

STATE OF 'UNCERTAINTY' OF PTH MEASUREMENT IN CKD

Jun-Ki Park
12/14/10

PTH and bone turnover in CKD

Hypoparathyroidism

Hyperparathyroidism

Very low PTH ← **Circulating PTH values ??** → Very high PTH

Low-turnover
bone disease

Adynamic bone disease
Osteomalacia

Normal bone
turnover

High-turnover
bone disease

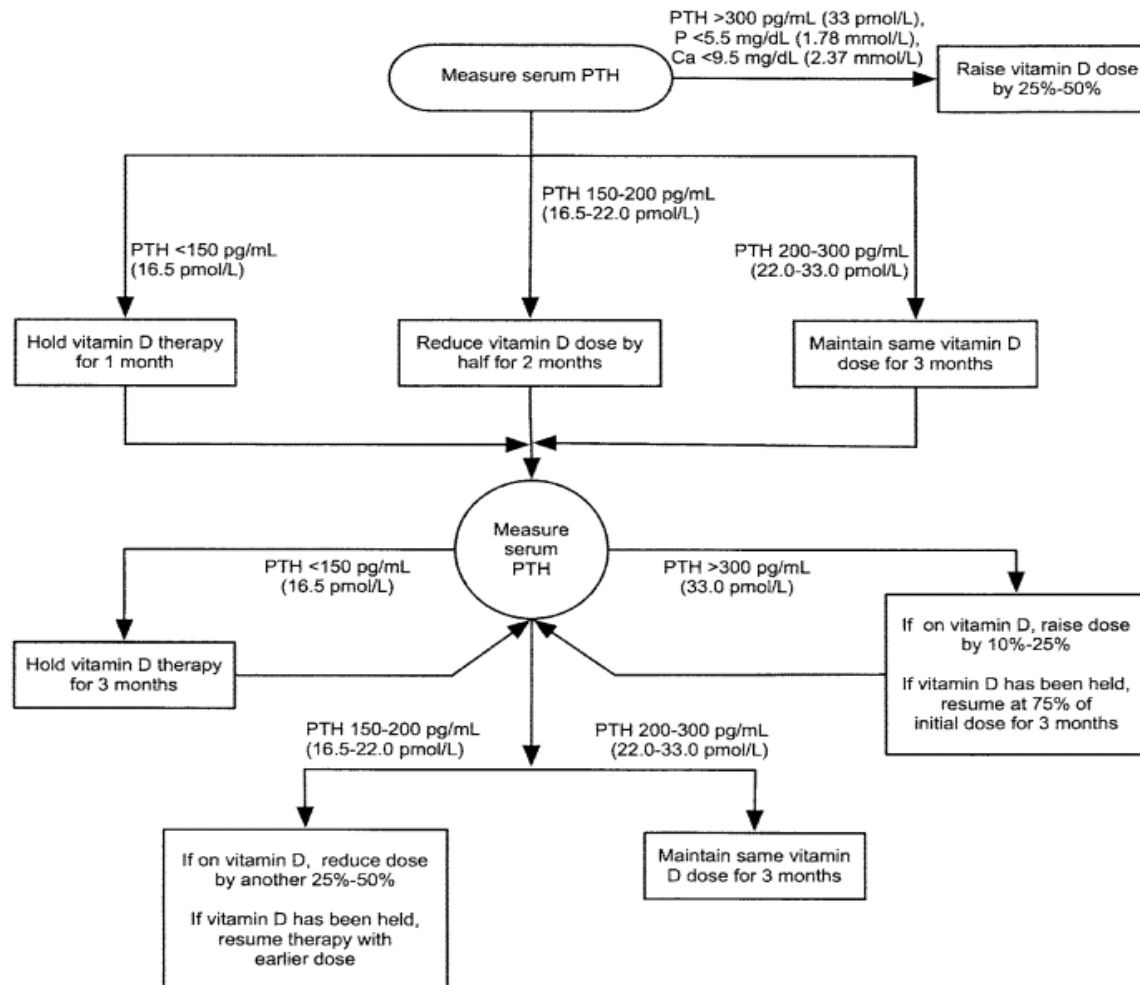
Osteitis fibrosa

Vascular calcification
Cardiovascular disease
Mortality

K/DOQI Guidelines

GUIDELINE STATEMENTS

S21



PTH

a

Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu
Gln
30 Asp
Val
Val-Asn-Asp-Glu-Lys-Lys-Arg-Pro-Arg-Gln-Ser-Ser-Gly-Asp-Arg-Tyr-Ala-Ile-Ser-Ala-Gly-Leu-Ala-Val-Phe-Asn-His
Leu
60 Val
Glu
Ser-His-Gln-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Ile-Lys-Ala-Lys-Pro-Gln

