Bone Biopsy Study

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Disclosure of Interests

Sanofi: Honoraria for lectures.

Abbott Laboratories: Honoraria for lectures.

KDIGO-Controversies Conference 2005

Definition, Evaluation, and Classification of Renal osteodystrophy: a position statement from KDIGO

CKD-MBD: A systemic disorder which include one or more elements of abnormal bone and mineral metabolism as follows:

- Laboratory-abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism
- **Abnormalities in bone turnover, mineralization, volume, linear growth, or strength,** and quantifiable by histomorphometry of bone biopsy (Renal Osteodystrophy)
- Vascular or other tissue calcification

Moe S, et al. Kidney Int. 2006 Jun;69(11):1945-53

Bone abnormalities in CKD

- Bone abnormalities start early during the course of CKD and may progress with loss of kidney function
- The histologic abnormalities are heterogeneous, and patients may develop different lesions as CKD progresses
- Bone biochemical markers, single or in combination, have limitations for a complete assessment of renal osteodystrophy (ROD)
- Therefore, bone histomorphometric analysis remains the *gold standard* to diagnose the type of ROD in CKD

Classical description of histologic abnormalities in CKD based on bone turnover

Low turnover

–

"PTH, surrogate indicator of bone turnover"

KDOQI: PTH 150-300 pg/ml

KDIGO: PTH 2-9 x ULN

High turnover

+

Adynamic bone disease
Osteomalacia

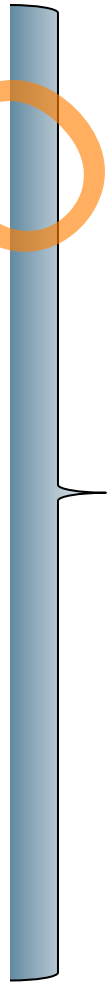
Mixed uremic osteodysthrophy

Hyperparathyroid bone disease

Modified from Salusky et al.

Other potential factors affecting bone in CKD

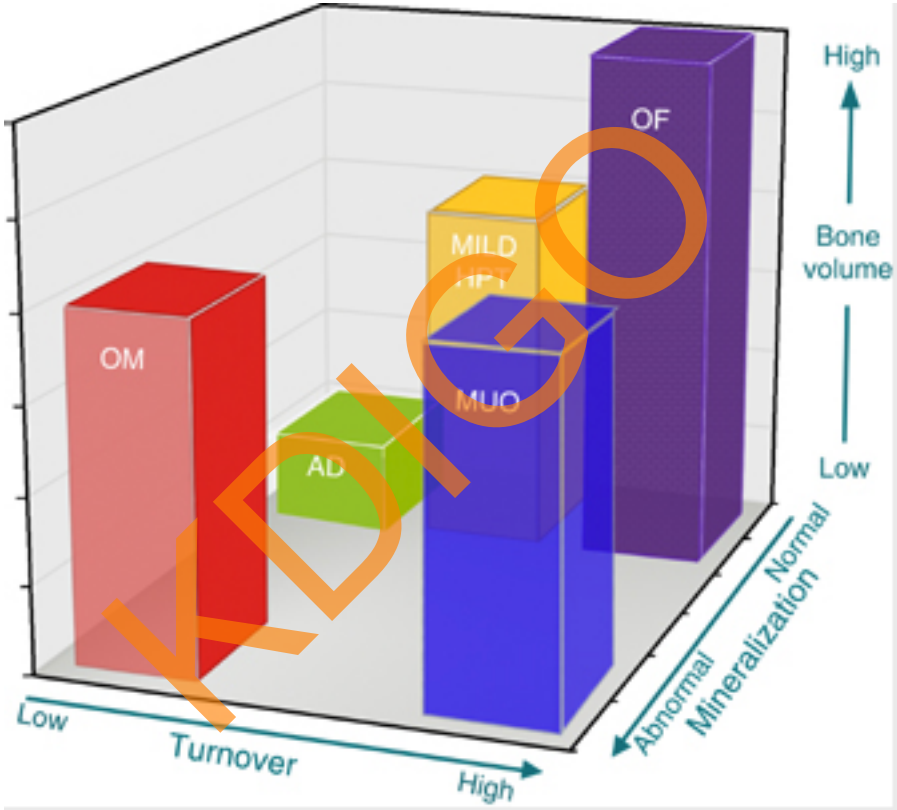
- Inheritance
- Age
- Nutrition
- Postmenopausal status
- Corticosteroid induced bone loss
- Vitamin D status
- FGF23
- Other…



