Hope for CKD-MBD Patients: New Diagnostic Approaches for Better Treatment of CKD-MBD

Berthold Hoher^{a–d} Andreas Pasch^e

^aInstitute of Nutritional Science, University of Potsdam, Potsdam-Rehbrücke, and ^bIFLB, Institut für Labormedizin Berlin, Berlin, Germany; ^cDepartment of Embryology, Medical School of Jinan University, and ^dDepartment of Nephrology, Guangzhou Overseas Chinese Hospital, The First Affiliated Hospital of Jinan University, Jinan University, Guangzhou, China; ^eDepartment of Clinical Research, University Hospital Bern, University of Bern, Bern, Switzerland

Keywords

Biomarker · Calcification propensity · Chronic kidney disease-mineral and bone disorder · Nonoxidized parathyroid hormone · Morbidity · Mortality · T₅₀ test

Abstract

Background: Chronic kidney disease-mineral and bone disorder (CKD-MBD) patients have a huge morbidity and mortality. Only relatively minor progress in therapeutic strategies has been made in the past decades. This is at least partially due to a lack of predictive diagnostic tools allowing personalized treatment of CKD-MBD patients. **Summary:** In this review we describe recent progress in the diagnosis of disturbances of calcium and phosphate metabolism in patients with CKD-MBD, measuring biological active nonoxidized parathyroid hormone as well as the overall likelihood of a patient to get calcified. **Key Message:** There is hope. The new tools have the potential of allowing personalized therapy for the treatment of CKD-MBD and hence improving outcome.

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Patients with chronic kidney disease (CKD) have a high morbidity and mortality that resembles the morbidity and mortality of patients with cancer. For certain cancer entities, big progress was made in the past decades with regards to the likelihood of improving outcome with new therapeutic strategies. Such progress is unfortunately missing in patients with CKD. This is at least partially due to a lack of adequate diagnostic tools for CKD-mineral and bone disorder (CKD-MBD) patients [1]. Without precise diagnostic tools a personalized therapy is not possible. This, however, was part of the success story in oncology. However, there is hope; in this review we describe recent progress in the diagnosis of disturbances of the calcium and phosphate metabolism in patients with CKD. These new tools have the potential of allowing personalized therapy for the treatment of CKD-MBD and hence improving outcome. These are tools to better analyze bioactive parathyroid hormone (PTH) as well as the overall likelihood of a patient to get calcified. PTH is a key player in the pathogenesis of CKD-MBD. However, measurements of PTH using the current state of the art PTH sandwich assay systems often fail to describe the CKD-MBD status (a description of the development of current tools to measure PTH is given in Fig. 1). Preclinical stud-

Fig. 1. Currently used assays only give a signal when antibodies have bound to two regions of the parathyroid hormone (PTH) molecule. This ensures that most of the degradation products of the entire PTH molecule are not measured. These assays are thus called intact PTH (iPTH) assays. However, they are not able to distinguish between oxidized and nonoxidized PTH. Mid/C-PTH, mid/carboxyl terminus of PTH; N-PTH, amino terminus of PTH; RIA, radioimmunoassay. Adapted from Moorthi and Moe [70].

ies clearly demonstrated that both too low and too high PTH concentrations are causal for the development of both bone and cardiovascular diseases [2–5]. Similar findings were seen in human observational studies [6–9]. Based on these data, guidelines for the treatment of too low and in particular too high PTH status in CKD patients were developed [10, 11] and subsequently for the development of pharmaceutical tools to treat these abnormal statuses, such as PTH analogues [12], for conditions of low PTH-related situations. The clinical management of CKD patients thus needs reliable analytical tools to measure PTH. However, this seems to be particularly tricky. As of today, we are unable to define a clear cutoff value for PTH clearly associated with secondary hyperparathyroidism. We have instead very complicated guidelines regarding what PTH in patients on dialysis should look like (PTH should be 2–9 times higher than the upper detection limit of the individual assay, and physicians need to consider PTH trends when adjusting therapy) [10, 11]. These wordings on their own clearly indicate that the current tools to measure PTH are insufficient. No diabetologist would accept such a definition for glucose or HbA1c as a biomarker for the definition and treatment monitoring of diabetes mellitus.

The poor performance of current PTH assays is due to the posttranslational modification of PTH, in particular

