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Low serum levels of alkaline phosphatase of bone origin: a good marker of adynamic bone disease in haemodialysis patients

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Abstract

Background. Adynamic bone disease was recently described to be increasingly prevalent in the dialysis population. At present the diagnosis of this type of renal osteodystrophy can only be made by bone histomorphometry. We assessed the value of different biochemical serum markers in the diagnosis of adynamic bone disease.

Methods. In 103 haemodialysis patients a bone biopsy was performed after double tetracycline labelling, and the serum levels of intact PTH, osteocalcin, and the bone isoenzyme of alkaline phosphatase were determined. Bone alkaline phosphatase was measured by an optimized agarose gel electrophoretic method, recently shown to have a high accuracy, precision and reproducibility, also in the lower range.

Results. In 38 (37%) of the patients the diagnosis of adynamic bone disease was histologically established. Constructing receiver operator curves optimal cut-off levels for the diagnosis of adynamic bone disease were determined, being $\leqslant 27$ U/litre for the bone isoenzyme of alkaline phosphatase, $\leqslant 14$ μg/litre for osteocalcin and $\leqslant 150$ pg/ml for intact PTH. Concentrations of bone alkaline phosphatase or intact PTH below these cut-off levels, were shown to be the best performing tests in the detection of adynamic bone disease as indicated by a sensitivity of 78.1 and 80.6% and a specificity of 86.4 and 76.2% respectively. Applying Bayes' theorema, it was calculated that in the current haemodialysis population in which a prevalence of adynamic bone disease up to 35% has been described, the positive predictive values for the proposed cut-off values are 75% for bone alkaline phosphatase, 65% for intact PTH and 55% for osteocalcin. Moreover, in this population, levels of bone alkaline phosphatase and intact PTH below the optimal cut-off excluded hyperparathyroid bone disease.

Conclusion. In view of the relative easy and accurate methodology for bone alkaline phosphatase determination, the closer physiological link with osteoblast function and the lesser expense for its determination we suggest that this marker is a useful tool in the non-invasive diagnosis of the adynamic type of bone disease in the individual patient.

Key words: adynamic bone disease; bone alkaline phosphatase; haemodialysis; osteocalcin; parathyroid hormone

Introduction

Renal osteodystrophy encompasses a variety of skeletal disorders [1] including high-turnover lesions of mild secondary hyperparathyroidism and osteitis fibrosa and low-turnover lesions of osteomalacia and adynamic bone. Mixed or transitional disorders which display selected histological features of both high- and low-turnover lesions may also be seen. In recent years an increasing prevalence of adynamic bone disease (ABD) all over the world has been reported [2–4]. The physiopathological mechanism behind this type of renal osteodystrophy is largely unknown and is most likely multifactorial. In the past, aluminium deposition at the mineralization front accounted for most low turnover lesions of renal osteodystrophy (both osteomalacia and ABD) in patients undergoing regular dialysis [5,6]. However, ABD with no evidence of aluminium deposition at the mineralization front seems to be more prevalent in the dialysis population in recent years [2–4,7,8]. Patients with diabetic nephropathy have also been described to be more prone to this type of bone disease than non-diabetics [9]. It has been suggested that in some patients the disorder can be attributed to iron overload [10,11]. Different groups [2–4,7,8], have shown, however, that not all cases of ABD can be explained by these factors. What all the patients with ABD do have in common is a relatively low level of intact PTH (iPTH) [2,3,7,8,12–15].

Neither the clinical symptoms, nor the long-term

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consequence of this disease have yet fully been elucidated. Recently it has become apparent that ABD is not as innocuous as was previously thought: it has been suggested that these patients are more susceptible to develop hypercalcaemia [3,4,16]. Meric *et al.* [17] have pointed towards the increased risk for hypercalcaemia when prescribing $CaCO_3$ in patients with low bone turnover. The increased risk for metastatic calcifications with this disease has been reported [18]. Some authors [12,13] also advocate that vitamin D analogues should be avoided in ABD in order to have the parathyroids not 'oversuppressed'.

