Bone Specific Alkaline Phosphatase and Bone Turnover in African American Hemodialysis Patients

Lenar Yessayan,[1,2] Carol Moore,[3] Mei Lu,[5] Jerry Yee[3,4]

[1]Division of Nephrology, [2]Department of Medicine, University of Michigan, Ann Arbor, MI

[3]Division of Nephrology, [4]Department of Internal Medicine, and [5]Department of Public Health Sciences, Henry Ford Hospital, Detroit, Michigan, USA

Corresponding author: Lenar Yessayan, MD MS, Clinical Associate Professor of Medicine, Division of Nephrology, University of Michigan

Address: 1500 E Medical Center Drive, 3916 C, Taubman Center, Ann Arbor, MI 48109

Phone: 1-734-936-6489; Fax: 1-734-232-4160; Email: lenar@med.umich.edu

Running head: Bone alkaline phosphatase in ESRD

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Abstract

Introduction: Noninvasive measures of bone activity include intact parathyroid hormone (iPTH) and bone-specific alkaline phosphatase (BSAP). Whether BSAP measurement alone or in combination with other biochemical data provides more reliable information about bone turnover than iPTH alone in African Americans on hemodialysis is unknown.

Methods: This cross-sectional study aimed to determine the optimal predictor and cutoff points for BSAP, iPTH, calcium and phosphorus in classifying bone biopsy findings. Forty-three African American hemodialysis patients were available for analysis. Biochemical data on the day of biopsy across a spectrum of qualitative histologic bone features were compared. Classification and regression tree analysis was used to determine both the optimal predictor and cutoff points for BSAP, iPTH, calcium and phosphorus in identifying bone turnover status.

Findings: Seven subjects had adynamic disease, 31 had mild/moderate hyperparathyroid bone features, and 5 had severe hyperparathyroid bone disease. BSAP was the optimal predictor of bone biopsy with a cutoff point of 22 ng/mL. Calcium and phosphorus had no predictive value. At BSAP ≤ 22 ng/mL, subjects had either adynamic bone disease or mild/moderate hyperparathyroid bone disease but iPTH was not useful in further classifying biopsy findings. When BSAP was >22 ng/mL, subjects had either mild/moderate or severe hyperparathyroid bone disease, and iPTH was useful in further classifying biopsy findings. With BSAP > 22 ng/mL and iPTH < 726 pg/mL, all subjects had mild/moderate bone turnover features.

Discussion: Compared to iPTH, BSAP was shown to be the optimal predictor of biopsy findings with an optimal cutoff at 22 ng/ml.

Key words: bone biopsy, bone specific alkaline phosphatase, adynamic bone disease, high

turnover bone disease, low turnover bone disease

INTRODUCTION

Renal bone disease manifests in derangements in bone histologic findings such as abnormalities in bone turnover, mineralization, and volume.[1] Bone histology is the "gold standard" for definitive diagnosis of the osteodystrophic lesion; however, because it is a painful, costly procedure, nephrologists rely mainly on intact parathyroid hormone (iPTH) measurements to predict bone histology and guide treatment. The 2009 Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease state that measurements of iPTH and bone-specific alkaline phosphatase (BSAP) may be used to evaluate bone disease, because high or low values predict underlying bone turnover. KDIGO recommends maintaining iPTH approximately 2 to 9 times the upper limit of normal, but no specific target value or range has been proposed for BSAP.[2] Although iPTH is used more often as a marker of bone turnover in clinical practice, use of BSAP has been suggested recently because of its lower biologic variability and better clinical outcome predictability.[3-5]

Ethnicity has an important role in many aspects of bone and mineral metabolism including the relationship between markers of bone turnover and bone histology. Serum iPTH is higher in African Americans irrespective of the type of bone remodeling. Of several studies that compared BSAP and iPTH with bone histomorphometry, all except 1 were

predominantly in white patients. African Americans in particular have a lower bone turnover than whites across a wide range of iPTH levels,[6] a condition that should not be taken lightly given the association of low turnover bone disease with cardiovascular calcification[7] and vertebral and hip fractures in the end-stage renal disease population.[8, 9] Serum iPTH shows significant correlations to bone formation in white patients but not in African American patients.[10] Therefore, it is important to explore the role of markers other than iPTH for the assessment and treatment of bone and mineral disorders in this population.

