

Editorial

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Need for better PTH assays for clinical research and patient treatment

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Parathyroid hormone (PTH) – a 84-amino acids containing peptide hormone secreted by the parathyroid gland – regulates the blood calcium level via GPCR binding and subsequent activation of intracellular signaling cascades [1]. Animal models of chronic kidney disease (CKD) clearly demonstrated that both too low and also too high PTH concentrations in these CKD model are causal for the development of both bone and cardiovascular diseases [2–5].

Similarly, low PTH concentrations and in particular high PTH concentrations in CKD patients are associated with excess morbidity and mortality [6–9]. These studies led to the development of guidelines for the treatment of CKD patients [10, 11] and the development of pharmaceutical tools to treat these abnormal status such as PTH analogues [12] for conditions of low PTH related situations as well a vitamin D analogues, calcimimetics and phosphorus binders for conditions with high PTH [13–16].

Clinical research to develop new drugs for CKD-MBD patients, clinical use of these drugs and the monitoring of the efficacy requires reliable analytical tools to measure PTH. However, as of today, we are not able to define a clear cut-off value for PTH that is clearly associated with secondary hyperparathyroidism. We have instead complicated guidelines how PTH in patients on dialysis should look like (PTH should be 2–9 times higher than the upper detection limit of the individual assay used and physicians needs to consider PTH trends when adjusting therapy [10, 11]). This complicated wording is simply necessary, because current PTH assays are unable to provide general applicable exact cut-offs. Moreover, in daily practice it is well known that PTH measurements in individual cases are often not fitting to the clinical situation [17]. One underlying reason that might contribute to the poor performance of current PTH assays is potential posttranslational modification of PTH – in particular oxidation at Met8 and Met18 of the PTH-1-84 molecule, since patients with CKD and in particular CKD patients on dialysis have a huge burden of oxidative stress [18].

Oxidation of PTH has a huge impact on the biological activity of PTH. Studies performed by leading research

groups worldwide done about two decades ago clearly indicated that oxidized PTH (oxPTH) and non-oxidized PTH (n-oxPTH) have completely different biological properties (see reviews [19, 20]). All these studies – over 20 in independent groups worldwide – indicate that PTH oxidation is critical for the biological activity of PTH. PTH oxidation, however, has been so far ignored in the development of PTH assays that are used in current clinical practice.

Therefore, assay system separating oxPTH from n-oxPTH were developed recently and it was demonstrated that bioactive, n-oxPTH, but not iPTH nor oxPTH, is associated with mortality in CKD patients on dialysis [21–24]. The currently used PTH assays are called intact PTH (iPTH) assays. These are sandwich assays detecting the entire (intact) PTH molecule, but ignore PTH oxidation. Huge clinical studies like the EVOLVE trial [13] used iPTH measurements as key inclusion criteria to identify patients with secondary hyperparathyroidism. Intact PTH (iPTH) measurements in these patients, however, do more describe oxidative stress rather than biological active PTH (iPTH correlates much better with biologically non-active oxidized PTH [oxPTH] than n-oxPTH, see references [19, 20]). Thus including patients on the basis of iPTH is a poor clinical tool to select patients with secondary hyperparathyroidism. We speculate that this fact may explain at least partially the failure of the huge EVOLVE trial [13] aiming to show clinical benefits of calcimimetics in patients with secondary hyperparathyroidism. Using iPTH assays simply did not identify patients with secondary hyperparathyroidism. This is illustrated in the Figure 1. The current guidelines for target PTH in CKD stage five patients [6] were applied to the EVOLVE population. When using the n-oxPTH assay, most of the included patients should not receive calcimimetics because these patients were at target for PTH according to the guidelines. Some of the EVOLVE patients do even have too low biologically active n-oxPTH concentrations at study entry. We thus speculate that calcimimetics might have been beneficial only in patients who had really elevated biologically n-oxPTH at study entry, in the other patients this drug might have even caused harm to the patient – and the overall trial result was thus neutral [13]. This is just

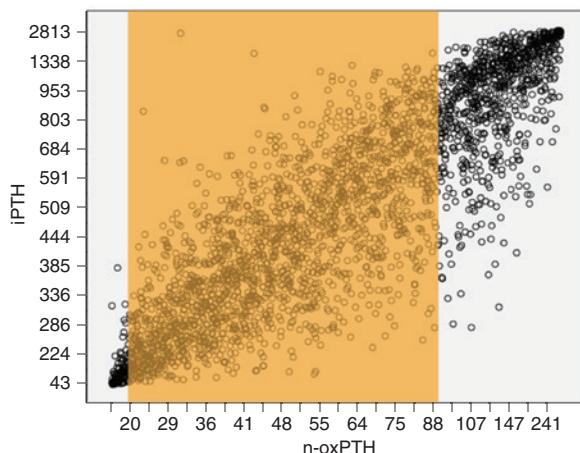


Figure 1: Correlation of intact PTH (iPTH) and non-oxidized biologically active PTH (n-oxPTH) in the EVOLVE trial population at study entry. Measuring n-oxPTH in the EVOLVE trial population at study entry [13] is shown. The shadowed area indicated patient at PTH target when applying KDIGO guidelines [16]. A large proportion of the EVOLVE trial population at study entry was at PTH target for n-oxPTH (20–90 pg/mL) and should not have been treated with the study drug. Only a minor part of the EVOLVE trial population did have high n-oxPTH concentrations at study entry. KDIGO, Kidney disease improving global outcomes; GLs, guidelines; n-oxPTH, non-oxidized PTH. For normal values for n-oxPTH, see Hocher et al. [21].

an example showing how important are reliable assays for clinical research. In the current issue of the journal, Ursem et al. [25] analysed an important but so far not adequately addressed issue of the n-oxPTH assay system: Could it be possible that PTH oxidation is an *ex vivo* phenomenon? In other words, is it possible that PTA oxidation is a preanalytical artifact – and does not occur *in vivo*? The authors addressed this topic very carefully [25] and provided convincing evidence that PTH oxidation does not occur after blood taking and is thus a real biological phenomenon. New assays need always clear independent validation. The current study [25] is a good example for this.

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