

Original article

Differences between 2nd and 3rd generation seric parathormone determination methods on mortality in haemodialysis patients[☆]

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ARTICLE INFO

Article history:

Received 13 January 2016

Accepted 17 November 2016

Available online 23 August 2017

Keywords:

Haemodialysis

Parathormone

PTH bio

Intact PTH

Mortality

Cardiovascular

ABSTRACT

Parathormone plays a key role in controlling mineral metabolism. PTH is considered a uremic toxin causing cardiovascular damage and cardiovascular mortality in dialysis patients. There are two different assays to measure PTH called 2nd generation or intact PTH (iPTH) and 3rd generation or bioPTH (PTHbio).

Objective: To evaluate the differences in mortality of dialysis patients between both assays to measure PTH, as well as the possible prognostic role of the PTHbio/iPTH ratio.

Methods: 145 haemodialysis patients were included with 2-year monitoring including baseline laboratory test and annually thereafter.

Results: 21 patients died in the first year and 28 in the second. No correlation was found between PTH, PTHbio and PTHbio/iPTH ratio with mortality. Both PTH have a perfect correlation between them and correlate similarly with other molecules of the mineral metabolism. The extreme baseline values of PTH are those of higher mortality. In survival by iPTH intervals (according to guidelines and COSMOS study), a J curve is observed. When iPTH increases, the ratio decreases, possibly when increasing fragments no. 1–84. There is no greater

[☆] Please cite this article as: Rodríguez-Osorio L, de la Piedra C, Rubert M, Martín-Fernández M, González Casaus ML, Gracia-Iguacel C, et al. Diferencias entre los métodos de determinación de 2.^a y 3.^a generación de la parathormona sérica sobre la mortalidad en el paciente en hemodiálisis. Nefrología. 2017;37:389–396.

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prognostic approximation on mortality with PTHbio than PTHi. There was also no difference in mortality when progression ratio PTHbio/PTHi was analysed.

Conclusions: We didn't find any advantages to using bioPTH vs. PTHi as a marker of mortality. BioPTH limits of normality must be reevaluated because its relationship with iPTH is not consistent. Not knowing these limits affects its prognostic value.

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Diferencias entre los métodos de determinación de 2.^a y 3.^a generación de la parathormona sérica sobre la mortalidad en el paciente en hemodiálisis

R E S U M E N

Palabras clave:
Hemodiálisis
Parathormona
PTH bio
PTH intacta
Mortalidad
Cardiovascular

La parathormona tiene un papel fundamental en el control del metabolismo mineral. Además es considerada como una toxina urémica al originar daño cardiovascular e influir en la mortalidad cardiovascular del paciente en diálisis. Existen dos métodos de medición denominados de 2.^a generación o PTH intacta (PTHi) y de 3.^a generación o bioPTH (PTHbio). **Objetivo:** Evaluar las diferencias en la mortalidad del paciente en diálisis entre ambas formas de medición de PTH, así como el posible papel pronóstico de su cociente.

Métodos: Se incluyeron 145 pacientes en hemodiálisis con un seguimiento de 2 años con determinación analítica basal y posteriormente de forma anual.

Resultados: Veintiún pacientes fallecieron el primer año y 28 el segundo. No se encontró correlación entre PTHi, PTHbio y cociente PTHbio/PTHi con la mortalidad. Ambas PTH tienen una buena correlación entre ellas y correlacionan de manera similar con otras moléculas del metabolismo mineral. Los valores basales de PTH extremos son los de mayor mortalidad. En la supervivencia por tramos de PTHi (según guías y estudio COSMOS) se observa una curva en J. A mayor aumento de PTHi el cociente desciende, posiblemente al aumentar los fragmentos no 1-84. No existe una mayor aproximación pronóstica sobre mortalidad con PTHbio que con PTHi. No se observan diferencias en el valor predictivo del cociente sobre la mortalidad. Tampoco hubo diferencias en mortalidad cuando se analiza la progresión del cociente PTHbio/PTHi.

Conclusiones: No encontramos ventajas en la utilización de PTHbio sobre la PTHi como marcador de mortalidad. Se deben reevaluar los límites de la PTHbio pues su relación con la PTHi no es constante. El no conocer esos límites condiciona su utilidad pronóstica.