The aim of this study was to describe the relationship of BSAP to bone histology across the spectrum of renal osteodystrophy in prevalent African American end-stage renal disease patients on hemodialysis. We also aimed to determine the optimal predictor and cutoff points for the 2 noninvasive measures of bone activity, iPTH and BSAP, in the classification of biopsy findings in African American hemodialysis patients.

MATERIALS AND METHODS

This cross-sectional study utilized data collected for a previous study that evaluated the relationship of bone histology to iPTH and other biochemical markers.[11] The original study, conducted at 3 urban Detroit hemodialysis units, was approved by the Henry Ford Hospital Institutional Review Board and informed consent was obtained from each subject. Approval was also granted for the current study and informed consent for this secondary analysis was waived. The study followed the Declaration of Helsinki on medical protocol and ethics. Inclusion/exclusion criteria, clinical data collection,

biochemical data collection, and bone biopsy methods were described previously.[11] Briefly, bone biopsy was performed on consenting subjects within the 3 dialysis units, and biochemical measurements were collected at the time of biopsy and correlated to biopsy findings. Qualitative histologic assessment was made using the conventional classification.[12]

Statistical analyses

For this study we used classification and regression tree (CART) analysis to develop a prediction model for bone histology.[4] The prediction model was generated using BSAP, iPTH variables, corrected calcium and phosphorus obtained on the day of biopsy. TAP was not included in the model given BSAP is a subset of TAP and more specific to bone than TAP. CART analysis, or binary recursive partitioning, uses a nonparametric analytical approach that identifies the variables most predictive of the outcome of interest and develops a predictive model for classifying future subjects. CART analysis starts with the root node (all subjects) and then determines which variable has the highest predictive ability. Subsequently, CART determines the optimal cut point for this variable that partitions the population into 2 child nodes with explicitly different outcomes. Every value of the predictor variable is evaluated for its potential as a split value, and the program determines the most predictive splitter. Each child node can then itself become a parent node that produces its own child nodes. The process continues to classify the subjects until no further variables of predictive importance are observed.[13] CART analysis was conducted using CART 6.0 software (Salford Systems, San Diego, CA). Continuous variables are presented as mean with standard deviation or median with

interquartile range where appropriate. Categorical variables are expressed as frequencies and percentages. Continuous variables were compared using one-way Analysis of Variance (ANOVA), and categorical variables were compared using the Fisher exact test. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

A total of 43 African American hemodialysis subjects were enrolled into the study. Qualitative bone histology classified subjects in three groups: 7 with adynamic bone disease (ABD), 31 with mild/moderate hyperparathyroid bone disease (mild/moderate HPT) and 5 with severe hyperparathyroid bone disease (severe HPT). There were no cases of normal bone histology, osteomalacia, or mixed uremic renal osteodystrophy. Bone formation rate/bone surface (mm^3/cm^2 per yr) was 1.66 ± 0.66 in the adynamic category, 4.53 ± 1.94 in mild/mod HPT and 8.67 ± 2.30 in severe HPT (normal values 1.80 to 3.80). Activation frequency (yr^{-1}) was 0.27 ± 0.11 in the adynamic category, 0.89 ± 0.32 in mild/mod HPT and 1.67 ± 0.20 in severe HPT (normal value 0.49 to 0.72).

Population characteristics did not differ by bone turnover status.[11] Mean age was 53.7 ± 11.6 years, dialysis vintage was 40.4 ± 24.5 months, 23 were male (51%), 13 were diabetic (30%) and 7 had history of fractures (16%). Most subjects were receiving intravenous vitamin D therapy (95%) and phosphate binder therapy (93%). Ten subjects (23%) were on calcium acetate, 28 subjects (65%) were on sevelamer and two subjects (5%) were on both. There was no association between bone turnover and calcium-based binder use (adynamic 43%, mild/mod HPT 23%, severe HPT 40%; *P* value = 0.4101), noncalcium-based binder use (adynamic 57%, mild/mod HPT 71%, severe HPT 40%; *P*

value = 0.3591) , average vitamin D analogue use [pg/treatment] (adynamic 5.3 ± 3.9, mild/mod HPT 4.6 ± 3.0, severe HPT 3.6 ± 1.3; *P* value = 0.6200) or cumulative vitamin D analogue [pg] in previous 3 weeks (adynamic 41 ± 27, mild/mod HPT 38 ± 27, severe HPT 35 ± 14 ; *P* value = 0.918).