Many attempts for obtaining good performing biochemical markers of renal osteodystrophy replacing the invasive bone biopsy have appeared in recent literature. iPTH was indeed described to have good predictive values for diagnosing either ABD or severe hyperparathyroidism [15], or for distinguishing patients with low-turnover lesions of renal osteodystrophy from those with secondary hyperparathyroidism [14,19]. Recently however, Quanle *et al.* [20] pointed towards the weakness of iPTH as a marker of bone turnover in renal osteodystrophy. Moreover, Goodman *et al.* [21] documented persistence of high iPTH levels in patients in whom vitamin D induced a reduction of bone formation rate, thus raising the question for markers reflecting more directly the osteoblast activity. Osteocalcin, a secretion product of the osteoblast, has been shown to correlate with histological parameters of bone formation in chronic dialysis patients [22,23]. Also for the newer markers of bone collagen synthesis (e.g. the carboxy-terminal propeptide of type I procollagen) and degradation (e.g. collagen type I C-terminal cross-linked telopeptide) correlations with histological indices of bone turnover have been studied [24]. The value of all these markers for the diagnosis of renal osteodystrophy in the individual patient however is not documented. Finally, total alkaline phosphatase (TAP) as a marker of osteoblastic function was described to have poor diagnostic performance for renal osteodystrophy in comparison with iPTH [14,20,22]. Taking into account the contribution of non-osseous sources (e.g. liver, intestinal) of alkaline phosphatase to its total serum level, this poorer diagnostic performance is not unexpected. Moreover, to be of value in the diagnosis of adynamic renal osteodystrophy, biochemical markers should have good specificity and sensitivity especially in the lower concentration range.

We recently described an optimized immunoelectrophoretic method for the quantification of the isoenzymes of alkaline phosphatase [25]. This assay was shown to quantify the different isoenzymes of alkaline phosphatase in a highly reproducible and accurate way [25,26]. In the current study, alkaline phosphatase of bone origin (BAP) was determined in serum, and the value of a low level of BAP for the diagnosis of adynamic bone disease was evaluated; results were compared with another biochemical index of bone formation and turnover: the serum level of osteocalcin (OC), and with iPTH.

Subjects and methods

Patients

In 103 chronic adult haemodialysis patients from different countries (Belgium ($n=43$), Greece ($n=42$), Egypt ($n=5$), Argentina ($n=3$), Slovakia ($n=7$) and Luxembourg ($n=3$)) a transiliac bone biopsy was taken. There were 53 female and 50 male patients included with a mean age of 59.7 ± 1.3 (SEM) years and a mean time on dialysis of 4.4 ± 0.4 (SEM) years. Eight patients had undergone a parathyroidectomy, and eight had had a previous renal transplantation in their medical history. In 89 patients, data on phosphate binding therapy were available: it consisted of $CaCO_3$ in 33 patients, of $Al(OH)_3$ in 31 patients, of both $CaCO_3$ and $Al(OH)_3$ in 21 patients. Four patients did not receive phosphate binders at all. Forty-two patients were taking a vitamin D preparation.

The indication for performing a bone biopsy were diverse: suspicion of aluminium intoxication, abnormal levels of iPTH (either high or low), diagnostic work-up before parathyroidectomy, a very low level of BAP, or suspicion of a combination of either of these pathologies. Patients with impaired hepatic function as indicated by increased sGOT, sGPT decreased level of total protein and or β-γ 'bridging' in electrophoresis were excluded.

Prior to the biopsy a double tetracycline labelling was carried out: demeclocyclin (2×300 mg) was given orally on 3 consecutive days. After 17–20 days, tetracycline HCl (2×500 mg) was administered orally for another 3 consecutive days. On the days of labelling the patients were instructed not to take any phosphate binder. Three to 7 days after the second labelling session, the patients were hospitalized for 24 h and a bone biopsy was taken. At the time of biopsy a serum sample was taken for the determination of alkaline phosphatase and its isoenzymes, iPTH, OC, serum aluminium, and ferritin. The serum samples were stored at -80°C until analysis in the central laboratory.