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Introduction

The Parathyroid Hormone (PTH) is a 84 amino acids protein with a established role in the regulation of mineral metabolism; main target organs are the bone and kidneys among others. In spite of being a hormone, in the case of the renal patient, it is classified as a true uremic toxin because it increases progressively with the progression chronic kidney disease (CKD), and it responsible for multiple systemic effects.^{1,2} It is included as one of the uremic toxins that cause cardiovascular damage.^{3,4}

The control of PTH is important for the adequate treatment of the abnormalities of mineral metabolism in CKD. Therefore much of the therapeutic efforts aimed at controlling these alterations are dedicated to an adequate control of PTH. Nevertheless, in hemodialysis patients PTH is a poor marker of mortality. Only large studies have revealed that high PTH values that are related to higher mortality. And

in these studies the results are variable.⁴⁻⁸ However, other molecules such as calcium and phosphorus have shown a better prognostic significance on mortality, along with vitamin D, although with the later the results have been less conclusive.^{9,10}

Circulating PTH includes a mixture of peptides such as the whole molecule 1-84 and smaller fragments resulting from the catabolism of PTH, these are called non-1-84 or carboxyterminal fragments. Only PTH 1-84 (whole protein) exert biological activity. Some of the carboxyterminal fragments have a PTH antagonistic action. PTH is cleared by the kidney and the proportion of these PTH peptides varies according to the CKD stage. The methods currently used to measure PTH in clinical practice are of two categories. The so-called 2nd generation that measures the intact 1-84 PTH (PTHi) and multitude of fragments no 1-84. The 3rd generation method, also called PTHbio, recently marketed, quantify the PTH 1-84 only, although its use is not generalized yet.^{11,12}

In an attempt to integrate the information provided by the measurement of both molecules, some authors have advocated the utility of the proportion (ratio) of these molecules by the ratio PTH_{bio}/PTH_i as a marker of the bone remodeling^{13,14} and mortality.¹⁵

In clinical practice, there are currently two criteria to define the normal values of PTH. The first, and most classic, is based on histomorphometry as defined by the Guides KDOQI¹⁶ and the Guidelines SEN.¹⁷ At that time, based on bone biopsies, bone abnormalities were present if PTH_i was lower than 150 pg/ml or above 300 pg/ml. The range of normality were established according to relationship of iPTH with the lesions observed in the bone biopsy.¹⁸ These values still exist in most of the recommendations. The other criterion is mortality. There are several studies that find increased mortality with high iPTH values; much higher than 300 pg/ml. Thus, the more recent KDIGO Guidelines¹⁹ defined adequate iPTH as 2–9 times above the normal values. However, not all nephrologists were completely convinced with this recommendation. The recent COSMOS study has found that in a large cohort of patients followed for 5 years, the PTH values associated with less mortality are in the range of 168–674 pg/ml,²⁰ very similar to the KDIGO recommendations. The authors of the COSMOS study recommend modifying the reference values of PTH_i based on bone histomorphometric studies, by PTH values based on survival. Although there are contradictory results, it seems that the mortality associated with serum PTH describes a J shape curve, with higher mortality in patients with lower and higher PTH values. But there is also significant variability between the different PTH 2nd generation commercial kits²¹ which makes it difficult to interpret the results.

The variability in PTH measurements poses several problems when interpreting their values, and deciding the target values to be achieved when treating a renal patient. What would be the normal values?, those based on histomorphometric studies?, the ones based on mortality?, both?; which method use to measure the PTH, 2nd or 3rd generation?

The present study evaluated the differences in mortality prediction using PTH values obtained with measurement of 2nd and 3rd generation in a group of hemodialysis patients followed for 2 years. In addition, we have evaluated the PTH_{bio}/PTH_i ratio as a possible marker of mortality, in case the ratio of the circulating molecules could be a marker. In addition, we have analyzed the association between mortality and serum iPTH levels recommended by KDOQI (150–300 pg/ml) and the range obtained by COSMOS (168–674 pg/ml). So the values of PTH that best predict mortality were determined.