BSAP was positively correlated with iPTH (r = 0.64; $P < 0.001$) and TAP (r = 0.76; $P < 0.001$). Correlations between BSAP and bone histomorphometric parameters were previously reported by Moore et al.[11] Plasma iPTH, BSAP and TAP increased with bone activity. Boxplots with individual values of iPTH, BSAP and TAP by bone turnover status are shown in Figures 1-3 respectively.

Using CART analysis, the strongest predictor of biopsy findings was BSAP with a cutoff point of 22 ng/mL. Corrected calcium and phosphorus had no additional predictive value and therefore did not appear in the decision tree. At BSAP ≤ 22 ng/mL, subjects had either ABD or mild/moderate HPT. Serum iPTH, corrected calcium and phosphorus were not useful in further classifying the biopsy type. In those with BSAP > 22 ng/mL, subjects had either mild/moderate HPT or severe HPT, and iPTH was useful in further classifying biopsy findings. With BSAP > 22 ng/mL and iPTH < 726 pg/mL, all subjects had mild/moderate HPT (Figure 4).

DISCUSSION

We used CART analysis to identify the individual cutoff levels of BSAP and iPTH, or the particular combination of both, that could best provide the clinician with additional insight into determining particular bone histology in African American dialysis patients. The main finding of the CART analysis was that BSAP was the better classification

variable and has an optimal cutoff at 22 ng/mL. The analysis also identified the values of each that correspond best with predicted bone histology. BSAP was the primary splitter and could clearly separate patients with ABD from high bone turnover disease. In addition, as determined by the CART analysis, the 2 tests in combination can provide a clinician with additional insight into the likelihood of high turnover disease.

Assays for BSAP have been available for many years, but BSAP has been largely overlooked in clinical practice as a marker of chronic kidney disease-mineral bone disorder. BSAP is a homodimeric glycoprotein anchored to the membrane of osteoblasts through glycosylphosphatidylinositol.[14, 15] It is cleaved from glycosylphosphatidylinositol and released into the circulation as a soluble homodimer by the action phospholipases C and D.[16, 17] The homodimer is eliminated through hepatic degradation. It is neither filtered by the kidney nor dialyzable.[18] It has a relatively long half-life of ~1.1-4.9 days.[19, 20] Many techniques have been used to measure BSAP, including differential heat denaturation,[21] electrophoresis,[22] and isoelectric focusing,[23] and more recently by a number of rapid and reproducible immunoassays.[24-27] However, some of these assays may exhibit a certain degree of cross-reactivity between BSAP and liver alkaline phosphatase. Therefore in patients with high liver alkaline phosphatase, BSAP measurements may be falsely elevated.[28]

BSAP has several advantages compared to iPTH measurement. It does not accumulate with progressive loss of glomerular filtration rate,[29] has lower biological variation,[3] exhibits better agreement across methods of measurement,[30] and is stable ex vivo and

does not require strict preanalytical handling procedures.[24]

BSAP is also closely linked to clinical outcomes. It has been shown to be a good predictor of future fracture risk in dialysis patients[31] and is strongly associated with 6-month cardiovascular and non-cardiovascular mortality in dialysis patients.[32] Another study identified BSAP as an independent predictor of all-cause mortality in men receiving hemodialysis, whereas there was no evidence of an association between iPTH concentration and mortality in the same cohort.[33] TAP has also been shown to be associated with clinical outcomes. Large cohort studies have shown that TAP is independently associated with increased hospitalization,[34] cardiovascular mortality,[34, 35] and all-cause mortality among dialysis patients.[36] Serum TAP activity has also been shown be a predictor of coronary calcification.[37] However, there is no direct evidence as to whether the association with mortality and vascular calcification is promoted by BSAP.