Volume

Mineralization

KDIGO: TMV classification of ROD



Moe S, et al. Kidney Int. 2006 Jun;69(11):1945-53

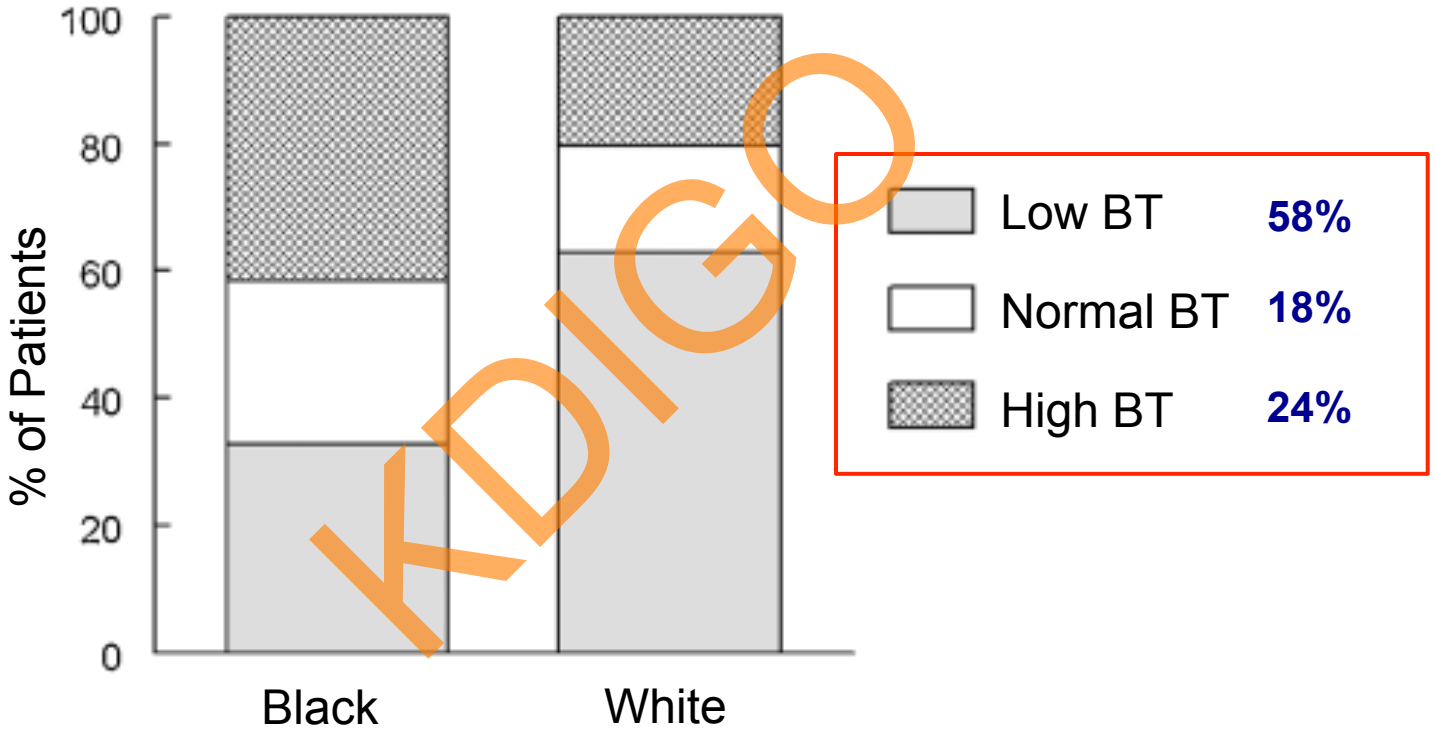
TMV classification

Renal Osteodystrophy in the first Decade of the New Millennium: Analysis of 630 Bone Biopsies in Black and White Patients

H. Malluche, HW Mawad, and Marie-Claude Monier-Faugere
J Bone and Miner Res 2011, 26: 1368-1376

Patients: 630 (USA: 316 and Europe: 314)
Age ≥ 18 years. Chronic maintenance HD for at least 6 months
Black: 83 patients
White: 543 pts.

Prevalence of low, normal, and high bone turnover in black and white patients with CKD 5D



No difference between gender, diabetics vs. non diabetics, and treatment with active vitamin D analogs

Malluche et al. J Bone Miner Res 2011; 26:1368-76

Clinical and biochemical characteristics in patients with and without mineralization defect

