



Inter-method variability in bone alkaline phosphatase measurement: Clinical impact on the management of dialysis patients



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ABSTRACT

Background: Bone-specific alkaline phosphatase (BAP) is now recommended to assess bone turnover in hemodialysis (HD) patients. However, little is known about potential variability between methods available to measure BAP.

Methods: We measured BAP in 76 HD patients with six different assays (Beckman-Coulter Ostase IRMA, Beckman-Coulter Ostase Access, IDS iSYS Ostase, IDS Ostase enzyme immunoassay, DiaSorin Liaison Ostase and Quidel MicroVue BAP).

Results: We observed a high correlation between all the assays ranging from 0.9948 (IDS iSYS vs. IDS EIA) to 0.9215 (DiaSorin Liaison vs. Quidel MicroVue). However, using the regression equations, the equivalent concentration of a Beckman-Coulter Access value of 10 µg/L can range from 7.7 to 14.4 µg/L and of 20 µg/L can range from 16.9 to 27.9 µg/L with other assays. According to Beckman-Coulter Access, 13%, 50% and 37% of the patients presented BAP values ≤10, between 10 and 20 and ≥20 µg/L, respectively. Discrepancies are observed when other assays are used (concordance from 10 to 100%).

Conclusions: Analytical problems leading to inter-method variation should be overcome to improve the usefulness of this marker in clinical practice. According to correlation results, recalibration of BAP assays is necessary but should not be a major issue.

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Introduction

Assessing bone turnover is of great importance in hemodialyzed (HD) patients and several therapeutic strategies are directly related to this assessment, such as phosphorus chelators, active vitamin D or calcimimetics [1]. Besides bone biopsy histomorphometric data, the reference method, serum biological markers of bone formation or resorption are more and more used to evaluate bone turnover in patients suffering from chronic kidney diseases (CKD), including HD patients. These markers are generally considered as candidate surrogate to bone biopsy because they are not invasive, can be repeated for the follow-up of the patients and are relatively inexpensive [2]. The 2009 Kidney Disease Improving Global Outcomes (KDIGO) guidelines suggested that “measurements of serum parathyroid hormone (PTH) or bone-specific alkaline phosphatase can be used to evaluate bone disease because markedly high or low values predict underlying bone turnover” (paragraph 3.2.3; grade 2B) [3]. This proposition to add bone-specific

alkaline phosphatase (BAP) to the panel of biological measurements already recommended in HD patients is supported by the fact that PTH is not a “true” bone marker [1], and has been reinforced by studies focusing on PTH measurements that have underlined an important inter-method variability and its potential impact on bone turnover diagnosis [4].

Different publications have proposed BAP cut-offs of 10 and 20 µg/L to diagnose low and high bone turnover in HD patients [5,6]. However, these cut-offs have been mostly determined using an assay, the Tandem-R-Ostase assay, which is not available anymore in its initial version. From an analytical point of view, the measurement of BAP has several advantages compared to PTH measurement: better stability, better analytical coefficient of variation (CV) and especially better intra-individual CV [7,8]. It should be noted however that in 2009, when the KDIGO guidelines were published, BAP measurement was not possible in many laboratories worldwide due to a lack of easily available automated assays. Things have changed and several automated BAP assays are now available on several fully automated platforms so that the recommendation to perform BAP measurements routinely is now possible worldwide. However, little is known about the inter-method variability in BAP measurement.

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In this study, we compared six methods and estimated the clinical impact on the diagnosis of bone turnover.

Subjects and methods

Patients

Seventy-six patients (mean age: 65.8 ± 15.8 years old, 45 males) from the Dialysis department of the University Hospital of Liège agreed to participate to the study and gave their informed consent. Samples were obtained before the dialysis session and centrifuged within an hour. Aliquots were constituted and were frozen at $-80\text{ }^{\circ}\text{C}$ until determination.

Assays

BAP was determined with one immunoradiometric (IRMA) assay (Beckman-Coulter Ostase), three automates (Beckman-Coulter Access Ostase, DiaSorin Liaison BAP Ostase and IDS iSYS Ostase BAP), and two immune-enzymatic assays (MicroVue BAP EIA, Quidel and IDS BAP Ostase EIA).