iPTH alone does not adequately predict underlying histology.[2] Few studies have compared the relationship of both BSAP and iPTH levels to histologic bone assessment[5, 38-40] and BSAP appears to be at least as closely linked to histologic parameters as iPTH and perhaps a slightly better predictor of low turnover bone disease in some studies. Two studies have also described the diagnostic accuracy of BSAP for high and low turnover bone disease.[5, 38] Both studies were conducted in an era when aluminum overload was common, and the identified thresholds for predicting bone histology may not be applicable to modern day hemodialysis patients. Notwithstanding this limitation, the 2 studies showed that BSAP may improve the predictive value of

PTH for both ABD[5, 38] and high turnover disease.[5]

In the present study, at BSAP level > 22 ng/mL no subject had ABD. These subjects had either mild/moderate bone disease or severe HPT. iPTH was useful in further classifying these subjects. In those with iPTH < 726 pg/mL, none had severe HPT. However, in those with iPTH > 726 pg/mL, severe HPT was common. At the very least, these cutoffs may be useful in determining those patients at highest risk for high turnover bone disease. Attention could be focused on decreasing bone turnover in these patients by employing measures to allow the iPTH to decrease to < 726 pg/mL. This may be achieved by increasing dosages of vitamin D or its analogues and/or by increasing dosages of phosphorus binders.

A BSAP level of < 22 ng/mL identified all subjects with either ABD or mild/moderate HPT and ruled out severe HPT. No other laboratory measurement (calcium, phosphorus, iPTH) was useful in further differentiating adynamic bone disease from mild/moderate bone turnover disease. In these subjects, strategies to prevent adynamic bone disease could be employed that target those at highest risk for ABD; as in those with history of vascular calcification,[41] hip[8] and vertebral fractures.[9] This could be achieved by decreasing dosages of vitamin D or its analogues and by decreasing dosages of calcium-based phosphorus binders or lowering of the dialysate calcium concentration. While the results of this study may support such an approach, there are no randomized controlled trials that have evaluated patient outcomes based on BSAP targeted approach. Indeed, a prospective cohort study of incident dialysis patients in the

Netherlands showed low levels of BSAP were associated with improved survival.[32] However, in contradistinction to our study, this study was conducted in Caucasians and in patients with dialysis vintage of 12 months (versus 40.4 ± 24.5 months in our study). Furthermore, the authors contend that results may potentially differ in long-term dialysis patients in whom suppression of bone turnover is more common. They also acknowledge that BSAP levels in incident dialysis patient may rather represent the general quality of care before ESRD.

The study has a few limitations. The major limitation is the small number of patients. Furthermore, the results could only be applicable to African American patients on hemodialysis as there are ethnic differences in the relationship between bone turnover markers and bone histology. Finally, this study focuses on qualitative bone histology to assess bone turnover status, without reference to mineralization or bone volume. The KDIGO Consensus statement recommends the use of the TMV (turnover, mineralization, volume) classification system to assess bone status in chronic kidney disease. This system expands the possibilities of outcomes that describe underlying bone histology; therefore, requires a considerably larger sample size to determine optimal cut-offs.

In conclusion, this study, together with other published literature, advocates the use of BSAP as a marker of chronic kidney disease-mineral bone disorder, with additional potential value in the risk stratification of African American hemodialysis patients. The cutoffs determined by this analysis to best predict biopsy findings warrant further study

and validation.