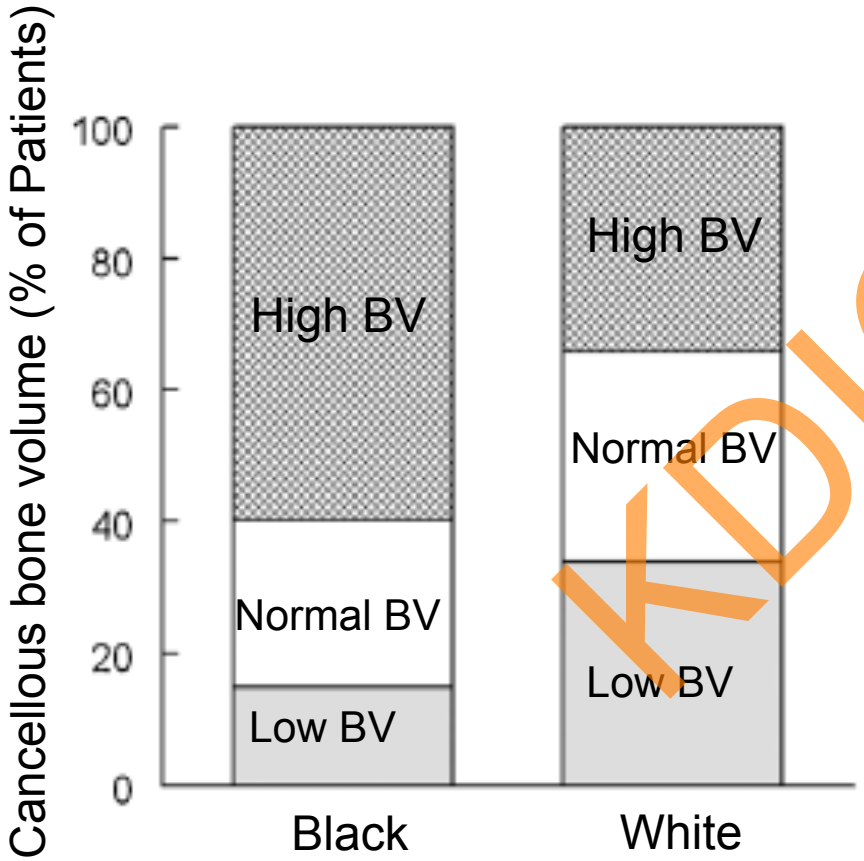
(Oth and MLT)

Characteristics	Mineralization defect (*n* = 21)	No mineralization defect (*n* = 609)	*p* Value
Age (years)	51 ± 4	56 ± 4	.168
Males (%)	52.4	47.6	.668
Dialysis vintage (months)	75 ± 9	49 ± 2	.005
Diabetics (%)	14.3	21.4	.202
Vitamin D treatment (%)	30.0	23.5	.502
Phophate binders			.354
Calcium-containing phosphate binders (%)	57.9	68.5	
Noncalcium, nonaluminum phosphate binders (%)	21.1	21.7	
No phosphate binder (%)	21.1	9.8	
Serum calcium level (mg/mL)	8.70 ± 0.22	9.22 ± 0.04	.010
Serum phosphorus level (mg/mL)	4.76 ± 0.30	5.39 ± 0.07	.097
Serum alkaline phosphatase level (UI/L)	206 ± 30	148 ± 7.61	.005
Plasma parathyroid hormone level (pg/mL)	502 ± 110	320 ± 15.5	.046

None of the patients showed stainable aluminum or iron

Malluche et al. J Bone Miner Res 2011; 26:1368-76

Prevalence of low, normal, and high cancellous bone volume (BV) in black and white patients with CKD 5D

