

27th BCLF Meeting, 29 October 2019, Antalya, Turkey

# Significance of the determination of biomarkers of bone resorption and formation in patients with end stage renal disease



Teaching Assistant, Neda Milinković, PhD  
Specialist of medical biochemistry

Faculty of Pharmacy  
University of Belgrade

## • SPECIFIC BIOMARKERS

1. Bone isoenzyme of alkaline phosphatase (**BALP**)
2. Tartaric resistant acid phosphatase (**TRAP**)
3. Beta-carboxy terminal telopeptide of collagen type I, (**beta-CrossLaps, beta-CTx**)

## • RECOMMENDED BIOMARKERS

1. Alkaline phosphatase (**ALP**)
2. Total calcium (**Ca**)
3. Inorganic phosphate (**P**)
4. Calcium-phosphate product (**Ca $\times$ P**)
5. Magnesium (**Mg**)
6. Intact parathyroid hormone (**iPTH**)
7. 25-hydroxy vitamin D (**25D**)

## ➤ Aims of this study:

1. Distribution of the analyzed biomarkers in ESRD patients in relation to gender, age, duration and type of dialysis
2. Analysis of the concentrations of specific and recommended biomarkers in relation to iPTH and 25D
3. Diagnostic value of the analyzed biomarkers for the assessment of adynamic bone disease

## ❑ Material and methods

- ❑ Predialysis patients (PD) (N=40) (18 women, 22 men)  
GFR  $<15 \text{ mL} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-1}$
- ❑ Hemodialysis patients (HD) (N=112)  
(53 women, 59 men) (duration ~ 76 months)
- ❑ Patients on continuous ambulatory peritoneal dialysis (CAPD)  
(N=114) (49 women, 65 men) (duration ~ 25 months)
- ❑ Control group participants (CG) (N=50) (27 women, 23 men)

# Biomarkers concentrations in ESRD patients in relation to gender

## PD patients

## HD patients

## CAPD patients

Biomarker	Women	Man	P*
ALP, IU/L	82 ± 63,1	80 ± 32,3	0,169
BALP, IU/L	38 ± 17,3	32 ± 15,2	0,706
TRAP, IU/L	7 ± 1,4	8 ± 1,1	0,178
Beta-CTx, ng/mL	3,51 ± 0,803	3,74 ± 0,872	0,852
Ca, mmol/L	2,13 ± 0,244	2,06 ± 0,166	0,077
Mg, mmol/L	0,86 ± 0,132	0,84 ± 0,153	0,604
P, mmol/L	1,44 ± 0,271	1,67 ± 0,367	0,275
CaxP, mmol <sup>2</sup> /L <sup>2</sup>	3,5 ± 0,63	3,7 ± 0,65	0,762
iPTH, pg/mL	<b>408,5 ± 211,62</b>	<b>348,8 ± 173,72</b>	<b>&lt; 0,05</b>
25D, nmol/L	30,7 ± 12,31	31,7 ± 9,39	0,101

Biomarker	Women	Men	p*
ALP, IU/L	57 ± 17,4	58 ± 18,2	0,796
BALP, IU/L	21 ± 12,1	22 ± 11,7	0,607
TRAP, IU/L	8 ± 1,6	8 ± 1,4	0,495
Beta-CTx, ng/mL	4,03 ± 1,184	3,90 ± 1,073	0,560
Ca, mmol/L	2,38 ± 0,132	2,38 ± 0,151	0,963
Mg, mmol/L	0,98 ± 0,117	1,01 ± 0,126	0,157
P, mmol/L	<b>2,03 ± 0,274</b>	<b>1,40 ± 0,221</b>	<b>&lt; 0,05</b>
CaxP, mmol <sup>2</sup> /L <sup>2</sup>	4,1 ± 1,15	3,8 ± 1,04	0,240
iPTH, pg/mL	<b>88,6 ± 71,97</b>	<b>136,7 ± 86,64</b>	<b>&lt; 0,05</b>
25D, nmol/L	30,9 ± 20,48	31,6 ± 21,62	0,866

Biomarker	Women	Men	P*
ALP, IU/L	53 ± 16,1	57 ± 16,4	0,239
BALP, IU/L	20 ± 11,3	23 ± 10,2	0,140
TRAP, IU/L	6 ± 1,7	6 ± 2,6	0,660
Beta-CTx, ng/mL	3,47 ± 0,680	3,56 ± 0,831	0,574
Ca, mmol/L	2,29 ± 0,192	2,26 ± 0,154	0,426
Mg, mmol/L	0,93 ± 0,186	0,93 ± 0,147	0,967
P, mmol/L	1,60 ± 0,424	1,49 ± 0,309	0,099
CaxP, mmol <sup>2</sup> /L <sup>2</sup>	<b>3,7 ± 0,85</b>	<b>3,4 ± 0,69</b>	<b>&lt; 0,05</b>
iPTH, pg/mL	<b>167,2 ± 132,4</b>	<b>174,5 ± 143,3</b>	<b>&lt; 0,05</b>
25D, nmol/L	46,2 ± 10,64	47,6 ± 12,76	0,540

\*P<0,05, level of significance

# Biomarkers concentrations in ESRD patients in relation to age

## PD patients

Biomarker	Age <60	Age >60	P*
ALP, IU/L	72 ± 30,2	84 ± 59,4	0,353
<b>BALP, IU/L</b>	<b>34 ± 17,5</b>	<b>32 ± 14,7</b>	<b>&lt; 0,05</b>
TRAP, IU/L	7 ± 1,1	7 ± 1,8	0,555
Beta-CTx, ng/mL	3,49 ± 0,835	3,81 ± 0,844	0,282
Ca, mmol/L	2,16 ± 0,247	2,13 ± 0,188	0,696
Mg, mmol/L	0,88 ± 0,151	0,86 ± 0,142	0,635
P, mmol/L	1,52 ± 0,334	1,52 ± 0,347	0,985
CaxP, mol <sup>2</sup> /L <sup>2</sup>	3,1 ± 0,61	3,1 ± 0,63	0,974
iPTH, pg/mL	337,3 ± 233,91	367,8 ± 140,61	0,627
25D, nmol/L	30,3 ± 8,85	32,6 ± 12,67	0,507

## HD patients

Biomarker	Age <60	Age >60	P*
ALP, IU/L	59 ± 18,3	55 ± 17,5	0,226
<b>BALP, IU/L</b>	<b>23 ± 11,3</b>	<b>20 ± 12,7</b>	<b>&lt; 0,05</b>
TRAP, IU/L	8 ± 1,1	8 ± 1,4	0,310
Beta-CTx, ng/mL	4,03 ± 1,145	3,91 ± 1,108	0,595
Ca, mmol/L	2,38 ± 0,151	2,38 ± 0,143	0,770
Mg, mmol/L	1,02 ± 0,122	0,98 ± 0,117	0,071
P, mmol/L	1,63 ± 0,404	1,70 ± 0,398	0,357
CaxP, mmol <sup>2</sup> /L <sup>2</sup>	4,0 ± 0,98	3,9 ± 1,20	0,686
iPTH, pg/mL	116,1 ± 80,33	113,8 ± 85,76	0,900
25D, nmol/L	32,6 ± 21,522	29,4 ± 20,31	0,441