oxidation at Met8 and Met18 of the PTH molecule [1, 13] (Fig. 2), since patients with CKD have a huge burden of oxidative stress [14–17]. About two to three decades ago, it was convincingly demonstrated by independent leading research teams worldwide that oxidized PTH (oxPTH) and nonoxidized PTH (n-oxPTH) have completely different biological properties [18–47]. Only n-oxPTH is a full PTH receptor ligand, whereas oxPTH does not stimulate the PTH receptor. PTH oxidation, however, has not been considered in the development of PTH assays so far. Therefore, we recently developed an assay system separating oxPTH from n-oxPTH [1, 48–50]. Children with stage 2–4 CKD had the highest mean n-oxPTH concentrations compared with adult patients (adults on dialysis as well as kidney transplant recipients) [48–50]. Analysis of the subgroup of children with intact PTH (iPTH) >250 ng/L demonstrated a close to linear correlation between iPTH and oxPTH ($r^2 = 0.997$; $p < 0.001$), but a much weaker correlation between iPTH and n-oxPTH ($r^2 = 0.718$; $p < 0.05$) [1]. An observational study showed that the predictive power of n-oxPTH and iPTH on the mortality of hemodialysis patients differs substantially. Multivariable-adjusted Cox regression showed that higher age increased the odds for death, whereas higher n-oxPTH reduced the odds for death [49]. Analysis of iPTH, oxPTH, and n-oxPTH in a second independent cohort (2,867 participants of the EVOLVE trial at study entry) [51] revealed that n-oxPTH, but not oxPTH nor iPTH, had a predictive value for cardiovascular events and all-cause mortality. The patients were followed for up to 64 months. The primary composite end point was the time until death, myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event [51]. Pearson correlation analyses showed a very strong relationship between iPTH and oxPTH ($r = 0.996$; $p < 0.001$) and a weaker relationship between iPTH and n-oxPTH ($r = 0.82$; $p < 0.001$) (Fig. 3); see also Hoher et al. [52]. A multivariate Cox regression model adjusted for patient characteristics, cardiovascular comorbidities, and baseline characteristics revealed that n-oxPTH, but not oxPTH nor iPTH, was associated with the EVOLVE primary end point (time until death, myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event [51], cardiovascular mortality, and all-cause mortality [52]).

The linear correlation [1, 50, 52] between oxPTH and iPTH seen in patients with secondary hypertension – adults (Fig. 3, [52]) and children [1, 50] – indicates that the currently used iPTH assays primarily describe oxidative stress in CKD patients, but not PTH bioactivity.

Fig. 2. a Under conditions of oxidative stress, the methionine residues at positions 8 and 18 may be oxidized to methionine sulfoxide and methionine sulfone. Oxidation to methionine sulfoxide is reversible, whereas the second oxidation step to methionine sulfone is irreversible. Oxidized parathyroid hormone (PTH) changes its three-dimensional structure. This blocks the interaction of

PTH with its receptor. **b** Schematic diagram of the full length PTH(1-84) molecule (“bioactive” intact PTH). Oxidation at position Met8 and/or Met18 (red) alters the receptor binding site of PTH. Oxidized PTH does not bind the PTH receptor anymore and is thus biologically inactive. Adapted from Hocher and Yin [1]. hPTH, human parathyroid hormone.

Fig. 3. Pearson correlation analyses of 2,867 participants of the EVOLVE trial at study entry showed a very strong relationship between intact parathyroid hormone (iPTH) and oxidized parathyroid hormone (oxPTH) ($r = 0.996$; $p < 0.001$) – suggesting that iPTH is a measure of oxidative stress rather than PTH bioactivity – and a weaker relationship between iPTH and nonoxidized parathyroid hor-

mon (n-oxPTH) ($r = 0.82$; $p < 0.001$) [52]. A multivariate Cox regression model adjusted for patient characteristics, cardiovascular comorbidities, and baseline characteristics revealed that n-oxPTH, but not oxPTH or iPTH, was associated with the EVOLVE primary end point (time until death, myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event) [51].

Fig. 4. Schematic illustration of the test principle. The addition of 10 mM calcium and 6 mM phosphate to serum triggers the formation of primary calciprotein particles (CPPs; <100 nm), reflecting a serum-intrinsic defense mechanism against calcification. Primary CPPs contain fetuin-A and albumin as well as amorphous calcium phosphate. Primary CPPs undergo spontaneous transformation into secondary CPPs, which contain crystalline calcium phosphate and an extended spectrum of serum proteins. The transformation time (T_{50}) is specific for individual sera and depends – among other factors – on the concentration of the serum constituents indicated in the figure. The transformation from primary to secondary CPPs is detected by time-resolved nephelometry and provides an estimate of the integrated calcification-inhibitory capacity present in this particular serum sample. Adapted from Pasch et al. [56].

The iPTH measures describe very well protein oxidative stress in patients with renal failure (Fig. 3), but not PTH bioactivity. Guidelines and hence patient treatment for CKD-MBD can only be improved by measuring real bioactive n-oxPTH, but not a surrogate of oxidative stress, i.e., iPTH, since PTH bioactivity and oxidative stress will require different treatment approaches [1].

We need, however, to keep in mind that also n-oxPTH assays might have limitations. Potential other posttranslational PTH modifications such as phosphorylation of certain amino acids of the PTH molecule [53] will not be detected by this type of assay system [48–50]. What we really need is a true PTH bioassay suitable for routine testing – a challenge for scientists working in the field of assay development.