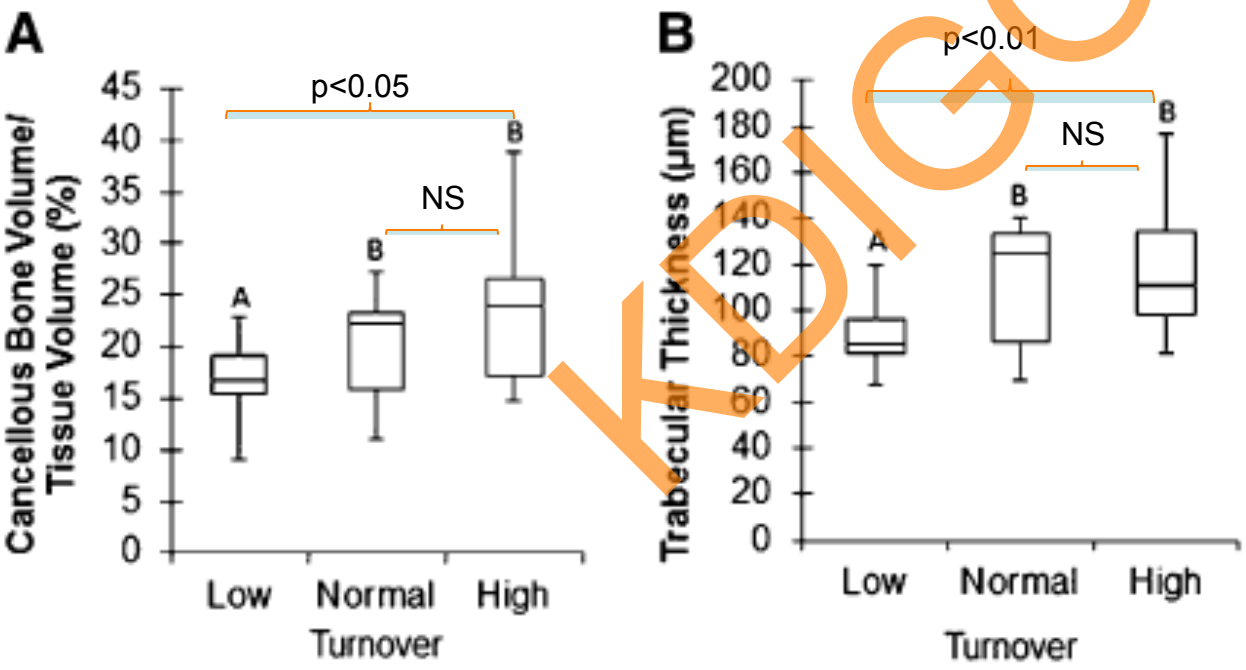


- This study based on TMV calls the attention on the importance of race in the type of bone alteration in CKD, and suggests that this should be taken into account to decide treatment.

Malluche et al. J Bone Miner Res 2011; 26:1368-76

Microstructural parameters in patients with low and high bone turnover

CKD 5D patients: 32	Normal controls: 12
High bone turnover: 17 Low bone turnover: 15	Normal bone turnover: 12



High turnover:

Showed material and nanomechanical abnormalities such as reduced mineral to matrix ratio and lower stiffness.

Conclusion:

Turnover related alterations in bone quality may contribute to diminish mechanical competence of bone in CKD

Malluche et al. J Am Soc Nephrol 2012, 23: 525-532

Bone classification system in pediatric renal osteodysthrophy

Patiens: 161 pediatric patients on CCPD (13 ± 3 months)
Age: 14.1 ± 1.2 years (0.7-20 years)
Bone biopsy between years 1990 and 2005

Turnover	Serum Ca mg/dl	Serum P mg/dl	Alk Phosph IU/L	PTH pg/ml
Low (n=7; 4.34%)	9.1 ± 0.6	8.2 ± 0.6[a]	212 ± 40	163 ± 48
Normal (n=62; 38.5%)	9.3 ± 0.1	6.0 ± 0.2	214 ± 18	356 ± 36
High (n=92, 57.1%)	9.0 ± 0.1	6.4 ± 0.1	436 ± 28[a]	781 ± 49[a]

[a]P < 0.01 from other two groups

Bakkaloglu SA, et al 2010, CJASN

Histomorphometry according to turnover and mineralization with corresponding biochemical values

Bone volume: normal 73%, increased 27%

Abnormal mineralization: 48% of all patients

58% of high turnover patients
38% of normal turnover patients
29% of low turnover patients

Turnover (BFR/BS)	Mineralization (OV/BV+ OMT)	Serum Ca mg/dl	Serum P mg/dl	Alk Phosp IU/ml	PTH Pg/ml
Low (n=7)	Normal (5)	9.6 ± 0.4	8.2 ± 0.6	197 ± 26	116 ± 15
	Abnormal (2)	8.1 ± 2.0	8.2 ± 2.2	250 ± 160	282 ± 162
Normal (n=62)	Normal (39)	9.6 ± 0.1	6.0 ± 0.2	198 ± 16	286 ± 38
	Abnormal (23)	8.9 ± 0.2[a]	5.9 ± 0.3	243 ± 41	477 ± 68[a]
High (n=92)	Normal (39)	9.2 ± 0.2	6.2 ± 0.2	340 ± 31	587 ± 58
	Abnormal (53)	8.8 ± 0.1	6.5 ± 0.2	506 ± 39	924 ± 67[a]

[a]P < 0.01 from subjects with normal mineralization

Bakkaloglu SA, et al 2010, CJASN

Summary of: TMV classification system in pediatric renal patients

- The TMV classification allowed the identification of mineralization defects that were previously underecognized.
- PTH and ALP may be useful in discriminating patients with normal turnover and defective mineralization from those with normal mineralization
- No single marker provides a complete assessment of ROD, but combination of markers may improve assessment of turnover and mineralization abnormalities in pediatric patients on dialysis.

Bakkaloglu Clin J Am Soc Nephrol 5: 1860–1866, 2010

Relationship between Bone Biomarkers and Bone Histology in End-Stage Kidney Disease

Stuart M. Sprague, Ezequiel Bellorin-Font, Vanda Jorgetti, Aluizio B. Carvalho, Hartmut H. Malluche, Aníbal Ferreira, Patrick C. D'Haese, Tilman B. Drüeke, Hongyan Du, Thomas Manley, Eudocia Rojas, and Sharon M. Moe

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The funding organizations had no role in the design, conduct, analysis, or interpretation of the study.