## CAPD patients

Biomarker	Age <60	Age >60	P*
ALP, IU/L	55 ± 15,4	55 ± 17,2	0,816
<b>BALP, IU/L</b>	<b>23 ± 11,5</b>	<b>19 ± 9,8</b>	<b>p &lt; 0,05</b>
TRAP, IU/L	6 ± 2,1	6 ± 1,7	0,866
Beta-CTx, ng/mL	3,55 ± 0,753	3,51 ± 0,824	0,819
Ca, mmol/L	2,29 ± 0,177	2,26 ± 0,168	0,220
Mg, mmol/L	0,96 ± 0,154	0,90 ± 0,171	0,066
P, mmol/L	1,55 ± 0,344	1,54 ± 0,375	0,944
CaxP, mmol <sup>2</sup> /L <sup>2</sup>	3,5 ± 0,78	3,6 ± 0,77	0,350
iPTH, pg/mL	173,9 ± 125,72	163,1 ± 147,34	0,691
25D, nmol/L	47,4 ± 12,22	46,4 ± 10,76	0,647

\*P<0,05, level of significance

# Biomarkers concentrations in ESRD patients in relation to maintenance dialysis

## HD patients

Biomarker	< 30 months	> 30 months	P*
ALP, IU/L	64 ± 17,4	55 ± 17,3	< 0,05
BALP, IU/L	26 ± 14,2	20 ± 11,6	< 0,05
TRAP, IU/L	8 ± 1,1	7 ± 1,4	0,682
Beta-CTx, ng/mL	4,16 ± 0,916	3,92 ± 1,174	0,395
Ca, mmol/L	2,40 ± 0,123	2,37 ± 0,156	0,350
Mg, mmol/L	0,99 ± 0,114	1,00 ± 0,122	0,694
P, mmol/L	1,65 ± 0,461	1,67 ± 0,383	0,787
CaxP, mmol <sup>2</sup> /L <sup>2</sup>	4,2 ± 1,15	3,9 ± 1,08	0,208
iPTH, pg/mL	127,4 ± 60,75	111,7 ± 88,96	0,497
25D, nmol/L	33,7 ± 23,24	30,7 ± 20,47	0,540

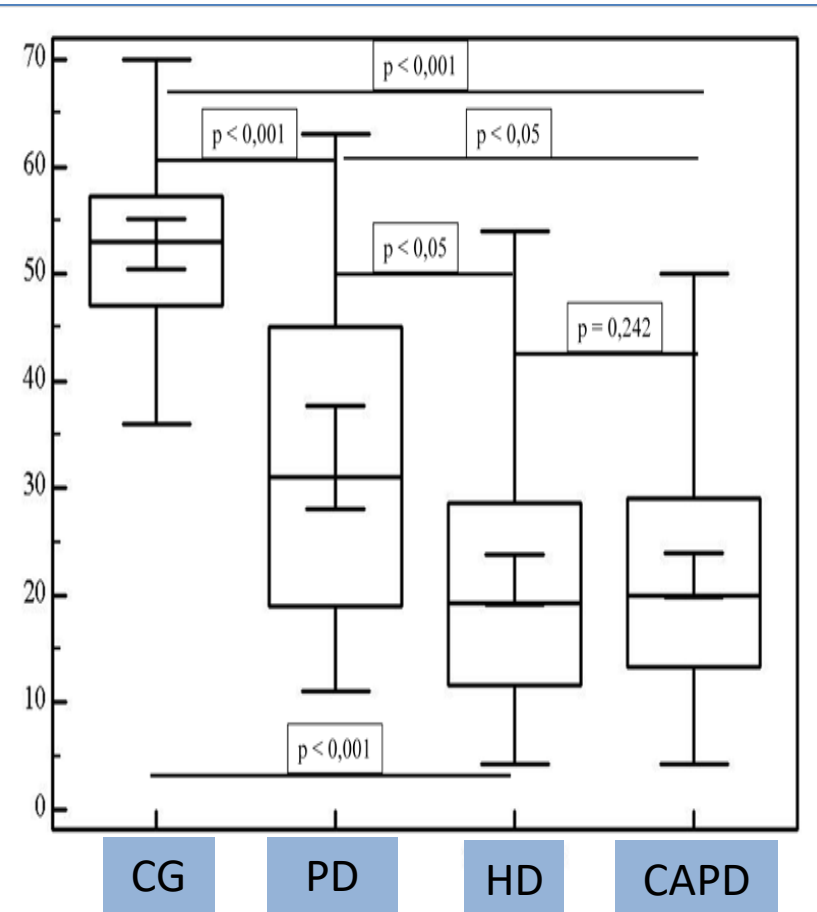
## CAPD patients

Biomarker	< 30 months	> 30 months	P*
ALP, IU/L	54 ± 17,4	57 ± 14,7	0,399
BALP, IU/L	21 ± 10,2	22 ± 10,6	0,476
TRAP, IU/L	6 ± 1,6	6 ± 1,1	0,320
Beta-CTx, ng/mL	3,58 ± 0,797	3,43 ± 0,755	0,389
Ca, mmol/L	2,29 ± 0,167	2,26 ± 0,193	0,383
Mg, mmol/L	<b>0,95 ± 0,156</b>	<b>0,88 ± 0,167</b>	<b>&lt; 0,05</b>
P, mmol/L	1,55 ± 0,355	1,53 ± 0,377	0,810
CaxP, mmol <sup>2</sup> /L <sup>2</sup>	3,5 ± 0,82	3,5 ± 0,66	0,963
iPTH, pg/mL	174,9 ± 144,75	154,8 ± 116,12	0,493
25D, nmol/L	46,5 ± 11,71	47,6 ± 11,23	0,657

