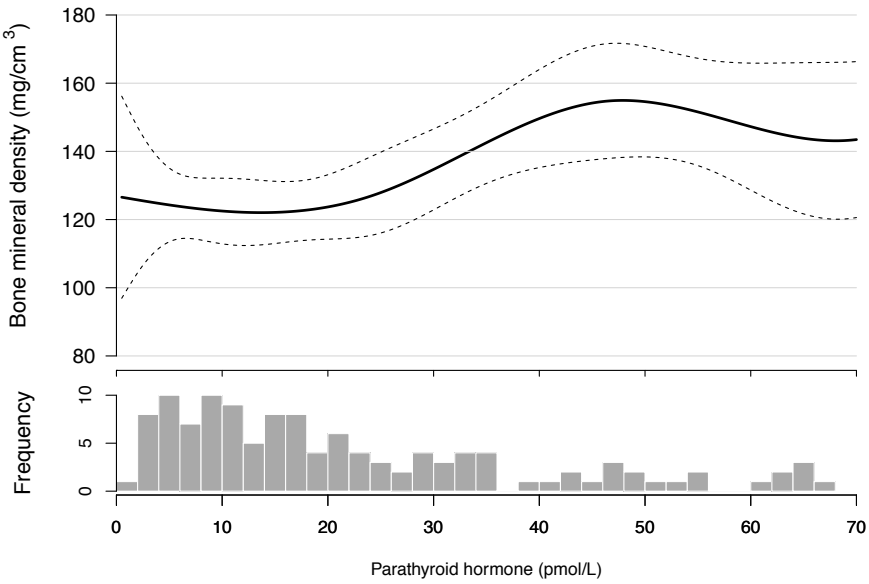
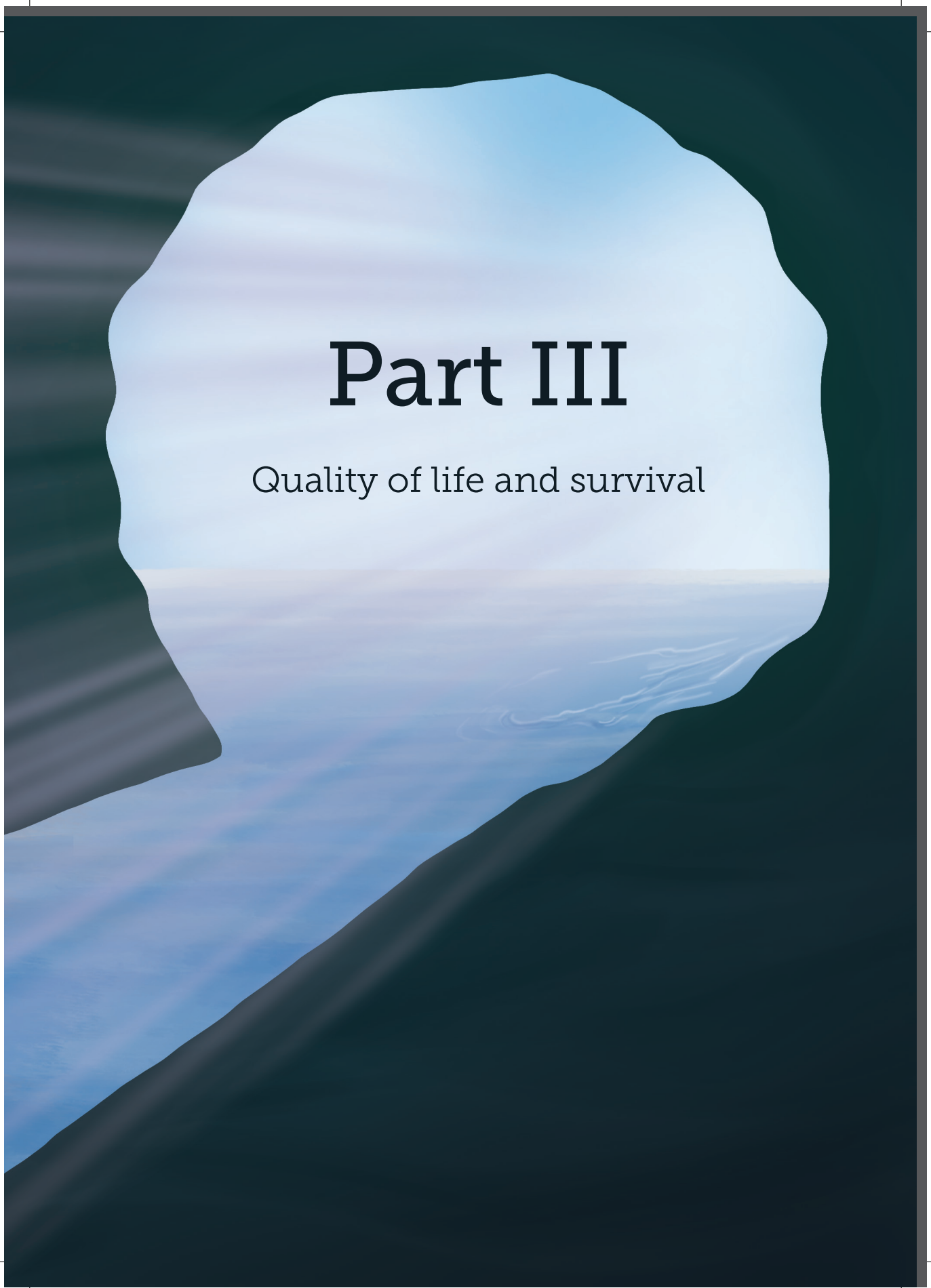


Figure S1. Continuous associations of parathyroid hormone with vertebral trabecular bone mineral density in 131 patients with end-stage renal disease adjusted for age- and sex. A histogram of parathyroid hormone is shown at the bottom.



Part III

Quality of life and survival

Chapter 7

Long-term clinical parameters after switching to nocturnal hemodialysis: a Dutch propensity-score-matched cohort study comparing patients on nocturnal hemodialysis with patients on 3-times weekly hemodialysis/ hemodiafiltration

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Abstract

Background

Nocturnal hemodialysis (NHD), characterized by 8-hr sessions ≥3x weekly, is known to improve clinical parameters short-term compared with conventional-schedule hemodialysis (HD), generally 3 x 3.5–4 hrs weekly. We studied long-term effects of NHD and used patients on conventional HD/ hemodiafiltration (HDF) as controls.

Methods

A total of 159 patients starting with NHD since 2004, aged 56.7 ±12.9 years, with median dialysis vintage 2.3 (0.9–5.1) years were followed during 4 years. One hundred of these patients were propensity-score matched with 100 control patients on HD/HDF, treated in 28 Dutch dialysis centers. Outcome measures were control of hypertension (pre-dialysis blood pressure, number of antihypertensives), phosphate (phosphate, number of phosphate binders), nutritional status and inflammation (albumin, CRP and post-dialysis weight), and anemia (erythropoiesis-stimulating agent [ESA] resistance).

Results

Switching to NHD was associated with a non-significant reduction of antihypertensives compared with HD/HDF (OR <2 types 2.17, 95% CI 0.86; 5.50, p=0.11); and a prolonged lower need for phosphate binders (OR <2 types 1.83, 95% CI 1.10; 3.03, p=0.02). NHD was not associated with significant changes in blood pressure, or phosphate. NHD was associated with significantly higher albumin over time compared with HD/HDF (0.70 g/L/year, 95% CI 0.10; 1.30, p=0.02). ESA resistance decreased significantly in NHD compared with HD/HDF, resulting in a 33% lower ESA dose in the long-term.

Conclusions

After switching to NHD, the lower need for antihypertensives, phosphate binders and ESA persists for at least 4 years. These sustained improvements in NHD contrast significantly with the course of these parameters during continued treatment with conventional-schedule HD and HDF. NHD provides an optimal form of dialysis, also suitable for patients expected to have a long waiting time for transplantation or those remaining indefinitely dialysis.

Introduction

Nocturnal hemodialysis (NHD) has increasingly become subject of research in recent years, as a potential solution for the high cardiovascular morbidity and mortality among hemodialysis (HD) patients[1-3]. Several factors are deemed responsible for this high risk. Recurrent states of hypervolemia are known to cause left ventricular hypertrophy and cardiac remodeling. The consequent necessity of rapid fluid removal during dialysis may cause hypotension and compromise tissue perfusion[4-6]. Furthermore, continuous hyperphosphatemia, often in conjunction with hypercalcemia and hyperparathyroidism, leads to vascular calcifications[7-10].

NHD provides opportunity for slower fluid removal and increased clearance of solutes such as urea and phosphate[11], due to twice as long (7–8 hours) dialysis sessions[12]. Studies have shown beneficial effects of NHD on hypertension[13-18] and hyperphosphatemia[13, 14, 16-20], yet data on nutritional status, anemia control and mortality are not consistent[14, 16, 19-21]. In addition, as these parameters have not been investigated beyond 12 months of NHD treatment, it is not yet known whether these improvements last in the long run.

We followed a large cohort of patients that switched to NHD prospectively, and compared long-term control of hypertension, phosphate and anemia, as well as nutritional status to data collected in the same patients before switch to NHD. Secondly, we compared the long-term course of these parameters in NHD to a cohort of patients treated with conventional (3 x 3.5–4 hours weekly) HD and hemodiafiltration (HDF).

Methods

Outcomes

We studied the following four domains as primary outcomes: hypertension control (pre-dialysis systolic and diastolic blood pressure and number of different antihypertensive agents), phosphate control (phosphate and number of different phosphate binding agents), nutritional status and inflammation (albumin, C-reactive protein [CRP] and post-dialysis weight), and anemia control (erythropoiesis-stimulating agent [ESA] resistance). We investigated all-cause mortality as secondary outcome.

Cohorts

We prospectively followed all patients that had switched to NHD at two major Dutch centers that offered NHD as well as HD and HDF, either in-center or at home with ≥7-hour hemodialysis sessions. We defined baseline as the date of first NHD treatment, which was any time after April 2004. We collected data from electronic and paper records, from initiation until discontinuation of NHD, or until February 1, 2016. Ethical approval for this study was waived by the Medical Ethics Committee of the VU University Medical Centre, Amsterdam.

As reference, we used patient data from the CONvective TRAnsport STudy (CONTRAST, NCT00205556), a randomized trial designed to compare online HDF with low-flux HD regarding cardiovascular morbidity and mortality. CONTRAST randomized adult patients, treated with low-flux HD 2–3 times weekly for at least 2 months with a single-pool Kt/V_{urea} ≥1.2 per treatment, in a 1:1 ratio to treatment with low-flux HD or on-line HDF and followed for 1–7 years[22]. We used data of patients treated in the Netherlands (26 centers) with at least 3 sessions per week between June 2004 and January 2011.

Dialysis regimens

We defined NHD treatment as 3–5 x 8 hours weekly either in-center or at home, with a lower blood flow (150–220 ml/min), lower dialysate flow (300 ml/min) and a somewhat lower bicarbonate concentration compared with conventional hemodialysis, adjusted depending on laboratory results. Anticoagulation was performed with low-molecular weight heparin (dalteparin or nadroparin) for in-center NHD and unfractionated heparin for home NHD.

We defined conventional HD and HDF treatment as 3 x 3.5–4 hours weekly (incidentally 5 hours), with blood flow rates between 300–400 ml/min. All patients used double-needle cannulation. Online HDF was performed in post-dilution mode with a mean convection volume of 20.7 ±6.0 L/h[23]. Anticoagulation was performed with low-molecular weight heparin[23].

Data collection

Apart from demographics and medical history, we collected data on clinical parameters and medication use at switch to NHD (baseline), and at 3, 6, 12, 18, 24, 36 and 48 months. Also, we recorded reasons for discontinuation of NHD. We recorded deaths that occurred within 3 months after cessation of NHD.

In CONTRAST, quarterly measured data similar to the NHD cohort were available for up to 48 months. Although mortality follow-up of CONTRAST was obtained irrespective of censoring, we adjusted these data to a follow-up similar to the NHD cohort (within 3 months of censoring).

In both cohorts, mean values of pre-dialysis blood pressure and post-dialysis weight from the last week before a selected time point were taken. Laboratory parameters were measured with routine assays. We converted albumin, when measured with bromcresol purple assays, to bromcresol green with the following formula: $Albumin_{bromcresol\ green} = Albumin_{bromcresol\ purple} + 5.5\ g/^{24}$. We converted averaged weekly ESA doses of one month to defined daily doses (DDD) with conversion factors provided by the World Health Organization Drug Classification[25].

Statistical analysis

We performed longitudinal analyses with generalized linear mixed models[26], with time as continuous variable and random intercepts and slopes when appropriate. CRP and ESA resistance were skewed and therefore natural-log-transformed. For practical reasons, we dichotomized number of antihypertensive and phosphate binding agents into 0–1 and ≥2 types; we present odds ratios for having a lower number of drugs compared with baseline (<2 types). We performed survival analysis with Cox proportional hazards regression[27]. We tested for proportional hazards assumptions with Schoenfeld's residuals.

To compare the NHD cohort with the HD/HDF cohort, we used propensity-score matching. We imputed data missing at baseline 25 times[28] with multivariate imputations by chained equations[29] in order to estimate propensity scores[28]. All variables had ≤1% missing values at baseline, apart from albumin (1.8%), dialysis prescription (2.4%), diabetes (2.5%), smoking (2.9%), residual GFR (5.3%), cholesterol (10.8%) and CRP (30.7%). In each of the 25 imputed datasets, we subsequently matched on propensity of NHD treatment[30], logistically regressed on 22 variables affecting outcome or treatment selection (see supplemental material). We matched nearest neighbors in a 1:1 ratio, without replacement, within a 0.1 caliper. We considered covariates balanced when standardized mean differences were <0.1.

We used multiple imputations only for propensity score matching; we performed longitudinal analyses for each matched cohort in the non-imputed dataset. We restricted analyses to the follow-up duration of each outcome available in the HD/HDF cohort. We pooled effect measures from the 25 analyzed matched cohorts using Rubin's rules[28, 31]. P values <0.05 (two-sided) were considered statistically significant. All analyses were performed with R 3.3.3[32].

Sensitivity analysis

As sensitivity analysis, we repeated the multivariate imputations, propensity score matching and subsequent analyses for NHD patients versus HD patients and NHD patients versus HDF patients separately, to account for potential benefits of HDF. As a *post-hoc* sensitivity analysis, we adjusted for cause of ESRD (being end-organ damage due to cardiovascular disease and diabetes mellitus), as this remained unbalanced after matching.

Results

NHD cohort at baseline (*n=159*)

One hundred and fifty-nine patients from 2 large Dutch NHD centers (*n=76* and *n=83*) were included, representing an urban as well as a rural population. None of the patients had been treated with HDF previously. At baseline, mean age was 52.0 ±14.6 years, 32% of patients were female, and median dialysis vintage was 2.5 (IQR 0.9–5.5) years (Table 1).

29% of patients were treated with home NHD, 86% of which used single-needle cannulation; 34% of in-center NHD patients used single-needle cannulation. Median standard Kt/V (calculated with Leypoldt's formula[33]) was 3.02 (IQR 2.69–3.91). Seventy-eight patients discontinued NHD treatment within 2 years, due to renal transplantation (45%), medical reasons (23%), sleeping difficulties (14%), social reasons (6%), switch to diurnal home hemodialysis (4%), and death (8%).

Table 1. Baseline characteristics of the complete (unmatched) nocturnal hemodialysis (NHD) and hemodialysis/hemodiafiltration (HD/HDF) cohorts

	NHD (n=159)	HD/HDF (n=560)	*Standardized mean difference*
Demographics			
Age (yr)	52.0 ±14.6	64.0 ±15.2	0.79
Male (%)	68	62	0.12
BMI (kg/m²)	26.1 ±6.2	25.2 ±4.4	0.18
Medical history			
Dialysis vintage (yr)	2.5 (0.9–5.5)	2.0 (1.0–3.8)	0.22
Cause of ESRD (%)			0.63
Glomerulonephritis	24	13	
Interstitial nephritis	14	10	
Cystic kidney disease	15	8	
Congenital, other	5	1	
Renovascular	17	30	
Diabetes mellitus	9	15	
Multi system disease	6	5	
Other	4	11	
Unknown	6	9	
Current smoker (%)	16	21	0.13
Comorbidities (%)			
Diabetes mellitus	21	21	0.00
Cardiovascular disease	28	41	0.42
Transplant waiting list listed (%)	23	33	0.21

Table 1. Continued.

	NHD (n=159)	HD/HDF (n=560)	*Standardized mean difference*
Phosphate control			
Phosphate (mmol/l)	1.73 ±0.53	1.64 ±0.50	0.16
Different phosphate binding agents	1.46 ±0.65	1.27 ±0.74	0.27
Vitamin D usage (%)	85	67	0.43
Hypertension control			
Systolic blood pressure (mmHg)	140.4 ±21.2	148.1 ±22.2	0.36
Diastolic blood pressure (mmHg)	79.9 ±12.9	76.1 ±12.4	0.30
Different antihypertensive agents	0.99 ±0.93	1.55 ±1.28	0.50
Nutritional status / inflammation			
Post-dialysis weight (kg)	77.9 ±19.3	72.4 ±13.9	0.33
Creatinine (µmol/l)	892 ±275	886 ±249	0.02
Albumin (g/l)	40.8 ±2.9	40.0 ±3.8	0.22
CRP (mg/l)	5.0 (2.2–12.7)	4.1 (1.4–10.8)	0.05
Cholesterol (mmol/l)	3.9 ±1.1	3.6 ±1.0	0.26
Anemia control			
Hemoglobin (mmol/l)	7.1 ±0.8	7.4 ±0.8	0.33
ESA dose (DDD)	7.8 (3.9–13.3)	8.9 (4.4–13.3)	0.18
ESA resistance index (DDD/Hb/kg/week)	0.01 (0.01–0.03)	0.02 (0.01–0.03)	0.21
Use of iron supplementation (%)	88	75	0.34
Dialysis treatment parameters and residual kidney function			
Residual diuresis > 100ml/24h (%)	45	50	0.11
Residual GFR (ml/min)	0 (0–2.1)	0.2 (0–2.4)	0.05
Central venous catheter (%)	10	1	0.38
Weekly dialysis sessions	3.2 ±0.8	3.0 ±0.1	0.33
Weekly dialysis hours	12.4 ±2.6	11.3 ±1.2	0.54

BMI: body mass index; ESRD: end-stage renal disease; CRP: C-reactive protein; Hb: hemoglobin; ESA: erythropoiesis-stimulating agent; DDD: defined daily dose; GFR: glomerular filtration rate, mean of urea/creatinine clearance, 0 when residual diuresis < 100ml/24h. We report data as means ±SD, median (IQR) or proportions where appropriate. Standardized mean differences < 0.1 are considered balanced.

Baseline comparison of NHD and HD/HDF cohorts

There were notable differences between the complete NHD and HD/HDF cohorts at baseline (Table 1). We matched 200 (IQR 198–202) NHD and HD/HDF patients on propensity of NHD treatment. In the matched NHD cohort, mean age was 56.7 ±12.9 years and median dialysis vintage was 2.3 (0.9–5.1) years at baseline. 21% of patients had

diabetes mellitus, and 31% a history of cardiovascular disease. Similar to the complete NHD cohort, patients were treated 7.8 ±0.4 hours 3.5 (IQR 3.0–4.0) times weekly. After matching, the NHD and HD/HDF cohorts were largely similar (Table 2): age, sex, dialysis vintage, diabetes, history of cardiovascular disease, transplant waitlist status, BMI and residual diuresis were balanced across both cohorts. Of note, cause of ESRD remained unbalanced after matching. We could not match 37% of the NHD cohort, mainly due to young age compared with the HD/HDF cohort. Hereafter, we refer to the matched cohorts as the NHD and HD/HDF cohorts.

Table 2. Baseline characteristics of the nocturnal hemodialysis (NHD) and hemodialysis/hemodiafiltration (HD/HDF) cohorts after propensity score matching

	NHD (n = 100)*	HD/HDF (n = 100)*	*Standardized mean difference*
Demographics			
Age (yr)	56.7 ±12.9	56.3 ±15.1	0.03
Male (%)	68	67	0.02
BMI (kg/m²)	25.9 ±5.6	25.9 ±5.1	0.01
Medical history			
Dialysis vintage (yr)	2.3 (0.9–5.1)	2.1 (1.1–4.0)	< 0.01
Cause of ESRD (%)			
Glomerulonephritis	24	16	
Interstitial nephritis	13	6	
Cystic kidney disease	16	9	
Congenital, other	4	1	
Renovascular	19	27	
Diabetes mellitus	9	16	
Multi system disease	5	6	0.54
Other	6	10	
Unknown	5	9	
Current smoker (%)	19	19	0.02
Comorbidities (%)			
Diabetes mellitus	21	22	0.02
Cardiovascular disease	30	29	0.02
Transplant waiting list listed (%)	28	28	< 0.001
Phosphate control			
Phosphate (mmol/l)	1.72 ±0.52	1.69 ±0.53	0.05
Different phosphate binding agents	1.40 ±0.74	1.43 ±0.64	0.05
Vitamin D usage (%)	84	67	0.40

Table 2. Continued.