1-84 PTH

b

Phe-Met-His-Asn-Leu-Gly-Lys-His-Leu-Ser-Ser-Met-Glu-Arg-Val-Glu-Trp-Leu-Arg-Lys-Lys-Leu
Gln
30 Asp
Val
Val-Asn-Asp-Glu-Lys-Lys-Arg-Pro-Arg-Gln-Ser-Ser-Gly-Asp-Arg-Tyr-Ala-Ile-Ser-Ala-Gly-Leu-Ala-Val-Phe-Asn-His
Leu
60 Val
Glu
Ser-His-Gln-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Ile-Lys-Ala-Lys-Pro-Gln

7-84 PTH

Different PTH assays and what they measure - First Generation PTH Assay

- Radioimmunoassays
- **Polyclonal Ab's** directed toward synthetic C-terminal (such as 53-84 PTH) or mid-region (such as 44-68 PTH) PTH fragments.
- In addition to PTH 1-84, biologically inactive fragments are measured, which have a longer half-life than 1-84 PTH, and accumulate in CKD patients.
- Consequence: PTH concentrations were always increased.
- Currently considered obsolete for clinical practice.

Second Generation PTH Assay

- First 2nd-gen. PTH assay: '**Allegro**' intact PTH assay:
- 2 different Ab's:
 - **Capture Ab**: towards 39-84 portion of the PTH
 - **125-I-labeled Ab**: towards 15-20 portion.
(Thus unable to measure the C-terminal or mid-fragments, such as 53-84 or 44-68).
- Fully automated immunoanalyzers allowed better analytical performances and shorter turnaround time.
- 'Allgro' 15-20 portion; Elecsys/modular 26-32 portion.
- Globally called '**intact**' **PTH assays** as they were thought to measure only the full-length 1-84 PTH.

Second Generation PTH Assay

- More clinically satisfying data than 1st gen. PTH assays (better correlation with bone biopsy parameters in CKD pts; clearly low values in 'non-parathyroid' hypercalcemia or hypoparathyroidism)

However:

- Possibly overestimate degree of secondary HPT in CKD pts (Quarles et al. JCEM 1992; Wang et al. AJKD 1995)
- Leading to increased effort to suppress PTH
- Concern for the development of adynamic bone dz and erroneous referrals for parathyroidectomy.

Second Generation PTH Assay

Possible explanation:

- Cross-reactivity (from approximately 50 to 100%) for 7-84 PTH fragments (Lepage et al. Clin Chem 1998)
- 7-84 PTH increases when the GFR decreases, it's variable from one pt to another.
- Animal and cellular models demonstrated that 7-84PTH exerts opposite effects to those of 1-84 PTH; mediated by a receptor different of PTH/PTHrP receptor (PTHR1) (Murray et al. Endocr Rev 2005)

Third Generation PTH Assay

- **'Whole PTH assay'**
- Capture Ab towards 39-84 portion
N-terminal Ab towards 1-4 portion
Thus, does **not** measure the 7-84 PTH.

- Does 3rd gen. PTH assay improve the detection of altered bone turnover?
 - 4 bone biopsy studies:
 - 1 showed that the ratio 1-84 /7-84PTH discriminated between high- and low-turnover bone dz significantly better than either 2nd or 3rd alone. (Monier-Faugere et al. KI 2001)
 - 3 other studies did not find diagnostic improvement with either this ratio or 3rd gen. assay alone. (Coen et al. AJKD 2002; Salusky et al. KI 2003; Lehmann et al. KI 2005)

Table 1 | The main circulating fragments of PTH, and whether they are measured (Yes) or not (No) by the various PTH assay generations

	First-generation assays	Second-generation assays	Third-generation assays
Most common identifications	C-PTH assays, Mid-PTH assays	'Intact' PTH assays	Whole PTH assay, Ca-PTH assay, BioIntact PTH assay
Methodology	Competition (mostly RIA)	Immunometry ('sandwich' assays)	Immunometry ('sandwich' assays)
1-84 PTH	Yes	Yes	Yes
7-84 PTH	Yes	Yes (with various cross-reactivity)	No
C-terminal fragments	Yes	No	No
'Amino' PTH	Yes	Depends on the epitope of the anti-N-terminal Ab: No if the epitope is proximal (13-24) and Yes if the epitope is distal (26-32)	Yes

Ab, antibody; Ca-PTH, calcium-PTH; PTH, parathyroid hormone; RIA, radioimmunoassay.