The Beckman-Coulter Ostase IRMA is a “sandwich” type assay that uses two monoclonal antibodies directed against 2 different epitopes of BAP. This assay determines the *mass* of the BAP in $\mu\text{g/L}$. The kit was formerly known as “Tandem-R Ostase” and was produced by Hybritech. This version of the assay uses coated tubes whereas the “Hybritech” version used coated beads. The coefficient of variation (CV) of the method is $\leq 13.6\%$ according to the manufacturer.

The Beckman-Coulter Access Ostase is a one-step immunoenzymatic assay using a single monoclonal antibody specific to BAP that captures the enzyme on a magnetic bead. Then, a chemiluminescent substrate for the enzyme is added and the light generated is measured. This assay thus measures the *activity* of the enzyme, but it has been calibrated on the Tandem-R Ostase to give a result in *mass*. In 2000, the regression equation obtained in 172 patients (88 osteoporotic and 84 Paget's) between the 2 methods was: Access Ostase = $0.9756 \times \text{Tandem-R Ostase} - 0.5987$ ($r = 0.9895$) [9]. The CV of the assay is $<6.5\%$.

The IDS iSYS Ostase uses the monoclonal anti-BAP antibody and the calibrators provided by Beckman-Coulter. The principle of the procedure is the same as the Access, but the substrate is different (p-nitrophenol phosphate) as well as the detection method (spectrophotometry). The iSYS also measures the *activity* of the enzyme and has been calibrated against the Access to provide results in *mass*. The CV of the assay is $<9\%$.

The IDS Ostase BAP enzyme immunoassay (EIA) also uses the monoclonal anti-BAP antibody and the calibrators provided by Beckman-Coulter. It is a manual immunoenzymatic assay in which the biotin-labeled monoclonal anti-BAP is incubated with serum in a plastic well coated with streptavidin. The complex solid phase/anti-BAP antibody/BAP is then incubated with the substrate of the enzyme (p-nitrophenol phosphate) and the coloration at 405 nm in a microplate reader. This assay also measures the *activity* of the enzyme and has been calibrated against the Access to provide results in *mass*. The CV of the assay is $<6.4\%$.

The DiaSorin Liaison Ostase uses two monoclonal antibodies specific for BAP in a “sandwich” feature. The capture antibody and the BAP antigen come from Beckman-Coulter. The second antibody is bound to isoluminol to induce a chemiluminescence reaction. This assay thus measures the *mass* of the enzyme and has been calibrated against the Beckman-Coulter Access. The CV of the assay is $<8.1\%$.

The Quidel MicroVue BAP uses a monoclonal anti-BAP antibody and BAP antigen not provided by Beckman-Coulter. After capture of the enzyme, the substrate (p-p-nitrophenol phosphate) is added and the coloration, proportional to the *activity* of the BAP, is read spectrophotometrically. The CV is $<7.6\%$.

The methods are summarized in Table 1.

Method comparison analysis

We used the weighted Deming regression method to assess the relationship between values provided by the different assays. For the Quidel MicroVue BAP, that provides results of the activity in Units, we used a factor as provided by the Manufacturer ($1\text{ U} = 0.488\text{ }\mu\text{g/L}$) [10]. All the values provided for this method in this work will be expressed in $\mu\text{g/L}$ according to this factor.

In the present study, we used the Beckman-Coulter Access assay as the comparator method because a regression equation between the Access assay and the initial version of the Tandem-R-Ostase assay had previously been published, showing that Access = $0.997\text{ Tandem R Ostase} + 0.7$.

According to the Deming equations, we calculated, for each method, the value corresponding to an Access value of 10 and 20 $\mu\text{g/L}$. We then calculated the bias for each method at 10 and 20 $\mu\text{g/L}$ and the mean bias and we compared the percentage of patients with BAP values ≤ 10 and $\geq 20\text{ }\mu\text{g/L}$ with each assay. The Beckman-Coulter Access method was chosen as the comparative method.

Table 1
Summary of the methods used in the study.