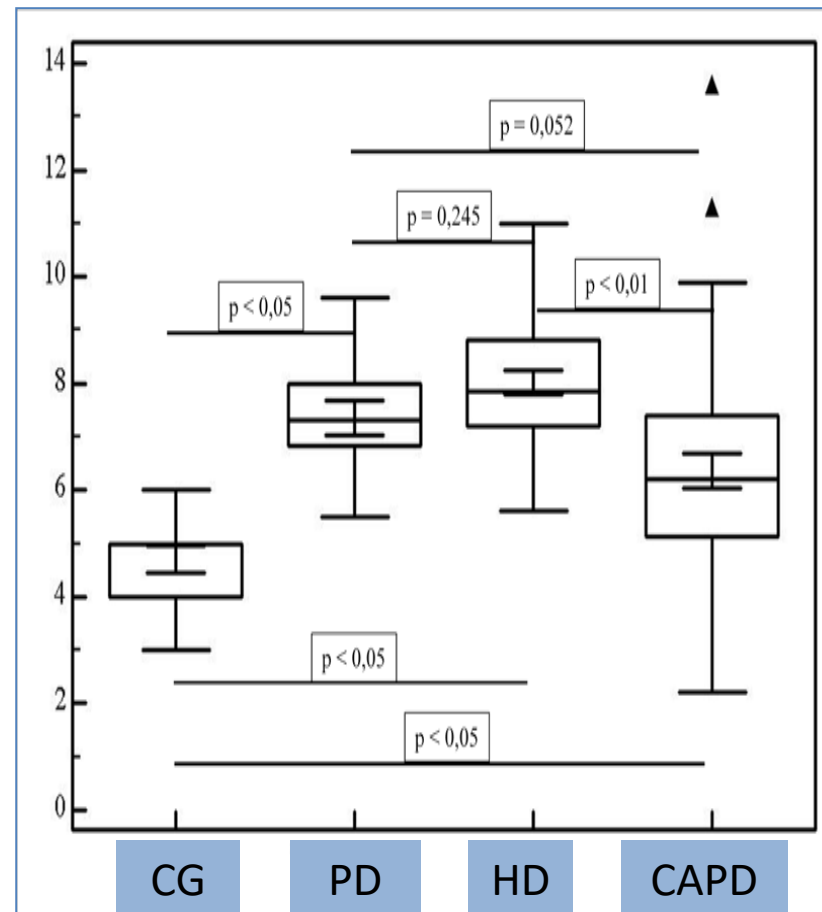
\*P<0,05, level of significance

# ESRD specific biomarkers concentrations in relation to control group (CG)