The protocol was approved by the committee for Medical Ethics of the University Hospital Antwerp.

Bone biopsy

The biopsies were taken under local anaesthesia using a Bordier–Meunier needle with an internal diameter of 7 mm, at a site 2 cm posterior and 2 cm inferior to the anterior iliac spine. The specimen was cut into two pieces. The largest part, destined for histomorphometry, was kept for 24 h in Burkhardt's solution, after which it was transferred into 70% ethanol until processing. Biopsy specimens were prepared for quantitative histology as previously described [27]. Five-micrometre section of non-decalcified bone were stained by the modified Goldner technique for light-microscope examination and by the aurine tricarboxylic acid method (Aluminon®) for the histochemical detection of bone surface aluminium [27,28]. Perl's staining was applied for detection of iron. Ten-micrometre sections were mounted unstained in 10% glycerol and examined by fluorescence microscopy for the evaluation of tetracycline labels [27]. All measurements of length, width, and area were done using a digitizer interfaced with a microcomputer, and the results represent two-dimensional variables.

Bone histological data as well as dynamic parameters are reported using standardized nomenclature and definitions [29]. On the basis of results obtained in normal controls, the different histological diagnoses are defined according to the

Table 1. Histological classification of renal osteodystrophy according the criteria described by Salusky *et al.* [14]

Type of lesion	Osteoid area (% of total bone area)	Fibrosis	BFR_1 ($\mu m^2/mm^2$ per day)
Osteitis fibrosa	<12	+	>97
Mild lesion	<12	−	>613
Normal	<12	−	>97 <613
Adynamic	<12	−	<97
Osteomalacia	>12	−	<97
Mixed lesion	>12	+	

amount of osteoid, the presence of fibrosis and the bone formation rate as previously described [30] and outlined in Table 1. The second, smaller, part of the biopsy was wet weighted and sent to the laboratory for bulk analysis of aluminium as soon as possible [31]. The diagnosis of aluminium overload was established on the presence of an amount of aluminium $\geqslant 15$ μg/g wet weight, and/or in the presence of a positive Aluminon® staining (>0%) at the calcification front [32].

Biochemical measurements

Total alkaline phosphatase (TAP) was determined with Baker Biochemicals according to the recommendations of the SSCC but at 25°C on Hitachi 705. With this method the normal range varies between 63 and 166 U/litre. The bone isoenzyme was determined by an agarose gel electrophoretic method described by Van Hoof *et al.* [25]. In short the method consists of an electrophoresis on a commercially available agarose gel (Isopal, Beckman, Brea, CA). The serum sample is pretreated with a polyclonal serum against placental and intestinal alkaline phosphatase. This pretreatment retards the migration of the isoenzymes of placental and intestinal origin and thus allows for better quantification of the bone and liver fractions. If the bone fraction appears to account for more than 50% of the total alkaline phosphatase, pretreatment with neuraminidase is applied to allow further separation of the liver and bone fractions in order to obtain an accurate determination of the bone isoenzyme (Figure 1). Quantification of the fractions is performed by scanning of the gel by a computerized densitometer (Appraise, Beckman Instr., Brea, CA). This method has been shown to have an intra-assay coefficient of variation of 2% and an inter-assay coefficient of variation of 7% when BAP is within reference ranges [25]. The mean value of BAP in subjects with normal renal function is 40 U/l, the 5th and 95th percentile being 23 and 80 U/l respectively [33].

iPTH was measured using the Nichols IRMA-kit (Nichols Institute, San Juan Capistrano, CA), the normal values for patients with normal renal function ranging between 10 and 65 pg/ml. Osteocalcin was determined by the RIA system of Sorin, Incstar (Incstar Corp., Stillwater, Minnesota) with normal values for patients with normal renal function between 1.8 and 6.6 μg/litre.

Serum aluminium was determined by graphite furnace atomic absorption spectrophotometry [34].