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Figure Legends

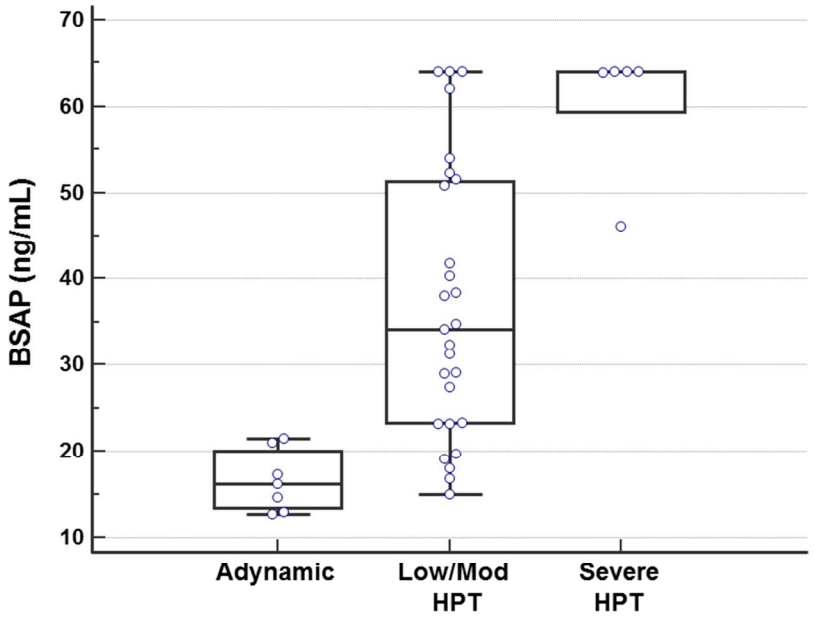
Figure 1. Box plots of iPTH level by bone turnover status. Open circles indicate individual values. The median iPTH levels were 207 pg/ml (IQR, 158-302 pg/ml; range, 41-384 pg/mL) for ABD, 430.8 pg/mL (IQR, 268-784 pg/ml; range, 75-2187 pg/mL) for mild/moderate HPT, and 941 pg/ml (IQR, 756-1110 pg/ml; range 75-2187 pg/mL) for severe HPT. Abbreviations: ABD, adynamic bone disease; iPTH, intact parathyroid hormone; HPT, hyperparathyroid bone disease; IQR, interquartile range; mod, moderate.

Figure 2. Box plots of BSAP by bone turnover status. Open circles indicate individual values. Median BSAP level was 16.2 ng/mL (IQR, 12.9-21.0 ng/mL; range, 12.6 to 21.5 ng/mL) for ABD, 34.1 ng/mL (IQR, 23.2-51.5 ng/mL; range, 15.0-64.0 ng/mL) for mild/moderate HPT, and 64 ng/mL (IQR, 63.8-64.0 ng/mL; range 46.1-64.0 ng/mL) for severe HPT. Abbreviations: ABD, adynamic bone disease; BSAP, bone specific alkaline phosphatase; HPT, hyperparathyroid bone disease; IQR, interquartile range; mod, moderate.

Figure 3. Box plots of TAP by bone turnover status. Open circles indicate individual values. Median TAP level was 68.0 ng/mL (IQR, 58.3-89.8 ng/mL; range, 49.0 to 113.0 ng/mL) for ABD, 131.0 ng/mL (IQR, 99.3-189.0 ng/mL; range, 18.0-307.0 ng/mL) for mild/moderate HPT, and 238.0 ng/mL (IQR, 173.5-310.0 ng/mL; range 146.0-376.0 ng/mL) for severe HPT. Abbreviations: ABD, adynamic bone disease; TAP, total alkaline phosphatase; HPT, hyperparathyroid bone disease; IQR, interquartile range; mod, moderate.

Figure 4. Binary recursive partitioning of BSAP and iPTH to identify biopsy findings. All subjects with adynamic bone disease had a BSAP $\leq$ 22 ng/mL. iPTH did not improve the classification of those with BSAP $\leq$ 22 ng/mL. When BSAP was > 22 ng/mL, the iPTH was

useful in further classifying biopsy findings. With BSAP > 22 ng/mL and iPTH < 726 pg/mL, all subjects had mild/moderate hyperparathyroid bone disease. Abbreviations: BSAP, bone specific alkaline phosphatase; iPTH, intact parathyroid hormone; mod, moderate.



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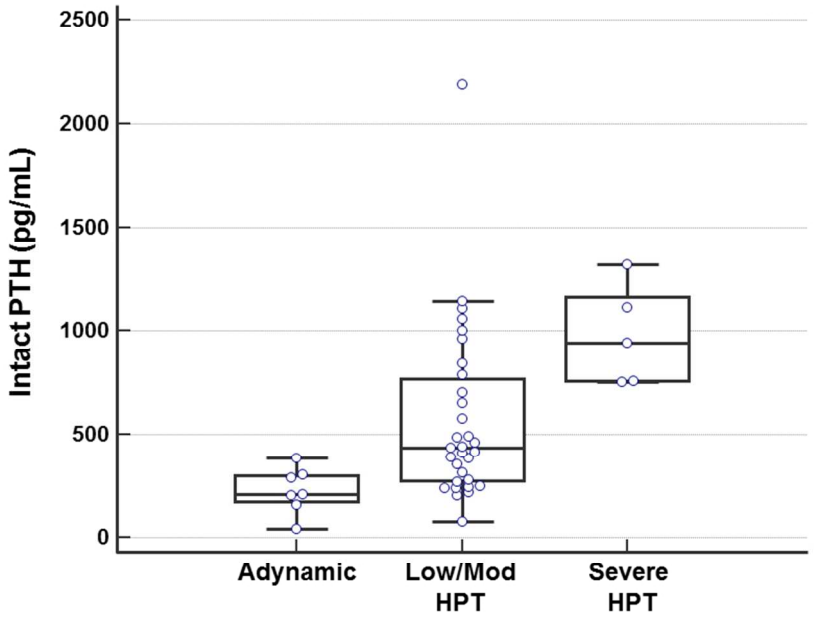