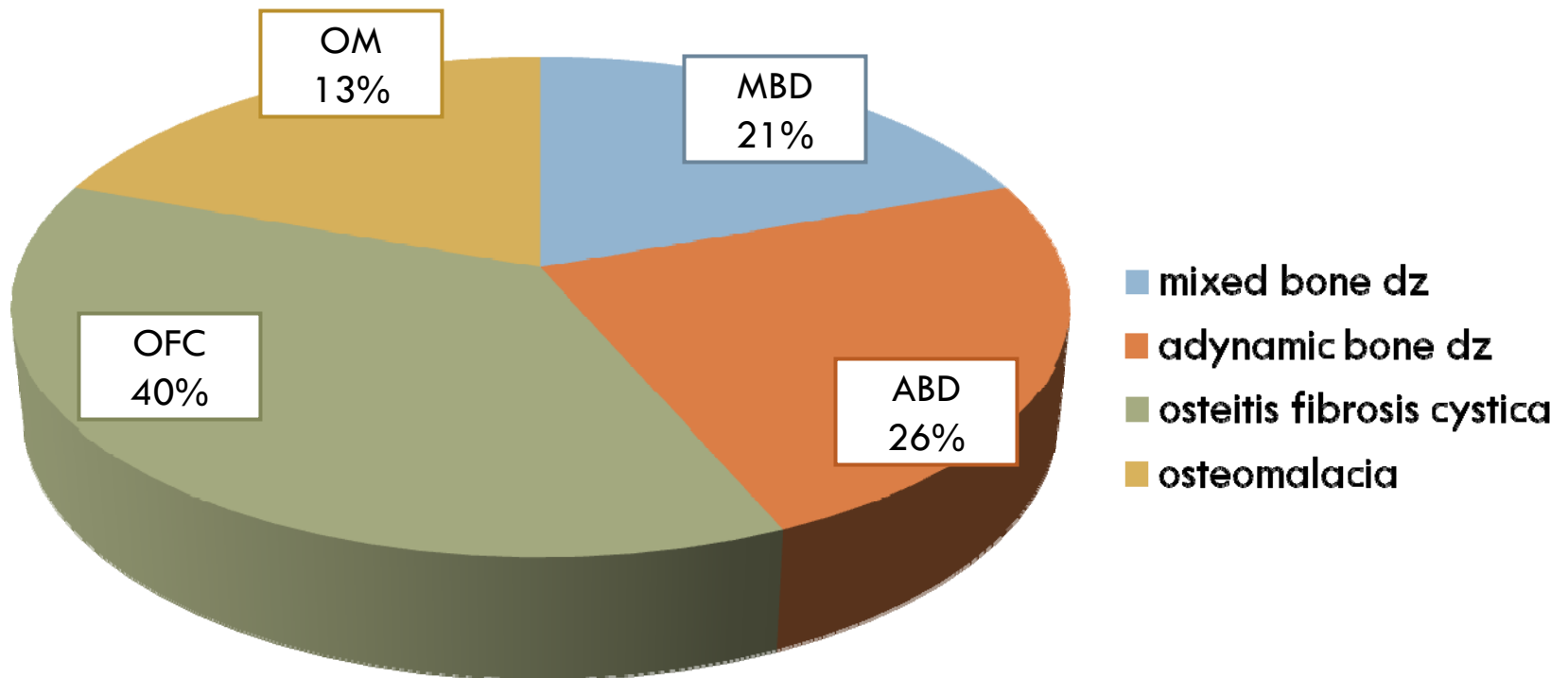
Bone Formation Rate and PTH Levels

- Bone specimens from 96 ESRD pts obtained
- In 52 pts PTH levels (2nd gen.) were obtained 1 week prior to bone biopsy.

Prior to bone biopsy:

- 3-day oral tetracycline hydrochloride (250mg tid) followed by free interval of 17 days, then 3-day oral decloxycline (300mg tid). Bone biopsy 3 days thereafter.
- $BFR/BS = MS/BS \times MAR$ mm³/mm²/day
- MS/BS (mineralized surface): is given by the total extent of double labels plus half the extent of single labels.
- MAR (mineral appositional rate): obtained by measuring mean distance between tetracycline labels and dividing by the number of days between doses of tetracycline.