Statistical analysis

The results were analysed with SPSS for Windows (release 6.0).

A logistic regression model was constructed that included the covariates age, sex, duration of dialysis, BAP, ferritin, iPTH, OC, and serum aluminium concentration. A covariate was considered to be predictive for ABD when its estimated coefficient was significantly different from 0 based on the Wald statistic ($P<0.05$).

The diagnostic tests with a good predictive value were further assessed by receiver–operator characteristic (ROC) curves, which are generated by plotting sensitivity or true positive rate (y axis) versus 1-specificity or false positive rate (x axis). The optimal discrimination limit for each test was determined at the maximum of the Youden's index [35]: J = sensitivity + specificity − 1.

To compare the performance of the different tests, the statistical methods for comparing the areas under the curve could not be applied since the ROC curves intersect. Therefore the significance of the difference in specificity found for the different tests at a single point was calculated according to Hanley and McNeil [36]. The index point of sensitivity was chosen at a level where the highest sensitivity with the highest specificity was found i.e. at 78.1% for BAP. A paired sample test for differences in proportions [37] was applied which takes into account only the samples that disagree with each other.

The positive predictive value (PPV) and the negative predictive value (NPV) were calculated according to Bayes' theorema.

In the explanatory analysis group covariates were first analysed with analysis of variance, followed, if significant differences were observed, by multiple comparisons with the Student–Newman–Keuls test.

Results

The different histological diagnoses found in the 103 bone biopsies are summarized in Table 2. Thirty-eight (37%) of the patients were diagnosed to have ABD. Out of these, only 12 patients displayed aluminium overload. Logistic regression analysis identified BAP, iPTH, and OC as predictors of the presence of ABD in the study population. Age, duration of dialysis,

Table 2. Distribution of types of renal osteodystrophy diagnosed. Hyperparathyroidism includes both cases of mild lesion and osteitis fibrosa

Diagnosis	Patients (*n*)
Normal	13
Hyperparathyroidism	21
Adynamic bone disease	38
Osteomalacia	10
Mixed disease	21