Method	Measures	Has been calibrated against	Origin of the antibodies	Origin of the antigen	CV
Beckman-Coulter Ostase IRMA	Mass of the enzyme. Provides results in $\mu\text{g/L}$	Hybritech Tandem-R Ostase	Beckman-Coulter	Beckman-Coulter	$<13.6\%$
Beckman-Coulter Ostase Access	Activity of the enzyme. Provides results in mass ($\mu\text{g/L}$) after calibration	Hybritech Tandem-R Ostase	Beckman-Coulter	Beckman-Coulter	$<6.5\%$
IDS iSYS Ostase	Activity of the enzyme. Provides results in mass ($\mu\text{g/L}$) after calibration	Beckman-Coulter Ostase Access	Beckman-Coulter	Beckman-Coulter	$<9\%$
IDS Ostase BAP EIA	Activity of the enzyme. Provides results in mass ($\mu\text{g/L}$) after calibration	Beckman-Coulter Ostase Access	Beckman-Coulter	Beckman-Coulter	$<6.4\%$
DiaSorin Liaison Ostase	Mass of the enzyme ($\mu\text{g/L}$)	Beckman-Coulter Ostase Access	Beckman-Coulter Ostase Access for the capture antibody, DiaSorin for the second, isoluminol bound, antibody	DiaSorin	$<8.1\%$
Quidel MicroVue	Activity of the enzyme (U/L) ^a	1 unit of BAP is defined as 1 μmol of p-nitrophenylphosphate hydrolyzed per minute at $25\text{ }^{\circ}\text{C}$	Quidel (Metra)	Quidel (Metra)	$<7.6\%$

^a U/L values were divided by 0.488 to yield $\mu\text{g/L}$.

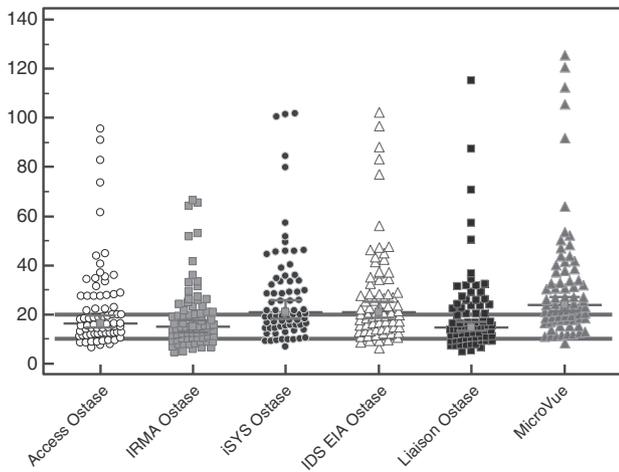


Fig. 1. Distribution of BAP levels observed in 76 hemodialyzed patients according to the different methods used in the study. The solid reference lines at 10 and 20 µg/L represent the different cut-offs proposed to define low and high bone-turnover in hemodialyzed patients, obtained with the former Hybritech Tandem Ostase assay.

Data were expressed as mean ± standard deviation (SD) when distribution was normal and as median and interquartile range [IQR] if not. Normality was assessed by the Kolmogorov–Smirnov test.

Results

Using the Beckman-Coulter Access, the median BAP concentration in the 76 patients was 16.3 µg/L (interquartile range: 14.8–19.3 µg/L) and ranged from 6.4 to 95.5 µg/L. The distribution of the results according to each method is presented in Fig. 1.

The equations of the weighted Deming regression lines between the different methods, the correlation coefficients and the mean difference calculated according to the Bland–Altman method are presented in Table 2.

We observed an excellent correlation, ranging from 0.9948 (IDS iSYS vs. IDS EIA) to 0.9215 (DiaSorin Liaison vs. Quidel MicroVue). In regression analyses, the slopes ranged from 0.73 (Beckman-Coulter IRMA vs. Access) to 1.83 (Quidel MicroVue vs. Beckman-Coulter IRMA) and the Bland–Altman plots showed a mean difference ranging from 0.7 ± 2.4

Table 3

Equivalent concentrations obtained with each BAP assay, when the value measured with the Beckman-Coulter Access is 10 or 20 µg/L.

Assay	BALP (µg/L)	BALP (µg/L)	Mean bias (%)
Beckman-Coulter Access Ostase	10	20	0
Beckman-Coulter IRMA Ostase	9.6	16.9	−9.8
IDS iSYS Ostase	13.2	24.7	27.8
IDS Ostase EIA	13.2	24.2	26.5
DiaSorin Liaison Ostase	7.7	17.4	−18
Quidel MicroVue	14.4	27.9	41.8

These values were calculated according to the equations presented in Table 2. The mean bias value, expressed in %, is, for a given method, the mean bias observed when the “X” of the Deming equation is replaced by 10 and 20 µg/L.