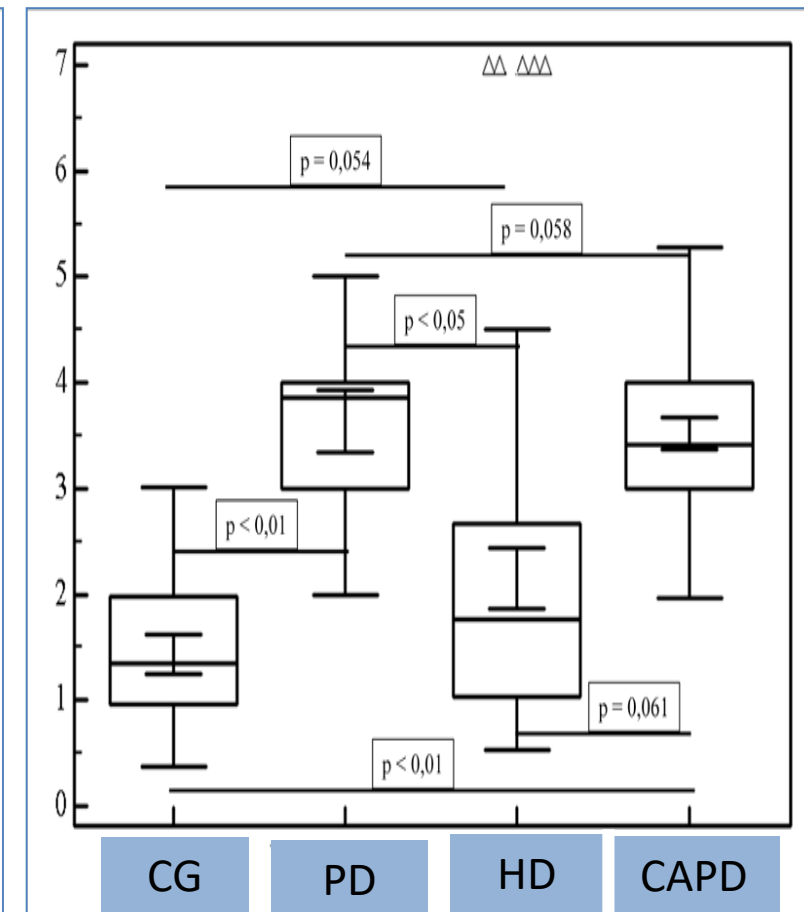
## BALP



## TRAP



## Beta-CTx

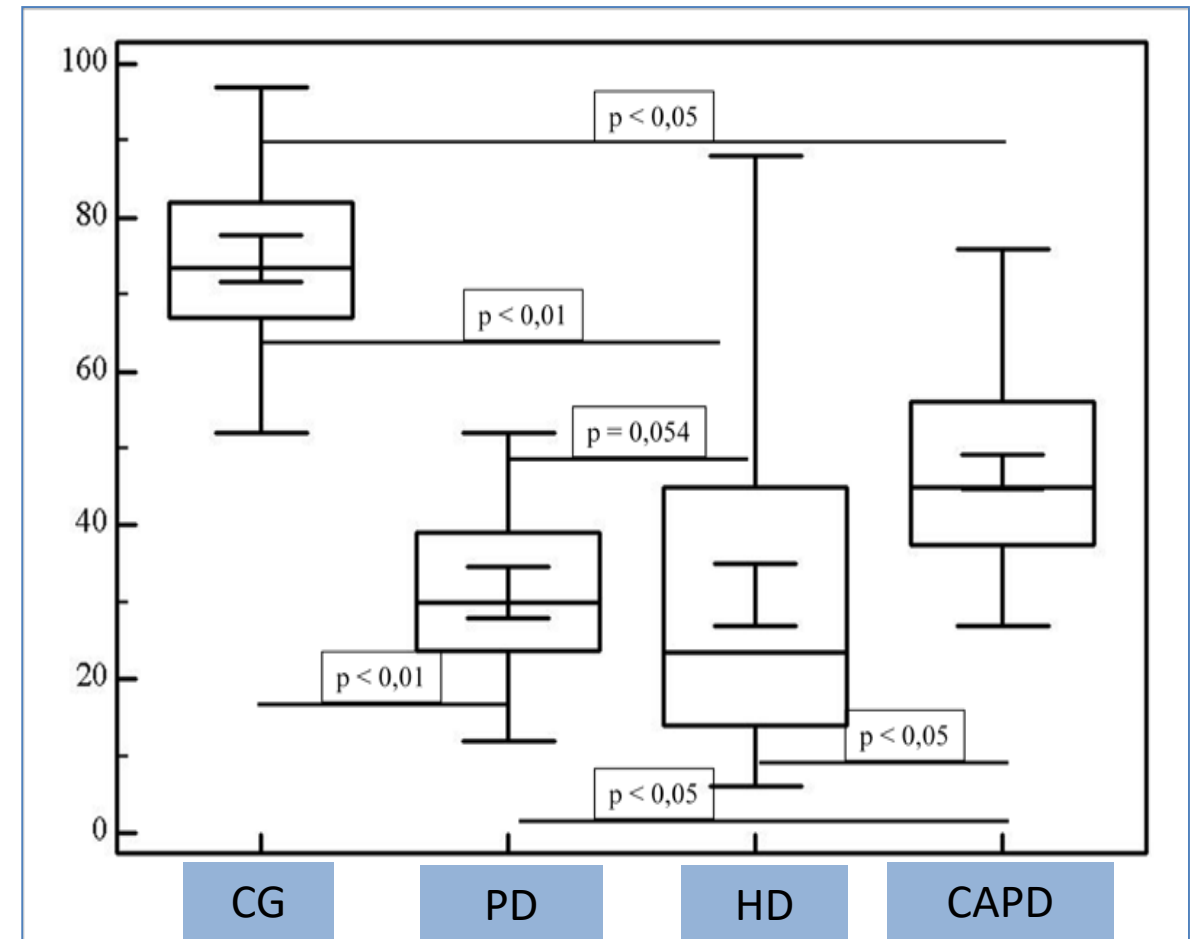
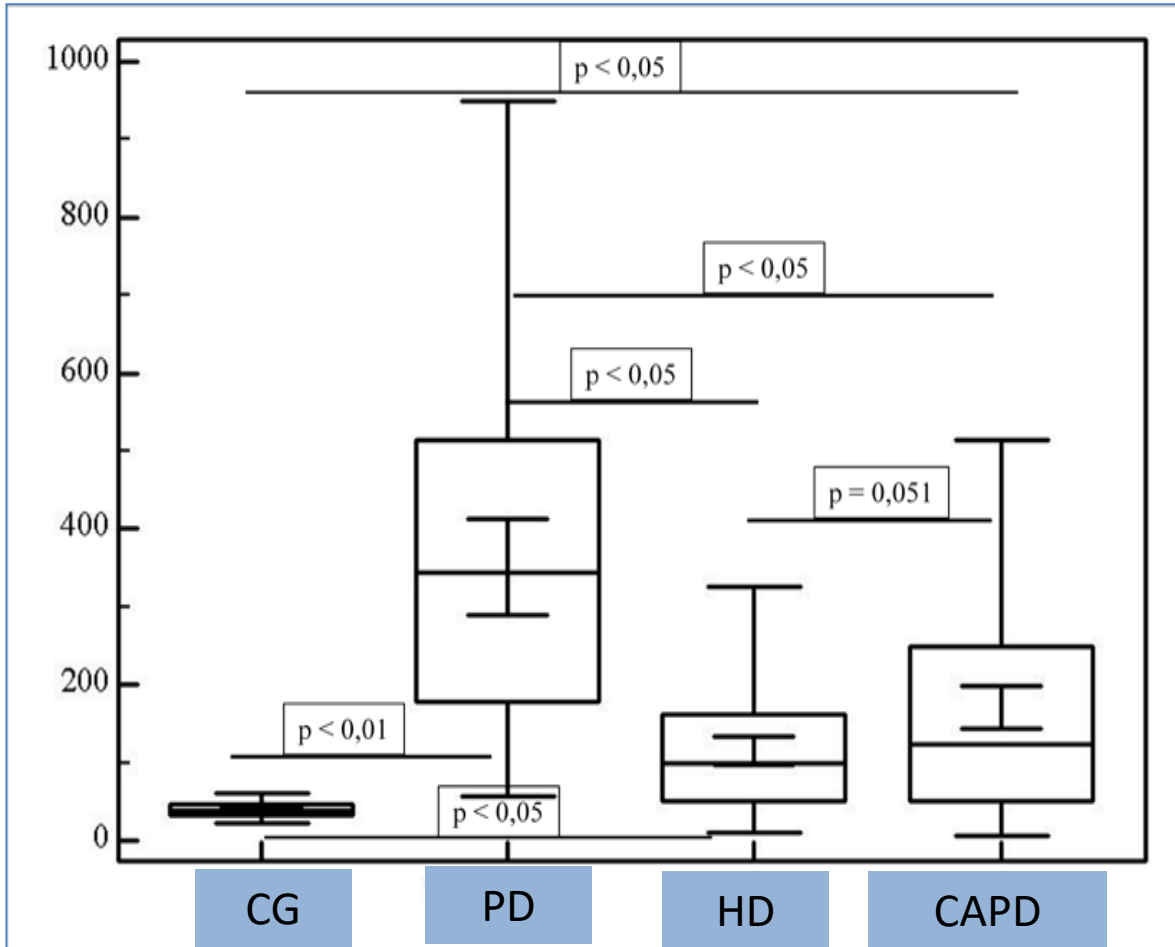