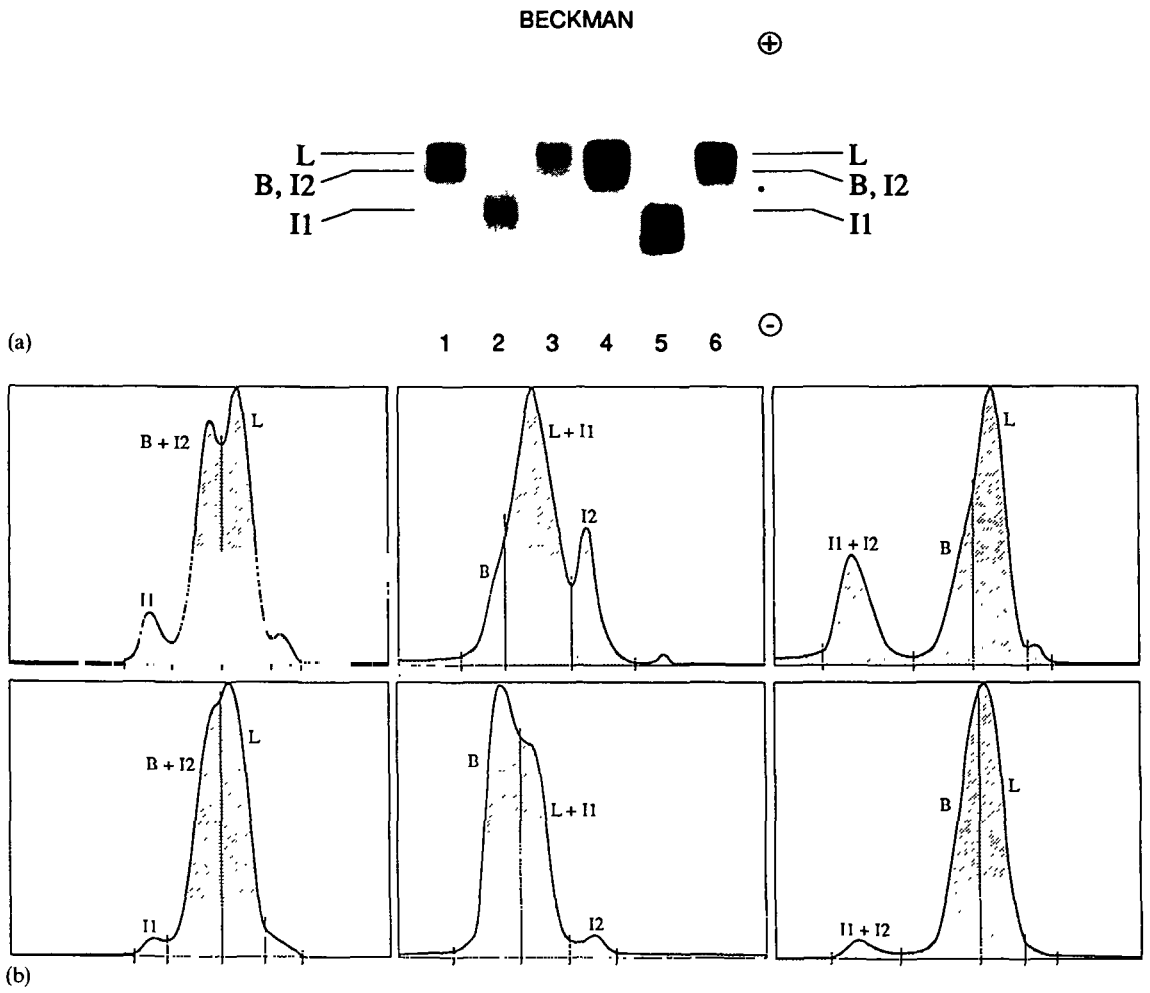

Fig. 1a. Typical ALP isoenzyme patterns obtained with the Isopal (Beckman) system in chronically dialysed patients. Lane 1, serum from a patient with adynamic bone disease: low bone ALP, high liver ALP, a trace of intestinal ALP and high intestinal variant ALP; lane 2, serum from lane 1 treated with neuraminidase for 30 min; lane 3, serum from lane 1 treated with polyclonal antiplacental antiserum for 5 min; lane 4, serum from a patient with secondary hyperparathyroidism: high bone ALP, high liver ALP, a trace of intestinal ALP and intestinal variant ALP; lane 5, serum from lane 4 treated with neuraminidase for 30 min; lane 6, serum from lane 4 treated with polyclonal antiplacental antiserum for 5 min.

Fig. 1b.Densitometric scannings of gel a. Top: lane 1, 2, and 3; bottom: lane 4, 5, and 6 (left, untreated samples; middle, samples treated with neuraminidase for 30 min; right, samples treated with polyclonal antiplacental antibody for 5 min). *Note*: the symbols indicate ALP fractions of untreated samples. L, liver ALP; B, bone ALP; I1, intestinal ALP; I2, intestinal variant ALP (anchor intestinal ALP).

ferritin, serum aluminium concentration, and sex did not possess a significant predictive value in the diagnosis of ABD. The sensitivity and specificity of BAP, OC, and iPTH in the diagnosis of ABD were calculated for different cut-off levels and are presented as receiver–operator curves in Figure 2.