Material and methods

This is a two-year prospective study performed under daily clinical practice conditions. The main objective of this study was to determine the association of serum PTH with hemodialysis patient survival. It included 145 hemodialysis patients from a single center, who underwent a baseline analytical determination, followed by a follow-up of two years. We determined clinical parameters and baseline analysis in which

PTH was determined by 2 different methods, 2nd and 3rd generation, as well as calcium, phosphorus, alkaline phosphatase and bone alkaline phosphatase, FGF23, 25 (OH)-vitamin D, and bone remodeling markers. The values of PTH-intact (PTH_i) or PTH of 2nd generation were determined by an electrochemiluminescence method using the Elecsys autoanalyzer (Roche); this method uses an antibody directed against amino acids 26–32 of the molecule and another against the aminoacids 55–64. Therefore it does not detect short carboxyterminal fragments, but this method measures PTH 1–84 and long PTH fragments 7–84 and amino-PTH11 (intra- and inter-assay coefficients of variation are <2.5 and <3% respectively and sensitivity of 1.2 pg/ml method). The PTH_i values were normalized to the Allegro PTH_i using the formulas given by the Spanish Society of Nephrology (SEN). The values of PTH of 3rd generation or PTH_{bio} were determined by an electrochemiluminescence method also using the Elecsys autoanalyzer (Roche), which uses an antibody directed against amino acids 1–4 of the molecule and another against aminoacids 55–64, thus measuring the molecule 1–84 and the amino-PTH11 (coefficients of intra- and inter-assay variation are <3 and <6% respectively and the sensitivity of the method is 5.50 pg/ml). Normal values of PTH_{bio} were defined as 50% of the value of PTH.¹⁵

The ratio of PTH_{bio}/PTH_i molecules was also calculated in order to determine the proportion of PTH fragments 7–84.

A registry of deceased patients (of any cause) was recorded at year 1 and 2, and general analytical parameters of mineral metabolism, inflammation and nutrition were determined. In both the annual and the two-year follow-up, the PTH was measured using the 2nd generation method.

The ADVIA CENTAUR 2400 autoanalyzer was used for the measurements of serum albumin (bromocresol green dye binding technique; intra- and inter-assay coefficients of variation were <1.3 and <2% and the sensitivity of the method was 1 g/dl). Serum calcium (arsenazo III technique, coefficients of intra- and inter-assay variation are <1.2 and <2%, and the sensitivity of the method is 0.5 mg/dl), serum creatinine (alkaline picrate technique, intra- and inter-assay variation are <3.1 and <4%, and the sensitivity of the method is 0.2 mg/dl), serum phosphate (UV phosphomolybdate technique; intra- and interassay coefficients of variation are <2, 2 and <3%, and the sensitivity of the method is 0.3 mg/dl), total serum proteins (biuret technique, intra- and inter-assay coefficients of variation are <1.3 and <2%, and sensitivity of the method is 2 g/dl), PCR (turbidimetric technique enhanced with latex, coefficients of intra- and interassay variation are <4.9 and <6%, and the sensitivity of the method is 0.003 mg/dl), and serum alkaline phosphatase (hydrolysis p-nitrophenyl phosphate; Coefficients of intra- and interassay variation are <1.9 and <2.4%, and the sensitivity of the method is 5 U/l).

Serum prealbumin values were determined by turbidimetry in a SPA plus autoanalyzer, with intra- and interassay coefficients of variation being <4.4 and <6% respectively and a sensitivity of 0.006 g/l.

The values of total vitamin D (25 [OH] vitamin D₂ and D₃) were determined by an electrochemiluminescence method performed on the Elecsys autoanalyzer (Roche) with coefficients of intra- and inter-assay variation respectively <7.5% and <8% and sensitivity of 3 ng/ml.

Plasma (C-terminal) fibroblast growth factor 23 (FGF-23) was determined by an ELISA method (Immutopics, USA) using 2 polyclonal antibodies directed to the C-terminal fraction of FGF-23 (coefficients of intra- and inter-assay variation are <1.7 and <3.5% respectively and the sensitivity of the method is 1.5 RU/ml).

The bone isoenzyme of alkaline phosphatase was determined in serum by ELISA (OSTASE BAP, IDS, UK) (coefficients of intra- and inter-assay variation are <4.5 and <6.4% respectively and the sensitivity of the method is 0.7 Mg/l).