Histological interpretation of bone biopsies

Participating laboratories:

- University of Kentucky, Lexington, KY USA (patients from Turkey)
- Federal University of São Paulo, São Paulo, Brazil
- University of São Paulo, São Paulo, Brazil
- University Hospital of Caracas, Caracas, Venezuela

Histomorphometric parameters utilized for analysis of TMV:

- Bone turnover: Bone formation/ bone surface (BF/BS)
- Bone mineralization: Mineralization lag time (MLT), Osteoid thickness O.Th
- Bone volume: Bone volume/tissue volume (BV/TV)

Each laboratory utilized their normative data to classify bone parameters in the TMV system.

Serum biochemistries and statistics

- Serum biomarkers were analyzed in a single central laboratory (Nordic Biochemical Research Laboratory, Denmark)
- Intact PTH (iPTH) and Procollagen type I N Propeptide (PINP) were measured by chemiluminescence immunoassay (Roche Elecsys® 2010 Analyzer)
- Whole PTH™ (1-84) (wPTH) was measured by an immunoradio-metric assay (Scantibodies Laboratories with the test performed at Nordic.
- BSAP was measured by immunoassay (Quidel)
- All sites used an intact assay, but the assay kit varied, and was not standardized in terms of assays or freeze/thaws (OiPTH)

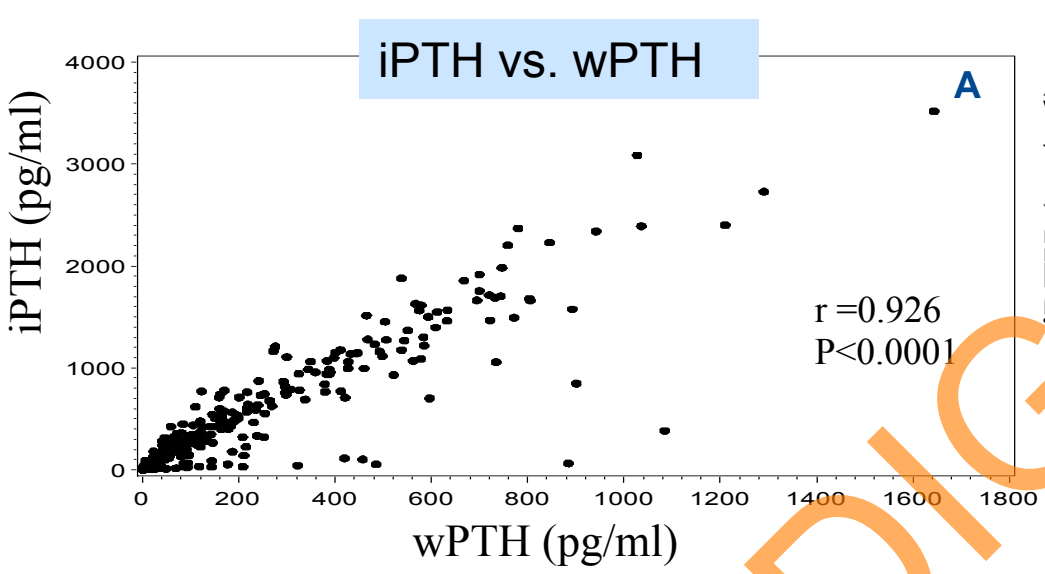
Statistics: Mean ± SD, Median. Spearman correlation coefficient
Logistic regression analysis to derive area under the Receiver operator characteristics (ROC) curve:

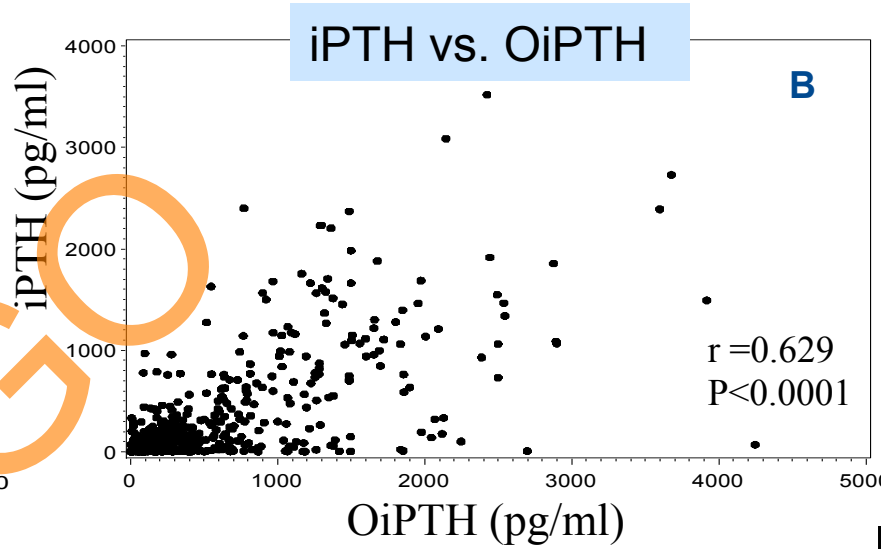
AUC>0.7: Very good; >0.8: Excellent; >0.9: Distinguished