CKD-MBD patients are characterized by high mortality and the propensity to calcify [54]. Both these problems have been related to disturbances in mineral metabolism. Classically, phosphate, calcium, and PTH have been assessed to characterize mineral metabolism in individual patients. The broad range of treatment aims provided in current clinical guidelines for these blood values are reflective of considerable uncertainty about the optimal state of this system. Given this problem, the calcium-phosphate product has been used in a first attempt to integrate single values into a functional system of a higher order [55]. Extending on this conceptual consideration, a novel blood test has recently been developed [56]. Blood is physiologi-

cally close to supersaturation with regard to the formation of hydroxyapatite crystals from calcium and phosphate. However, the crystallization cascade from calcium phosphate prenucleation clusters to amorphous calcium phosphate, octacalcium phosphate, to the final product hydroxyapatite is biologically controlled in serum [57]. The main inhibitors fetuin-A and albumin are functionally complemented by long-known small molecules and ions such as pyrophosphate and magnesium into a functional mineralization-regulating physiological system, which is able to keep in a soluble state (i.e., prevent from precipitation) surplus amounts of calcium and phosphate.

A recently developed new blood test, the T_{50} test, measures the integrated functional status of this systemic calcium phosphate-buffering system by measuring the transformation time from primary calciprotein particles (CPPs) to secondary CPPs (Fig. 4).

When uremic serum is challenged with high amounts of calcium and phosphate in vitro, the spontaneous formation of primary CPPs occurs. These particles are globular (diameter approximately 50 nm) and contain amorphous calcium phosphate. Upon incubation at 37°C, they undergo spontaneous transformation towards spindle-shaped particles (diameter >100 nm), called secondary CPPs, which contain crystalline calcium phosphate (i.e., hydroxyapatite) [58]. The transformation time point reflects the ability of a given serum to delay the crystallization cascade and is specific for individual patient sera.

Table 1. Associations between clinical characteristics and the T₅₀ test result

	Stage 3 and 4 CKD	RTR	HD
All-cause mortality	X ⁵	X ^{6,7}	X ⁸
Cardiovascular mortality		X ⁷	X ⁸
Myocardial infarctions			X ⁸
Peripheral vascular events			X ⁸
Renal transplant failure		X ⁷	
Progression of aortic stiffness	X ⁵		
Histologic changes in transplant kidneys		X ¹⁶	
Kidney oxygenation ^a	X ¹⁷		
Renal resistive index	X ^{17, 18}		

^a Measured by blood oxygen level-dependent magnetic resonance imaging. CKD, chronic kidney disease; HD, hemodialysis; RTR, renal transplant recipients.

Excitingly, the transformation time point has to date been shown to correlate with prognosis many years in advance in various large cohorts [59–62] including more than 5,000 patients. This indicates a considerable intraindividual and technical stability of the test result.

In more detail, the T₅₀ test has been related to all-cause mortality, cardiovascular mortality, and also to specific cardiovascular events (myocardial infarctions and peripheral vascular events) in renal patients (Table 1). Furthermore, a number of intermediate and physiologically relevant clinical links have been related to the T₅₀ test, lending plausibility to the concept of crystallization inhibition and its importance for clinical end points and events (Table 1).

Overall, the T₅₀ test measures the individual systemic mineralization setpoint in patients. This setpoint determines the overall propensity in the body to form calcium phosphate nanocrystals. While the precise mechanistic link between test result and outcome has not been firmly elucidated in detail yet, it appears reasonable that they might be based at least in part on the development of vascular calcification/soft tissue calcification, oxidative

stress, and inflammatory events, which may all be triggered alone or in combination by circulating nanocrystals. The occurrence of naturally occurring CPPs in the circulation has of note been demonstrated both indirectly (by measuring sedimentable fetuin-A) [59, 63] and directly (using cryo-TEM imaging) [64].

Primary CPPs are nontoxic when exposed to vascular smooth muscle cells in vitro, whereas the secondary type is highly toxic to vascular smooth muscle cells [65, 66]. The exposure of these cells to secondary CPPs results in the aforementioned triad of calcification, oxidative stress, and the release of inflammatory cytokines, a combination which is also commonly encountered in renal patients. Furthermore, macrophages exhibit a strong inflammatory response when exposed to secondary CPPs [67].

Given that the T₅₀ test measures the functional performance of a physiological system, the link between test result and outcome may well be of causal nature.

First pilot studies with CKD and hemodialysis patients have demonstrated that the T₅₀ value is amenable to improvements by therapeutic interventions. Specifically, interventions such as hemodialysis [67–69], increasing bicarbonate and magnesium, and lowering phosphate serum concentrations improve the T₅₀ value [56, 68]. Uniform interventions, even of moderate intensity, have been shown to improve the T₅₀ value by approximately 40 min in hemodialysis and CKD patients [69]. This is in the order of magnitude of half a standard deviation of T₅₀ in various clinical studies and, therefore, opens the exciting possibility of improving the long-term prognosis of kidney patients via individualized combinations of T₅₀-directed interventions. Finally, analysis of metabolomics in CKD-MBD patients will further provide useful information for personalized CKD-MBD treatment [15].

Conflict of Interest Statement

B. Hoher submitted a patent on an n-oxPTH assay system. A. Pasch is an inventor and holds a patent of the T₅₀ test; he is cofounder, stockholder, and current CEO of CALCISCON AG, which develops the T₅₀ test for commercialization.

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