The determination of the C-terminal propeptide of procollagen type I (PINP) and the β -Cross Laps (β -CTX) in serum was performed by electrochemiluminescence in the Elecsys auto-analyzer (Roche) (for PINP coefficients of intra- and interassay variation are <2.9 and <3.7% respectively and the sensitivity of the method is 5 μ g/l; for β -CTX, coefficients of intra- and interassay variation are <4.6 and <4.7% respectively and sensitivity of the method is 0.07 ng/ml).

Results

A prospective study including 145 hemodialysis patients was conducted for two year period; 49% were women. **Table 1** shows baseline clinical and biochemistry data of the patients studied. 85.1% of the patients was on some type vitamin D receptor activator, and 17.9% received cinacalcet.

Twenty one patients died during the first year follow-up, and 28 in the second year. Potential Clinical and biochemical variables analytical that correlate with mortality are shown in **Table 2**. The parameters related to mortality were albumin, prealbumin, C-reactive protein, ProBNP, creatinine, age, vascular access type, and bone alkaline phosphatase. However, the PTH values of both the 2nd (PTH_i) and the 3rd generation (PTH_{bio}) and the ration PTH_i/PTH_{bio} of the patients who died were not different than in patients that remained alive.

The main objective of this study is to define the effect on the mortality of PTH values of both 2nd and 3rd generation, as the ratio between both. Second generation PTH (PTH_i) and third generation PTH (PTH_{bio}) have a good correlation within each other. The correlations of PTH from 2nd and the 3rd generation, with other molecules of the mineral metabolism are similar (**Table 3**).

The ratio PTH_{bio}/PTH_i decreases as la PTH_i values increases suggesting that non 1-84 fragments increase secondary to a degradation of the PTH 1-84 (**Fig. 1**).

When patients were stratified by PTH intervals, a significant increase in mortality was observed in patients with extreme PTH_i values: greater than 678 pg/ml and less than 150 pg/ml, compared to the group of patients with PTH between 150 and 300 pg/ml (**Fig. 2**); although the mortality is higher among patients with PTH_i between 300 and 678 pg/ml than in those whose PTH_i is 150-300 pg/ml (**Fig. 2**), this difference did not reach statistical significance. With the PTH_{bio} calculated as 50% of the PTH_i the differences are not significant in the same intervals.

Comparison of mortality in the PTH range recommended by the current SEN and KDOQI PTH guidelines (150-300 pg/ml) and the values established by the COSMOS study (164-678 pg/ml) (**Table 4**), the relative risk of mortality

Table 1 – Demographic and clinical characteristics; and, baseline biochemistry of the study population.

Variable	
Age (years)	65.9 ± 14.6
Dry weight (kg)	65.5 ± 14.2
Vintage (years)	Mean 3 (P25-2; P75-6)
Males (%)	51
Vascular access (%)	
AVF	60.8
PTFE (politetrafluoroetileno)	19.2
Permanent catheter	20
Hemodialysis technique (%)	
Conventional	90
On-line	10
Hypertension (%)	86.9
Diabetes mellitus (%)	23.4
Dyslipemia (%)	35.2
	M ± SD
Hemoglobin (g/dl)	11.8 ± 1.6
Hematocrit (%)	35.5 ± 4.6
Urea (mg/dl)	117.7 ± 36.5
Proteins (g/dl)	6.5 ± 0.6
Albumin (g/dl)	3.6 ± 0.4
Iron (μ g/dl)	65.4 ± 28
Fe saturation index (%)	31.7 ± 14.3
Calcium (mg/dl)	9.2 ± 0.7
Phosphate (mg/dl)	4.7 ± 1.6
CO ₂	21.4 ± 3.5
Total cholesterol (mg/dl)	158 ± 35
Prealbumin (mg/dl)	33 ± 10
Ferritin (ng/dl)	406 ± 242
	Mean (P25;P75)
Bone alkaline phosphatase (μ g/l)	29.6 (20.7;41.9)
PINP (μ g/l)	255.3 (161.2;486.6)
β -CTX (ng/ml)	1.8 (1.3;2.5)
PTH 2. ^a gen (iPTH) (pg/ml)	205 (116.5;386.1)
PTH 3. ^a gen (bioPTH) (pg/ml)	119.5 (76.2; 240)
Ratio bioPTH/iPTH	0.61 (0.56;0.68)
25OH- D (ng/dl)	22.9 (12.7;34.2)
Creatinine (mg/dl)	7.6 (5.6;9.1)
Alkaline phosphatase (UI/l)	111 (89;145)
Triglycerides (mg/dl)	146 (102;193)
C reactive protein (mg/dl)	0.6 (0.25;1.97)
ProBNP (pg/ml)	8325 (3422;17450)
FGF-23 (RU/ml)	788 (384;3258)