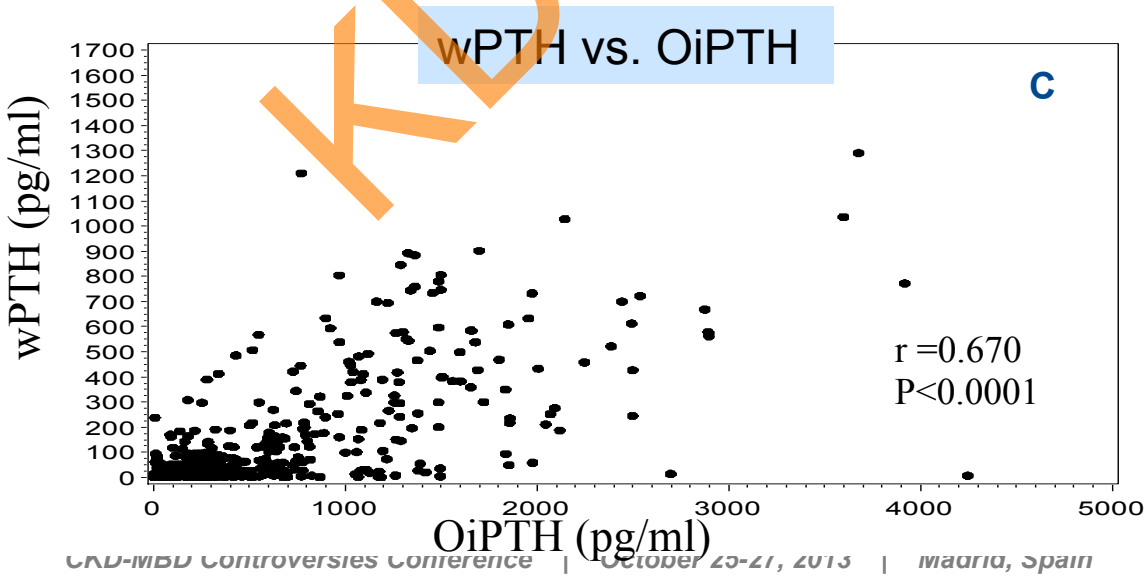
Patient characteristics

	Brazil N=156	Portugal N=106	Turkey N=196	Venezuela N=152	Entire Cohort N=610
Age (M±SE)	49±13	50±14	59±13	42±15	51±15
Gender (%Fem)	35	40	51	47	**44**
Race (%)					
Caucasian					**303 (49.7)**
Black					38 (6.2)
Asian					11 (1.8)
Hispanic					**201** (33.0)
Other					55 (9.0)
Unknown					2 (0.3)
Duration Dialysis	3.5 (0.4-19.5)	5.7 (0.6-19.9)	3.2 (0-21.1)	3.1 (0.2-11.2)	**3.7** (0-21.1)
Diabetes (%)	13	1	23	14	**14.4**
History of Transplant %	7	11	5	15	8.9
Active Vit D therapy	42	44	4	28	27