(IDS iSYS vs. EIA) to −12.8 ± 11.6 (Beckman-Coulter IRMA vs. Quidel MicroVue).

Using the regression equations, we calculated, for each assay, the equivalent concentration when the Beckman-Coulter Access was 10 and 20 µg/L (Table 3). The results show that, according to the assay Access, 10 µg/L can range from 7.7 to 14.4 µg/L and Access 20 µg/L can range from 16.9 to 27.9 µg/L.

According to Beckman-Coulter Access, 13%, 50% and 37% of the patients presented BAP values ≤10, between 10 and 20 and ≥20 µg/L, respectively. The concordance between Beckman-Coulter Access and the other assays to classify the patients accordingly is presented in Table 4.

Discussion

BAP determination is becoming one essential tool in the management of bone-related disorders observed in patients suffering from kidney diseases. BAP presents very interesting features, like liver clearance (thus concentrations not influenced by decreased GFR), relative high half-life in serum, storage stability and relatively low intra-individual variability [7, 8]. Different cut-offs have been proposed for patient management, but unfortunately, these cut-offs have been obtained with a method (Hybritech Tandem-R Ostase) that is no longer available in its original form (coated beads). Since then, different methods have emerged to routinely determine BAP. Among them, we used the Beckman-Coulter Access as the comparator method because a regression equation had previously been published, showing that the Access

Table 2

Lower (white) part: equations of the weighted Deming regression lines between the different methods: the Y of the equations [95% CI] corresponds to the methods in vertical whereas the X [95% CI] corresponds to the method in horizontal. Upper (gray) part: coefficient of correlation and mean difference ± 1 standard deviation calculated with the Bland–Altman method (method in vertical – method in horizontal).

		Beckman		IDS		DiaSorin	Quidel
		Access	IRMA	iSYS	EIA	Liaison	MicroVue
Beckman	Access		r = 0.9758 4.1 ± 5.9 µg/L	r = 0.9869 −5.2 ± 4.2 µg/L	r = 0.9891 −4.5 ± 3.3 µg/L	r = 0.9457 2.7 ± 5.9 µg/L	r = 0.9856 −8.6 ± 7.1 µg/L
	IRMA	Y = 0.73 [0.67; 0.79] X +2.3 [1.0; 3.5]		r = 0.9778 −9.2 ± 8.3 µg/L	r = 0.9785 −8.4 ± 7.3 µg/L	r = 0.9215 −1.3 ± 7.4 µg/L	r = 0.9794 −12.8 ± 11.6 µg/L
IDS	iSYS	Y = 1.15 [1.05; 1.25] X + 1.7 [−0.1; 3.5]	Y = 1.58 [1.62; 1.54] X −1.8 [−3.0; −0.6]		r = 0.9948 0.7 ± 2.4 µg/L	r = 0.9343 7.8 ± 14.9 µg/L	r = 0.9876 −3.4 ± 4.3 µg/L
	EIA	Y = 1.10 [1.00; 1.20] X + 2.2 [0.4; 4.0]	Y = 1.58 [1.62; 1.54] X −1.8 [−3.0; −0.6]	Y = 0.95 [0.89; 1.01] X +0.7 [−0.6; 1.9]		r = 0.9485 7.0 ± 6.4 µg/L	r = 0.9343 7.8 ± 14.9 µg/L
	DiaSorin Liaison	Y = 0.97 [0.68; 1.25] X −1.9 [−7.4; 3.5]	Y = 1.38 [0.93; 1.84] X −6.0 [−13.5; 1.4]	Y = 0.85 [0.57; 1.14] X −3.7 [−10.4; 3.0]	Y = 0.88 [0.63; 1.13] X −3.8 [−9.5; 1.9]		r = 0.9271 −11.3 ± 10.3 µg/L
	Quidel MicroVue	Y = 1.34 [1.20; 1.47] X +0.9 [−1.6; 3.4]	Y = 1.83 [1.68; 1.99] X −3.2 [−5.8; −0.6]	Y = 1.16 [1.09; 1.23] X −1.1 [−2.8; 0.6]	Y = 1.22 [1.09; 1.35] X −1.8 [−4.7; 1.1]	Y = 1.37 [0.81; 1.93] X +3.8 [−5.4; 13.1]	

Table 4

Concordance of the different methods to classify identically the patients when they present BAP values ≤ 10 , between 10 and 20 and ≥ 20 $\mu\text{g/L}$ with the Beckman-Coulter Access Ostase assay.