is only significant with PTH greater than 300 pg/ml and less than 150 pg/ml. This implies that patients with PTH_i greater than 300 have higher mortality only when patients with PTH_i greater than 678 pg/ml are included. In our study, iPTH showed a better predictive value of mortality than bioPTH, taking 150-300 (75-150) pg/ml as a reference, but it is due to the patients with highest PTH values (**Table 4**).

No differences were observed in bioPTH/iPTH ratio between patients who died and those who did not. Likewise, no differences in mortality were observed when the PTH_{bio}/PTH_i ratio was analyzed by quintiles, so bioPTH/iPTH ratio does not appear to be a marker of mortality (**Fig. 3**). The bioPTH/iPTH value higher than 0.8 has a higher mortality, but given the small group of patients this difference is not significant.

Table 2 – Variables significantly related with mortality.

Variable	No exitus	Exitus	p value
Age (years) M ± DE	62.8 ± 14;9	73.0 ± 10.6	p < 0.0001
Vintage Me (P25,P75)	3 (2;5.5)	4 (3;7)	p = 0.027
Permanent catheter (%)	9.9	36.4	p < 0.0001
Albumin (g/dl) M ± DE	3.72 ± 0.48	3.51 ± 0.42	p = 0.0138
Prealbumin (mg/dl) M ± DE	35.1 ± 9.6	28.1 ± 10	p = 0.0008
Bone alkaline phosphatase (μg/l) Me (P25,P75)	26.8 (19.7;31.7)	31.4 (22.4;45.5)	p = 0.037
Creatinine (mg/dl) Me (P25,P75)	8 (5.8;9.8)	6.6 (5.5;8.4)	p = 0.018
CRP (mg/dl) Me (P25,P75)	0.25 (0.25;1.37)	1.3 (0.25;3.4)	p = 0.0001
ProBNP (pg/ml) Me (P25,P75)	6590 (2778;14450)	14,100 (6580;34000)	p = 0.0001

Table 3 – Correlation of PTH 2.^a y 3.^a generation with other molecules of mineral metabolism.

Variable	iPTH		bioPTH	
	r	p	r	p
25OH-D (ng/dl)	-0.034	ns	-0.033	ns
FGF-23 (RU/ml)	0.296	0.0003	0.228	0.006
Alkaline phosphatase (UI/l)	0.268	0.0012	0.254	0.0022
Bone alkaline phosphatase (μg/l)	0.342	0.0000	0.338	0.0000
Calcium (mg/dl)	-0.001	ns	-0.050	ns
Phosphate (mg/dl)	0.326	0.0001	0.295	0.0003

Table 4 – Mortality in patients with PTH within reference values established by Guides SEN and KDOQI (150–300 pg/ml) and COSMOS (168–674 pg/ml) using iPTH and bio PTH methods.

Guides iPTH	Exitus No/yes	OR (IC95%)	p	Guides bioPTH	Exitus No/yes	OR (IC95%)	p
150–300	34/8			75–150	24/6		
<150	27/19	2.99 (1.14–7.88)	0.024	<75	29/19	2.62 (0.90–7.60)	ns
>300	27/20	3.15 (1.20–8.25)	0.017	>150	35/23	2.63 (0.93–7.42)	ns
Cosmos iPTH	Exitus No/yes	OR (IC95%)	p	Cosmos BioPTH	Exitus No/yes	OR (IC95%)	p
168–674	47/22			84–337	56/27		
<168	36/20	1.19 (0.56–2.50)	ns	<84	25/15	1.24 (0.57–2.74)	ns
>674	5/6	2.56 (0.71–9.32)	ns	>337	7/7	2.07 (0.66–6.51)	ns

Discussion

In renal patients, abnormalities of mineral metabolism are a risk for cardiovascular disease.²² However, among the

parameters of mineral metabolism PTH has a relatively low weight as a marker of morbidity and mortality in the renal patient. In our study we observed that PTH is not a marker of mortality when analyzed in conjunction with other factors