	NHD (n = 100)*	HD/HDF (n = 100)*	*Standardized mean difference*
Hypertension control			
Systolic blood pressure (mmHg)	143.0 ±21.7	143.7 ±21.1	0.03
Diastolic blood pressure (mmHg)	79.4 ±13.2	79.7 ±12.7	0.02
Different antihypertensive agents	1.22 ±1.18	1.15 ±0.95	0.07
Nutritional status / inflammation			
Post-dialysis weight (kg)	77.5 ±16.8	75.5 ±15.4	0.13
Creatinine (µmol/l)	863 ±260	938 ±273	0.28
Albumin (g/l)	40.6 ±3.0	40.6 ±3.8	0.02
CRP (mg/l)	5.0 (2.3–13.6)	3.7 (1.4–10.1)	0.02
Cholesterol (mmol/l)	3.9 ±1.0	3.9 ±1.0	< 0.01
Anemia control			
Hb (mmol/l)	7.1 ±0.8	7.4 ±0.7	0.35
ESA dose (DDD)	8.0 (4.4–13.3)	6.7 (4.0–13.3)	0.04
ESA resistance index (DDD/Hb/kg/week)	0.01 (0.01–0.03)	0.01 (0.01–0.03)	0.01
Use of iron supplementation (%)	87	86	0.01
Dialysis treatment parameters and residual kidney function			
Residual diuresis > 100ml/24h (%)	49	50	0.04
Residual GFR (ml/min)	0.0 (0.0–3.7)	0.0 (0.0–2.7)	0.08
Central venous catheter (%)	4	5	0.04
Weekly dialysis sessions	3.0 ±0.7	3.0 ±0.1	0.02
Weekly dialysis hours	11.7 ±2.2	11.6 ±1.1	0.05

BMI: body mass index; ESRD: end-stage renal disease; CRP: C-reactive protein; Hb: hemoglobin; ESA: erythropoiesis-stimulating agent; DDD: defined daily dose; GFR: glomerular filtration rate, mean of urea/creatinine clearance, 0 when residual diuresis < 100ml/24h. We report data as means ±SD, medians (IQR) or proportions where appropriate. Standardized mean differences < 0.1 are considered balanced. *median 200 (IQR 198–202) matched cases.

Longitudinal comparison of matched NHD and HD/HDF cohorts

In the NHD cohort, systolic and diastolic blood pressure did not change over time (Table 3). As can be seen from Figure 1, blood pressure fluctuated somewhat during the third and fourth year in the HD/HDF cohort, but tended to decrease overall. There was no significant difference in systolic or diastolic blood pressure change between the NHD and HD/HDF cohorts (systolic blood pressure change versus HD/HDF: 1.94 [95% CI -1.17; 5.06] mmHg/year, p=0.22, and diastolic blood pressure change versus HD/HDF: 0.31 [95% CI -1.31; 1.93] mmHg/year, p=0.71). In the NHD cohort, the number of antihypertensive agents diminished substantially after switching to NHD; the odds

of having less antihypertensive agents increased over time (Figure 1). Although the number of antihypertensive agents did not change in the HD/HDF cohort, the change in number of antihypertensive agents in the NHD cohort was not significantly different from the HD/HDF cohort (odds ratio of <2 types versus HD/HDF per year: 2.17 [95% CI 0.86; 5.50], p=0.11).

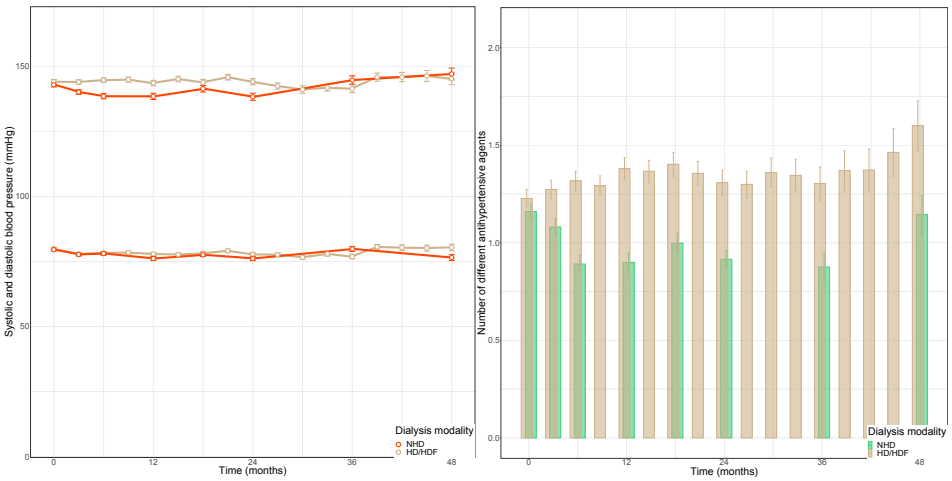

Figure 1. Hypertension control in nocturnal hemodialysis versus hemodialysis/hemodiafiltration. Left: systolic (upper two lines) and diastolic (lower two lines) blood pressure (mmHg) in propensity score matched nocturnal hemodialysis (NHD, dark lines) and hemodialysis/hemodiafiltration (HD/HDF, light lines) patients over the course of 48 months. Right: number of different antihypertensive agents in propensity score matched NHD (dark line) and HD/HDF (light line) patients over the course of 48 months. OR <2 types NHD compared with baseline p=0.02; OR <2 types NHD vs HD/HDF p=0.11. 95% confidence intervals are shown. Number of NHD / HD/HDF patients available for analysis at 0 months: 100/100; 12 months: 57/74; 24 months: 35/51; 36 months: 20/34; 48 months: 11/22.

Initially, phosphate levels decreased slightly in patients that switched to NHD, and remained stable during follow-up (Figure 2). In patients on HD/HDF, phosphate levels did not decrease significantly and fluctuated during follow-up. The course of phosphate in NHD was not significantly different from that in HD/HDF(-0.04 [95% CI -0.12; 0.03] mmol/L/year, p=0.23). However, the number of phosphate binding agents diminished sharply in patients that switched to NHD compared with patients on HD/HDF (odds ratio of <2 types versus HD/HDF per year: 1.83 [95% CI 1.10; 3.03], p=0.02, Figure 2). Moreover, the absolute number of phosphate binding pills decreased in the matched NHD cohort (from 6 at baseline [IQR 4–9] to 3 [IQR 0–5]) in the first 3 months after switching, and remained stable afterwards.

Table 3. Effect estimates per year in the propensity score matched nocturnal hemodialysis (NHD) and hemodialysis/hemodiafiltration (HD/HDF) cohorts, and difference between the propensity score matched NHD and HD/HDF cohorts (all outcomes 48 months, except for CRP 36 months, and ESA resistance index 12 months)

	NHD	
Hypertension control	Δ	*p*
Systolic blood pressure (Δ mmHg)	0.62 (-1.74; 2.99)	0.27
Diastolic blood pressure (Δ mmHg)	-0.82 (-2.10; 0.45)	0.21
Different antihypertensive agents (odds ratio < 2 types)	2.25 (1.12; 4.54)	0.02
Phosphate control	Δ	*p*
Phosphate (Δ mmol/l)	-0.04 (-0.10; 0.01)	0.14
Different phosphate binding agents (odds ratio < 2 types)	1.79 (1.13; 2.84)	0.01
Nutritional status / inflammation	Δ	*p*
Albumin (Δ g/l)	0.29 (-0.14; 0.72)	0.19
CRP (ratio*)	0.98 (0.91; 1.06)	0.64
Post-dialysis weight (Δ kg)	0.09 (-0.80; 0.98)	0.85
Anemia control	Δ	*p*
ESA resistance index (ratio*)	0.75 (0.62; 0.91)	< 0.01

Effect estimates are presented with 95% confidence intervals
CRP: C-reactive protein; ESA: erythropoiesis-stimulating agent
*CRP and ESA resistance were modelled with a gamma distributed log link function. Hence, the (exponentiated) coefficients should be interpreted multiplicatively, i.e. as a ratio.

In patients that switched to NHD, albumin was stable. Compared with patients on HD/HDF, NHD was associated with significantly higher albumin levels over time ((0.70 [95% CI 0.10; 1.30] g/L/year, p=0.02; Figure 3). CRP and post-dialysis weight did not change significantly after switching to NHD, which was not significantly different from patients on HD/HDF (Table 3).

ESA resistance decreased in patients that switched to NHD (ratio per year 0.61 [95% CI 0.47; 0.81], p < 0.001), while ESA resistance increased in patients on HD/HDF, of whom only one-year data were available (Figure 4). ESA resistance remained persistently low for up to 48 months after switching to NHD (ratio per year 0.89 [95% CI 0.83; 0.96], p <0.01). ESA dose was reduced from median 8.0 (IQR 4.4–13.3) to 5.6 (IQR 2.2–8.9) DDD after 4 years of NHD. Additional data on iron storage parameters in both cohorts (ferritin, transferrin saturation); intravenous iron dose and residual urine production in the NHD cohort are available as Supplementary Material. We provide data on the 4-year course of clinical parameters in the complete NHD cohort *(n=159)* as Supplementary Material.

HD/HDF		NHD vs HD/HDF	
Δ	*p*	Δ	*p*
-1.32 (-3.40; 0.76)	0.22	1.94 (-1.17; 5.06)	0.22
-1.13 (-2.18; -0.08)	0.04	0.31 (-1.31; 1.93)	0.71
1.04 (0.55; 1.97)	0.91	2.17 (0.86; 5.50)	0.11
Δ	*p*	Δ	*p*
0.00 (-0.05; 0.05)	0.90	-0.04 (-0.12; 0.03)	0.23
0.98 (0.73; 1.31)	0.90	1.83 (1.10; 3.03)	0.02
Δ	*p*	Δ	*p*
-0.41 (-0.85; 0.03)	0.07	0.70 (0.10; 1.30)	0.02
1.01 (0.90; 1.13)	0.89	0.97 (0.84; 1.12)	0.72
-0.08 (-0.86; 0.69)	0.84	0.17 (-1.04; 1.38)	0.78
Δ	*p*	Δ	*p*
1.23 (1.03; 1.46)	0.02	0.61 (0.47; 0.81)	< 0.001

33 NHD patients and 26 HD/HDF of the matched patients received a renal transplant, while 11 NHD patients and 23 HD/HDF patients died during a mean follow-up of 1.73 ±1.41 and 2.28 ±1.38 years. Hazard ratio for all-cause mortality was 0.64 (95% CI 0.28; 1.48; p=0.29) for NHD compared with HD/HDF patients.

Sensitivity analysis

When matching NHD with either HD (Supplemental Table 1) or HDF patients (Supplemental Table 2), similar results were obtained regarding hypertension, phosphate and anemia control, and nutritional status (Supplemental Table 3 and 4), and mortality (hazard ratio NHD versus HD 0.68 [95% CI 0.29; 1.59]; hazard ratio NHD versus HDF 0.65 [95% CI 0.28; 1.49]). Also, *post-hoc* sensitivity analysis with adjustments for causes of ESRD yielded similar results (Supplemental Table 5).

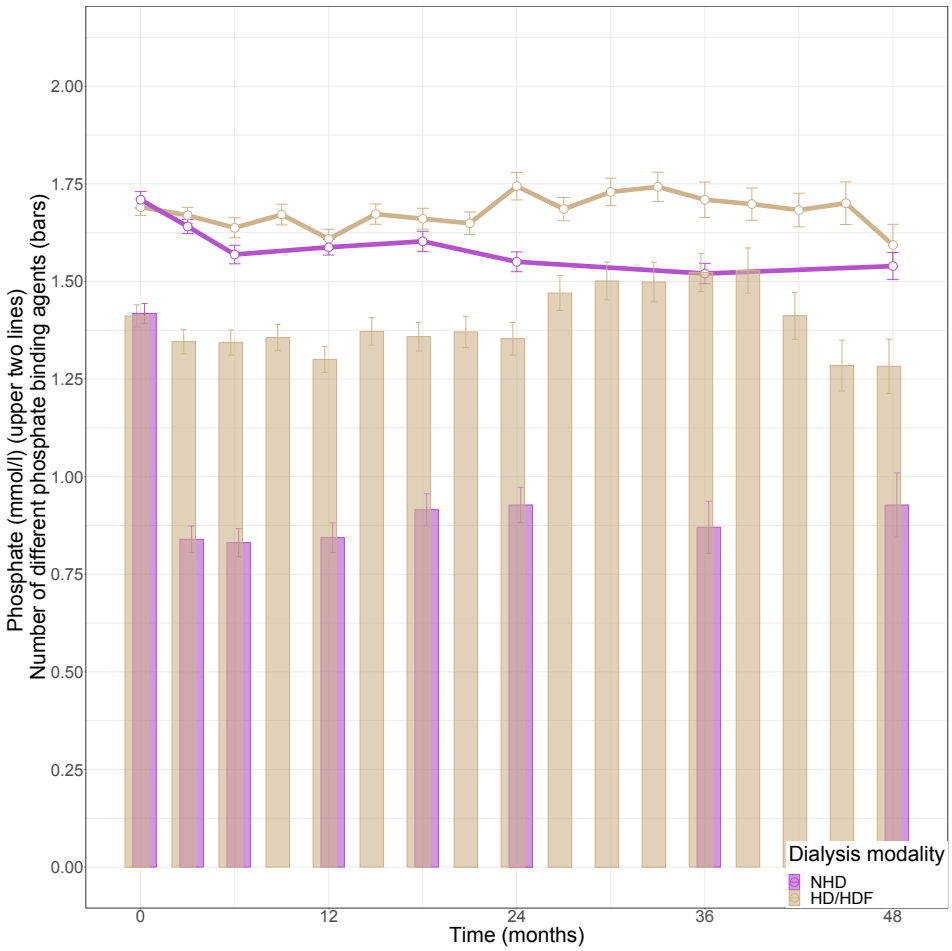

Figure 2. Phosphate control in nocturnal hemodialysis versus hemodialysis/hemodiafiltration. Phosphate (lines, mmol/l) and number of different phosphate binding agents (bars, same axis) in propensity score matched nocturnal hemodialysis (NHD, dark lines/bars) and hemodialysis/hemodiafiltration (HD/HDF, light lines/bars) patients over the course of 48 months. OR <2 types NHD compared with baseline p=0.01; OR <2 types NHD vs HD/HDF p=0.02. 95% confidence intervals are shown. Number of NHD / HD/HDF patients available for analysis at 0 months: 100/100; 12 months: 57/74; 24 months: 35/51; 36 months: 20/34; 48 months: 11/22.

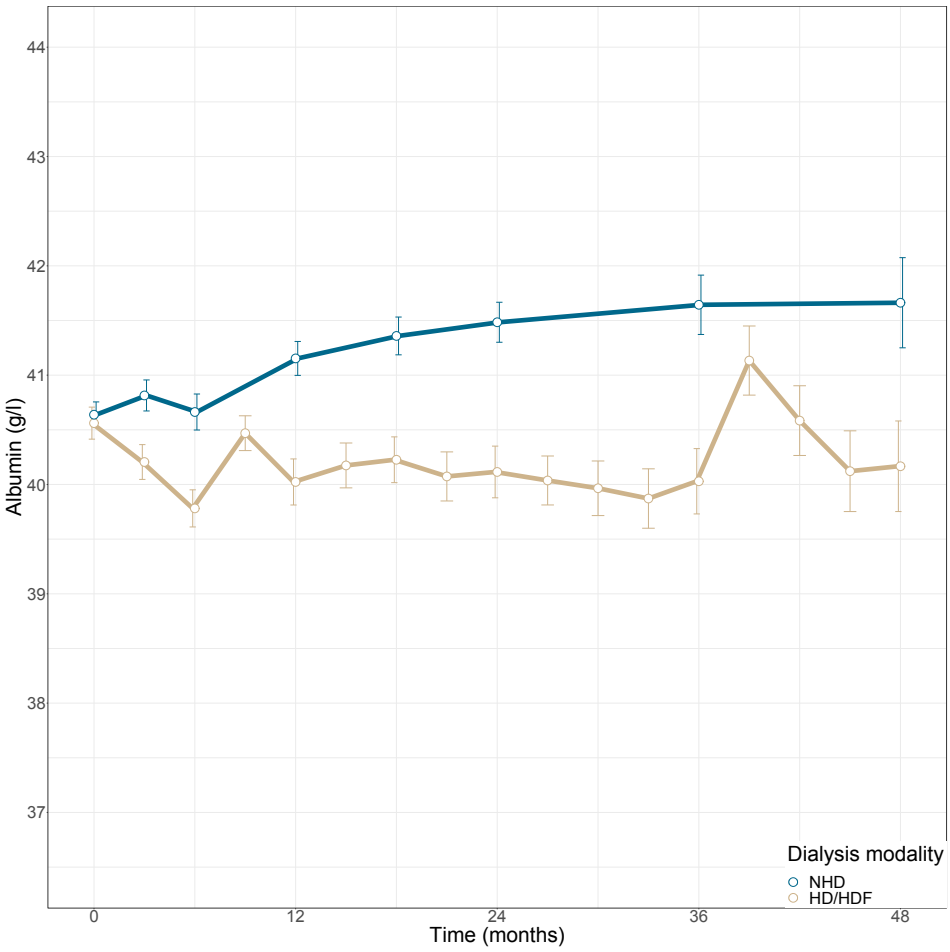

Figure 3. Albumin in nocturnal hemodialysis versus hemodialysis/hemodiafiltration. Albumin (g/l) in propensity score matched nocturnal hemodialysis (NHD, dark line) and hemodialysis/hemodiafiltration (HD/HDF, light line) patients over the course of 48 months. NHD compared with baseline p=0.19; NHD vs HD/HDF p=0.02. 95% confidence intervals are shown. Number of NHD / HD/HDF patients available for analysis at 0 months: 100/100; 12 months: 57/74; 24 months: 35/51; 36 months: 20/34; 48 months: 11/22.

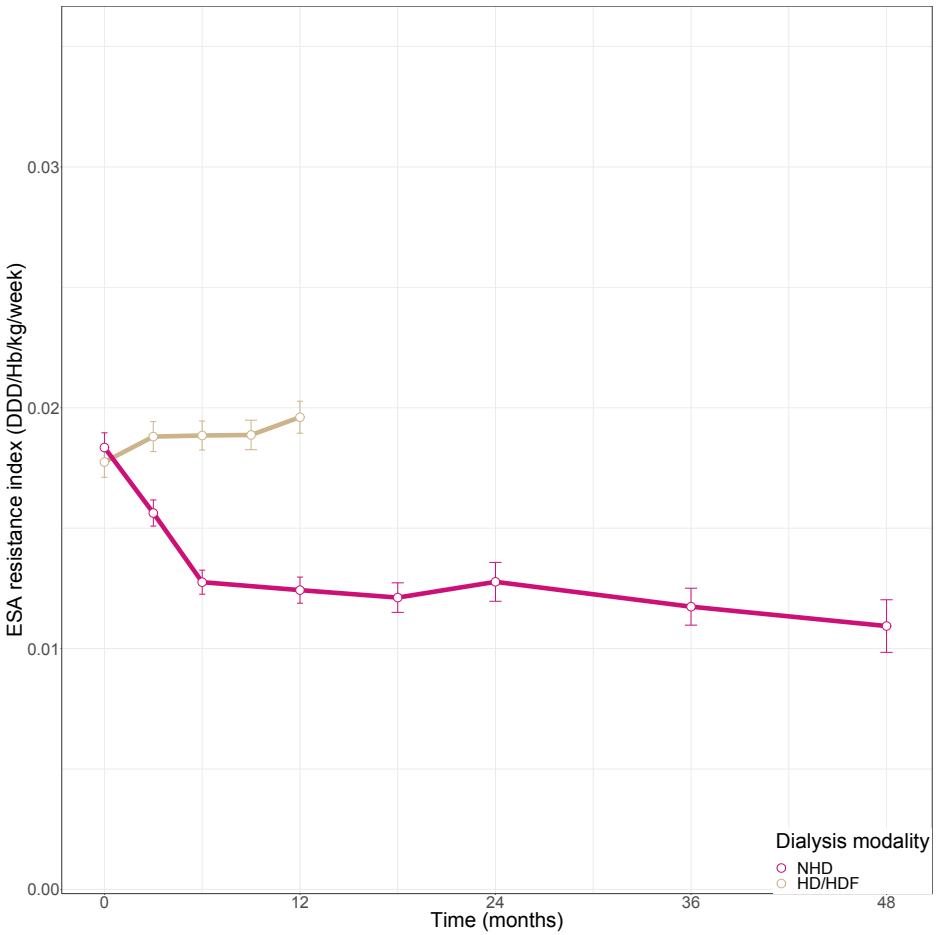

Figure 4. Erythropoiesis-stimulating agent (ESA) resistance in nocturnal hemodialysis versus hemodialysis/hemodiafiltration. ESA resistance (DDD/Hb/kg/week) in propensity score matched nocturnal hemodialysis (NHD, dark line) and hemodialysis/hemodiafiltration (HD/HDF, light line) patients over the course of 48 months. NHD compared to baseline $p < 0.01$; NHD vs HD/HDF $p < 0.001$. 95% confidence intervals are shown. Number of NHD / HD/HDF patients available for analysis at 0 months: 100/100; 12 months: 57/74; 24 months: 35/0; 36 months: 20/0; 48 months: 11/0.