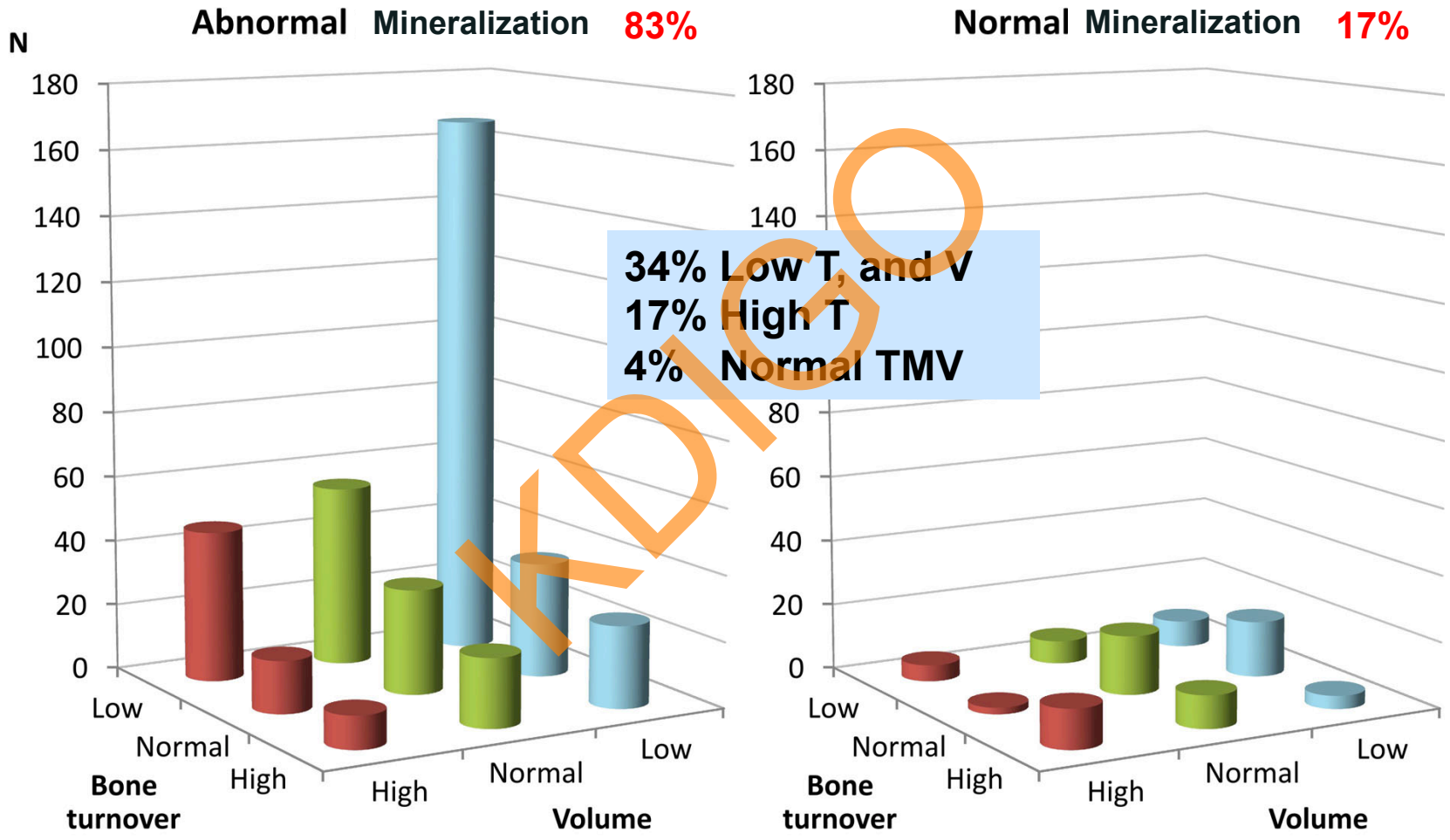
Correlation of central lab serum iPTH, and wPTH with original iPTH (OiPTH) at the time of the biopsy







Distribution of the histologic findings according to TMV classification



Total N= 489

iPTH, wPTH, BSAP and PINP stratified by bone turnover

	Bone Turnover (BFR/BS)		
	Low M±SE (Median)	Normal M±SE (Median)	High M±SE (Median)
iPTH (pg)ml)	180 ± 288 (69)	444.4 ± 576.4[a] (179)	594 ± 609[a,b] (382)
wPTH (pg/ml)	69 ± 121 (24)	202 ± 250[a] (69)	226 ± 260[a] (106)
BSAP (U/L)	51.4 ± 84.9 (28.1)	143.6± 229.3[a] (59.8)	99.0 ± 100.5[a] (63.3)
PINP	475 ± 509 (350)	914 ± 2454 (482)	828 ± 728[a,b] (787)

[a] Different from Low

[b] Different from Normal

AUC of circulating bone markers to predict bone turnover as assessed by BFR/BS

BFR/BS	Blood Marker	N	AUC[a]	Best Cut-Off[a]	AUC[b]	Best Cut-Off[b]	AUC[c]	AUC[d]
Low N=289	iPTH (pg/ml)	280	0.653	103.7			**0.702**	
	wPTH (pg/ml)	260	0.696	47.1				**0.726**
	BSAP (U/L)	273	**0.738**	37.4				
	S-PINP (ng/ml)	280	0.569	434.5				
	OiPTH (pg/ml)	285	0.615	280				
Normal N= 120	iPTH (pg/ml)	115						
	wPTH (pg/ml)	105						
	BSAP (U/L)	113						
	S-PINP (ng/ml)	116						
	OiPTH (pg/ml	119						
High N= 83	iPTH (pg/ml)	81						
	wPTH (pg/ml)	75						
	BSAP (U/L)	77						
	S-PINP (ng/ml)	81						
	OiPTH (pg/ml	81						

- Only BSAP achieved an acceptable level to discriminate between low from normal, or high from normal bone turnover (AUC[a])
- BSAP marginally improved the AUC for iPTH to discriminate low from normal (AUC[c])
- BSAP improved the AUC for wPTH to discriminate low from normal turnover (AUC[d])