Assay	Concordance with Access: BAP ≤ 10 $\mu\text{g/L}$	Concordance with Access: BAP comprised between 10 and 20 $\mu\text{g/L}$	Concordance with Access: BAP ≥ 20 $\mu\text{g/L}$
Beckman-Coulter IRMA Ostase	100%	79.5%	79.2%
IDS iSYS Ostase	70%	66.7%	100%
IDS Ostase BAP EIA	50%	61.5%	100%
DiaSorin Liaison Ostase	90%	80.5%	88.9%
Quidel MicroVue	10%	38.4%	100%

assay gave results that were very similar to those of the initial version of the Tandem-R-Ostase assay (Access = 0.997 Hybritech Tandem R Ostase + 0.7).

We demonstrated here for the first time that there was quite a large inter-method variation for BAP measurements. The practical implication of this finding is that, in HD patients, different diagnoses of bone turnover can be done clinically, and thus different decisions can be initiated according to the BAP method that was used to determine the levels (or activity) of the enzyme. For example, 24 patients might have been considered as having a high bone turnover because they would have presented BAP values >20 $\mu\text{g/L}$ with MicroVue whereas their Beckman-Coulter Access levels is actually below 20 $\mu\text{g/mL}$. One patient even presented BAP values <10 $\mu\text{g/L}$ with the Beckman-Coulter IRMA whereas its MicroVue equivalent was over 20 $\mu\text{g/L}$, leading to opposite diagnosis.

There are different reasons that might explain the differences that we observed. Among them, the lack of International Standard and reference method is certainly one of the most important. Second, as BAP determination is considered as a very robust method by laboratories, clinicians and manufacturers, it is not impossible that they did not pay as much attention as they do for other analytes, like 25-hydroxyvitamin D or parathormone, that are considered as more “sensible”. Third, there is, to our knowledge, only one institution proposing an external control for BAP (UK-NEQAS). Unfortunately the BAP values observed with this control are very low (generally <10 $\mu\text{g/L}$), which does not allow the users to detect a significant difference between the methods.

Our study has however some limitations. First, we arbitrarily decided to use the Beckman-Coulter Access as the reference method for the 10 and 20 $\mu\text{g/L}$ cutoffs. We considered this method as the “referent”, not as the “reference”. Once again, we decided to do so because Beckman-Coulter showed a perfect correlation between the Access and the Hybritech Tandem-R Ostase in 2000 [9]. This does not mean that the Access is a “gold standard” and that the other methods are less performing. Moreover, recently, Beckman-Coulter encountered problems with the stability of the raw material that was used for Access kits that forced the company to emit a recall of the device [11]. Thus, the fidelity to the perfect correlation observed in 2000 may significantly be compromised in 2013. Second, we used a factor of 0.488 (provided by Quidel) to transform the units into mass concentrations. The value of this factor can be discussed as well as the stability of this value over time. However, in the UK-NEQAS results of June and February 2013, we calculated that the mean factor between values provided by Quidel and mass users was 0.504, which is only 3% above the factor mentioned by Quidel. Third, we used cutoffs of 10 and 20 $\mu\text{g/L}$ in this study. However, we think that these cutoffs, obtained with a method that has

disappeared in its initial form, may not be accurate anymore or, at least, transposable to any of the techniques that are used today for BAP determination. To overcome these problems, we think that an important work on the standardization of BAP assays should be performed. We should define a reference method like, for instance, a capillary electrophoresis method, that would separate the BAP from the other alkaline phosphatase isoforms. Then, a set of human samples, aliquoted and kept at -80 $^{\circ}\text{C}$, should be constituted and a BAP value should be defined for these samples with the accepted reference method. Next, these samples should be made available to the manufacturers, which should calibrate their kits on these samples, with a defined tolerance. According to the good coefficient of correlation that we observed between the different methods, this standardization of BAP should not be an issue in the future. After that, new studies involving bone biopsies should be performed to define new cut-offs for the management of CKD patients.

In conclusion, while we think that BAP determination presents an important added value in the management of bone diseases of CKD patients, analytical problems leading to inter-method variation should be overcome to improve the usefulness of this marker in clinical practice.

Disclosure

Pierre Delanaye is consultant for IDS. Etienne Cavalier is consultant for IDS and DiaSorin.

The results presented here are original and have not been published previously.

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