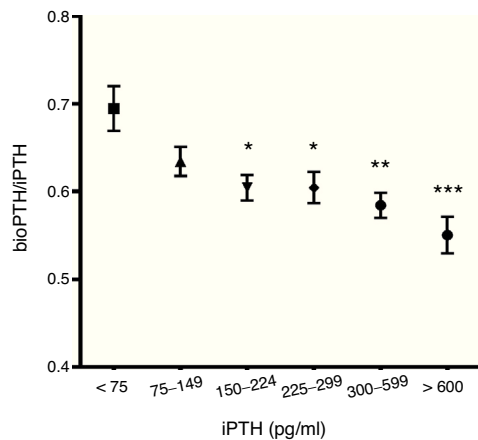
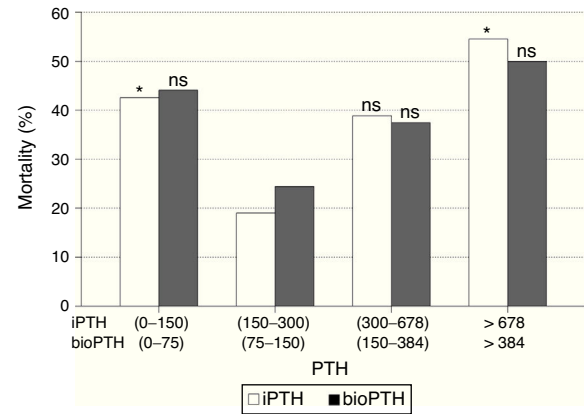


Fig. 1 – Changes in PTHbio/PTH_i, in relation to the iPTH values (2.^a generation) (pg/ml). (*): p < 0.05; (): p < 0.01; (***): p < 0.001. Y axis bioPTH/iPTH. X axis iPTH (pg/ml).**



* p < 0.05 (as compared with 150–300)

Fig. 2 – Mortality according to the PTH reference values established by SEN guidelines, KDOQI guidelines and the COSMOS study.

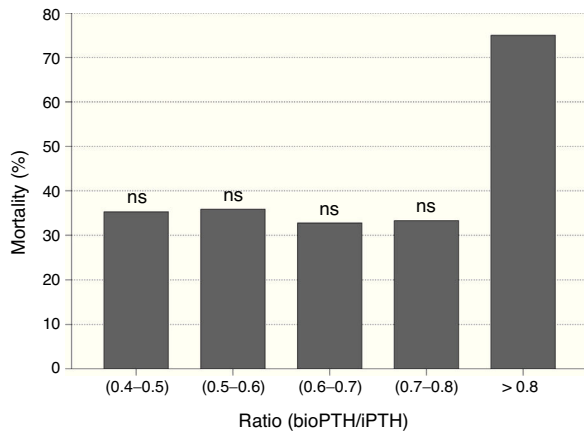


Fig. 3 – Mortality in relation to the bioPTH/iPTH ratio. Y axis: mortality (%); X axis: mortality (%); X axis: (0.4–0.5)(0.5–0.6)(0.6–0.7)(0.7–0.8)(0.8–0.9); ratio(bioPTH/iPTH).

of greater influence such as age, years on dialysis, albumin or vascular access. However, it is an important modulator of all alterations of mineral metabolism, so its control should not be neglected. As we observed, PTHb and PTHi correlate very well with other parameters such as phosphorus, FGF23, bone alkaline phosphatase and years of dialysis. All these parameters are associated with morbimortality. In fact, many research groups find a greater interest in combining several parameters of mineral metabolism as a marker of mortality.⁹

The main objective of this work has been to define PTH as a marker of mortality and the reference values that should be used in daily practice.

The relationship between iPTH and mortality is controversial. Although most studies find that PTHi is a marker of mortality some authors did not.⁹ The established range of 150–300 pg/ml were based on histomorphometric criteria, but these values are also the inflection points in mortality. The meta-analysis of Natoli et al.⁸ described PTH related mortality as a J curve. We also a J-shaped mortality curve where values with lower mortality are within the PTHi range of 150–300 pg/ml.