From these curves the optimal cut-off level of the different markers in the diagnosis of ABD could be derived and confirmed by calculating the Youden's indices at different cut-off levels. It appeared that in the diagnosis of ABD the best performance of BAP in terms of both sensitivity and specificity could be reached at a level of BAP $\leqslant$27 U/litre (the intra- and inter-assay coefficient of variation in this range being 4 and 3% respectively), of OC $\leqslant$14 µg/litre and of iPTH $\leqslant$150 pg/ml. The sensitivities, specificities, their respective confidence limits, and the Youden's indices of the different markers at these cut-off levels are presented in Table 3. It is shown that a low level of OC has an excellent sensitivity but a low specificity giving rise to a high number of false positives. BAP and iPTH display comparable 'sensitivities' though a low BAP test has 10% less false positives. The same calculations were made for TAP: the optimal cut-off level being at $\leqslant$123 U/l we found a sensitivity of 75% and a specificity of 83%. In order to compare the performance of the different tests, we performed a statistical paired sample evaluation of the specificities found for each marker at a level of sensitivity where the highest level of specificity was found from all the tests performed in this population: this level is reached at a sensitivity of 78% for a level of BAP $\leqslant$27 U/l (Figure 2). As shown in Table 4, only the difference in specificity between BAP and OC reaches a statistically significant level (in casu 17.2%).

As can be expected combining two markers decreases

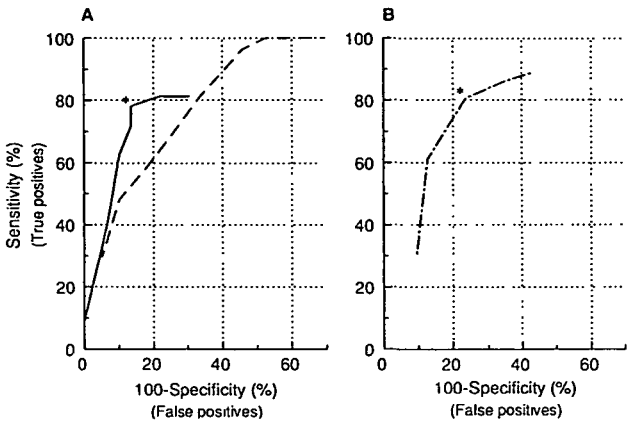

Fig. 2. Receiver–operator characteristic (ROC) curves for bone alkaline phosphatase (—), osteocalcin (– – –), and iPTH (–·–) in the diagnosis of adynamic bone disease: for different cut off levels the sensitivity is related to the percentage of false positives. The optimal cut-off level (symbol (o)) is defined as the level where the highest sensitivity is reached for the lowest number of false positives.

Table 3. Sensitivities, specificities and Youden's index of the different markers tested in the diagnosis of adynamic bone disease

Marker (*n*)	Sensitivity (95% CI)	Specificity (95% CI)	Youden's index
BAP ⩽27 U/litre (91)	78.1 (63.8↔92.4)	86.4 (77.7↔95.1)	0.64
OC ⩽14 µg/litre (86)	96.3 (89.2↔100)	54.2 (47.7↔60.9)	0.50
iPTH ⩽150 pg/ml (99)	80.6 (67.7↔93.5)	76.2 (56.3↔79.3)	0.57
TAP ⩽123 U/l (91)	75.0 (66.2↔83.8)	83.1 (76.1↔90.1)	0.58
BAP ⩽27 U/litre and iPTH ⩽150 pg/ml (90)	67.7 (51.2↔84.2)	91.5 (84.4↔98.6)	0.59
BAP ⩽27 U/litre and OC ⩽14 µg/ml (86)	75.0 (59.0↔91.0)	86.7 (78.1↔95.3)	0.62
iPTH ⩽150 pg/ml and OC ⩽14 µg/ml (86)	81.5 (73.5↔89.5)	76.3 (67.3↔85.3)	0.58

n=number of patients in whom the test was performed; 95% CI, 95% confidence intervals; Youden's index=sensitivity+specificity−1.

the sensitivity. However, only the combined finding of a level of BAP ⩽27 U/litre and of a iPTH ⩽150 pg/ml entails a considerable increase in specificity and thus decrease of false positives, as is also displayed in Table 3. The combination of a low BAP with a low OC level loses less sensitivity than the combination of BAP ⩽27 U/litre with iPTH ⩽150 pg/ml, but it does not reach a specificity superior to a low level of BAP ⩽27 U/litre alone. Finally, also the combination of a low iPTH level with a low OC level does not increase the specificity at a level superior to the one obtained for iPTH or BAP as individual test.