Discussion

To our knowledge, this is the first study to evaluate clinical parameters in detail for several years of NHD treatment. Our findings suggest that patients who switch to NHD experience long-term improvements of important clinical parameters, while benefiting from a greatly reduced pill burden and a lower need for ESA. As a randomized controlled trial for such a long follow-up is not feasible, with most patients refusing randomization between such different dialysis modalities, we employed propensity score matching, the next-best method, for a well-founded comparison. Compared with patients that continued conventional treatment with HD/HDF, the above conclusions remained valid.

Our findings support more easily controlled hypertension in patients that switch to NHD, as they tend to develop a prolonged lower need for antihypertensive agents. Although the difference was not statistically significant compared with HD/HDF patients (p=0.11), we consider the effect size clinically meaningful: NHD patients were over two times more likely to have their antihypertensives reduced from ≥2 to 0 or 1 types. The lack of statistical significance may be due to relatively small patient numbers, and a loss of information by dichotomization into 0-1 and ≥2 types of antihypertensives. We did not find explicit differences in blood pressure between NHD and HD/HDF patients, probably because systolic blood pressure did not decrease significantly in our NHD cohort, contrasting with some previous studies[13, 14, 16]. This may be explained by the heterogeneous NHD frequencies in our cohort: we observed significantly lower 1-year blood pressures in NHD patients who dialyzed >3 times/week compared to NHD patients who dialyzed ≤3 times/week (data not shown). Consistent with this, a recent trial also did not find differences in blood pressure between extended-hours HD (≥24 hours during mean 3.4 ±0.5 sessions weekly) and conventional treatment[34].

Evidently, NHD patients also had more easily regulated phosphate levels. Compared with HD/HDF patients, patients in the NHD cohort experienced a significantly lower need for phosphate binding agents during the 48 month follow-up period. Also, the coinciding halving in absolute number of phosphate binding pills in the NHD cohort corroborates the lower need for phosphate binding agents in NHD patients, posing a great relief for their daily pill burden. As phosphate levels are often kept within target ranges[35] by attending nephrologists, no outstanding differences in phosphate were observed. Thirty-four % of NHD patients had near-normal phosphate levels (≤1.50 mmol/L) at baseline, compared with 52% at 12 months (with some receiving phosphate added to the dialysate).

As to nutritional status, we found striking differences between NHD and HD/HDF patients over the course of 48 months. In our study, NHD was associated with significantly higher albumin levels over time, compared with HD/HDF. This is in line with previous studies that described stable or even increasing albumin levels following initiation of NHD[14, 16, 19]. CRP levels did not differ significantly between the NHD and HD/HDF patients. Post-dialysis weight remained stable in NHD patients over the years. Contrary to what we had expected based on previous reports[16, 19], this was not significantly different from HD/HDF patients, which might be explained by patients adopting a more active lifestyle after several years on NHD. NHD patients also achieved superior anemia control compared with HD/HDF patients, contrasting with previous conflicting reports[13, 14, 16, 18, 19]. Although hemoglobin did not change in NHD patients, presumably due to adjustments of ESA and iron dosages according to guidelines, ESA resistance diminished evidently in NHD patients and remained persistently low for up to 4 years, against a clear increase in ESA resistance in the HD/HDF cohort.

Amidst conflicting data, a recently published large study showed a lower mortality risk in patients treated with extended-hours dialysis compared with conventional HD (hazard ratio 0.67 [95% CI 0.49, 0.93])[36]. We found a remarkably similar lower mortality risk in NHD (hazard ratio 0.64 [95% CI 0.28–1.48]), although our study was not powered to prove mortality differences due to its design and consequent sample size. It should be noted that no safety events (i.e. vascular access complications) were recorded, as we did not expect additional complications because of the habit of single-needle cannulation in frequent NHD.

Remarkably, more NHD patients were transplanted compared with matched HD/HDF patients (32.6 vs 26.3%), despite similar rates of transplant waitlist listing at baseline. As transplanted, presumably healthier patients dropped out more frequently in the NHD cohort, this could have resulted in a somewhat less healthy matched cohort, which could have led to an overestimation (but surely no underestimation) of mortality hazard in the NHD cohort. The reasons for the disparate transplantation rates remain unclear. Possibly, clinical improvement of non-waitlisted NHD patients resulted in more transplant waitlist listed patients.

There were limitations to this study and the results should be interpreted within the limitations of this study. First, this study was non-randomized, and as a result, patients in our NHD cohort were from a selected population, as with many other studies on NHD[17, 19]. We used propensity score matching to reduce this selection bias, so that the groups were similar in baseline characteristics. Although causes of ESRD remained unbalanced between the NHD and HD/HDF cohorts after matching, a *post-hoc* sensitivity analysis

with adjustments for cause of ESRD yielded similar results, supporting the conclusions of this study. Second, not all NHD patients could be matched to HD/HDF counterparts, and this study had to compromise somewhat on sample size and was underpowered to evaluate mortality. On the other hand, we gathered a large cohort of NHD patients that can be considered demographically representative for the Dutch NHD population. Third, as data on the HD/HDF cohort were collected in a randomized trial as opposed to the observational NHD cohort, a potential risk of information bias exists, albeit minor as clinical parameters in the NHD cohort were assessed at standardized intervals as part of routine care. Also, we aimed to minimize interference due to different health care systems, by including patients on HD/HDF only when they were treated in Dutch dialysis centers. Fourth, the NHD cohort was drawn from only 2 centers; however, we deem the potential influence of individual prescribing practices (e.g. nephrologists preferentially prescribing a single type of phosphate binder) insignificant, considering the equal number of phosphate binding agents at baseline and the coinciding halving in number of pills in the NHD cohort, and otherwise uniform treatment patterns in The Netherlands. Fifth, the preferable method for hypertension monitoring is ambulatory blood pressure monitoring or self-reported home measurements, while we only measured pre-dialysis blood pressure. Finally, our NHD cohort comprised patients who performed any kind of extended-hours NHD, which resulted in heterogeneity of frequency and location. On the other hand, we accounted for potential benefits of HDF treatment by comparing NHD with HD and HDF separately, i.e. sensitivity analyses. Earlier data from CONTRAST showed that HDF helped to improve phosphate control[37, 38], yet not ESA resistance[39]. These sensitivity analyses yielded similar results.

Overall, our study highlights the long-term improvements in important clinical parameters in patients switching to NHD, compared with conventional HD/HDF treatment. Translated to the clinic, our findings suggest that patients considering NHD can expect an increase in albumin, which is associated with lower mortality[40], a lower dose of ESA, and a sizable reduction in pill burden, which is significant for their well-being. Our findings imply that NHD is more than a hype: it offers the prospect of enduring improvements for patients on hemodialysis, also suitable for patients expected to have a long waiting time for transplantation or those convicted to indefinite dialysis.

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Supplemental material

Table S1. Baseline characteristics after matching with hemodialysis patients only

	NHD (n=84)*	HD (n=84)*	*Standardized mean difference*
Demographics			
Age (yr)	57.3 ±12.9	57.1 ±15.0	0.01
Male (%)	67	66	0.02
Height (cm)	172.7 ±11.8	170.4 ±9.5	0.21
BMI (kg/m²)	25.8 ±5.4	25.7 ±4.9	0.01
Medical history			
Dialysis vintage (yr)	2.3 (0.8–4.8)	2.3 (1.2–4.5)	< 0.01
Cause of ESRD (%)			0.65
Glomerulonephritis	26	18	
Interstitial nephritis	14	7	
Cystic kidney disease	15	6	
Congenital, other	3	0	
Renovascular	20	25	
Diabetes mellitus	7	13	
Multi system disease	5	5	
Other	6	14	
Unknown	5	11	
Current smoker (%)	21	21	0.01
Comorbidities (%)			
Diabetes mellitus	20	19	0.02
Cardiovascular disease	32	32	0.02
Active transplant waitlist status (%)	31	31	0.01
Phosphate control			
Phosphate (mmol/l)	1.70 ±0.51	1.69 ±0.52	0.02
Different phosphate binding agents	1.41 ±0.70	1.42 ±0.64	0.02
Vitamin D usage (%)	84	73	0.28
Hypertension control			
Systolic blood pressure (mmHg)	143.5 ±22.7	144.8 ±21.0	0.06
Diastolic blood pressure (mmHg)	79.1 ±13.5	79.5 ±11.7	0.03
Different antihypertensive agents	1.20 ±1.14	1.19 ±0.95	0.01

Table S1. Continued.

	NHD (n=84)*	HD (n=84)*	*Standardized mean difference*
Nutritional status / inflammation			
Post-dialysis weight (kg)	76.6 ±15.9	74.8 ±15.1	0.12
Creatinine (μmol/l)	854 ±264	956 ±260	0.39
Albumin (g/l)	40.6 ±3.0	40.7 ±3.7	0.03
CRP (mg/l)	5.0 (2.3–13.1)	4.1 (1.4–10.1)	0.01
Cholesterol (mmol/l)	3.8 ±1.0	3.8 ±1.1	0.03
Anemia control			
Hb (mmol/l)	7.1 ±0.8	7.4 ±0.7	0.32
ESA dose (DDD)	8.3 (4.4–13.3)	6.7 (4.4–13.3)	0.07
ESA resistance index (DDD/Hb/kg/week)	0.02 (0.01–0.03)	0.01 (0.01–0.02)	0.02
Ferritin (μg/L)	226 (110–485)	320 (170–592)	0.24
Transferrin saturation (%)**	13 (8–26)	26 (19–32)	0.48
Use of iron supplementation (%)	85	84	0.02
Dialysis treatment parameters and residual kidney function			
Residual diuresis > 100ml/24h (%)	48	49	0.02
Residual GFR (ml/min)	0.0 (0.0–4.6)	0.0 (0.0–2.4)	0.27
Central venous catheter (%)	4	4	0.02
Weekly dialysis sessions	3.0 ±0.7	3.0 ±0.1	0.03
Weekly dialysis hours	11.6 ±2.2	11.6 ±1.1	0.03

NHD: nocturnal hemodialysis; HD: hemodialysis; BMI: body mass index; ESRD: end-stage renal disease; CRP: C-reactive protein; Hb: hemoglobin; ESA: erythropoiesis-stimulating agent; DDD: defined daily dose; GFR: glomerular filtration rate, mean of urea/creatinine clearance, 0 when residual diuresis < 100ml/24h. Means ±SD, medians (IQR) or proportions are reported where appropriate.

* median 168 (IQR 166–172) matched cases per dataset.

Table S2. Baseline characteristics after matching with hemodiafiltration patients only

	NHD (n=84)*	HDF (n=84)*	*Standardized mean difference*
Demographics			
Age (yr)	58.8 ±11.3	57.7 ±14.8	0.08
Male (%)	66	65	0.01
Height (cm)	173.3 ±11.4	170.7 ±10.4	0.23
BMI (kg/m²)	25.7 ±5.1	25.7 ±4.6	0.01
Medical history			
Dialysis vintage (yr)	2.3 (0.8–4.8)	1.9 (1.0–3.8)	0.06
Cause of ESRD (%)			0.51
Glomerulonephritis	23	12	
Interstitial nephritis	13	7	
Cystic kidney disease	16	9	
Congenital, other	2	2	
Renovascular	20	28	
Diabetes mellitus	9	17	
Multi system disease	5	8	
Other	7	10	
Unknown	5	8	
Current smoker (%)	20	19	0.02
Comorbidities (%)			
Diabetes mellitus	22	24	0.05
Cardiovascular disease	34	33	0.03
Active transplant waitlist status (%)	29	31	0.04
Phosphate control			
Phosphate (mmol/l)	1.70 ±0.51	1.72 ±0.55	0.02
Different phosphate binding agents	1.39 ±0.75	1.39 ±0.63	< 0.01
Vitamin D usage (%)	85	61	0.56
Hypertension control			
Systolic blood pressure (mmHg)	142.5 ±22.0	144.6 ±21.4	0.10
Diastolic blood pressure (mmHg)	78.4 ±13.3	79.3 ±12.4	0.07
Different antihypertensive agents	1.29 ±1.22	1.16 ±0.94	0.11
Nutritional status / inflammation			
Post-dialysis weight (kg)	76.9 ±15.0	74.8 ±14.6	0.15
Creatinine (µmol/l)	858 ±263	918 ±264	0.23
Albumin (g/l)	40.5 ±3.0	40.5 ±3.6	0.02
CRP (mg/l)	5.0 (2.3–13.1)	4.0 (1.6–10.4)	0.04
Cholesterol (mmol/l)	3.8 ±1.0	3.8 ±0.9	0.01

Table S2. Continued.

	NHD (n=84)*	HDF (n=84)*	*Standardized mean difference*
Anemia control			
Hb (mmol/l)	7.1 ±0.8	7.4 ±0.8	0.37
ESA dose (DDD)	8.0 (4.4–13.3)	8.0 (3.3–13.3)	< 0.01
ESA resistance index (DDD/Hb/kg/week)	0.01 (0.01–0.03)	0.01 (0.01–0.03)	0.02
Ferritin (µg/L)	226 (110–478)	266 (160–553)	0.21
Transferrin saturation (%)**	13 (8–26)	24 (19–31)	0.46
Use of iron supplementation (%)	87	88	0.05
Dialysis treatment parameters and residual kidney function			
Residual diuresis > 100ml/24h (%)	48	51	0.06
Residual GFR (ml/min)	0.0 (0.0–4.6)	0.3 (0.0–2.8)	0.18
Central venous catheter (%)	3	3	0.03
Weekly dialysis sessions	3.0 ±0.7	3.0 ±0.1	0.01
Weekly dialysis hours	11.7 ±2.3	11.6 ±1.1	0.07

NHD: nocturnal hemodialysis; HDF: hemodiafiltration; BMI: body mass index; ESRD: end-stage renal disease; CRP: C-reactive protein; Hb: hemoglobin; ESA: erythropoiesis-stimulating agent; DDD: defined daily dose; GFR: glomerular filtration rate, mean of urea/creatinine clearance, 0 when residual diuresis < 100ml/24h. Means ±SD, medians (IQR) or proportions are reported where appropriate.
*median 168 (IQR 164–170) matched cases per dataset.

Table S3. Effect estimates in the matched nocturnal hemodialysis (NHD) and hemodialysis (HD) cohorts, and difference between the matched NHD and HD cohorts (CRP 36 months, ESA resistance index 12 months, all other outcomes 48 months)

	NHD		HD		NHD vs HD
Hypertension control	Δ	*p*	Δ	*p*	*p*
Systolic blood pressure (Δ mmHg)	0.67 (-1.92; 3.26)	0.61	-1.08 (-3.49; 1.33)	0.38	0.33
Diastolic blood pressure (Δ mmHg)	-0.67 (-2.08; 0.74)	0.35	-0.85 (-1.95; 0.25)	0.13	0.84
Different antihypertensive agents (odds ratio < 2 types)	2.80 (0.97; 8.02)	0.06	1.15 (0.67; 1.97)	0.61	0.14
Phosphate control	Δ	*p*	Δ	*p*	*p*
Phosphate (Δ mmol/l)	-0.03 (-0.09; 0.02)	0.26	-0.02 (-0.06; 0.03)	0.45	0.66
Different phosphate binding agents (odds ratio < 2 types)	1.73 (1.01; 2.98)	0.05	1.03 (0.76; 1.40)	0.85	0.09
Nutritional status / inflammation	Δ	*p*	Δ	*p*	*p*
Albumin (Δ g/l)	0.30 (-0.15; 0.74)	0.19	-0.49 (-0.86; -0.13)	0.01	0.01
CRP (ratio*)	0.98 (0.91; 1.06)	0.62	1.09 (0.95; 1.25)	0.21	0.21
Post-dialysis weight (Δ kg)	0.19 (-0.74; 1.13)	0.69	-0.38 (-1.16; 0.40)	0.34	0.34
Anemia control	Δ	*p*	Δ	*p*	*p*
ESA resistance index (ratio*)	0.79 (0.64; 0.97)	0.03	1.25 (1.04; 1.50)	0.02	< 0.01

Effect estimates are presented with 95% confidence intervals

CRP: C-reactive protein; ESA: erythropoiesis-stimulating agent

*CRP and ESA resistance were modelled with a gamma distributed log link function. Hence, the (exponentiated) coefficients should be interpreted multiplicatively, i.e. as a ratio.

Table S4. Effect estimates in the matched nocturnal hemodialysis (NHD) and hemodiafiltration (HDF) cohorts, and difference between the matched NHD and HDF cohorts (CRP 36 months, ESA resistance index 12 months, all other outcomes 48 months)

	NHD		HDF		NHD vs HDF
Hypertension control	*Δ*	*p*	*Δ*	*p*	*p*
Systolic blood pressure (Δ mmHg)	1.09 (-1.69; 3.86)	0.59	-1.99 (-4.10; 0.12)	0.07	0.09
Diastolic blood pressure (Δ mmHg)	-0.65 (-2.16; 0.86)	0.40	-1.70 (-2.81; -0.59)	< 0.01	0.26
Different antihypertensive agents (odds ratio < 2 types)	2.36 (1.03; 5.42)	0.04	1.10 (0.57; 2.10)	0.78	0.11
Phosphate control	*Δ*	*p*	*Δ*	*p*	*p*
Phosphate (Δ mmol/l)	-0.03 (-0.09; 0.03)	0.31	-0.01 (-0.05; 0.04)	0.82	0.51
Different phosphate binding agents (odds ratio < 2 types)	1.79 (1.02; 3.12)	0.04	1.23 (0.84; 1.81)	0.29	0.29
Nutritional status / inflammation	*Δ*	*p*	*Δ*	*p*	*p*
Albumin (Δ g/l)	0.29 (-0.23; 0.80)	0.28	-0.46 (-0.90; -0.02)	0.04	0.03
CRP (ratio*)	1.00 (0.92; 1.08)	0.94	1.02 (0.91; 1.15)	0.80	0.80
Post-dialysis weight (Δ kg)	0.07 (-0.87; 1.01)	0.89	-0.08 (-0.90; 0.74)	0.85	0.82
Anemia control	*Δ*	*p*	*Δ*	*p*	*p*
ESA resistance index (ratio*)	0.80 (0.65; 0.97)	0.03	1.25 (1.03; 1.52)	0.02	< 0.01

Effect estimates are presented with 95% confidence intervals

CRP: C-reactive protein; ESA: erythropoiesis-stimulating agent

*CRP and ESA resistance were modelled with a gamma distributed log link function. Hence, the (exponentiated) coefficients should be interpreted multiplicatively, i.e. as a ratio.

Table S5. Effect estimates per year in the propensity score matched nocturnal hemodialysis (NHD) and hemodialysis/hemodiafiltration (HD/HDF) cohorts, and difference between the propensity score matched NHD and HD/HDF cohorts (CRP 36 months, ESA resistance index 12 months, all other outcomes 48 months), adjusted for cause of end-stage renal disease**.

	NHD		HD/HDF		NHD vs HD/HDF
Hypertension control	Δ	*p*	Δ	*p*	*p*
Systolic blood pressure (Δ mmHg)	0.54 (-1.81; 2.90)	0.65	-1.09 (-3.14; 0.95)	0.30	0.31
Diastolic blood pressure (Δ mmHg)	-0.74 (-1.96; 0.48)	0.24	-1.04 (-2.03; -0.06)	0.04	0.71
Different antihypertensive agents (odds ratio < 2 types)	2.30 (1.15; 4.58)	0.02	0.94 (0.53; 1.67)	0.84	0.08
Phosphate control	Δ	*p*	Δ	*p*	*p*
Phosphate (Δ mmol/l)	-0.04 (-0.09; 0.02)	0.20	0.00 (-0.05; 0.05)	0.94	0.31
Different phosphate binding agents (odds ratio < 2 types)	1.85 (1.13; 3.04)	0.02	0.98 (0.66; 1.46)	0.93	0.08
Nutritional status / inflammation	Δ	*p*	Δ	*p*	*p*
Albumin (Δ g/l)	0.29 (-0.14; 0.73)	0.19	-0.45 (-0.85; 0.06)	0.03	0.01
CRP (ratio*)	0.97 (0.90; 1.05)	0.51	1.02 (0.91; 1.14)	0.77	0.55
Post-dialysis weight (Δ kg)	0.09 (-0.74; 0.93)	0.83	-0.14 (-0.89; 0.61)	0.71	0.68
Anemia control	Δ	*p*	Δ	*p*	*p*
ESA resistance index (ratio*)	0.76 (0.63; 0.92)	< 0.01	1.22 (1.00; 1.48)	0.05	< 0.001

Effect estimates are presented with 95% confidence intervals

CRP: C-reactive protein; ESA: erythropoiesis-stimulating agent

*CRP and ESA resistance were modelled with a gamma distributed log link function. Hence, the (exponentiated) coefficients should be interpreted multiplicatively, i.e. as a ratio.

**Hazard ratio for mortality in the NHD cohort adjusted for cause of end-stage renal disease: 0.65 (95% CI 0.28; 1.53, p=0.33).