# ESRD recommended biomarkers concentrations in relation to control group

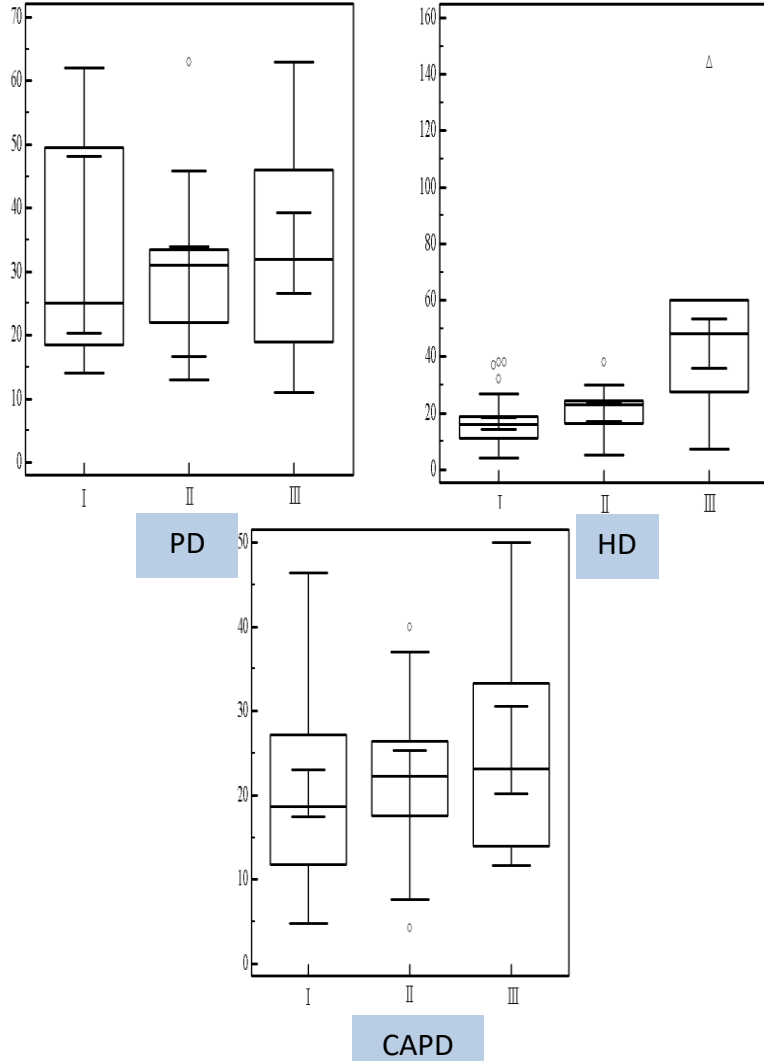
## iPTH

## 25D

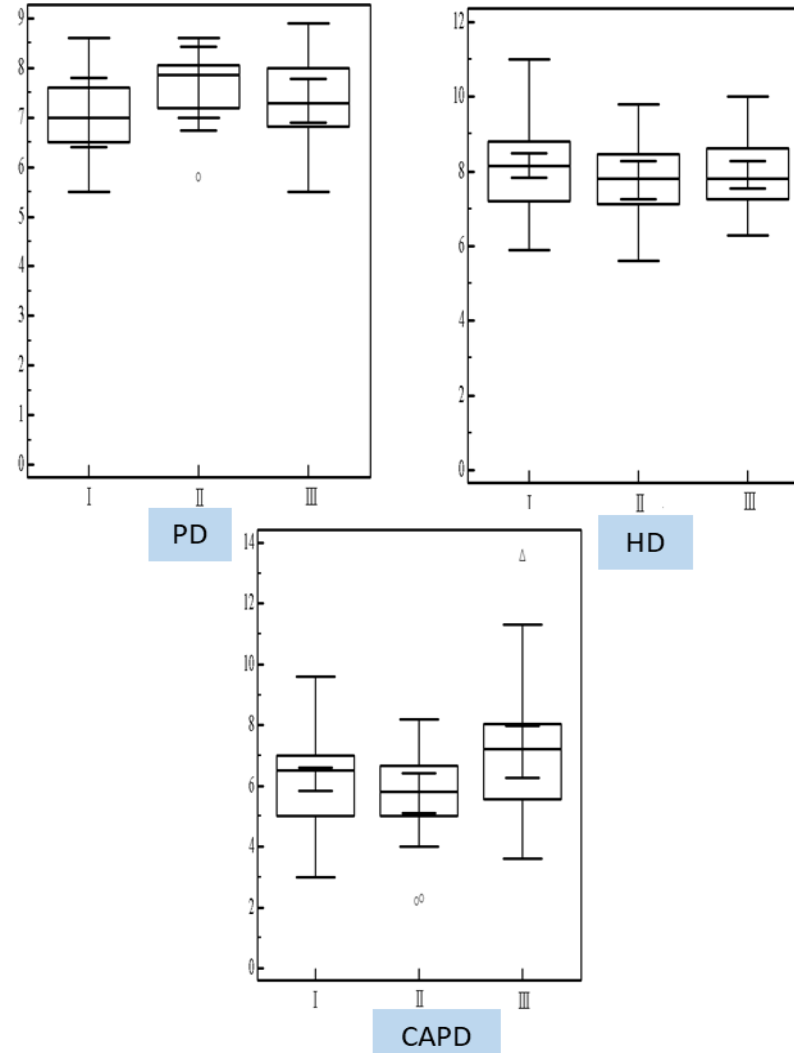


# BALP, TRAP and beta-CTx concentrations in relation to iPTH critical levels (I <150 pg/mL; II 150–300 pg/mL; III >300 pg/mL)