Distribution of Different Forms of Renal Osteodystrophy



Distribution of Different Forms of Renal Osteodystrophy As Related To Subgroups of PTH Levels

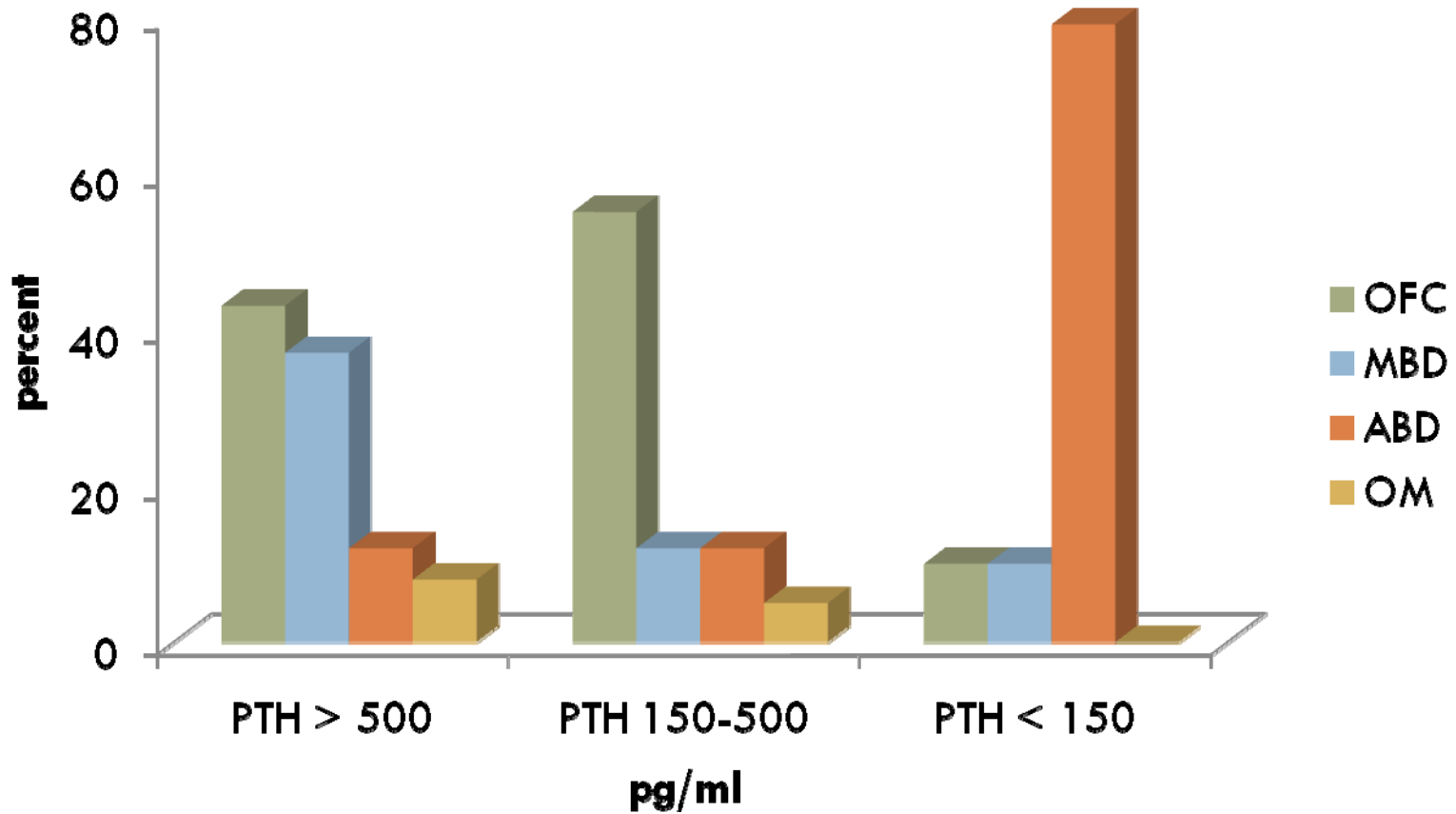


Table 2. Correlation between intact parathyroid hormone levels and BFR/BS

PTH range	BFR range	n	r	p
0-150	0.0 - 0.022	12	0.84	< 0.05
150-500	0.04 - 0.16	22	0.027	NS
500-1200	0.04 - 0.54	18	0.21	NS
0-1200	0.0 - 0.54	52	0.28	NS

Lack of Standardization

- Few of the currently used PTH assays have been calibrated against the international reference preparation for PTH immunoassays (WHO79/500; prepared in 1981 with purified human PTH)
- Most assays are calibrated against synthetic 1-84 PTH from various origins. Currently no recognized international standard made of synthetic PTH.