Supplemental information

Variables used in the propensity score model

The following covariates were used in the propensity score model: age, sex, dialysis vintage, active transplant waitlist status, residual diuresis, history of cardiovascular disease, diabetes, smoking status, body mass index, blood pressure, number of antihypertensive agents, phosphate, number of phosphate binding agents, cholesterol, albumin, CRP, use of iron supplementation, ESA resistance, weekly dialysis hours, and central venous catheter as vascular access.

Complete (unmatched) NHD cohort from switch to follow-up

In the complete NHD cohort *(n=159)*, the 4-year course of clinical parameters was similar to the matched NHD cohort *(n=100)*. Improvements in two parameters in the complete cohort exceeded the matched cohort: phosphate decreased significantly (-0.05 [95% CI -0.08; -0.03] mmol / year, $p < 0.01$ and albumin increased significantly (0.35 [95% CI 0.16; 0.54] g/l/year, $p < 0.01$) compared with baseline, whereas they were not significantly different from baseline when the patients who could not be matched were left out.

Data on iron storage parameters, iron doses and residual function

Ferritin levels were median 226 (IQR 112–437) and 316 (IQR 182–604) µg/L in the NHD and HD/HDF cohorts at baseline, respectively. In the NHD cohort, ferritin levels increased significantly over time (ratio per year 1.23, 95% CI 1.12; 1.34, $p < 0.001$), while they did not change significantly in the HD/HDF cohort (ratio per year 0.99, 95% CI 0.93; 1.06, p=0.81). The increase in the NHD cohort was significantly different from the HD/HDF cohort (ratio of difference per year 1.24, 95% CI 1.11; 1.38, $p < 0.001$, supplemental figure 1). Transferrin saturation levels were mean 20 ±16 (median 13, IQR 8–25) and 28 ±13 (median 25, IQR 18–31) % in the NHD and HD/HDF cohorts at baseline, respectively. In both the NHD cohort (-0.28%/year, 95% CI -2.17; 1.60) and HD/HDF cohort (-0.77%/year, 95% CI -1.92; 0.38) transferrin saturation did not change significantly over time. There was no significant difference in change over time between the groups (difference 0.48%/year, 95% CI -1.73; 2.70, p=0.67, supplemental figure 2).

Iron doses in the matched NHD cohort were median 50 (IQR 25–100) mg/week at baseline, 50 (IQR 25–100) at 12 months, 50 (IQR 33–50) at 24 months, 50 (IQR 33–50) at 36 months and 50 (IQR 25–100) at 48 months.

As a *post-hoc* analysis, we adjusted for ferritin levels in the analysis of ESA resistance; the associations between NHD and ESA resistance over time remained numerically similar. ESA resistance tended to increase in the HD/HDF cohort (ratio per year 1.20, 95% CI 0.96; 1.49, p=0.11), and decreased significantly in the NHD cohort (ratio per year 0.69, 95% CI 0.55; 0.87, p < 0.01). This difference was significant (ratio per year 0.58, 95% CI 0.41; 0.81, p < 0.01).

Residual urine production was present (≥100 mL/24h) in 49% of the (matched) NHD cohort at baseline, and in 25% at 12 months, 20% at 24 months, 20% at 36 months and 9% at 48 months. This was similar to the complete NHD cohort (45% at baseline, 26% at 12 months, 23% at 24 months, 9% at 48 months).

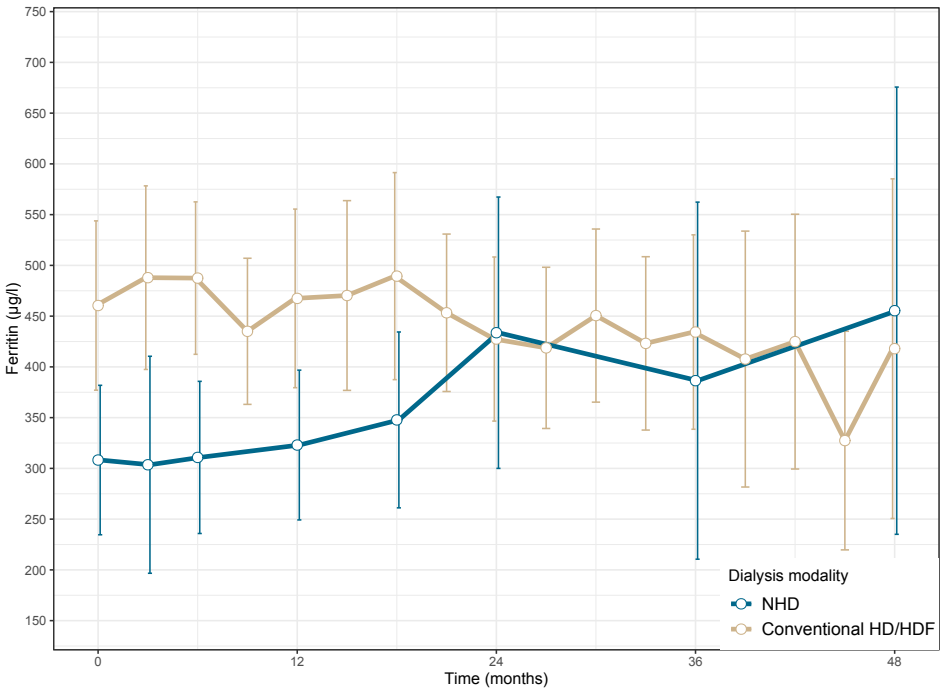

Figure S1. Ferritin levels in nocturnal hemodialysis versus hemodialysis/hemodiafiltration.

Ferritin (µg/L) in propensity score matched nocturnal hemodialysis (NHD, dark line) and hemodialysis/hemodiafiltration (HD/HDF, light line) patients over the course of 48 months. NHD compared to baseline $p < 0.001$; NHD vs HD/HDF $p < 0.001$. 95% confidence intervals are shown. Number of NHD / HD/HDF patients available for analysis at 0 months: 100/100; 12 months: 57/74; 24 months: 35/51; 36 months: 20/34; 48 months: 11/22.

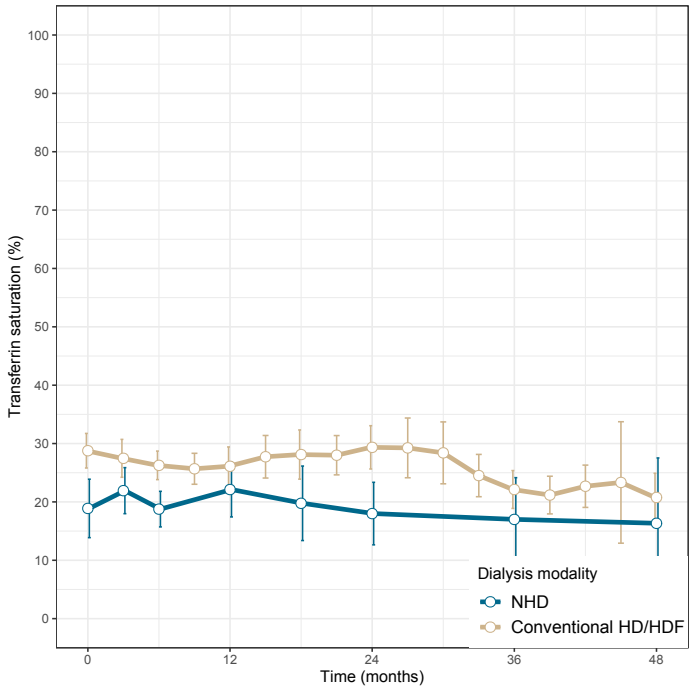

Figure S2. Transferrin saturation in nocturnal hemodialysis versus hemodialysis/hemodiafiltration.

Transferrin saturation (%) in propensity score matched nocturnal hemodialysis (NHD, dark line) and hemodialysis/hemodiafiltration (HD/HDF) patients, over the course of 48 months. NHD compared to baseline p=0.77; NHD vs HD/HDF p=0.67. 95% confidence intervals are shown. Number of NHD / HD/HDF patients available for analysis at 0 months: 44/100; 12 months: 26/74; 24 months: 17/51; 36 months: 9/34; 48 months: 6/22.

Chapter 8

Health-related quality of life compared between kidney transplantation and nocturnal hemodialysis

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Abstract

Background

Health-related quality of life (HRQOL) is an important outcome measure in patients with end-stage renal disease. HRQOL is assumed to improve with kidney transplantation and also with nocturnal hemodialysis compared to conventional hemodialysis. However, there is no evidence regarding HRQOL to support the optimal treatment choice for patients on nocturnal hemodialysis who hesitate opting for transplantation. We therefore compared HRQOL between patients who were treated with kidney transplantation or nocturnal hemodialysis for one year.

Methods

We assessed HQROL using the Kidney Disease Quality of Life–Short Form questionnaire in a cross-sectional sample of patients who were treated with kidney transplantation *(n=41)* or nocturnal hemodialysis *(n=31)* for one year. All patients on nocturnal hemodialysis were transplantation candidates. Using linear regression, we compared HRQOL between kidney transplantation and nocturnal hemodialysis, and adjusted for age, sex, dialysis duration, cardiovascular disease, and presence of residual urine production.

Results

At one year follow-up, mean age of the study population was 54 ±13 years, and median dialysis duration was 3.2 (IQR 2.1–5.0) years. Kidney transplantation was associated with significantly higher HRQOL on the domain "effects" compared to nocturnal hemodialysis (adjusted difference 12.0 points, 95% CI 3.9; 20.1). There were potentially clinically relevant differences between kidney transplantation and nocturnal hemodialysis on the domains "burden" (adjusted difference 11.1 points, 95% CI -2.6; 24.8), "social support" (adjusted difference 6.2, 95% CI -6.6; 19.1), and the physical composite score (adjusted difference 3.0, 95% CI -2.0; 8.1), but these were not significant.

Conclusions

After kidney transplantation, HRQOL is especially higher on the domain "effects of kidney disease" compared to nocturnal hemodialysis. This can be useful when counseling patients on nocturnal hemodialysis who may opt for transplantation.

Introduction

Health-related quality of life is an important indicator of well-being in patients with end-stage renal disease and is associated with survival and clinical outcomes[1-4]. Compared to the general population, patients with end-stage renal disease have severely diminished health-related quality of life, by some deemed even lower than in diseases such as congestive heart failure, chronic lung disease or cancer[5].

The preferred treatment for end-stage renal disease is kidney transplantation, which is associated with improved health-related quality of life and survival[6]. However, because of the limited availability of donor kidneys and because of transplant failure, many patients have to remain on dialysis.

An alternative to conventional dialysis modalities is frequent nocturnal hemodialysis. With this treatment, patients dialyze almost daily and twice as long (7–8 hours), generally at home. Thus, this treatment removes fluid more slowly and clears more solutes such as urea and phosphate[7]. Nocturnal hemodialysis may hence improve intermediate outcomes[8, 9] and possibly even survival, although mortality data remain inconsistent[10, 11]. By dialyzing at night, patients save time during the day, and nocturnal hemodialysis has thus been reported to improve health-related quality of life[12-14], to such an extent that some patients may even choose to forgo transplantation[15].

How clinicians should deal with this reluctance toward transplantation is unclear. Currently, there is no evidence to support the optimal treatment choice for these patients, particularly not regarding patient-reported outcome measures. To fill this gap, we compared health-related quality of life measured with the Kidney Disease Quality of Life—Short Form (KDQOL-SF) between kidney transplant recipients and transplantation-eligible patients treated with nocturnal hemodialysis.

Methods

Study population

We analyzed a cross-sectional cohort from the ongoing NOCTx study (NCT00950573), a prospective cohort study designed to compare progression of coronary artery calcification between kidney transplant recipients, patients on frequent nocturnal home hemodialysis, and patients on chronic peritoneal dialysis or conventional hemodialysis. Patients were eligible when aged between 18 and 75 years and were candidates for transplantation when on dialysis. All study participants gave written informed consent. NOCTx excluded patients with a life expectancy <3 months, pre-emptive transplantation, or non-adherence to dialysis regimens. NOCTx has been approved by the Medical Ethics Committee of the University Medical Center Utrecht and is conducted in accordance with the Declaration of Helsinki.

Between December 2009 and February 2016, NOCTx included 54 kidney transplant recipients and 39 patients on nocturnal hemodialysis who were referred for study participation to the University Medical Center of Utrecht, the Netherlands. For the present analyses, we included all kidney transplant recipients (*n=41*) and patients on nocturnal hemodialysis (*n=31*) who had one-year follow-up data. Most patients with a kidney transplant and on nocturnal hemodialysis entered NOCTx 2–3 months after switching to their respective treatment; thus, data from before switching were not available in these patients. We therefore analyzed data cross-sectionally after one year of treatment.

Treatment characteristics

Patients received treatment according to guidelines by the attending nephrologists. Kidney transplant recipients were treated in two tertiary centers, where standard immunosuppressant regimens consisted of a calcineurin inhibitor (tacrolimus), mycophenolate mofetil, and prednisone in tapering doses. Patients on nocturnal hemodialysis were trained and monitored in two dialysis centers that offered specialized training programs for nocturnal home hemodialysis. Patients dialyzed ≥ 4 x 8 hours per week at home, on a single needle, with a lower effective blood flow (150–220 mL/min), lower dialysate flow (300 mL/min), and a somewhat lower bicarbonate concentration compared to conventional hemodialysis, which was adjusted depending on laboratory results. Unfractionated heparin was used as anticoagulation.

Health-related quality of life

We assessed health-related quality of life with the validated KDQOL-SF version 1.2[16]. The KDQOL-SF consists of a general part and a disease-specific part. The general part, the Short Form with 36 questions (SF-36) version 1[17], consists of eight domains that can

be summarized in two scores. These summary scores are designed to reflect the general population in the United States when the means are 50 with a standard deviation of 10 points for physical functioning (physical composite score) and mental functioning (mental composite score)[18]. The composite scores were obtained from 12 questions in the SF-36 (PCS-12 and MCS-12)[1]. The disease-specific part of the KDQOL-SF consists of 44 kidney disease-targeted questions, grouped in 12 domains. We focused on the domains "symptoms of kidney disease", "effects of kidney disease", "burden of kidney disease", "cognitive function", "quality of social interaction", "sexual function", "sleep", "social support" and "overall health". We did not evaluate the domains "work status", "patient satisfaction" and "dialysis staff encouragement" in this study. The domains are scored from 0 to 100, with higher scores indicating better quality of life. Explanations of the disease-specific domains are available as Table 1 (adapted from Carmichael et al.[19]).

Table 1. Explanation of the Kidney Disease Quality of Life-Short Form (KDQOL-SF) kidney disease-specific domains.

Domains	Interpretation	
	Low score	High score
Symptoms of kidney disease	Extremely bothered by dialysis-related symptoms such as muscle cramps, pruritus, anorexia, and/or access problems	Not at all bothered
Effect of kidney disease on daily life	Extremely bothered by fluid and dietary restriction, by an inability to travel, and dependency on doctors	Not at all bothered
Burden of kidney disease	Extremely bothered by the time consumed by dialysis, its intrusiveness, and degree burden on family	Not at all bothered
Cognitive function	Affected all of the time by inability to concentrate, confused, with poor reaction time	Not at all affected
Quality of social interaction	Continual irritation and failure to get along with people with virtual isolation	No problems, socially interactive
Sexual function	Experiencing severe problems with enjoyment and arousal	No problems
Sleep	Very poor sleep with daytime somnolence	No problems with sleep
Social support	Very dissatisfied	Satisfied with level of social support
Overall health	Rates health as worst possible	Rates health as best possible

Adapted from Carmichael et al.[19]

Other variables

At time of questionnaire completion, study personnel recorded demographical and clinical parameters (pre-dialysis blood pressure and post-dialysis weight averaged from routine measurements during 3 hemodialysis sessions or 2 outpatient visits for kidney transplant recipients) and laboratory parameters (total calcium, phosphate, parathyroid hormone, total cholesterol, albumin, hemoglobin, and C-reactive protein) routinely measured at local treatment facilities. Study personnel assessed presence of comorbidities by chart review, and assessed residual urine production with the most recent 24h-urine collection, which we classified as present (≥100mL/24u) or absent. Smoking status, oral anticoagulant use, and educational level were self-reported.

We defined diabetes mellitus as use of oral anti-diabetic medication or insulin therapy, and cardiovascular disease as any history of angina, myocardial infarction, percutaneous coronary intervention, coronary artery bypass grafting, stroke, intermittent claudication, peripheral artery angioplasty or bypass grafting. We defined higher education as any tertiary education. We estimated glomerular filtration rate with the Chronic Kidney Disease-Epidemiology Collaboration equation 2009 for kidney transplant recipients.

Statistical Analyses

We reported data as number (proportion) for categorical data, mean ±standard deviation for normally distributed variables, and median (interquartile range [IQR]) for non-normally distributed variables. We presented patient characteristics and health-related quality of life by renal replacement therapy. We compared categorical data with chi-squared tests, normally distributed variables with t-tests, and non-normally distributed variables with Mann-Whitney-U tests.

We used multiple linear regression analyses to examine the associations between renal replacement therapy and health-related quality of life. We regarded 5-point differences clinically relevant in the disease-specific domains, and 3-point differences clinically relevant in the composite scores[17, 18]. We adjusted stepwise for potential confounders age (years), sex, educational level (high/low), dialysis duration (years), presence of diabetes mellitus, cardiovascular disease, and presence of residual urine production (≥100mL/24u or absent), and kept them in the model when coefficients changed >10%. In the final model, we adjusted for age, sex, dialysis duration, cardiovascular disease, and presence of residual urine production.

We reported regression coefficients with 95% confidence intervals (CI). We considered P-values ≤ 0.05 (two-tailed) statistically significant, did not attempt imputation for missing values, and performed all analyses with R 3.4.1[20].

Results

Study population

The mean age of the study population *(n=72)* was 54 ±13 years, 50 (69%) were male, median dialysis duration was 38 (IQR 25–60) months, and 17 (24%) had a history of cardiovascular disease. There were no significant differences in demographics or medical history between the kidney transplant recipients (*n=41*) and patients on nocturnal hemodialysis (*n=31*), but kidney transplant recipients had significantly lower phosphate levels and higher hemoglobin levels (Table 2). Kidney transplant recipients had an estimated glomerular filtration rate of 54.8 ±15.7 mL/min, while patients on nocturnal hemodialysis had median 0 (IQR 0–250) mL/day residual urine production. Patients on nocturnal hemodialysis dialyzed 38.3 ±7.2 hours per week in 4.8 ±0.8 sessions per week.

The current sample comprised 77% of all kidney transplant recipients and patients on nocturnal hemodialysis who entered NOCTx *(n=93)*. Seven kidney transplant recipients (3 were lost to follow-up, 2 withdrew consent, 2 died) and 7 patients on nocturnal hemodialysis (3 received a transplant, 2 withdrew consent, 1 was lost to follow-up, 1 died) did not complete follow-up at one year, while 6 kidney transplant recipients and 1 patient on nocturnal hemodialysis did not complete quality of life questionnaires at the one-year follow-up. Their mean age (*n=21*) was 49 ±14 years (P=0.15 versus study population), 12 (57%) were male (P=0.43 versus study population), median dialysis duration was 65 (IQR 42–84) months (P=0.03 versus study population), and 4 (19%) had a history of cardiovascular disease (P=0.89 versus study population). Kidney transplant recipients were not more likely to complete follow-up than patients on nocturnal hemodialysis (P=0.88).

Health-related quality of life at one year of treatment

The quality of life questionnaires were generally well-completed. In the following scales, one or more questionnaire items were missing resulting in a missing score: "sexual function" (5 respondents, 7%), SF-12 items (physical and mental composite scores; 2 respondents, 3%), "symptoms of kidney disease", "effects of kidney disease", "burden of kidney disease", and "overall health" (1 respondent each, 1%).

Table 2. Characteristics of the 72 kidney transplant recipients and patients on nocturnal hemodialysis at one year of follow-up.

	Kidney transplantation (n = 41)	Nocturnal hemodialysis (n = 31)	*P-value*
Demographics			
Age (yr)	54.0 ±13.8	53.9 ±12.5	0.97
Male (%)	31 (75)	19 (62)	0.29
Body mass index (kg/m²)	25.5 ±4.2	26.5 ±5.2	0.37
Systolic blood pressure (mmHg)	132 ±14	139 ±20	0.11
Diastolic blood pressure (mmHg)	80 ±10	75 ±12	0.12
Current smoker (%)	6 (15)	6 (19)	0.83
Oral anticoagulant use (%)	5 (13)	2 (7)	0.66
Higher education (%)	11 (28)	8 (26)	0.99
Medical history			
Dialysis duration (mo)	28 (24–58)	39 (28–66)	0.12
End-stage renal disease duration (mo)	28 (25–62)	39 (28–94)	0.15
Cause of end-stage renal disease (%)			0.23
Glomerulonephritis	9 (22)	11 (36)	
Interstitial nephritis	1 (2)	0 (0)	
Cystic kidney disease	14 (34)	5 (16)	
Renovascular	9 (22)	3 (10)	
Diabetes mellitus	1 (2)	2 (7)	
Other	3 (7)	5 (16)	
Unknown	4 (10)	5 (16)	
Comorbidities (%)			
Diabetes mellitus	3 (7)	4 (13)	0.70
Prior cardiovascular disease	7 (17)	10 (32)	0.22
Laboratory parameters			
Calcium (mmol/L)	2.41 ±0.10	2.37 ±0.20	0.30
Phosphate (mmol/L)	0.88 ±0.21	1.42 ±0.39	<0.001
Parathyroid hormone (pmol/L)	8.5 (6.4–12.0)	13.8 (7.6–22.8)	0.14
Cholesterol (mmol/L)	5.0 ±1.1	4.6 ±1.0	0.26
Albumin (g/L)	42.4 ±3.1	42.4 ±3.1	0.95
Hemoglobin (mmol/L)	8.9 ±1.0	7.0 ±0.8	<0.001
C-reactive protein (mg/L)	3.0 (2.0–8.3)	5.0 (3.0–10.0)	0.29

Results are presented as mean ±standard deviation, median (interquartile range), or number (proportion).