[a]low vs. normal
[b]high vs. normal
[c]combine iPTH and BSAP
[d]combine wPTH and BSAP

AUC of bone markers to predict low bone turnover from non-low turnover as assessed by BFR/BS

BFR/BS	Blood Marker	N	AUC	Best Cut-Off	AUC[a]	AUC[b]
Low (N289)	iPTH (pg/ml)	280	**0.701**	**103.7**	**0.728[a]**	**0.734[b]**
	wPTH (pg/ml)	260	**0.712**	**47.1**		
	BSAP (U/L)	273	**0.757**	**39.8**		
	S-PINP (ng/ml)	280	0.650	497.3		
	OiPTH (pg/ml)	285	0.661	306		
Non-Low (N= 203)	iPTH (pg/ml)	196				
	wPTH (pg/ml)	180				
	BSAP (U/L)	190				
	S-PINP (ng/ml)	197				
	OiPTH (pg/ml	200				

[a]combined iPTH and BSAP
[b]combined wPTH and BSAP

- **Cutoff value to predict low turnover:**
- iPTH <103.7 pg/ml would have an AUC of 0.701
- wPTH < 47.1 pg/ml, AUC of 0.712
- BSAP < 39.8 UI/L, AUC 0.757
- BSAP with either iPTH or wPTH would slightly improve the sensitivity / specificity of either iPTH or wPTH

AUC of bone biomarkers to predict high turnover from non-high turnover as assessed by BFR/BS

AUC: area under the ROC curve

BFR/BS	Blood Marker	N	AUC	Best Cut-Off	AUC[a]	AUC[b]
Non-High (N=409)	iPTH (pg/ml)	395	**0.724**	**242.7**	**0.710**	0.638
	wPTH (pg/ml)	365	0.628	61.5		
	BSAP (U/L)	386	**0.711**	**42.3**		
	S-PINP (ng/ml)	396	**0.743**	**622.6**		
	OiPTH (pg/ml)	404	0.681	359		
High (N= 83)	iPTH (pg/ml)	81				
	wPTH (pg/ml)	75				
	BSAP (U/L)	77				
	S-PINP (ng/ml)	81				
	OiPTH (pg/ml	81				

[a]**combined iPTH and BSAP**
[b]**combined** wPTH and BSAP

- **To predict high bone turnover:**
- iPTH > 242.7 pg/ml, AUC of 0.724
- wPTH did not predict high bone turnover
- BSAP > 42.3 U/L, AUC of 0.711
- PINP > 622.6 ng/ml, AUC of 0.743
- Combining BSAP or PINP with either iPTH or wPTH did not improve the sensitivity/ specificity of either parameter alone.

Sensitivity and specificity of PTH to predict bone turnover as assessed by BFR/BS

	Sensitivity	Specificity
iPTH<150 pg/ml for low turnover	68.6%	61.2%
iPTH>300 pg/ml for high turnover	58.0%	**77.7%**
iPTH <2 ULN for low turnover	65%	67.3%
iPTH >9 ULN for high turnover	37%	**85.8%**
wPTH <2 ULN for low turnover	73.5%	56.7%
wPTH >9 ULN for high turnover	30.7%	**87.9%**

BFR/BS, bone formation rate
i**PTH,** intact parathyroid hormone
wPTH, whole parathyroid hormone
ULN, upper limit of normal values

Utility of KDOQI and KDIGO PTH thresholds for diagnostic decision making

	KDOQI*				KDIGO+			
	Sensitivity %	Specificity %	PPV %	NPV %	Sensitivity %	Specificity %	PPV %	NPV %
Differentiating low tunover from non-low turnover bone disease or "When do I stop therapy"	68.5	61.2	71.6	57.7	65.7	65.3	**73**	**57**
Diferentiating high turnover from non-high turnover bone disease or "When do I start therapy"	58	**77.7**	34.8	90	37	**85.8**	34.9	**86.9**

NPV, negative predictive value; **PPV**, positive predictive value.

*Using serum iPTH <150 pg/ml for lower and >300 pg/ml for upper threshold

+Using serum iPTH <130 pg/ml for lower and >585 pg/ml for upper threshold (2X and 9X ULN limit of normal for assay)

Summary

- The present and other recent studies support the use of the TMV system to evaluate ROD
- There was excellent correlation between iPTH and wPTH
- None of the markers achieved acceptable minimal criteria (AUC >0.7) to discriminate low from normal or high from normal BFR/BS
- In contrast, the ability to discriminate low from non-low BFR/BF was achieved with iPTH, wPTH, BSAP, with AUC curve >0.7
- ROC based discrimination in differentiating low from non-low and high from non-high turnover was significant, but the clinical utility of these markers requires further prospective studies
- The identification of new biomarkers with higher discriminatory ability may help to improve diagnosis of each type of ROD


KDIGO