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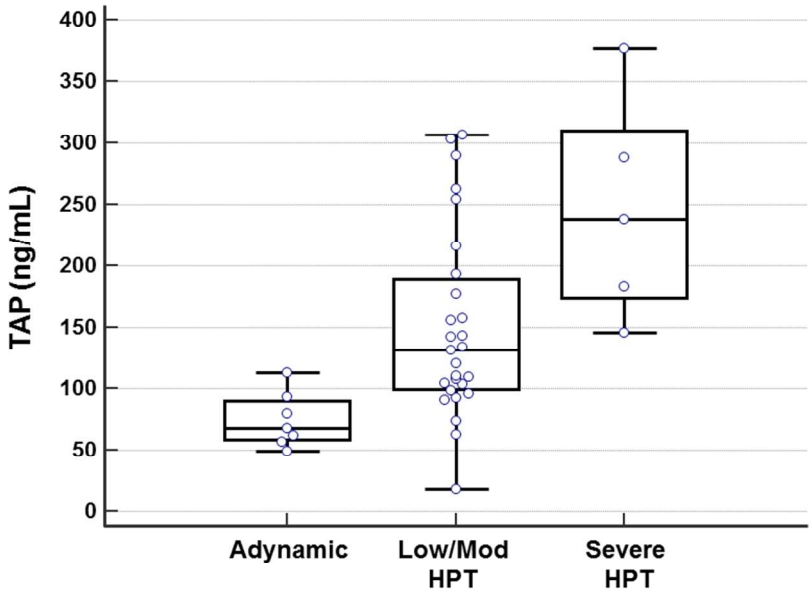

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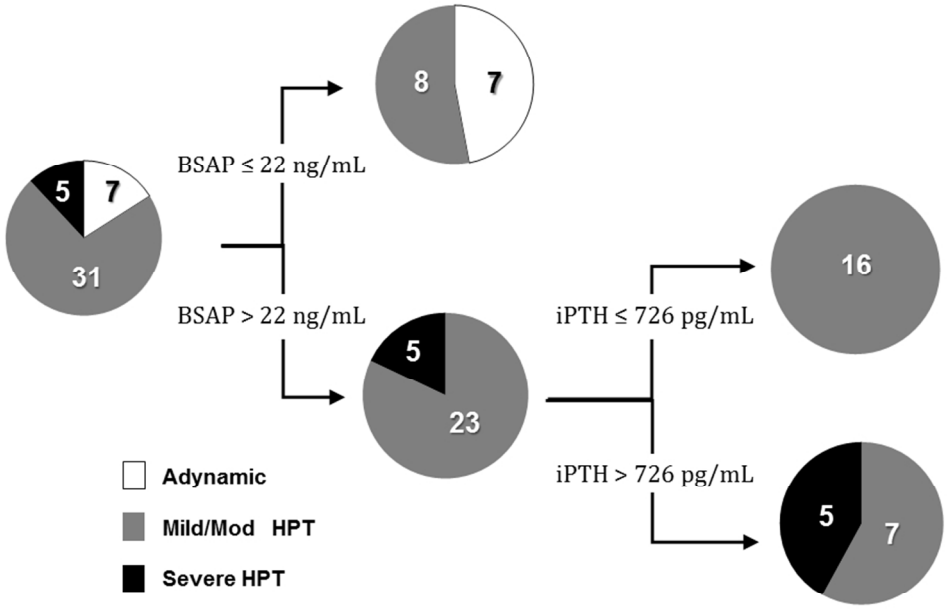

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