Overall, kidney transplant recipients had numerically higher scores on the kidney disease-specific domains of health-related quality of life and the physical composite score compared to patients on nocturnal hemodialysis (Figure 1). Kidney transplant recipients scored significantly higher on the domain "effects of kidney disease" compared to patients on nocturnal hemodialysis, both in crude and adjusted analyses (Table 3). There were no significant differences on the other kidney disease-specific domains or the composite scores in both crude and adjusted analyses. When adjusted for age, sex, dialysis duration, cardiovascular disease, and residual urine production, kidney transplant recipients had potentially clinically relevant higher scores on the domains "burden of kidney disease", "social support", and the physical composite score compared to nocturnal hemodialysis, but these differences were not significant.

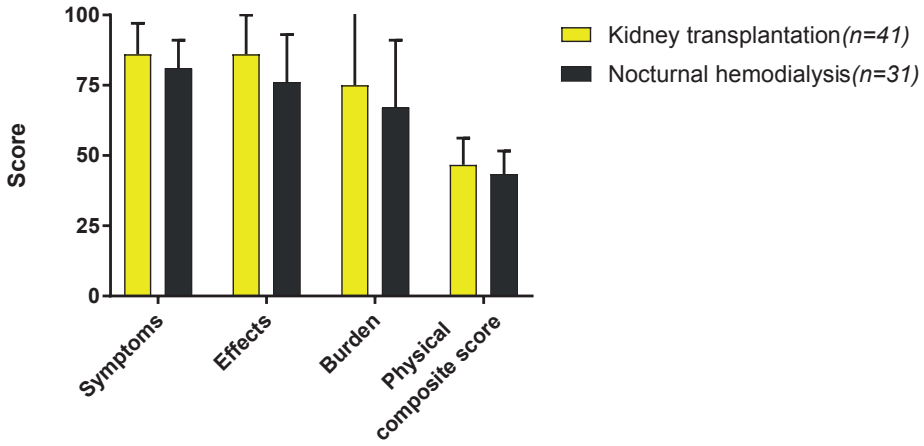

Figure 1. Disease-specific health-related quality of life scores and physical composite scores in the 72 kidney transplant recipients and patients on nocturnal hemodialysis. Mean health-related quality of life scores on the disease-specific domains "symptoms", "effects", "burden of kidney disease", and the physical composite scores as bar charts in the 72 kidney transplant recipients and patients on nocturnal hemodialysis. We presented 95% confidence intervals alongside the bars. Mean scores for kidney transplantation and nocturnal hemodialysis: "symptoms" 86 and 81; "effects" 86 and 76; "burden" 75 and 67; physical composite score 47 and 43 points, respectively.

Table 3. Health-related quality of life scores and differences in scores between the 72 kidney transplant recipients and patients on nocturnal hemodialysis at one year of follow-up.

	Kidney transplantation (n = 41)	Nocturnal hemodialysis (n = 31)	*Crude difference (95% CI)*	*Adjusted* difference (95% CI)*
Kidney disease-related quality of life				
Symptoms of kidney disease	86 ±11	81 ±10	-5.7 (-10.7; -0.7)	-4.6 (-10.6; 1.3)
Effects of kidney disease	86 ±14	76 ±17	-9.8 (-16.9; -2.6)	-12.0 (-20.1; -3.9)
Burden of kidney disease	75 ±27	67 ±24	-8.0 (-20.1; 4.1)	-11.1 (-24.8; 2.6)
Cognitive function	81 ±19	78 ±18	-2.5 (-11.3; 6.3)	-4.3 (-14.2; 5.6)
Quality of social interaction	79 ±15	77 ±14	-1.3 (-8.3; 5.8)	1.4 (-6.7; 9.5)
Sexual function	72 ±30	64 ±33	-7.8 (-23.1; 7.5)	-2.0 (-19.1; 15.0)
Sleep	66 ±23	63 ±16	-2.8 (-12.3; 6.8)	-3.3 (-14.5; 8.0)
Social support	87 ±21	82 ±25	-4.7 (-15.5; 6.0)	-6.2 (-19.1; 6.6)
Overall health	70 ±16	65 ±17	-4.3 (-12.3; 3.6)	-4.9 (-14.1; 4.3)
SF-12 composite scores				
Physical composite score	47 ±10	43 ±8	-3.4 (-7.7; 0.9)	-3.0 (-8.1; 2.0)
Mental composite score	51 ±10	52 ±11	0.6 (-4.2; 5.5)	1.2 (-4.4; 6.8)

Abbreviations: SF-12: short form-12 items. Scores are presented as mean ±standard deviation, and differences with 95% confidence intervals.

*Adjusted for age (years), sex (male/female), dialysis duration (years), cardiovascular disease, and presence of residual urine production (≥100mL/24u or absent).

Discussion

To our knowledge, this is the first study to compare health-related quality of life between kidney transplantation and nocturnal hemodialysis, demonstrating that kidney transplantation is associated with significantly higher quality of life on the domain "effects of kidney disease" compared to nocturnal hemodialysis. In addition, kidney transplant recipients have potentially clinically relevant higher quality of life on the domains "burden of kidney disease", "social support", and the physical composite score, although not significantly higher in this study. Together, these findings suggest that health-related quality of life is generally better after kidney transplantation than on treatment with nocturnal hemodialysis.

The differences in health-related quality of life are the most evident on the domain "effects of kidney disease". As this domain involves the restraints patients experience regarding their diet, ability to travel, and dependency on doctors, it is explainable that kidney transplant recipients score higher on this domain. After all, kidney transplant recipients are freer in terms of diet and travel than any patient on dialysis. Besides this domain, kidney transplant recipients have numerically higher adjusted scores on the domains "burden of kidney disease", "social support", and the physical composite score. Although not *statistically significant*, these differences may be *clinically relevant*[21]. The original KDQOL-SF manual reads that 5-point differences are clinically relevant regarding the disease-specific domains, and 3-point differences regarding the composite scores[17, 18], which has been adopted by others[22, 23]. Notably, a 3-point difference in the composite scores is associated with a mortality risk of approximately 6.0%[2, 3, 24]. Given the size and consistent direction of these differences, we consider them relevant, even though they do not reach statistical significance with the current sample size.

In our experience, some patients treated with nocturnal hemodialysis decline kidney transplantation and prefer to stay on treatment with nocturnal hemodialysis. The current findings suggest that kidney transplantation - in which quality of life is known to improve[25-27] - is a more favorable treatment option regarding health-related quality of life for transplantation-eligible patients on nocturnal hemodialysis, although individual outcomes may differ importantly.

For patients that are unlikely to receive a kidney transplant (e.g. HLA-sensitized patients), potential benefits of nocturnal hemodialysis remain relevant, such as an improvement of quality of life. Importantly, health-related quality of life has been shown to improve after conversion to nocturnal hemodialysis from conventional hemodialysis in several observational studies[12, 14] and on selected domains in a randomized trial[28]. This is despite

the fact that nocturnal hemodialysis is performed almost daily and requires considerable patient involvement. Notably, patients on nocturnal hemodialysis in our cohort have somewhat higher health-related quality of life scores compared to North-American cohorts[12, 13, 29], which may be because all patients were transplantation candidates in our study. Remarkably, nocturnal hemodialysis does not seem to deteriorate sleep quality: patients on nocturnal hemodialysis have similar scores on the domain "sleep" to kidney transplant recipients in our study.

The results of this study should be interpreted within the context of some limitations. First, our study is not powered to demonstrate significance of all potentially relevant differences in kidney disease-specific health-related quality of life domains. For example, we would have needed 161 patients per group to show significance of an 8-point difference (as currently found) in the disease-specific domain "burden of kidney disease". Second, the current data are cross-sectional after one year of treatment with kidney transplantation or nocturnal hemodialysis. A before/after comparison of health-related quality of life was not possible as patients were included in this study shortly after they had started treatment with either kidney transplantation or nocturnal hemodialysis. Third, we do not know the reasons why individual patients converted to nocturnal hemodialysis – there may have been patient selection. As noted in previous studies, healthier and more motivated patients may have been preferentially selected for nocturnal hemodialysis[30], which could influence health-related quality of life. Also, the current data are observational, although it should be noted that randomization to kidney transplantation would be unethical.

Our study has several strengths. First, questionnaire response rate in this study is high (91%) as compared to large studies on patients on hemodialysis[1, 2]. The responders' demographic characteristics are largely similar to that of non-responders; therefore, we consider our findings generalizable to patients on nocturnal hemodialysis who may opt for kidney transplantation. Second, we focus on kidney disease-specific domains of health-related quality of life alongside the physical and mental composite scores, which increases the ability to detect more specific differences in patients' well-being. Finally, this study has only included patients on nocturnal hemodialysis who were transplantation candidates; simultaneously, no kidney transplant recipients had been transplanted pre-emptively, i.e. all recipients had a history of dialysis treatment. Both of these inclusion criteria enable valid comparisons between the treatment groups.

In conclusion, health-related quality of life is higher after kidney transplantation especially on the domain "effects of kidney disease" compared to nocturnal hemodialysis. This can be useful when counseling patients on nocturnal hemodialysis who may opt for transplantation.

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Chapter 9

Survival of patients treated with extended-hours hemodialysis in Europe – an analysis of the European Renal Association-European Dialysis and Transplant Association Registry

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Abstract

Background

Previous US studies have indicated that hemodialysis with ≥6-hour sessions (extended-hours hemodialysis) may improve patient survival. However, patient characteristics and treatment practices vary between the US and Europe. We therefore investigated the effect of extended-hours hemodialysis three times weekly on survival compared with conventional hemodialysis among European patients.

Methods

We included patients who were treated with hemodialysis between 2010 and 2017 from 8 countries providing data to the ERA-EDTA Registry. Hemodialysis session duration and frequency were recorded once every year or at change of hemodialysis prescription and categorized into three groups: conventional hemodialysis (3x weekly, 3.5-4 hours/treatment), extended-hours hemodialysis (3x weekly, ≥6 hours/treatment), or other. In the primary analyses, we attributed death to the treatment at time of death, and in secondary analyses to extended-hours hemodialysis if ever initiated. We compared mortality risk for extended-hours hemodialysis to conventional hemodialysis with causal inference from marginal structural models, using Cox proportional hazards models weighted for the inverse probability of treatment and censoring and adjusted for potential confounders.

Results

From a total of 142,460 patients, 1,338 patients were ever treated with extended-hours hemodialysis (3x 7.1±0.8 hours/week) and 89,819 patients exclusively with conventional hemodialysis (3x 3.9 ±0.2 hours/week). Crude mortality rates were 6.0 and 13.5 per 100 person-years. In the primary analyses, patients treated with extended-hours hemodialysis had an adjusted hazard ratio of 0.73 (95% confidence interval 0.62 to 0.85) compared with patients treated with conventional hemodialysis. When we attributed all deaths to extended-hours hemodialysis after initiation, the hazard ratio for extended-hours hemodialysis was comparable to the primary analyses (0.80, 95% confidence interval 0.71 to 0.90).

Conclusions

Extended-hours hemodialysis is associated with better survival in European patients treated with hemodialysis three times weekly.

Introduction

Patients on dialysis have poor survival when compared with age-matched individuals from the general population[1-4]. Interestingly, various studies have shown lower mortality risks associated with hemodialysis treatment times of >4 hours compared with shorter treatment times in patients from the United States (US), Australia/New Zealand, and Europe[5-7]. This has prompted research into the effects of hemodialysis with session durations far beyond the conventional 3.5-4 hours, i.e. extended-hours hemodialysis, with ≥6-hour sessions.

Several previous studies have demonstrated survival benefits of extended-hours hemodialysis. Two observational studies found better survival among patients treated with frequent extended-hours hemodialysis (≥4 times weekly) compared with conventional hemodialysis[8, 9]. Yet, this finding could not be confirmed by another observational study[10], a randomized controlled trial that had a small sample size due to recruitment difficulties[11], and two post-trial observational studies[12, 13]. However, these studies did not distinguish between the effects of hemodialysis session duration and treatment frequency. Several observational studies investigating extended-hours hemodialysis three times weekly also demonstrated survival benefits compared with conventional hemodialysis three times weekly[14-17]. However, most of these studies were limited to the US. In general, US patients treated with hemodialysis more often have diabetes, have shorter hemodialysis session durations with higher blood flow rates, and less often use an arteriovenous fistula compared with European patients treated with hemodialysis[5, 18]. Thus far, no study has evaluated whether extended-hours hemodialysis three times weekly (with ≥6-hour sessions) affects survival in European patients.

We aimed to study the effect of extended-hours hemodialysis three times weekly on survival compared with conventional hemodialysis among European patients. To this end, we used data from the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Registry.

Methods

Study population

The study cohort consisted of prevalent patients who were treated with hemodialysis between 2010 and 2017, derived from the ERA-EDTA Registry. The ERA-EDTA Registry collects data on renal replacement therapy via national and regional renal registries in Europe on an annual basis. The core dataset includes date of birth, sex, primary kidney disease, date of start of renal replacement therapy, dialysis modality at dialysis initiation and during follow-up, and date and cause of death. In addition, several renal registries also provided additional clinical and biochemical data. For this study, we included patients aged ≥20 years at any time during follow-up from the following eight national and regional registries that provided data including hemodialysis session duration and frequency: Austria, Catalonia (Spain), France, and Scotland (United Kingdom) (2010 to 2017); the French-speaking part of Belgium, Finland, Norway, and Sweden (2010 to 2016). We excluded patients that were treated with hemodialysis for less than 120 days (four months) in total, as mortality risk is highest in this early period after starting hemodialysis[19]. All patients were followed until death or censoring (i.e. recovery of renal function, transfer to peritoneal dialysis, kidney transplantation, loss to follow-up, or end of administrative follow-up). End of administrative follow-up was December 31, 2016 for the French-speaking part of Belgium, Finland, Norway, and Sweden; and December 31, 2017 for Austria, Catalonia (Spain), France, and Scotland (United Kingdom).

Hemodialysis session duration and frequency

In the ERA-EDTA Registry, hemodialysis session duration and frequency were recorded once every year (Austria, the French-speaking part of Belgium, Catalonia (Spain), Finland, Norway, Sweden, and Scotland (United Kingdom)) or at every change in hemodialysis prescription (France). To investigate exclusively the association of extended hemodialysis session duration with survival, we categorized hemodialysis treatment into three groups: *1)* conventional hemodialysis (3x weekly, 3.5-4 hours/treatment), *2)* extended-hours hemodialysis (3x weekly, ≥6 hours/treatment), or *3)* other (not in any of the previous categories). We did not distinguish between hemodialysis, hemofiltration, and hemodiafiltration. One registry (Austria) only recorded hemodialysis session frequency for treatments ≥18 hours/week. For this registry, we therefore categorized hemodialysis treatment of 10.5-12 hours/week as conventional hemodialysis (assuming treatment 3x weekly) and any other treatment <18 hours/week as other.

Mortality

In the primary analyses, we attributed mortality to the last recorded hemodialysis treatment. If a patient's last event was "limited care / stopped treatment (without recovery of renal function)" we assumed that the patient died shortly thereafter. In secondary analyses, we attributed all deaths after initiation of extended-hours hemodialysis, to extended-hours hemodialysis. We calculated person-time by summing all time attributed to each treatment.

Other variables

We calculated age at the first record of hemodialysis session duration and frequency. Primary kidney disease was classified according to the coding system of the ERA-EDTA[20]. We grouped patients into eight classes of primary kidney disease: glomerulonephritis, pyelonephritis, polycystic kidney disease, diabetes, hypertension, renal vascular disease, miscellaneous, and unknown. We defined renal replacement therapy vintage as the time between the first day of renal replacement therapy and the first record of hemodialysis session duration and frequency. Dialysis vintage was defined as the time on renal replacement therapy minus the time with a functioning kidney transplant. Definitions of comorbidities are available as Appendix 1.

Statistical analyses

We reported normally distributed numerical variables as mean ± standard deviation (SD), non-normally distributed numerical variables as median with interquartile range (IQR), and categorical variables as number (percentage). We tabulated patient characteristics at time of their first record of hemodialysis session duration and frequency, stratified for patients exclusively treated with conventional hemodialysis, patients ever treated with extended-hours hemodialysis, and patients never treated with extended-hours hemodialysis but ever treated with other hemodialysis regimens.

We assessed the effect of extended-hours hemodialysis on survival with causal inference from marginal structural models, using hemodialysis treatment as time-varying exposure. Marginal structural models are a class of causal models that can be used to estimate the causal effect of a time-varying exposure or treatment[21]. They are a powerful method for confounding control in longitudinal study designs with time-varying information on exposure and outcome[22]. In this study, this means that we assessed the association of total time spent on extended-hours dialysis with survival, even if treatment modality had been changed before death. Marginal structural models use estimators weighted for the inverse probability of treatment (IPTW) and censoring (IPCW), which we calculated with multinomial logistic regression models including the variables age (years), sex, primary renal disease, country, previous kidney transplantation (yes/no), dialysis vintage

(years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy)[23]. Weights were truncated at the 2nd and 98th percentiles. We adjusted the IPTW- and IPCW-weighted Cox proportional hazard models for age (years), sex, primary renal disease, country, previous kidney transplantation (yes/no), dialysis vintage (years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy), with conventional hemodialysis as reference group. We also present results from unweighted, unadjusted Cox proportional hazard models with hemodialysis treatment as time-varying exposure. We present hazard ratios for extended-hours hemodialysis only, because of the heterogeneity of the other hemodialysis regimens and the ensuing limited interpretability.

The completeness of comorbidity data varied, ranging from 11% missing for diabetes to 22% missing for congestive heart failure. We therefore imputed missing comorbidity data using the R aregImpute function with 10 imputations with predictive mean matching. Variables in the imputation model included age at each record (years), age at the start of dialysis (years), sex, primary renal disease, previous kidney transplantation (yes/no), dialysis vintage (years), time on renal replacement therapy (years), time on transplantation (years), indicators of censoring, transplantation, and death at each record and at any time during follow-up, indicators of treatment at each record and during follow-up, and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy). We analyzed each of the imputed datasets separately and pooled the results according to Rubin's rules[24].

As patients from France constituted most of the study population (71% of all patients), we repeated all analyses excluding data from this registry. Furthermore, we repeated all analyses excluding patients with missing comorbidity data, excluding patients that were ever treated with home hemodialysis, and exclusively including incident patients treated with hemodialysis (renal replacement therapy vintage less than 180 days). Finally, we conducted a propensity-score matched analysis, matching patients ever treated with extended-hours hemodialysis with up to 20 patients exclusively treated with conventional hemodialysis using propensity scores as calculated for the IPTWs, within a 0.1 caliper.

We reported hazard ratios (HRs) with 95% confidence intervals (95% CI). Furthermore, we created Kaplan-Meier curves weighted for the product of IPTW and IPCW[25]. We considered P-values ≤0.05 (two-tailed) statistically significant and used R 3.5.1 (R Foundation Statistical Computing) for all analyses.

Results

Our cohort included a total of 142,640 prevalent patients from 8 European countries treated with hemodialysis between 2010 and 2017. Of them, 89,819 were exclusively treated with conventional hemodialysis, 1,338 were ever treated with extended-hours hemodialysis, and 51,483 were ever treated with other hemodialysis regimens but never with extended-hours hemodialysis (Table 1). Of note, the latter group included 109 patients ever treated with frequent extended-hours hemodialysis (≥4x weekly, ≥6 hours/treatment). These treatments were also classified as other hemodialysis regimens because of their limited occurrence. Compared with patients exclusively treated with conventional hemodialysis, patients ever treated with extended-hours hemodialysis were generally younger (mean 55 versus 67 years), had been on dialysis longer (median 1.7 versus 0.4 years), were more often treated at home (6% versus 0%), and more often had a previous kidney transplantation (26% versus 9%). Also, patients ever treated with extended-hours hemodialysis less often had comorbidities, such as diabetes and cardiovascular diseases, and less often had diabetes or hypertension as primary renal disease.

Table 1. Patient characteristics of patients exclusively treated with conventional hemodialysis, patients ever treated with extended-hours hemodialysis, and patients treated with other hemodialysis regimens, at time of first record of hemodialysis session duration and frequency.