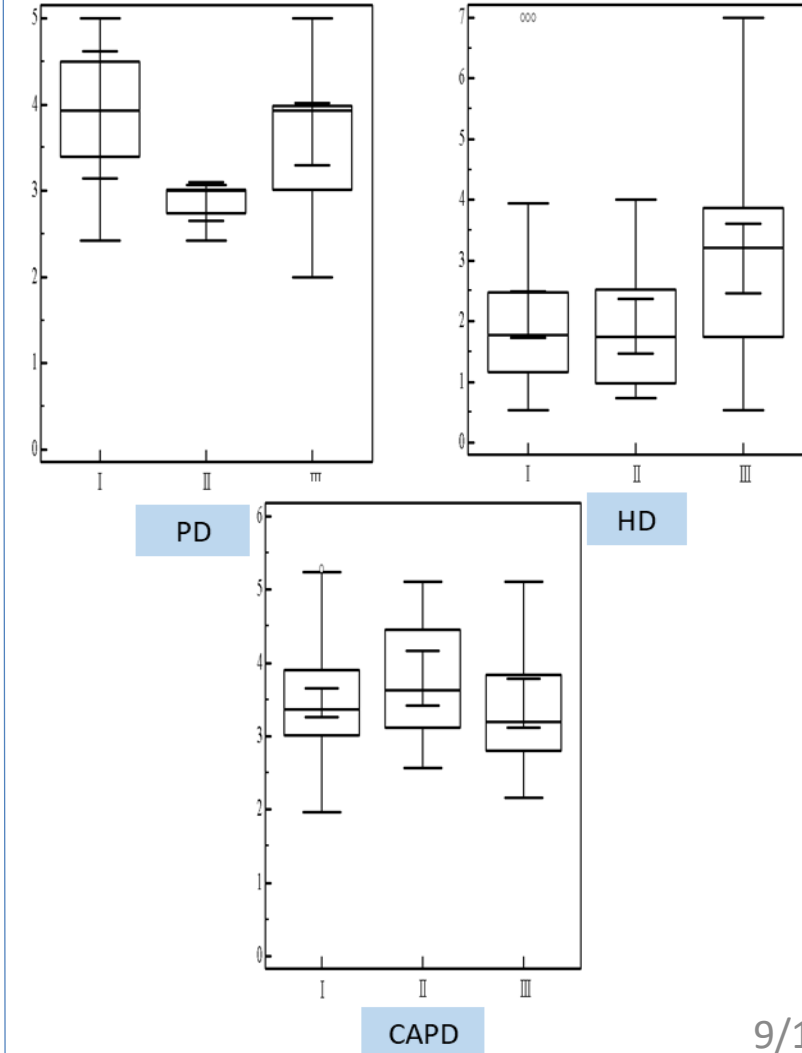
## BALP



## TRAP

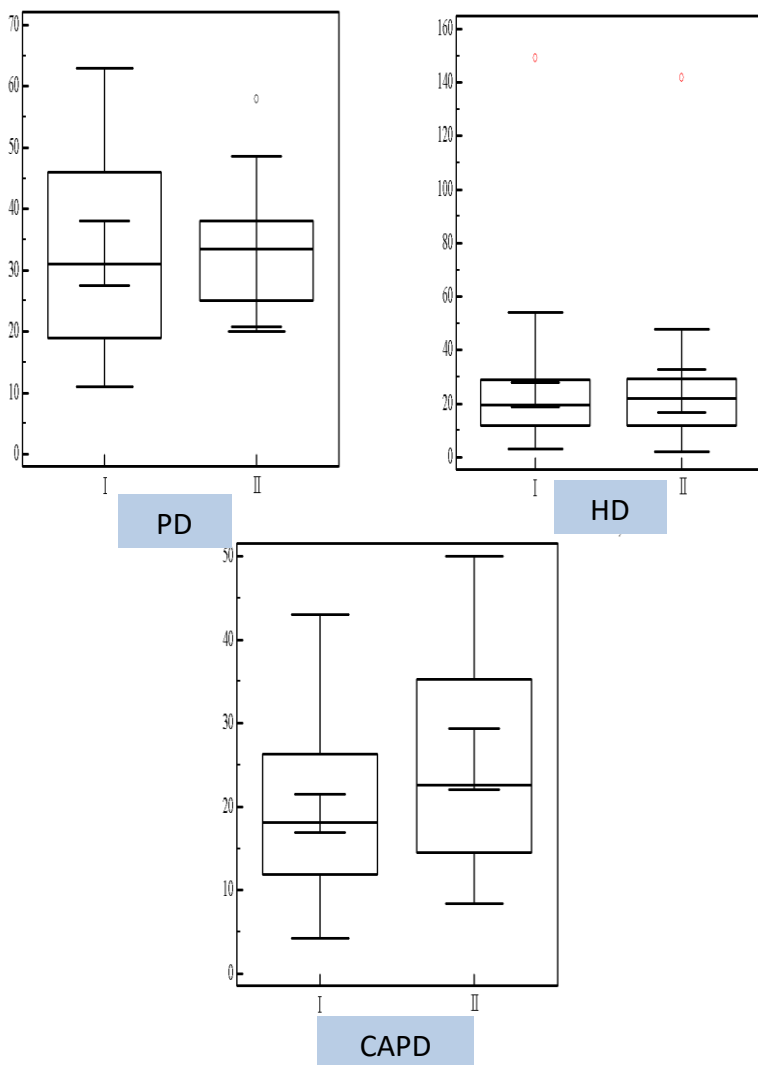


## beta-CTx

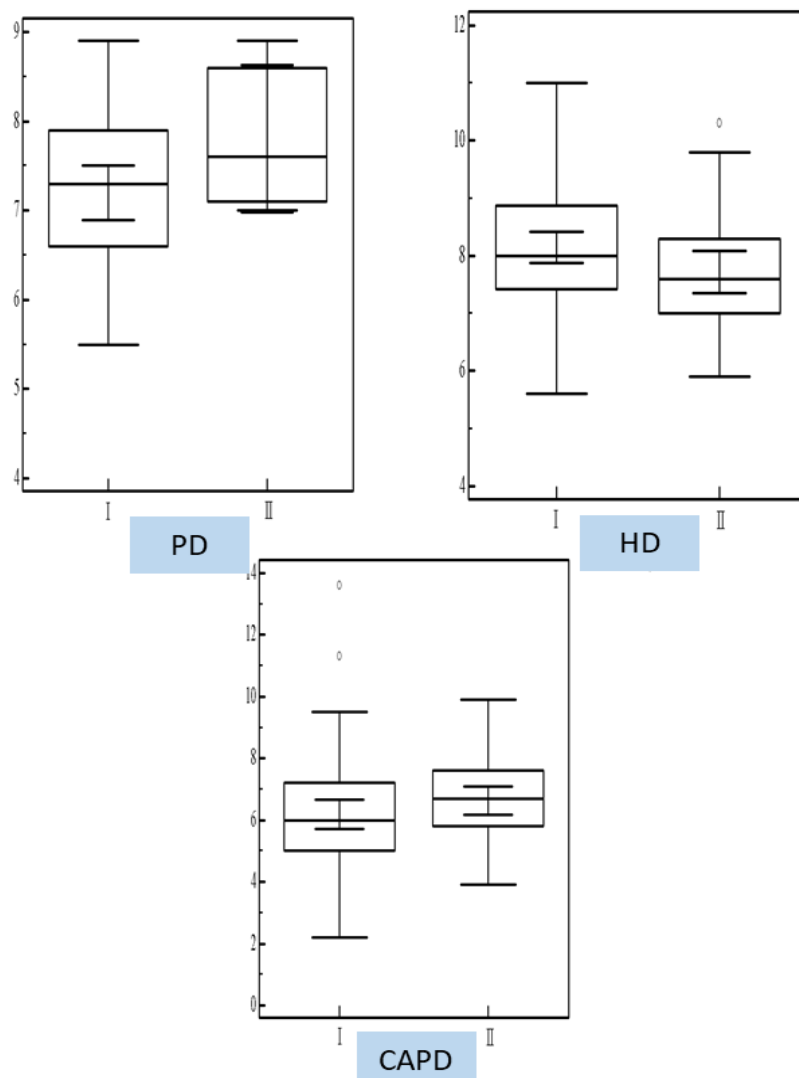


# BALP, TRAP and beta-CTx concentrations in relation to 25D critical levels (I < 50 nmol/L; II > 50 nmol/L)

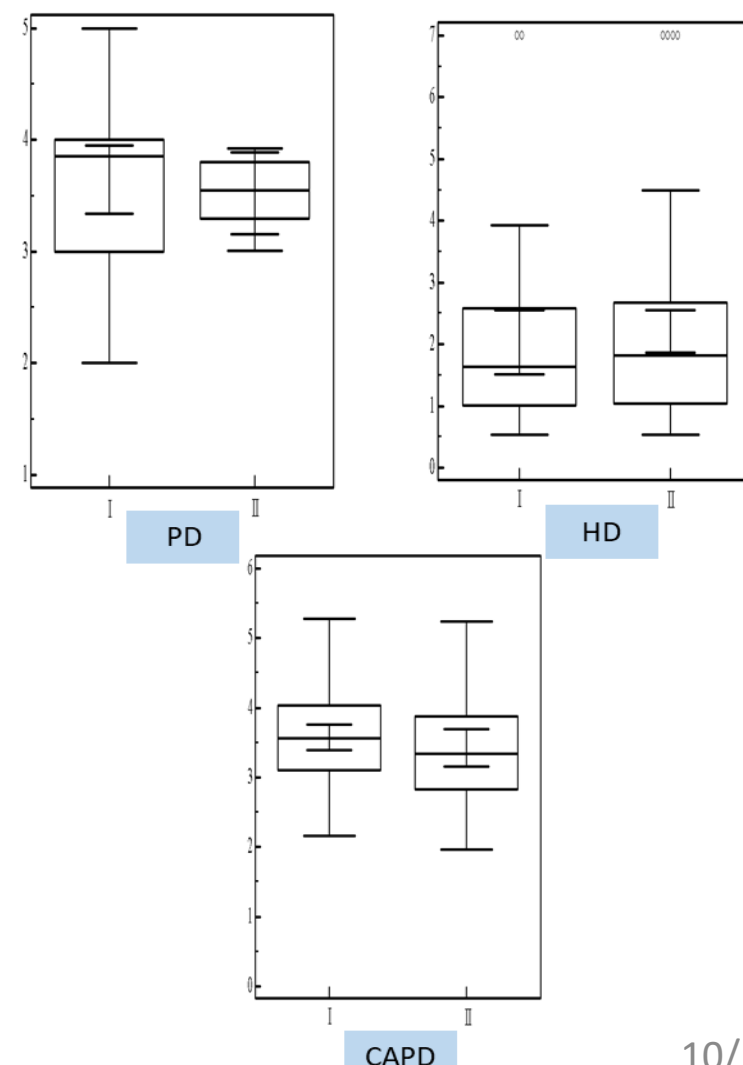