Lack of Standardization

- K/DOQI target ranges initially established early 1990's; bone biopsy data compared with PTH concentration measured with 'Allegro' assay. However 'Allegro' assay is no longer available since 2006.
- Clinically significant inter-method variability

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Table 3 | Proposed correcting factors for PTH results based on previous publications^{36,45,48}

No correction:

Bayer PTH Advia Centaur^{36,45}
Access Intact PTH (Beckman Coulter)³⁶
Roche Elecsys PTH⁴⁵
Schering CisBio ELSA⁴⁵
Scantibodies Total Intact PTH⁴⁵

Divide by following factor:

Architect PTH Abbott: /1.3⁴⁸
Beckman Coulter PTH IRMA: /1.2⁴⁵
DiaSorin Intact PTH IRMA: /0.55⁴⁵
LIAISON N-tact PTH: /0.90⁴⁵
Scantibodies Ca-PTH IRMA: /0.55 (the only third-generation assay)⁴⁵

Special case:

DPC Immulite 2000 intact PTH³⁶
If in serum, no change; if in plasma, divide by 1.25

Ca-PTH, calcium-PTH; ELISA, enzyme-linked immunosorbant assay; IRMA, immunoradiometric assay; PTH, parathyroid hormone.

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Table 4 | Effect of correcting PTH values in the French National Phosphorus and Calcium Observatory (June 2008 values) according to the measurement kit; correcting factors suggested in Table 3 were applied to raw PTH values.

Kit	<i>n</i>	Raw values	Corrected
1 Architect PTH Abbot	684	397.6 ± 359.4	305.8 ± 276.4
2 Bayer PTH Advia Centaur	652	342.3 ± 363.7	No correction
3 Beckman Coulter PTH IRMA	268	326.6 ± 314.4	272.2 ± 262.0
4 DPC Immulite 2000 intact PTH	542	316.7 ± 306.0	253.3 ± 244.8
5 DiaSorin Intact PTH IRMA	165	271.1 ± 222.2	492.9 ± 403.9
6 DiaSorin LIAISON N-tact PTH	953	320.8 ± 284.7	356.5 ± 316.4
7 Access Intact PTH (Beckman Coulter)	107	404.6 ± 329.3	No correction
8 Roche Elecsys PTH	1958	307.4 ± 297.4	No correction
9 Scantibodies Total Intact PTH	282	238.8 ± 197.3	No correction

IRMA, immunoradiometric assay; PTH, parathyroid hormone.

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Table 5 | Number of patients classified within three PTH targets before and after correction in 2612 patients: 2179 patients did not change categories, whereas 433 (17%) could have been misclassified

PTH (pg/ml)	Corrected PTH		
	<150	150-300	≥300
<i>Raw PTH</i>			
< 150	672	61	0
150-300	138	549	91
≥300	0	143	958

PTH, parathyroid hormone.

Future Tasks

- To calibrate all existing assays against a unique international reference preparation made of recombinant 1-84 PTH
- Limit inter-assay discrepancies.
- To avoid cross reactivity with 7-84 PTH / other N-truncated fragments, exclusive use of 3rd gen. PTH assays may be considered.
- Association of PTH measurement with one bone marker (such as bone alkaline phosphatase) may improve diagnosis of altered bone turnover.

THANK YOU




















Indication for bone biopsy: severe bone pain, extraosseous calcifications, spontaneous fractures, hypercalcemia, excessive PTH levels, suspected aluminum overload.

Exclusion Criteria: CAPD, pts w/ malignancies, liver diseases, granulomatous diseases, pts s/p parathyroidectomy, pts taking steroids, immunosuppression, antiepileptics.




Figure 1 | Amino-acid sequences.

1–84 PTH (a), 7–84 PTH (b), N-PTH (c).

Both second- and third-generation PTH assays use two antibodies directed toward different epitopes. In both generations of assays, the capture antibody (coated to a solid phase) is directed toward the 39–84 portion.

In 3rd gen. assay, the labeled antibody recognizes the 1–4 portion and, thus, may bind to 1–84 PTH, but not to the 7–84 PTH

In most second-generation assays, the labeled antibody is directed toward the 15–20 portion of the 1–84 PTH molecule and, thus, may bind to both 1–84 PTH and 7–84 PTH. In some second-generation assays (such as the Roche assay), the labeled antibody is directed toward the 26–32 portion of the 1–84 PTH molecule and, thus, may bind to 1–84 PTH, 7–84 PTH