	Exclusively treated with conventional hemodialysis (n = 89,819)	Ever treated with extended-hours hemodialysis (n = 1,338)	Ever treated with other hemodialysis regimen* (n = 51,483)
Age, years (mean ±SD)	67 ±15	55 ±15	67 ±16
Male (%)	56,360 (63%)	993 (74%)	32,229 (63%)
Primary renal disease (%)			
• Glomerulonephritis	12,500 (14%)	363 (27%)	7,244 (14%)
• Pyelonephritis	5,080 (6%)	103 (8%)	3,078 (6%)
• Polycystic kidney disease	5,675 (6%)	129 (10%)	3,295 (6%)
• Diabetes	20,871 (23%)	235 (18%)	11,870 (23%)
• Hypertension	18,675 (21%)	158 (12%)	10,334 (20%)
• Renal vascular disease	2,053 (2%)	15 (1%)	1,113 (2%)
• Miscellaneous	13,672 (15%)	243 (18%)	8,233 (16%)
• Unknown	11,293 (13%)	92 (7%)	6,316 (12%)
Dialysis vintage, years (median, IQR)	0.4 (0.0–2.4)	1.7 (0.0–5.3)	0.5 (0.0–2.6)
Time on RRT, years (median, IQR)	0.4 (0.0–3.0)	2.4 (0.0–10.5)	0.5 (0.0–3.2)
Previous transplantation (%)	8,348 (9%)	347 (26%)	5,321 (10%)

Table 1. Continued.

	Exclusively treated with conventional hemodialysis (n = 89,819)	Ever treated with extended-hours hemodialysis (n = 1,338)	Ever treated with other hemodialysis regimen* (n = 51,483)
Ever treated with home hemodialysis (%)	233 (0%)	77 (6%)	1407 (3%)
Comorbidities**			
• Diabetes mellitus (%)	35,201 (39%)	390 (29%)	20,323 (40%)
• Cerebrovascular disease (%)	10,015 (11%)	63 (5%)	5,934 (12%)
• Ischemic heart disease (%)	21,323 (24%)	197 (15%)	12,246 (24%)
• Peripheral vascular disease (%)	17,015 (19%)	150 (11%)	9,205 (18%)
• Congestive heart failure (%)	19,582 (22%)	165 (12%)	11,752 (23%)
• Malignancy (%)	9,475 (11%)	106 (8%)	5,540 (11%)
Country (%)			
• Austria	5,792 (6%)	28 (2%)	3,313 (6%)
• Belgium, French-speaking	3,166 (4%)	112 (8%)	1,048 (2%)
• Catalonia (Spain)	6,769 (8%)	95 (7%)	3,076 (6%)
• Finland	1,443 (2%)	22 (2%)	2,009 (4%)
• France	66,552 (74%)	910 (68%)	33,418 (65%)
• Norway	1,329 (2%)	0 (0%)	1,725 (3%)
• Sweden	2,409 (3%)	73 (5%)	4,599 (9%)
• Scotland (United Kingdom)	2,359 (3%)	98 (7%)	2,295 (5%)

Abbreviations: SD, standard deviation; IQR, interquartile range; RRT, renal replacement therapy.
*Patients in this category were never treated with extended-hours hemodialysis but were ever treated with other hemodialysis regimens.
**Comorbidity data were incomplete and missing comorbidity data were therefore imputed. Percentage missing comorbidity data: diabetes mellitus, 11%; cerebrovascular disease, 13%, ischemic heart disease, 13%; peripheral vascular disease, 13%; congestive heart failure, 22%; malignancy, 13%.

Hemodialysis session duration and frequency

Conventional hemodialysis was delivered 3 times weekly for on average 3.9 ±0.2 hours per session (11.8 ±0.5 hours weekly), whereas extended-hours hemodialysis was delivered 3 times weekly for 7.1 ±0.8 hours per session (21.4 ±2.5 hours weekly) (Figure 1). Other hemodialysis regimens were delivered on average 3.0 ±0.8 times weekly (range 1 to 7 times weekly) for a mean of 3.9 ±0.8 hours per session (range 0.1 to 10 hours per session), amounting to a mean of 11.6 ±3.5 hours weekly (range 0.1 to 56 hours weekly).

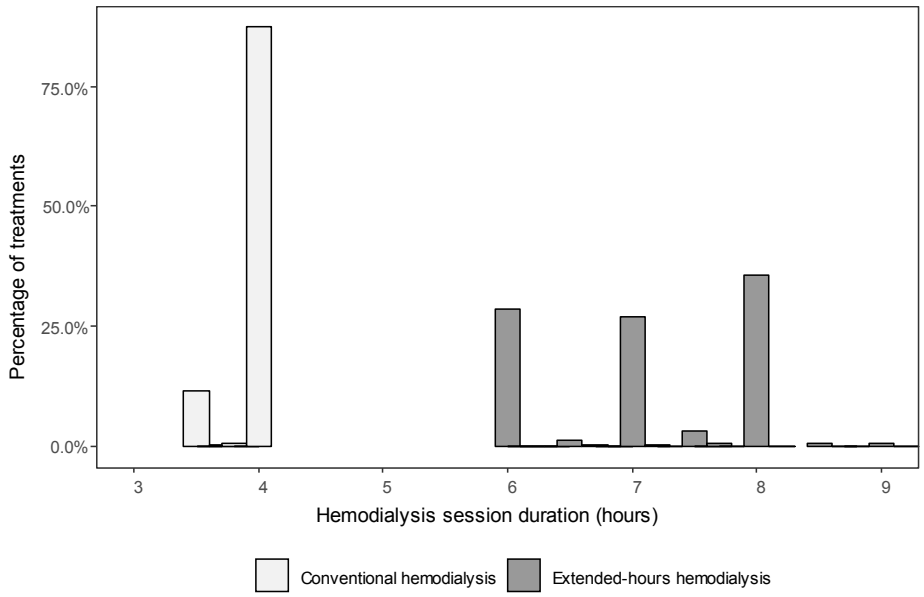

Figure 1. Distribution of hemodialysis session duration in treatment with conventional hemodialysis (grey bars, 383,204 records) and extended-hours hemodialysis (black bars, 3,111 records), as percentage of the total number of records per treatment group.

Mortality

A total of 41,892 patients died while treated with conventional hemodialysis (13.5/100 person-years), whereas 179 patients died while treated with extended-hours hemodialysis treatment (6.0/100 person-years), and 16,421 patients while treated with other hemodialysis regimens (16.1/100 person-years). A total of 17,963 patients received a kidney transplant while treated with conventional hemodialysis (5.8/100 person-years), whereas 308 received a kidney transplant while treated with extended-hours hemodialysis (10.4/100 person-years), and 5,977 while on other hemodialysis regimens (5.9/100 person-years). From the total of 1,338 patients ever treated with extended-hours hemodialysis, 468 (35%) transferred from extended-hours hemodialysis to conventional hemodialysis or other hemodialysis regimens, after an average recorded treatment duration of 2.5 ±1.8 years. The mortality rates (with deaths attributed to extended-hours hemodialysis after initiation) were similar for patients that initiated extended-hours hemodialysis but transferred from extended-hours hemodialysis to conventional hemodialysis or other hemodialysis regimens (7.5/100 person-years), and for patients that initiated extended-hours hemodialysis and did not transfer (7.5/100 person-years).

In the primary analyses, we attributed death to the treatment at time of death. In an ordinary unweighted, unadjusted Cox proportional hazard model, extended-hours hemodialysis was associated with a mortality HR of 0.41 (95% CI 0.36 to 0.48) compared with conventional hemodialysis. Using marginal structural models adjusted for case-mix factors and treatment history, we found that patients treated with extended-hours hemodialysis had a mortality HR of 0.73 (95% CI 0.62 to 0.85) compared with patients treated with conventional hemodialysis (Figure 2 and Table 2).

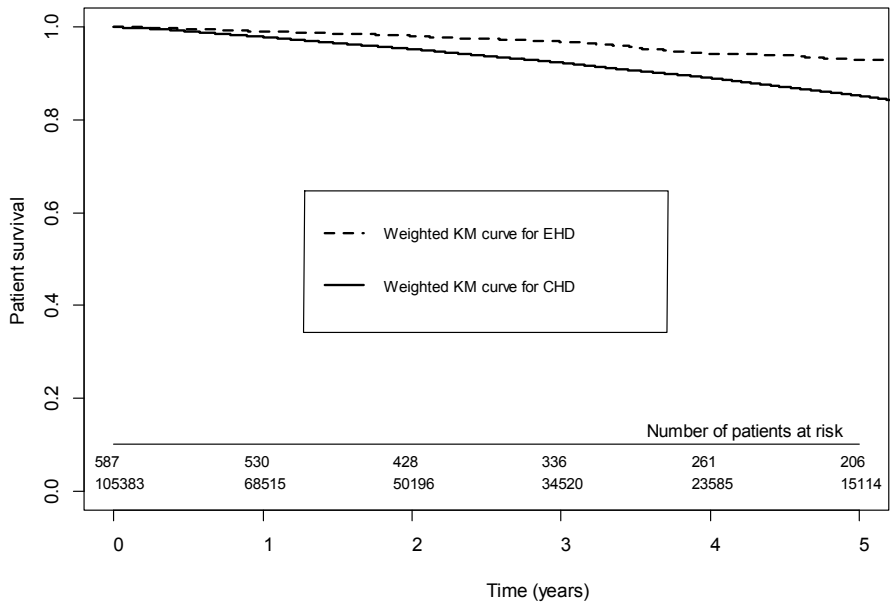

Figure 2. Weighted Kaplan-Meier (KM) curves for extended-hours hemodialysis (EHD, dashed line) and conventional hemodialysis (CHD, solid line), weighted for the inverse probability of treatment and censoring. We show the number of patients at risk of death per year at the bottom of the graph for extended-hours hemodialysis (top row) and conventional hemodialysis (bottom row).

In the secondary analyses, we attributed all deaths that occurred after a patient had ever been treated with extended-hours hemodialysis to extended-hours hemodialysis, regardless of the treatment at time of death. In the unweighted, unadjusted Cox proportional hazard model, extended-hours hemodialysis was associated with a mortality HR of 0.50 (95% CI 0.45 to 0.56) compared with conventional hemodialysis. Using marginal structural models adjusted for case-mix factors and treatment history, we found that patients treated with extended-hours hemodialysis had a mortality HR of 0.80 (95% CI 0.71 to 0.90) compared with patients treated with conventional hemodialysis.

Table 2. Mortality risk in extended-hours hemodialysis compared with conventional hemodialysis in prevalent patients treated with hemodialysis in 8 European countries between 2010 and 2017 (*n=142,640*).

	Number of deaths	Person-years	Mortality rate*	Adjusted hazard ratio (95% CI)**
Death attributed to treatment at time of death (primary analysis)				
• Conventional hemodialysis	41,892	310,712	13.5	1.0 (*ref.*)
• Extended-hours hemodialysis	179	2,966	6.0	0.73 (0.62 to 0.85)
All deaths attributed to extended-hours hemodialysis after initiation (secondary analysis)				
• Conventional hemodialysis	41,832	310,275	13.5	1.0 (*ref.*)
• Extended-hours hemodialysis	303	4,039	7.5	0.80 (0.71 to 0.90)

*Per 100 person-years.

**Hazard ratio from marginal structural model with Cox regression, adjusted for age (years), sex, primary renal disease, country, previous kidney transplantation (yes/no), dialysis vintage (years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy). Reference group is conventional hemodialysis.

Sensitivity analyses excluding data from France confirmed our finding that patients treated with extended-hours hemodialysis had a lower mortality risk compared with patients treated with conventional hemodialysis (Table S1). Although the effect estimates for extended-hours hemodialysis were even larger when excluding data from France, characteristics of patients treated with conventional hemodialysis or extended-hours hemodialysis were largely similar in the French registry and other registries (data not shown). Furthermore, we repeated the primary and secondary analyses adjusted for renal replacement therapy vintage instead of dialysis vintage, which yielded numerically similar results (data not shown). Our finding of lower mortality risk with extended-hours hemodialysis was also confirmed by sensitivity analyses excluding patients with missing comorbidity data (Table S2), sensitivity analyses excluding patients ever treated with home hemodialysis (Table S3), sensitivity analyses exclusively including incident patients treated with hemodialysis (Table S4), and a propensity-score matched analysis (Table S5 and S6).

Discussion

In this cohort of prevalent patients from eight European countries treated with hemodialysis three times weekly, we found that patients treated with extended-hours hemodialysis had a significantly lower mortality risk than patients treated with conventional hemodialysis. Our findings extend those of previous studies in patients from the US to European patients treated with hemodialysis and support the hypothesis that extended-hours hemodialysis improves survival.

To our knowledge, this is the first study to investigate the effect of extended-hours hemodialysis (3x weekly, ≥6 hours/treatment) on survival in a European population. Previously, the Frequent Hemodialysis Network (FHN) Nocturnal trial investigated the effect of frequent nocturnal hemodialysis (6x weekly, ≥6 hours/treatment) on survival, which was non-significant[11]. However, this trial was underpowered due to recruitment difficulties. Other trials with different endpoints include a Canadian trial and the ACTIVE dialysis trial. The Canadian trial randomized patients to in-center or home hemodialysis three times weekly and nocturnal hemodialysis six times weekly and found reductions in left ventricular mass, systolic blood pressure, serum phosphate, and parathyroid hormone[26]. The ACTIVE dialysis trial randomized patients to hemodialysis 12-18 hours/week and ≥24 hours/week and found reductions in serum phosphate and prescriptions of antihypertensives, but no improvements in quality of life[27]. A recent analysis of the ACTIVE dialysis trial showed no survival benefit for extended-hours hemodialysis 4 years post-intervention. However, the ACTIVE trial was not powered to detect mortality differences, and only very few patients in the extended-hours hemodialysis group were still treated for ≥24 hours/week post-intervention[13]. Moreover, these three trials were not designed to assess the impact of hemodialysis session duration separately from frequency. These limitations emphasize the need for thorough analyses of hemodialysis cohorts.

Several observational studies have investigated the effect of extended-hours hemodialysis three times weekly among hemodialysis cohorts in the US[14-16] and Turkey[17], with greatly varying effect estimates, from 10% to 72%. Our data show an about 20% lower mortality risk for extended-hours hemodialysis three times weekly compared with conventional hemodialysis. This variation may be due to differences in study design, analytical approach, health care systems, and population. For example, our estimates are smaller than a large recent study that used a similar analytical approach but investigated patients from the US[16]. Nevertheless, estimates of this study and previous studies are all in the same direction, indicating a robust effect of extended-hours hemodialysis.

The reduced mortality in extended-hours hemodialysis may develop through several mechanisms. Several studies have shown associations of extended-hours hemodialysis with lower phosphate levels[28]. High phosphate levels are associated with vascular calcification and arterial stiffness[29], which are risk factors for left ventricular dysfunction and heart failure among patients with chronic kidney disease[30], and thus mortality. Indeed, some studies including one randomized trial have suggested reductions of left ventricular mass with extended-hours hemodialysis[17, 26, 31-33], although this was not confirmed in two other randomized trials[11, 27]. Furthermore, high phosphate levels are associated with endothelial dysfunction, which may predispose to atherosclerosis[34]. Extended-hours hemodialysis is also associated with higher removal of fibroblast growth factor 23[35], which is associated with left ventricular hypertrophy and mortality[36]. On the other hand, extended-hours hemodialysis allows for a substantially lower ultrafiltration rate than conventional hemodialysis. High ultrafiltration rates in conventional hemodialysis are associated with myocardial stunning, which over time results in impaired segmental and global left ventricular function[37]. Slower fluid removal is associated with lower blood pressure and reduced mortality[38], an association that was also observed by Charra et al., who were one of the first to propagate extended-hours hemodialysis reporting low mortality rates among patients treated with extended-hours hemodialysis[39].

Our findings support the hypothesis that extending hemodialysis hours during treatment three times weekly improves survival. Still, all prior studies[14-17], including ours, have been observational, which cannot prove causation. Importantly, patients opting for extended-hours hemodialysis are generally a selected subgroup. Although there may be various reasons for initiating extended-hours hemodialysis, such as pregnancy or calciphylaxis, often patients initiating extended-hours hemodialysis are younger, treated at home, and healthier, more motivated, and more likely to adhere to treatment. Indeed, patients treated with extended-hours hemodialysis had higher transplantation rates and less often had comorbidities such as cerebrovascular disease and ischemic heart disease compared with patients treated with conventional hemodialysis. We therefore accounted for censoring and comorbidities in our analyses, which yielded much more conservative estimates compared with unadjusted, unweighted analyses. Nevertheless, unmeasured confounders may have led to improved survival independent of the treatment, such as fitness for which transplantation eligibility could be a proxy. Although randomized controlled trials could overcome this issue, a previous trial that randomly assigned patients to frequent nocturnal hemodialysis failed to recruit sufficient patients[11], as did another trial that randomly assigned patients to hemodialysis and peritoneal dialysis[40]. This indicates that patients are generally reluctant to be randomized to treatments such as dialysis

modalities, which have a tremendous impact on daily life. It is therefore questionable whether an adequately powered randomized controlled trial investigating the effect of extended-hours hemodialysis on mortality will take place.

In this study, we investigated the effect of extended-hours hemodialysis separate from treatment frequency. Two observational studies investigating more frequent (≥4x weekly) extended-hours hemodialysis compared with conventional hemodialysis reported larger mortality risk reductions compared with our study (45% and 66%)[8, 9]. However, these estimates are not directly comparable to ours due to differences in population and study design. Moreover, frequent hemodialysis increases the risk of vascular access complications[41]. Therefore, further study into the added value of frequent treatment in extended-hours hemodialysis would be useful.

The major strength of this study is that by using data from population-based national and regional registries contributing to the ERA-EDTA Registry, our cohort covered all adult patients treated with hemodialysis in the respective countries and regions, thus representing a large, unselected population. We therefore believe our results are generalizable to a broad population of European patients treated with hemodialysis. Also, we used marginal structural models for causal inference, which are sophisticated statistical methods to account for the propensity of healthier patients to survive to transfer to extended-hours hemodialysis. Nevertheless, our findings should also be viewed within the context of certain limitations. The ERA-EDTA Registry depends on data provided by national and regional registries, which did not include hemodialysis session duration and frequency in many registries. In addition, we studied prevalent patients treated with hemodialysis, which may have introduced survivor bias. Furthermore, data on reasons for transfer to extended-hours hemodialysis were not available. This may have included a full-time job or other daily activities that are accompanied by more favorable outcomes, introducing potential indication bias. Other limitations include unavailable data on clinical information including vascular access type, type of dialysis membrane, ultrafiltration rate, actual amount of delivered hemodialysis, and transplantation eligibility. Also, we did not distinguish between hemodialysis and hemodiafiltration, which could have led to lower estimates due to potentially more frequent hemodiafiltration in conventional regimens, which may improve survival[42]. Nevertheless, every observational study is limited by potentially unmeasured confounders and selection bias.

In conclusion, European patients treated with extended-hours hemodialysis three times weekly have a lower mortality risk compared with patients treated with conventional hemodialysis. This indicates that extending hemodialysis hours to ≥6 hours during treatment three times weekly may improve survival. Further studies could investigate the added value of frequent treatment in extended-hours hemodialysis.

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Supplementary material

Appendix 1. Definitions of comorbidities in the European Renal Association – European Dialysis and Transplant Association Registry.

Comorbidity	Definition
Diabetes mellitus	Metabolic disorder characterized by chronic hyperglycemia
Cerebrovascular disease	Any history of a cerebrovascular event or transient ischemic attack, cerebrovascular accident, or carotid surgery
Ischemic heart disease	Clinical diagnosis of angina or myocardial infarction, evidence of coronary artery disease on imaging or electrocardiography, or history of coronary artery bypass grafting
Peripheral vascular disease	Clinical history of claudication, evidence of peripheral vascular disease on imaging, or amputation or bypass graft for ischemic disease *(Austria: any peripheral vascular disease including aortic aneurysm, diffuse vessel sclerosis, or documented stenosis of vessels; Finland: symptomatic peripheral vascular disease, vascular surgery because of peripheral vascular disease, or amputation)*
Congestive heart failure	Prior diagnosis of congestive heart failure, a history of pulmonary edema, or a history of hospitalization for congestive heart failure within the past 12 months *(Austria: Congestive heart-failure with ejection fraction <30%; Finland: chronic clinical heart failure)*
Malignancy	Any history of malignancy except non-melanoma skin cancer *(Austria and Spain, Catalonia: any history of malignancy)*

Non-standard definitions per registry are described in italics between brackets. Adapted from Ceretta et al.[42]

Table S1. Mortality risk in extended-hours hemodialysis compared with conventional hemodialysis excluding data from the French registry* (*n=41,760*).

	Number of deaths	Person-years	Mortality rate**	Adjusted hazard ratio (95% CI)***
Death attributed to treatment at time of death				
• Conventional hemodialysis	11,175	67,501	16.6	1.0 (*ref.*)
• Extended-hours hemodialysis	48	816	5.9	0.55 (0.42 to 0.73)
All deaths attributed to extended-hours hemodialysis after initiation				
• Conventional hemodialysis	11,155	67,376	16.6	1.0 (*ref.*)
• Extended-hours hemodialysis	91	1,163	7.8	0.62 (0.51 to 0.76)

*Data from the following registries: Austria, the French-speaking part of Belgium, Catalonia (Spain), Finland, Norway, Sweden, and Scotland (United Kingdom).