## BALP



## TRAP



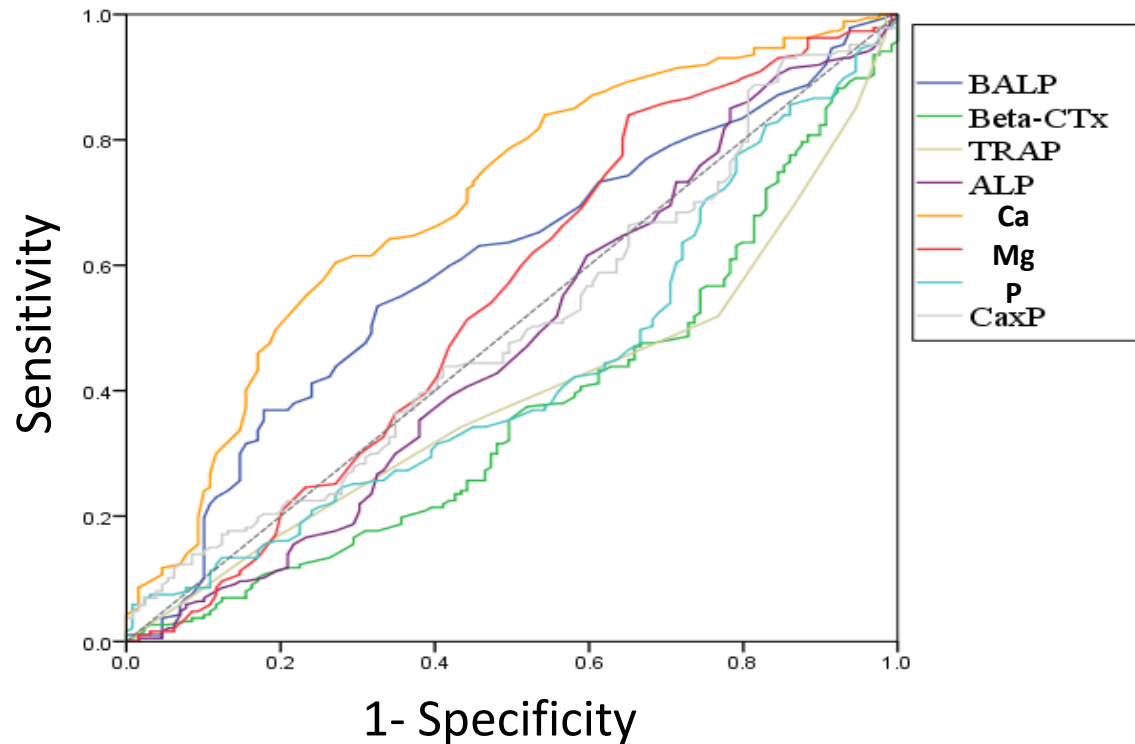
## beta-CTx



# Diagnostic value of the analyzed biomarkers for the assessment of adynamic bone disease

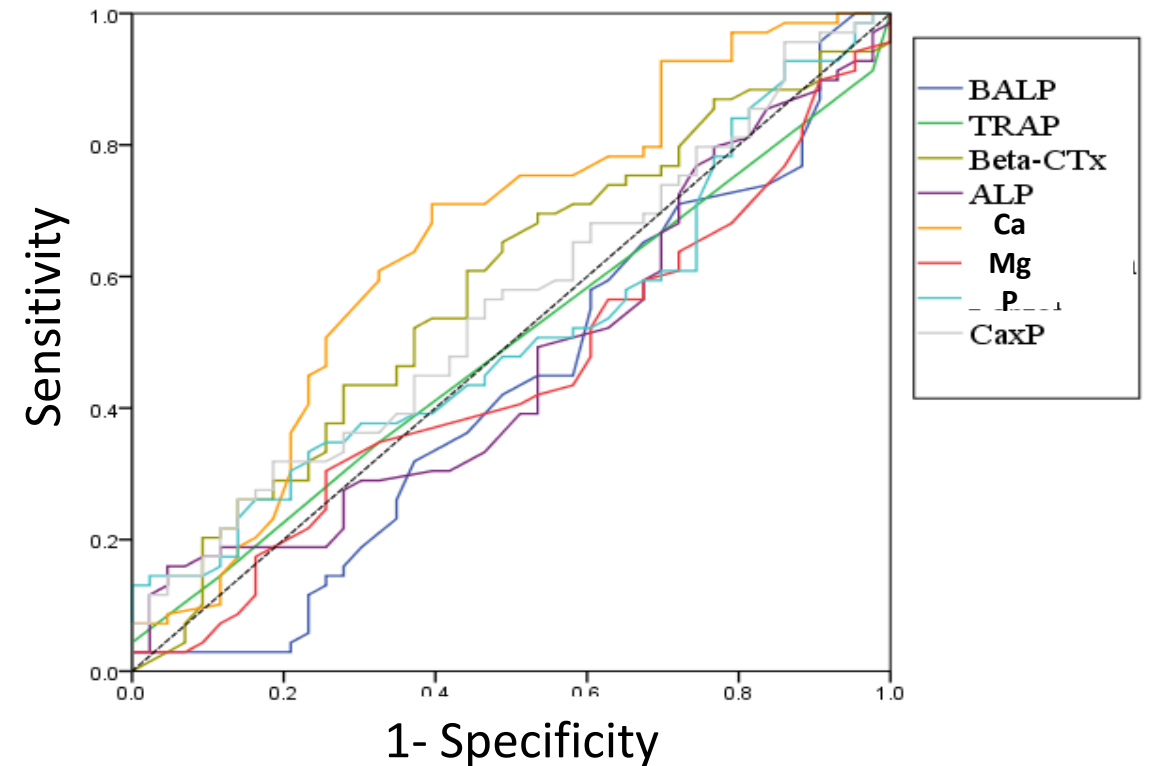
Critical iPTH concentration of <100 pg/mL for adynamic bone disease

ESRD patients



Ca cut off - 2,34 mmol/L; AUC (0,701)  
(67,9%, 73,8%) (p=0,0001)