Like other authors,²³ we found a correlation between iPTH and bioPTH. Thus once adjusted, the values of normality could be used interchangeably. In addition, both determinations correlate with the same clinical and analytical parameters, so it appears that one method does not have an advantage over the other. It is known that as the glomerular filtration rate decreases there is an increase in non-1–84 fragments.²⁴ It is remarkable to see that as the 1–84 PTH increases, the fragments are also increased even to a greater extent than the 1–84 PTH molecule itself. As non-1–84 fragments are antagonists of 1–84 PTH it could be assumed that at a higher iPTH, the systemic effects, especially bone, would be reduced by these molecules. This aspect deserves to be investigated in order to know its relevance. The changes in the proportion of these fragments are important when defining the normal values of bioPTH.

However, in our study we did not observe that patients with a low PTHb/PTHi ratio had a lower mortality due to an increase

in the 7–84 fragments, suggesting that the PTH 1–84 molecule has a relevant role in the mortality of these patients.

The normal values of bioPTH are defined as 54% of iPTHi.¹⁵ However, in our study, it was observed that this proportion varies according to the iPTH values. That is, at higher 1–84 PTH the non-1–84 PTH fragments increase to a greater extent than the 1–84 PTH itself. Therefore, the normal values of 1–84 PTH measured by 3rd generation methods in the hemodialysis patient are not known at present, as they are not yet formally established in any guideline recommendations. According to our results, limits of bioPTH should be narrow, since elevations of 1–84 PTH are accompanied by an even greater proportional increase non 1–84 PTH fragments; this should be considered when establishing normal values for bioPTH. It is not known how to interpret the values of bioPTH after eliminating the distortion factor of fragments non 1–84 PTH, since there are no studies to that end. The assumption of bioPTH as 50–54% of the iPTH does not seem correct, as it does not correspond to reality. A multicenter study should be performed with a large number of patients to define these values.

The COSMOS study finds that a iPTH of 674 ng/ml may be established as the upper limit of the reference interval in terms of mortality. Like other authors²⁵ we did not find, that a higher mortality in patients with iPTH between 300 and 674 than in those with iPTH between 150 and 300 ng/ml. These results should make us cautious before establishing the value of 678 ng/ml as the upper reference value. Mortality is significant above 678 pg/ml, but we should never consider that figure as a safe value. Statistical significance should not make us think that an increase in iPTHi up to 9 times normal values is safe. Such a high iPTH value may cause alterations of the mineral metabolism that may have negative repercussion in the renal patient. There is a confidence zone between 300 and 678 pg/ml in which mortality is low but we think that being near 300 is safer than 678 pg/ml.

The bioPTH (1–84)/PTHi (1–84 PTH + non 1–84 PTH) ratio has been evaluated as a marker of bone histomorphometric changes and as a predictor of mortality. However the evidence is still sparse. Like in the study by Melamed et al.,¹⁵ our results show that the ratio is not associated with mortality. Other authors have found that ratio bioPTH/iPTH is a good marker of mortality in men on hemodialysis.²⁶ Those patients with higher values of ratio bioPTH/iPTH ratio would have a higher mortality.

Our study has several limitations. The sample of patients studied is small, since a larger group of extreme PTH values is necessary. Another limitation of our study is to have performed bio PTH at baseline only and not being repeated in successive time points.

Conclusions

According to our results the values of PTHi defined by histomorphometric criteria are well-established limits of mortality in hemodialysis patients, the 300 pg/ml value defines better the cutoff point of mortality than the 674 value defined in the COSMOS study, although the mortality between 150–300 and 300–678 pg/ml is the same. The 3rd generation of PTH measurement method that define the bioPTH do not add more

precision to define defining mortality. The normal values of iPTH are not well defined, so the iPTH and bioPTH may not be compared with the values currently established. The ratio bioPTH/iPTH ratio is not a good marker of mortality.

Conflicts of interest

Roche Spain has sponsored the measurements of bioPTH, and has had no role in the design or the preparation of this publication.

Acknowledgements

Our thanks to Maribel Villarino for the help with the development of the study. L.R.-O. is a Health Professional on Research Training "Rio Hortega r" (CM13/00131), Ministry of Education, Government of Spain. R.V.B. is a professional with postdoctoral contract "Sara Borrell" (CD14/00198) and a project (SAF2014-60699-JIN) of the Ministry of Economy (MINECO) and FEDER funds. PI14/00386. PI16/01298. FEDER funds ISCIII-RETIC RED-inREN/RD06/0016, RD12/0021.

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