**Per 100 person-years.

***Hazard ratio from marginal structural model with Cox regression, adjusted for age (years), sex, primary renal disease, country, previous kidney transplantation (yes/no), dialysis vintage (years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy). Reference group is conventional hemodialysis.

Table S2. Mortality risk in extended-hours hemodialysis compared with conventional hemodialysis excluding patients with missing comorbidity data (*n=107,027*).

	Number of deaths	Person-years	Mortality rate*	Adjusted hazard ratio (95% CI)**
Death attributed to treatment at time of death				
• Conventional hemodialysis	31,181	246,211	12.7	1.0 (*ref.*)
• Extended-hours hemodialysis	135	2,136	6.3	0.82 (0.68 to 0.98)
All deaths attributed to extended-hours hemodialysis after initiation				
• Conventional hemodialysis	31,147	245,888	12.7	1.0 (*ref.*)
• Extended-hours hemodialysis	208	2,849	7.3	0.84 (0.72 to 0.97)

*Per 100 person-years.

**Hazard ratio from marginal structural model with Cox regression, adjusted for age (years), sex, primary renal disease, country, previous kidney transplantation (yes/no), dialysis vintage (years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy). Reference group is conventional hemodialysis.

Table S3. Mortality risk in extended-hours hemodialysis compared with conventional hemodialysis excluding patients ever treated at home (*n=140,923*).

	Number of deaths	Person-years	Mortality rate*	Adjusted hazard ratio (95% CI)**
Death attributed to treatment at time of death				
• Conventional hemodialysis	41,800	308,712	13.5	1.0 (*ref.*)
• Extended-hours hemodialysis	174	2,798	6.2	0.74 (0.63 to 0.87)
All deaths attributed to extended-hours hemodialysis after initiation				
Conventional hemodialysis	41,743	308,295	13.5	1.0 (*ref.*)
Extended-hours hemodialysis	293	3,744	7.8	0.81 (0.72 to 0.92)

*Per 100 person-years.

**Hazard ratio from marginal structural model with Cox regression, adjusted for age (years), sex, primary renal disease, country, previous kidney transplantation (yes/no), dialysis vintage (years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy). Reference group is conventional hemodialysis.

Table S4. Mortality risk in extended-hours hemodialysis compared with conventional hemodialysis exclusively among incident patients treated with hemodialysis (*n=72,650*).

	Number of deaths	Person-years	Mortality rate*	Adjusted hazard ratio (95% CI)**
Death attributed to treatment at time of death				
• Conventional hemodialysis	18,005	152,765	11.8	1.0 (*ref.*)
• Extended-hours hemodialysis	46	693	6.6	0.76 (0.56 to 1.02)
All deaths attributed to extended-hours hemodialysis after initiation				
• Conventional hemodialysis	17,994	152,632	11.8	1.0 (*ref.*)
• Extended-hours hemodialysis	68	946	7.2	0.72 (0.57 to 0.92)

*Per 100 person-years.

**Hazard ratio from marginal structural model with Cox regression, adjusted for age (years), sex, primary renal disease, country, dialysis vintage (years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy). Reference group is conventional hemodialysis.

Table S5. Patient characteristics of patients ever treated with extended-hours hemodialysis propensity score-matched to patients exclusively treated with conventional hemodialysis.

	Exclusively treated with conventional hemodialysis (n = 24,046)	Ever treated with extended-hours hemodialysis (n = 1,336)	*Standardized mean difference*
Age, years (mean ±SD)	56 ±16	55 ±15	0.09
Male (%)	17,506 (73%)	991 (74%)	0.03
Primary renal disease (%)			0.08
• Glomerulonephritis	5,934 (25%)	362 (27%)	
• Pyelonephritis	1,705 (7%)	103 (8%)	
• Polycystic kidney disease	2,495 (10%)	129 (10%)	
• Diabetes	4,606 (19%)	235 (18%)	
• Hypertension	3,074 (13%)	158 (12%)	
• Renal vascular disease	279 (1%)	15 (1%)	
• Miscellaneous	4,187 (17%)	242 (18%)	
• Unknown	1,766 (7%)	92 (7%)	
Dialysis vintage, years (median, IQR)	1.0 (0.0–4.1)	1.7 (0.0–5.3)	0.17
Previous transplantation (%)	5,108 (21%)	345 (26%)	0.11
Comorbidities*			
• Diabetes mellitus (%)	7,496 (31%)	390 (29%)	0.04
• Cerebrovascular disease (%)	1,168 (5%)	62 (5%)	0.01
• Ischemic heart disease (%)	3,667 (15%)	195 (15%)	0.02
• Peripheral vascular disease (%)	2,714 (11%)	146 (11%)	0.01
• Congestive heart failure (%)	3,113 (13%)	166 (12%)	0.02
• Malignancy (%)	2,041 (9%)	109 (8%)	0.01
Country (%)			0.08
• Austria	518 (2%)	28 (2%)	
• Belgium, French-speaking	1,764 (7%)	112 (8%)	
• Catalonia (Spain)	1,806 (8%)	95 (7%)	
• Finland	426 (2%)	22 (2%)	
• France	16,981 (71%)	910 (68%)	
• Norway	0 (0%)	0 (0%)	
• Sweden	1,195 (3%)	73 (5%)	
• Scotland (United Kingdom)	1,354 (6%)	96 (7%)	

Abbreviations: SD, standard deviation; IQR, interquartile range.

*Comorbidity data were incomplete and missing comorbidity data were therefore imputed. Percentage missing comorbidity data: diabetes mellitus, 11%; cerebrovascular disease, 13%, ischemic heart disease, 13%; peripheral vascular disease, 13%; congestive heart failure, 22%; malignancy, 13%.

Table S6. Mortality risk in extended-hours hemodialysis compared with conventional hemodialysis in a propensity-score matched cohort* (*n=25,348*).

	Number of deaths	Person-years	Mortality rate**	Hazard ratio (95% CI)
Death attributed to treatment at time of death				
• Conventional hemodialysis	5,293	59,977	8.8	1.0 (*ref.*)
• Extended-hours hemodialysis	179	2,966	6.0	0.64 (0.55 to 0.74)
All deaths attributed to extended-hours hemodialysis after initiation				
• Conventional hemodialysis	5,224	59,541	8.8	1.0 (*ref.*)
• Extended-hours hemodialysis	303	4,039	7.5	0.79 (0.65 to 0.96)

*Patients ever treated with extended-hours hemodialysis were matched to up to 20 patients exclusively treated with conventional hemodialysis with propensity scores, logistically regressed on age (years), sex, primary renal disease, country, previous kidney transplantation (yes/no), dialysis vintage (years), and comorbidities (diabetes, cerebrovascular disease, ischemic heart disease, peripheral vascular disease, congestive heart failure, and malignancy).

**Per 100 person-years.

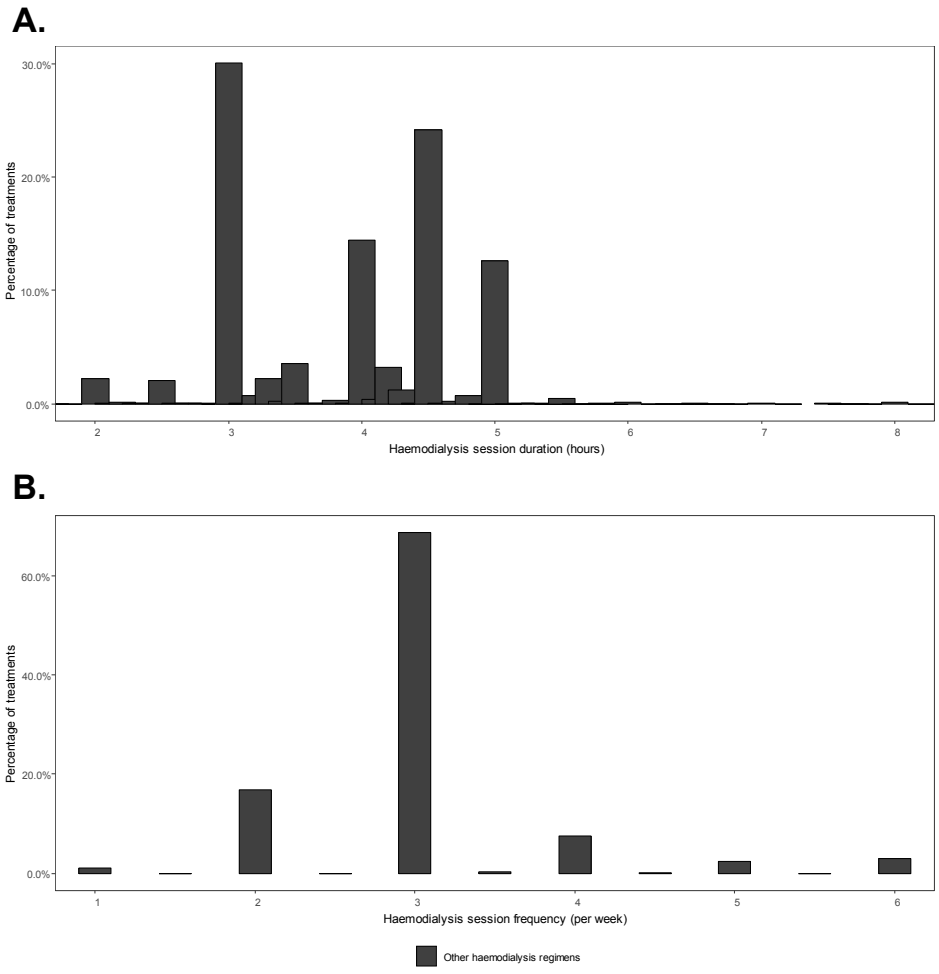

Figure S1. Distribution of hemodialysis session duration (panel A) and hemodialysis session frequency (panel B) in treatment with conventional hemodialysis (grey bars, 117,521 records), as percentage of the total number of records in this treatment group.

Chapter 10

Summary and general discussion

Summary and general discussion

Among patients on dialysis, five-year survival rates are as low as 42%[1]. Most of these deaths are due to cardiovascular disease[2], which is strongly associated with vascular calcification[3]. An important role is played by the disturbed mineral metabolism in end-stage kidney disease, which also affects the bone[4]. As a result, patients on dialysis are at high risk of skeletal fractures as well[5].

Several aspects and consequences of this disturbed mineral metabolism are poorly understood. Furthermore, a better understanding is needed of how different renal replacement therapies impact the vessels and the bone. This may help to identify optimal renal replacement therapies and areas where improvement of current treatment is desirable. In this thesis, we therefore investigated whether renal replacement therapies that evidently improve serum abnormalities of mineral metabolism, i.e. nocturnal hemodialysis (≥4x 8 hours weekly) and kidney transplantation[6, 7], have beneficial effects on the vessels and the bone. The keystone of this thesis is the NOCTx study. In this prospective study, we investigated the progression of coronary artery calcification in patients treated with nocturnal hemodialysis, conventional hemodialysis, kidney transplantation, and peritoneal dialysis. Additionally, the NOCTx study collected data on vertebral fractures and quality of life.

What have we learned from this thesis about the impact of end-stage kidney disease on the vessels and the bone, and how this is influenced by renal replacement therapies such as nocturnal hemodialysis and kidney transplantation? In this chapter, we summarize our findings and discuss these questions.

Is vascular calcification influenced by type of renal replacement therapy?

Vascular calcification in end-stage kidney disease is often of a different nature than in the general population. Patients with end-stage kidney disease do not only develop vascular calcification in the intimal layer, but also develop vascular calcification in the medial layer of the vessel wall. This is thought to be due to elevated phosphate and calcium levels, which are often present because of the disturbed mineral metabolism in end-stage kidney disease[8, 9]. In addition, this propensity for vascular calcification may be amplified in patients with end-stage kidney disease by deficiencies of inhibitors of vascular calcification, such as vitamin K. This vitamin is needed for the activation of calcification inhibitor Matrix Gla Protein[10]. Because of the potential impact of nocturnal hemodialysis and kidney transplantation on serum phosphate and vitamin K status, we hypothesized that these therapies might mitigate vascular calcification.

Our aim was to investigate the impact of different renal replacement therapies on vascular calcification. We, therefore, first systematically reviewed the literature on progression of vascular calcification in the coronary arteries comparing patients treated with different renal replacement therapies (*Chapter 2*). We focused on the coronary arteries, because vascular calcification is commonly measured at this site. In the general population, coronary artery calcification and its progression have been shown to predict coronary artery disease[11-13], and coronary artery calcification is also associated with cardiovascular disease risk in patients with chronic kidney disease[14].

Our systematic review showed that coronary artery calcification is common and progresses rapidly in patients treated with any of the studied renal replacement therapies. However, methodological issues precluded direct comparisons of progression of coronary artery calcification among renal replacement therapies based on the literature. Coronary artery calcification is measured with calcium scores, which have a mathematical distribution that cannot be transformed to a normal (Gaussian) distribution due to the skewness and the fact that many people have a calcium score of zero. It was, therefore, not possible to pool results from previous studies to compare progression of coronary artery calcification among renal replacement therapies in a meta-analysis.

We, therefore, conducted the NOCTx study. In this study, we analyzed progression of vascular calcification in the coronary arteries using two approaches that take into account the skewed distribution of calcium scores with many zeros[15, 16]. Contrary to other previously employed methods, our approaches allow to numerically quantify progression and to adjust for confounders. Our analyses showed that conventional hemodialysis was not associated with more progression of coronary artery calcification compared to peritoneal dialysis (*Chapter 4*). Furthermore, we found that conventional hemodialysis was not associated with more progression of coronary artery calcification compared to nocturnal hemodialysis (*Chapter 3*). Also, we did not find that kidney transplantation was associated with significantly less progression of coronary artery calcification compared to conventional hemodialysis (*Chapter 3*).

At the same time, data from the NOCTx study indicate better vitamin K status in kidney transplant recipients (*Chapter 5*). Furthermore, these data indicate that the use of phosphate binders such as sevelamer is associated with more severe vitamin K deficiency among dialysis patients (*Chapter 5*). Thus, even though nocturnal hemodialysis and kidney transplantation could have a favorable impact on serum phosphate and vitamin K status, we did not find that these therapies were associated with less progression of coronary artery calcification compared to conventional hemodialysis.

Does vascular calcification matter, and how?

Our findings regarding the progression of coronary artery calcification in different renal replacement therapies are contrary to our expectations. An interpretation of our findings could be that vascular calcification progresses regardless of the type of renal replacement therapy. This contrasts with the prevailing paradigm that kidney transplant recipients have lower cardiovascular morbidity and mortality in part due to mitigating effects of kidney transplantation. Rather, based on our findings, one might say that kidney transplant recipients have lower cardiovascular morbidity and mortality *despite* progressive vascular calcification. This raises the question whether vascular calcification is harmful in itself.

This question is not new and concerns both intimal and medial calcification. Some have proposed that from an evolutionary perspective, calcification constitutes an adaptive response to perceived threats, such as by encapsulating helminths, abscesses, and foreign bodies[17]. Hence, intimal calcification may be seen as a maladaptive response to inflammatory lipoprotein particles, which may resemble bacterial pathogens[18]. It has also been suggested that intimal calcification may reinforce atherosclerotic plaque[19], although this is still a matter of debate[20]. Studies using intravascular ultrasound have shown that patients with acute coronary syndrome have more and smaller calcium deposits (termed spotty calcification), compared to patients with stable angina, who have larger, continuous calcium deposits[21, 22]. Moreover, data from meta-analyses show that statin use does not slow down or reduce vascular calcification despite moderation of coronary stenosis[23]. This indicates that intimal calcification may not be the culprit of atherosclerotic disease, even though it may reflect atherosclerotic burden and, therefore, correlates well with cardiovascular events[24]. An increase in the amount of intimal calcification may, therefore, not necessarily reflect increased atherosclerosis, but instead also a process of vascular healing.

Similar to intimal calcification, medial calcification may be an adaptive response to damage to the vascular media. Indeed, medial calcification can be induced by widely varying noxious stimuli, ranging from mineral metabolism disturbances in chronic kidney disease to aging and diabetes[25]. There is an ongoing debate whether medial calcification is harmful in itself. A proposed mechanism by which medial calcification could lead to cardiovascular disease is by increasing vascular stiffness and pulse pressure, leading to left ventricular hypertrophy and ultimately heart failure, arrhythmia, and death[26]. However, some argue that medial calcification is merely a harmless, secondary phenomenon to vascular damage, pointing out that there are important discrepancies between the effects of several drugs on vascular calcification and mortality in patients with end-stage kidney disease[27]. For example, several trials have demonstrated that drugs such as

cinacalcet or sevelamer may slow down progression of coronary artery calcification in patients on dialysis[28, 29] but lack benefit on mortality[29, 30]. Nevertheless, this alone cannot prove that medial calcification is not harmful. Clinically, we cannot distinguish between intimal and medial calcification, and the coronary arteries of patients with end-stage kidney disease may contain both intimal and medial calcification. Even more, what is regarded as medial calcification even concerns two separate entities, i.e. calcification of the internal elastic lamina and of the vascular smooth muscle cells, although they might have similar pathophysiological consequences[31]. In any case, we cannot attribute changes in clinical measures of vascular calcification (such as coronary artery calcification) to a specific subtype of vascular calcification. Without histological studies that validate the location within the vessel wall of vascular calcification in several clinical measures of vascular calcification, it will remain difficult to determine the clinical consequences of the subtypes of vascular calcification in epidemiological studies.

Another interpretation of our findings is that kidney transplantation does in fact reduce progression of vascular calcification, but that we were somehow unable to detect this. For one, this could be due to limited sample size. However, it may also be a consequence of the vascular bed in which vascular calcification was determined. The anatomical distribution of vascular calcification in the vessel wall may differ according to the measured vascular bed. Based on its effects on serum abnormalities of mineral metabolism, one would primarily expect an effect of kidney transplantation on medial calcification. However, it is sometimes suggested that the coronary arteries are relatively resistant to medial calcification, implying that mostly intimal calcification is measured there[25]. There has been one post-mortem study, which found relatively limited medial calcification in the coronary arteries of patients with end-stage kidney disease[32]. Nevertheless, patients in this study were selected for having known significant coronary artery disease and were therefore likely to have predominantly intimal calcification. Thus, it is unclear to which extent coronary artery calcification is medial and whether its progression reflects the effects of renal replacement therapies, such as nocturnal hemodialysis or kidney transplantation.

End-stage kidney disease and the bone: what should we measure?

Patients with end-stage kidney disease have brittle bones. Using data from the NOCTx study, we have shown that vertebral fractures, the most common type of fragility fracture, occur frequently in patients with end-stage kidney disease (*Chapter 6*), which is over twice as frequent as in the general population. This high fracture risk results from a disturbed bone metabolism, also known as renal osteodystrophy, which comprises a spectrum of loss of bone mass due to high bone turnover, and microstructural abnormalities due to suppressed bone turnover[33].

In the general population, high-risk patients for fracture can be identified by measuring bone mineral density. The recently updated Kidney Disease: Improving Global Outcomes (KDIGO) guidelines also suggest that fracture risk can be assessed by measuring bone mineral density in patients with chronic kidney disease[34]. However, we found that vertebral trabecular bone mineral density as measured with computed tomography is not associated with vertebral fracture risk (*Chapter 6*). Furthermore, our findings indicate that bone mineral density testing is redundant in patients on dialysis. The clinical purpose of bone mineral density is to identify high-risk patients for fracture, but with the vertebral fracture risk we report in a relatively healthy and young population (34% prevalence among patients on dialysis eligible for transplantation), patients on dialysis can all be regarded as high-risk patients for fracture and rather may benefit from radiographic screening for vertebral fractures.

It remains difficult to monitor effects of disease and treatment on the bone in patients with end-stage kidney disease. This involves measuring bone turnover, for which clinicians mostly rely on parathyroid hormone, the main driver of bone turnover. There appears to be an optimal parathyroid hormone range where the bone is the least disturbed in end-stage kidney disease. Indeed, we demonstrated a U-shaped association of parathyroid hormone with vertebral fracture (*Chapter 6*). However, there is much inter-person variation in this optimal parathyroid hormone range. This is because of skeletal parathyroid hormone resistance[35] and also biological inactivity of parathyroid hormone due to oxidation[36]. Thus, at similar measured parathyroid hormone values, two patients may have vastly different bone turnover statuses. Recently, an assay has been developed that can specifically measure biologically active, non-oxidized parathyroid hormone[37]. Further studies will have to determine the added value of non-oxidized parathyroid hormone in monitoring bone turnover in patients with end-stage kidney disease.

Renal osteodystrophy and vascular calcification: a bone-vascular axis?