HD patients

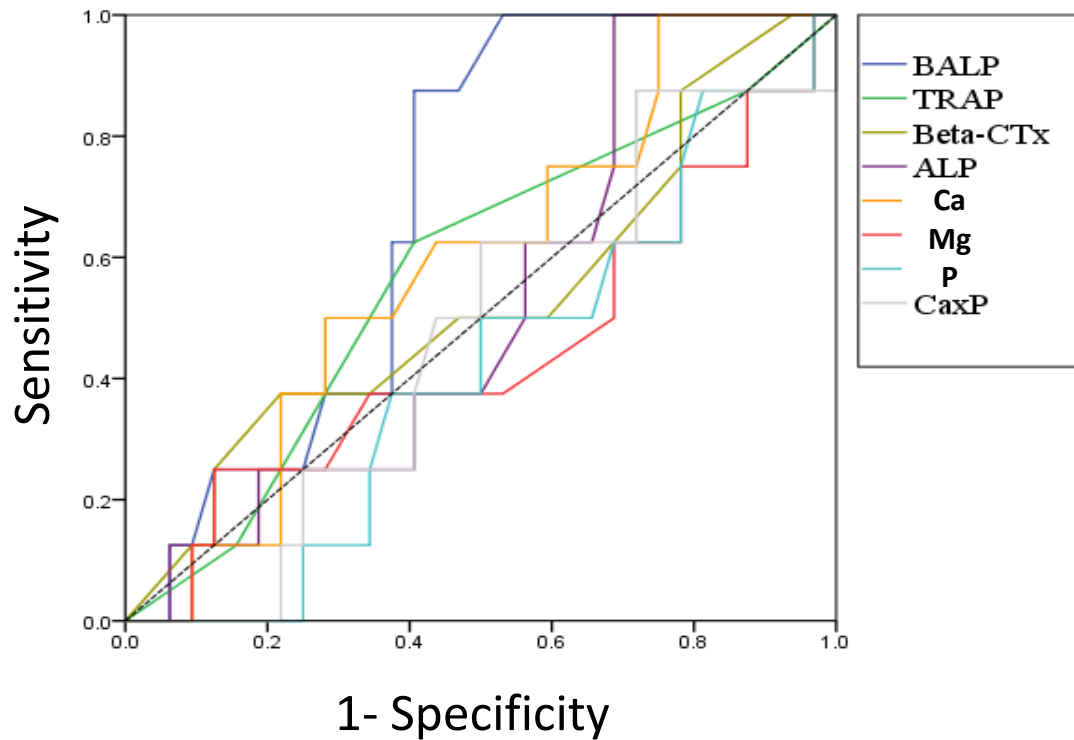


Ca cut off - 2,33 mmol/L; AUC (0,651)  
(69,2%, 73,4%) (p=0,0001)

# Diagnostic value of the analyzed biomarkers for the assessment of adynamic bone disease

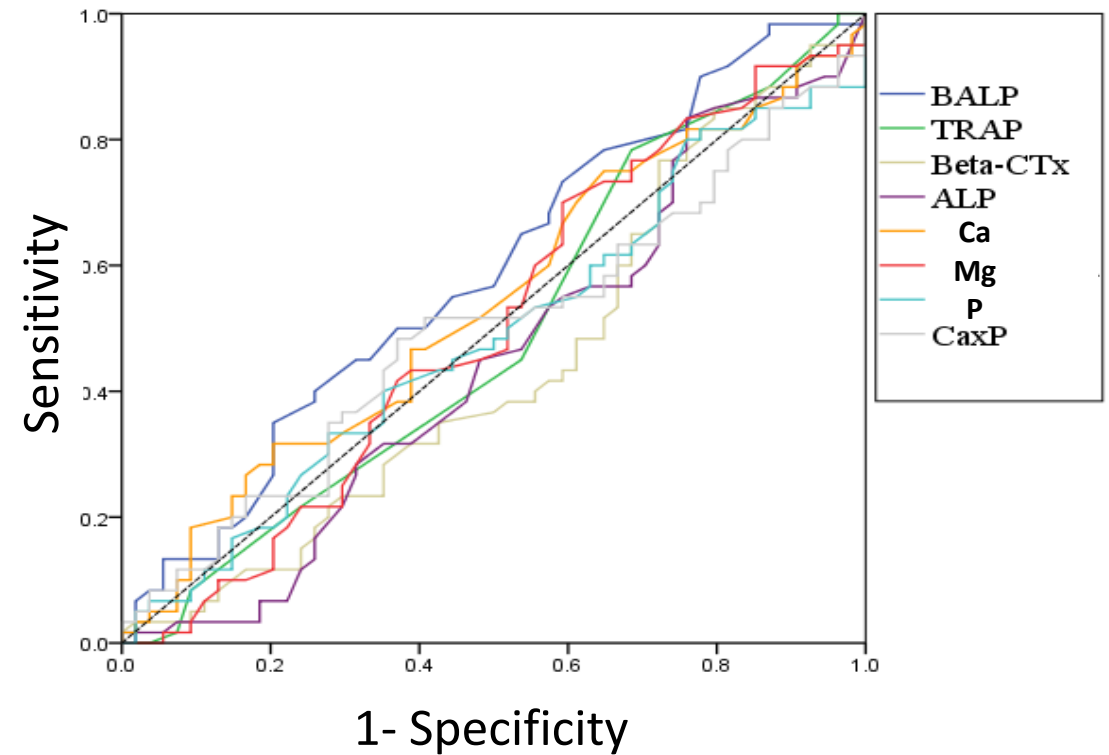
Critical iPTH concentration of <100 pg/mL for adynamic bone disease

Predialysis patients



**BALP** cut off - 27 IU/L; AUC (0,688)  
(84,4%, 58,2%) (p = 0,0001)

CAPD patients



**BALP** cut off - 25 IU/L; AUC (0,588)  
(64,1%, 62,6%) (p = 0,0001)

# Conclusion

- ✓ Determination of the analysed biomarkers - reliable
- ✓ Simultaneous evaluation of the specific and recommended biomarkers
- ✓ Harmonization of the used methods
- ✓ Necessary long-term studies
- ✓ Laboratory and physician collaboration

## ➤ Published literature:

1. Ležaić V, Tirmenstajn B, Bukvić D, Vujišić B, Perović M, **Novaković N**, Simić-Ogrizović S, Jovanović I, Marić I, Đukanović Lj. The role of calcium-phosphate abnormalities in the progression of chronic renal failure and development of bone and cardiovascular disease. *Hippokratia* 2006;10(Suppl 1) S6, IL8.
2. **Novaković N**, Dajak M, Pejanović S, Jovanović D, Majkić-Singh M. Poređenje biohemijskih markera koštanog prometa kod pacijenata na hemodijalizi i peritonealnoj dijalizi. *Jugoslov Med Biochem* 2007;26(3):215-219.
3. Ležaić V, Tirmenstajn-Janković B, Bukvić D, Vujišić B, Perović M, **Novaković N**, Dopsaj V, Marić I, Djukanović Lj. Efficacy of hyperphosphatemia control in the progression of chronic renal failure and the prevalence of cardiovascular calcification. *Clin Nephrol* 2009;71:21-29.
4. **Milinković N**, Majkić-Singh N, Mirković D, Beletić A, Pejanović S, Vujanić S. Relation between 25(OH)-vitamin D deficiency and markers of bone formation and resorption in haemodialysis patients. *Clin Lab* 2009;55:333–339.
5. **Milinković N**, Majkić-Singh N, Ignjatović S, Ležaić V, Pejanović S, Jovanović D. Correlation of bone alkaline phosphatase and iPTH with some basic biochemical markers in predialysis and dialysis patients. *Clin Lab* 2012;58:747–753.
6. **Milinković N**, Sarić Matutinović M, Pejanović S, Ignjatović. Comparison between bone alkaline phosphatase immunoassay and electrophoresis technique in hemodialysis patients. *J Med Biochem* 2019;38:1-6.

# Thanks for attention