Disturbances of bone metabolism often go hand in hand with vascular calcification, both in the general population[38] and in end-stage kidney disease[39]. Among patients with end-stage kidney disease, some studies have shown associations of vascular calcification with low bone volume[40-42], whereas others have reported associations of vascular calcification with low bone turnover[42-44]. Some authors have proposed the existence of a bone-vascular axis[39]. Indeed, regulatory factors involved in renal osteodystrophy are often also involved in vascular calcification. These include the receptor activator of NF-κB (RANK)/RANKL/osteoprotegerin (OPG) signaling pathway[45], inflammation[46], bone morphogenic protein 7[47], and vitamin K deficiency[48, 49]. It is poorly understood whether and how the bone and the vascular wall affect each other: vascular disease might compromise the blood flow to the bone, hormones secreted by bone cells may act on the vasculature, or both vascular

and bone disease may simply share common factors. This underlines the importance to consider both the vasculature and the bone, and their potential interplay in future research into chronic kidney disease – mineral bone disorder.

Quality of life and survival in end-stage kidney disease

Patients with end-stage kidney disease have poor quality of life, even worse than most other chronic diseases[50]. Several factors contribute to this poor quality of life: physical symptoms due to uremia and dialysis treatment[51], time constraints due to therapy regimens, rigorous dietary and fluid restrictions, and one of the highest pill burdens in any chronic disease state[52]. Switching to nocturnal hemodialysis may alleviate some of these constraints.

Using data from a historic cohort, we have shown that patients on nocturnal hemodialysis (≥21 hours/week) need significantly less phosphate binders compared to patients that are treated 3x 4 hours weekly (*Chapter 7*). Phosphate binders make up about half of the daily pill burden of patients on dialysis and negatively impact quality of life[52]. Phosphate binder reduction may therefore be an important aspect of how nocturnal hemodialysis improves quality of life. Furthermore, nocturnal hemodialysis significantly liberates dietary intake, with few if any restrictions on salt, water, potassium, phosphate, and protein intake[53]. Moreover, nocturnal hemodialysis significantly reduces recovery time after hemodialysis[54]. All of these changes contribute to improved quality of life when patients switch to nocturnal hemodialysis[55-57].

Longer hemodialysis sessions, such as in nocturnal hemodialysis, may also improve survival compared to conventional hemodialysis because of smaller plasma dialysate electrolyte gradients, less dramatic volume shifts and less sympathetic hyperactivity, which has been hypothesized to reduce the risk of sudden cardiac death[58]. Using data from the European Renal Association – European Dialysis and Transplant Association (ERA-EDTA) Registry we have shown that in hemodialysis 3x weekly, extended-hours hemodialysis (≥6 hours/treatment) is associated with an about 20% reduced mortality risk compared to conventional hemodialysis (3.5-4 hours/treatment) in European patients (*Chapter 9*). This was the first study to investigate the effect of extended-hours hemodialysis (≥6 hours/treatment) in European patients. Our estimates were smaller than those from a large recent study that investigated patients from the US using a similar analytical approach[59], and other previous studies into the effect of extended-hours hemodialysis on mortality in treatment 3x weekly[59-62] or more ≥4x weekly[63-66], which had all been limited to the US. In general, patients from the US treated with hemodialysis more often have diabetes, have shorter hemodialysis session durations with higher blood flow rates, and less often use an arteriovenous fistula compared to European patients. Patients from the US treated

with conventional hemodialysis may, therefore, have lower survival in general. Only one randomized trial studied the effect of frequent nocturnal hemodialysis on mortality, which was negative[66]. However, this study had an exceptionally low mortality rate in the control group and was underpowered.

Even though nocturnal hemodialysis may improve quality of life and mortality, kidney transplantation appears to be the most favorable type of renal replacement therapy. Using data from the NOCTx study, we have demonstrated that kidney transplantation is associated with the highest quality of life, even when compared to nocturnal hemodialysis (*Chapter 8*). This may be useful information when counseling patients on nocturnal hemodialysis who may opt for kidney transplantation. Furthermore, survival seems to be superior after kidney transplantation. A retrospective matched cohort study reported similar survival rates in American deceased-donor transplant recipients and Canadian patients on nocturnal hemodialysis[67], whereas a similar study in Canadian transplant recipients and Canadian patients on nocturnal hemodialysis found superior survival for deceased-donor transplant recipients compared to patients on nocturnal hemodialysis[68].

Future perspectives

In this thesis, we shed more light on the impact of end-stage kidney disease and the influence of different renal replacement therapies on the vessels, the bone, and quality of life and mortality. What are the gaps of knowledge that remain? And what kind of future research could help the field move forward?

The two main remaining questions regarding vascular calcification are *1)* what exactly we measure when studying coronary artery calcification, and *2)* what the clinical consequences of vascular calcification are. On the one hand, we do not know to what extent coronary artery calcification is intimal or medial, when measured with computed tomography in patients with end-stage kidney disease. The findings presented in this thesis raise the question whether coronary artery calcification is the appropriate measure to examine effects of nocturnal hemodialysis or kidney transplantation on vascular calcification. This limitation could be overcome in future research by measuring medial calcification specifically. This could help to study effects of treatments affecting mineral metabolism more accurately. One approach could be to use a recently developed computed tomography scoring method to distinguish intimal and medial calcification in the intracranial internal carotid artery[69]. It uses features that can be assessed visually, such as circularity, thickness, and morphology of calcification, to assess the dominant localization of vascular calcification (intimal or medial). This method may be investigated further for application in other vascular beds with computed tomography/histology correlation studies of vascular calcification. Another approach could be to measure vascular

calcification in vascular beds that are known to develop limited intimal calcification and thus likely contain predominantly medial calcification. These may include arteries in lower extremities[70], upper extremities[71], breasts[72], and intracranial internal carotid artery[73].

On the other hand, future research should also focus on the question whether vascular calcification is harmful in itself. Possible angles for such research could be to investigate whether presence and/or extent of medial calcification is associated with specific types of cardiovascular disease, such as peripheral artery disease or stroke. Possibly, we could get insights in pathophysiological consequences of medial calcification from certain rare monogenetic disorders. One such disease is pseudoxanthoma elasticum, an autosomal recessive disorder caused by mutations in the *ABCC6* gene. This gene defect results in low levels of inorganic pyrophosphate, a key inhibitor of calcification[74], causing among other things ectopic mineralization of the vascular media[75]. Patients with pseudoxanthoma elasticum have an increased risk of peripheral artery disease, vascular dementia, and ischemic stroke[76], suggesting that these entities may be consequences of medial calcification. Furthermore, a recent trial among patients with pseudoxanthoma elasticum demonstrated that progression of vascular calcification can be slowed down by administration of synthetic stable pyrophosphate (etidronate, one of the first bisphosphonates)[77]. Bisphosphonates are also used as treatment for calcification in other rare monogenetic disorders, such as generalized arterial calcification of infancy[78] and basal ganglia calcifications[79]. In uremic rats, subcutaneous and intraperitoneal administration of pyrophosphate has also been shown to slow down vascular calcification[80]. These promising results warrant studies into the effects of pyrophosphate on vascular (medial) calcification in patients with chronic or end-stage kidney disease. Such studies would however have to address potential adverse effects of pyrophosphate on bone metabolism, given the traditional concerns regarding possible worsening of low bone turnover, osteomalacia, or mixed uremic osteodystrophy due to bisphosphonates in patients with end-stage kidney disease[81].

This thesis also demonstrates that patients with end-stage kidney disease have a high risk of vertebral fracture. What is lacking, however, are non-invasive tests to monitor effects of disease and treatment in the bone. Currently, only bone biopsies can provide comprehensive information about bone architecture, turnover, mineralization, and volume, but they are invasive and expensive. A potential alternative may be ^{18}F-NaF PET/CT. This imaging technique can be used to quantify osteoblast activity[82] and thus may aid in the diagnosis of adynamic bone disease in end-stage kidney disease[83, 84]. As of 2019, several studies investigating the correlation of ^{18}F-NaF PET/CT with findings on bone biopsies are underway (a Dutch study in kidney transplant recipients, Amsterdam University Medical Center; a Danish study in patients with chronic kidney disease stage 5, NCT03716128; and a Finnish study in patients on hemodialysis, NCT02967042).

Finally, although quality of life and survival are superior after kidney transplantation, this thesis shows that longer hemodialysis sessions in nocturnal hemodialysis may also improve certain aspects of quality of life and survival. We, therefore, believe that nocturnal hemodialysis can provide an optimal dialysis therapy, and that we need to be able to identify patients that could benefit from nocturnal hemodialysis in clinical practice. Currently, our group is setting up a nation-wide registry of home and conventional hemodialysis, either at daytime or nocturnal (DOMESTICO). This study aims to identify patient characteristics associated with successful nocturnal hemodialysis treatment, to identify barriers to (nocturnal) home hemodialysis, and to implement best practices in the counseling process for renal replacement therapy choice. Ultimately, these and other future studies aim to work towards a decision-making process in the predialysis phase in which nocturnal hemodialysis can be delivered to all patients that may benefit.

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Appendix

Nederlandse samenvatting
Dankwoord
Curriculum Vitae

Appendix

Nederlandse samenvatting

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In Nederland hebben ruim 17,000 mensen eindstadium nierfalen. Dit houdt in dat hun eigen nieren zo slecht werken, dat ze niet kunnen overleven zonder een behandeling die de nierfunctie overneemt. Zo'n behandeling kan gebeuren met een transplantatienier of met dialyse. Grofweg kunnen we twee vormen van dialyse onderscheiden: peritoneale dialyse, waarbij het buikvlies (peritoneum) als membraan wordt gebruikt om afvalstoffen en overtollig vocht te verwijderen; en hemodialyse, waarbij het bloed gefilterd wordt door een machine. Hemodialyse vindt meestal 3x in de week gedurende 4 uur plaats in een dialysecentrum, maar kan ook vaker en langer gedaan worden, zoals bij nachtelijke hemodialyse. Dat kan dan ook thuis.

Helaas krijgen mensen met eindstadium nierfalen veel bijkomende lichamelijke ziekten en hebben ze een hoge sterftekans. De belangrijkste oorzaak van de hoge sterftekans is hart- en vaatziekten, wat bij meer dan de helft van deze mensen de doodsoorzaak is. We denken dat hier een belangrijke rol gespeeld wordt door de verstoorde mineraalhuishouding die optreedt bij eindstadium nierfalen. Als gevolg daarvan krijgen mensen met eindstadium nierfalen namelijk uitgebreide vaatverkalking, wat gepaard gaat met het optreden van hart- en vaatziekten. Daarnaast beïnvloedt deze verstoorde mineraalhuishouding de botten. Mensen met eindstadium nierfalen hebben daardoor ook een verhoogd risico op botbreuken. We hebben echter niet van alle aspecten en gevolgen van de verstoorde mineraalhuishouding in eindstadium nierfalen een goed beeld. Bovendien kan de verstoorde mineraalhuishouding beïnvloed worden door de vorm van nierfunctievervangende behandeling, zoals nachtelijke hemodialyse, maar is daar maar weinig over bekend.

Het doel van dit proefschrift is daarom het onderzoeken van de invloed van de vorm van nierfunctievervangende behandeling op verscheidene aspecten en gevolgen van de verstoorde mineraalhuishouding in eindstadium nierfalen. Hierbij hebben we gekeken naar de vaten, het bot, en kwaliteit van leven en overleving. Een belangrijk deel van dit proefschrift wordt gevormd door het NOCTx-onderzoek, een prospectief cohortonderzoek waarin we gedurende drie jaar mensen hebben gevolgd die nachtelijke hemodialyse gingen doen (≥4x in de week 8 uur), mensen die gewone hemodialyse deden, mensen die een niertransplantatie kregen, en mensen die peritoneale dialyse deden.

Deel I: eindstadium nierfalen en de vaten

De hoge sterftekans bij mensen met eindstadium nierfalen hangt sterk samen met vaatverkalking. Bij mensen met eindstadium nierfalen treedt vaatverkalking vaak op en neemt het snel toe onder invloed van de verstoorde mineraalhuishouding, in het

bijzonder verhoogde fosfaatspiegels. Deze fosfaatspiegels kunnen sterk verlaagd worden door bepaalde vormen van nierfunctievervangende behandeling, zoals door nachtelijke hemodialyse of niertransplantatie. Daarom zou de vorm van nierfunctievervangende behandeling vaatverkalking kunnen beïnvloeden.

In **Deel I** van dit proefschrift hebben we de invloed van vorm van nierfunctievervangende behandeling op vaatverkalking onderzocht. In de meeste onderzoeken wordt vaatverkalking bekeken in de kransslagaders rond het hart, wat wij daarom ook gedaan hebben. In *Hoofdstuk 2* hebben we stelselmatig alle tot nog toe verschenen onderzoeken op een rij gezet over toename van vaatverkalking in de kransslagaders bij mensen met eindstadium nierfalen. Verscheidene onderzoeken laten inderdaad toenemende vaatverkalking zien bij verschillende vormen van nierfunctievervanging, al konden we de resultaten niet samenvoegen om de verschillende vormen van nierfunctievervanging onderling te vergelijken. Dat kwam doordat er teveel verschillen waren in de analysemethoden gebruikt in de verschillende onderzoeken. De hoeveelheid vaatverkalking is namelijk lastig te analyseren vanwege de rekenkundige verdeling ervan, waardoor er tot nog toe geen gangbare standaardmethode was.

In het NOCTx-onderzoek hebben we daarom de toename van vaatverkalking in de kransslagaders vergeleken tussen vier vormen van nierfunctievervangende behandeling. Bij onze analyses hebben we een methode gebruikt die rekening houdt met de rekenkundige verdeling van de hoeveelheid vaatverkalking. In *Hoofdstuk 4* beschrijven we dat mensen die peritoneale dialyse doen niet minder vaatverkalking krijgen dan mensen die gewone hemodialyse doen. In *Hoofdstuk 3* laten we vervolgens zien dat mensen die nachtelijke hemodialyse doen of een niertransplantatie hebben gekregen ook niet minder vaatverkalking krijgen dan mensen die gewone hemodialyse doen. In *Hoofdstuk 5* hebben we dan het vitamine-K-tekort in de vaatwand bestudeerd. Vitamine K is nodig voor het activeren van een belangrijke remmer van vaatverkalking, het matrix-Gla-eiwit. We laten zien dat mensen die een niertransplantatie hebben gekregen veel minder vitamine-K-tekort hebben dan mensen die nog dialyseren, terwijl mensen die dialyseren en fosfaatbinders als sevelameer gebruiken meer vitamine-K-tekort hebben. Dus, ook al hebben nachtelijke hemodialyse en niertransplantatie een gunstige invloed op fosfaatspiegels en vermoedelijk op vitamine-K-tekort, kunnen we niet vinden dat deze behandelvormen gepaard gaan met minder toename van vaatverkalking van de kransslagaders dan gewone hemodialyse.

We hadden niet verwacht dat nachtelijke hemodialyse en niertransplantatie niet gepaard gaan met minder toename van vaatverkalking van de kransslagaders dan gewone hemodialyse. Dat past ook niet bij de heersende gedachte dat niertransplantatie-

ontvangers minder hart- en vaatziekten hebben doordat ze minder vaatverkalking krijgen dan mensen die blijven dialyseren. In plaats daarvan zou je nu juist misschien zeggen dat niertransplantatie-ontvangers minder hart- en vaatziekten hebben *ondanks* toenemende vaatverkalking. Dit roept de vraag op of vaatverkalking zelf dan wel schadelijk is. Niettemin zou het ook kunnen dat nachtelijke hemodialyse of niertransplantatie wel vaatverkalking beïnvloeden, maar dat wij dit niet hebben kunnen meten. Mogelijk komt dit doordat bij nachtelijke hemodialyse ook bepaalde remmers van vaatverkalking worden uitgespoeld, of doordat vaatverkalking in de kransslagaders een ander soort vaatverkalking is dan het soort vaatverkalking dat optreedt bij de verstoorde mineraalhuishouding in eindstadium nierfalen. Dit moet verder uitgezocht worden.

Deel II: eindstadium nierfalen en het bot

Door de verstoorde mineraalhuishouding hebben mensen met eindstadium nierfalen broze botten waardoor hun risico op botbreuken verhoogd is. De meest voorkomende botbreuk die voorkomt bij mensen met kwetsbare botten is een wervelinzakking. Echter, wervelinzakkingen worden vaak over het hoofd gezien en worden niet goed bijgehouden in dialysecohorten en -registraties. Daardoor is het niet bekend of mensen met eindstadium nierfalen ook vaker wervelinzakkingen hebben. Wij hebben daarom in *Hoofdstuk 6* onderzocht hoe vaak wervelfracturen voorkomen in deelnemers van het NOCTx-onderzoek. De resultaten laten zien dat wervelfracturen voorkomen bij één op de drie van deze groep relatief jonge en gezonde mensen met eindstadium nierfalen, en dat een nieuwe wervelfractuur optreedt bij een bijna even groot deel na mediaan 1.8 jaar. Dit is veel vaker dan beschreven is bij mensen uit de algemene bevolking. Gezien het vele voorkomen van wervelfracturen bij zelfs deze relatief jonge en gezonde mensen met eindstadium nierfalen, denken we dat het zinvol kan zijn alle mensen met eindstadium nierfalen te screenen op wervelfracturen.

We vonden ook dat een lage botdichtheid van de wervels gemeten op CT-scans niet met wervelinzakkingen samenhangt, terwijl zowel lagere als hogere bloedwaarden van het bijschildklierhormoon gepaard gaan met het voorkomen van wervelinzakkingen. Het bijschildklierhormoon meten we om te bepalen of het bot te snel of te traag wordt afgebroken en opgebouwd. Onze bevinding geeft aan dat in een groter onderzoek bekeken moet worden wat dan de optimale bloedwaarde is van het bijschildklierhormoon voor botbreuken. Een nadeel is alleen dat de bloedwaarden van het bijschildklierhormoon maar slecht voorspellen of het bot daadwerkelijk te snel of te traag wordt afgebroken en opgebouwd. Dit komt doordat een per individu wisselend deel van het bijschildklierhormoon in het bloed niet werkzaam meer is, en het bot ongevoelig kan zijn voor bijschildklierhormoon. Mogelijk dat we dankzij verder onderzoek in de toekomst beter kunnen bepalen of het bot te snel of te traag wordt afgebroken en opgebouwd,

waardoor we ook beter effecten van ziekte en behandeling op het bot kunnen vervolgen. Zo zijn er nieuwe meetmethoden ontwikkeld voor bijschildklierhormoon in het bloed die specifiek de hoeveelheid werkzaam bijschildklierhormoon meten, en wordt er onderzoek gedaan naar nieuwe scantechnieken (natriumfluoride PET-scans) die mogelijk kunnen bepalen of het bot te snel of te langzaam wordt afgebroken en opgebouwd, iets wat nu alleen nog betrouwbaar met botbiopten kan gemeten worden.

Deel III: kwaliteit van leven en overleving in eindstadium nierfalen

Mensen met eindstadium nierfalen hebben vaak een slechte kwaliteit van leven. Dit komt doordat hun leven beheerst wordt door de behandeling, klachten als gevolg van de ziekte, en leefregels met strenge diëten en veel medicijnen. In *Hoofdstuk 7* beschrijven we dat mensen die overstappen op nachtelijke hemodialyse veel minder pillen nodig hebben dan mensen die gewone hemodialyse doen, ook op de lange termijn. Dit kan bijdragen aan een betere kwaliteit van leven bij nachtelijke hemodialyse. In *Hoofdstuk 8* laten we dan in het NOCTx-onderzoek zien dat kwaliteit van leven wel hoger is bij mensen die een niertransplantatie hebben gekregen in vergelijking met mensen die nachtelijke hemodialyse doen. Als laatste hebben we in *Hoofdstuk 9* onderzocht of langere dialysesessies de sterftekans verlagen, in een grote groep Europese patiënten die hemodialyse deden. We vonden dat langere dialysesessies (meer dan 6 uur, 3x per week) een wel 20% lagere sterftekans gaven vergeleken met gewone dialysesessies (4 uur, 3x per week). Dit wijst erop dat een behandeling als nachtelijke hemodialyse met langere dialysesessies gunstig kan uitpakken voor patiënten. Wij denken daarom dat het belangrijk is dat nachtelijke hemodialyse aangeboden wordt aan alle mensen die moeten dialyseren en baat zouden kunnen hebben bij deze behandeling.

Appendix

Dankwoord

Dankwoord

Een proefschrift voltooien doet men niet alleen. Velen hebben hier direct en indirect aan bijgedragen. In dit hoofdstuk wil ik daarom graag een aantal mensen in het bijzonder bedanken. Beseffende dat dit hoofdstuk het meest gelezen gaat worden: excuus voor iedereen die ik hier ten onrechte niet noem!

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Appendix

Curriculum vitae

Curriculum vitae

Thijs Thomas Jansz was born on the 7th of September 1992 in Haarlem, the Netherlands. He obtained his gymnasium diploma summa cum laude at Gooi's Lyceum in Bussum, the Netherlands, in 2010. He then studied Medicine at Vrije Universiteit in Amsterdam, the Netherlands, and received his medical degree cum laude in 2016. Subsequently, he did his PhD at the Department of Nephrology and Hypertension at University Medical Center Utrecht under supervision of prof. dr. M.C. Verhaar, prof. dr. F.J. van Ittersum, and dr. B.C. van Jaarsveld. In August 2019 he started working as a resident in Internal Medicine (ANIOS) at Ziekenhuis Amstelland in Amstelveen, the Netherlands.