Based on the sensitivity and specificities found in our study population for the different markers, we calculated the positive and negative predictive values of these markers at different prevalences of the disease, applying Bayes' theorema. These data are outlined in Figure 3. In the current haemodialysis population in which a prevalence of ABD of 35% has been reported [3] it appears that a low level of BAP has a PPV and a NPV of 75 and 88% respectively, as compared to 55 and 97% for OC, and 65 and 88% for iPTH.

Considering the bone biopsies of the patients displaying a false positive low BAP level we found normal bone histology in five, osteomalacia (with very low BFR) in two and a mixed lesion in one. It is of note that none had signs of hyperparathyroidism. Also their

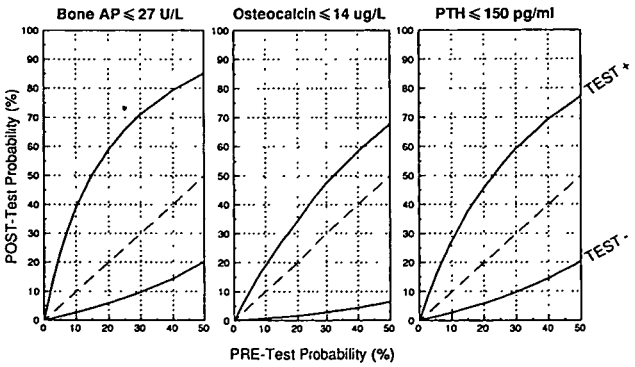

Fig. 3.Predictive values of a low level of bone alkaline phosphatase (⩽27 U/litre), osteocalcin (⩽14 µg/litre), and iPTH (⩽150 pg/ml) in the diagnosis of adynamic bone disease at different pre-test probabilities, i.e. different prevalences of the disease. The predictive values for both a positive and negative test result are given.

Table 4. Specificities (Spe) and their differences (Spe A−Spe B) for the different markers at a sensitivity of 78%, as well as the 95% confidence interval (95% CI) of the calculated differences

Marker A	Marker B	Spe A	Spe B	Spe A−Spe B	95% CI
BAP 27	OC 10.5	86.2	69.0	17.2	6.6↔17.2
BAP 27	iPTH 139	86.4	76.3	10.2	−2.9↔18.1
BAP 27	TAP 133	86.4	76.3	10.2	−2.9↔18.1
OC 10.5	iPTH 139	69.5	76.3	−6.7	−17.7↔7.0
OC 10.5	TAP 133	69.0	75.9	−6.9	−14.9↔5.2
iPTH 139	TAP 133	77.4	75.8	1.6	−10.4↔12.9

The cut-off level of the respective markers at a sensitivity of 78% is noted after the marker (in U/litre for BAP and TAP, in pg/ml for iPTH and in µg/litre for OC).

mean ferritin level (559 ng/ml ± 333) was significantly higher ($P < 0.05$) than the mean ferritin of the true positives (215 ng/ml ± 253). Comparing the mean ages, we also found the false positive patients to be significantly ($P < 0.05$) older than the patients showing no ABD with BAP >27 U/litre: 72.6 ± 10.1 vs 56.7 ± 12.0 years.

On the other hand, five of the seven false negative patients thus displaying ABD despite a BAP >27 U/litre had evidence of aluminium overload. Their means of the serum aluminium level (61.3 ± 10.5 ng/litre), of the aluminium content of the bone (26.9 ± 8.6 μg/g wet weight) and the surface stainable aluminium (13.5 ± 6.7%) tended to be higher than in the true positives presenting values of 46.5 ± 17.4 ng/litre, 12.3 ± 3.4 μg/g wet weight, 4.6 ± 2.3% respectively. These differences did not reach statistical significance, which is most probably due to the small numbers of patients. They all had iPTH levels ⩽150 pg/ml. Also their mean serum ferritin levels were considerably lower (91 ± 27 ng/ml) as compared to the true positives (215 ± 52 ng/ml), although this difference also did not reach statistical significance either.

Discussion

Renal osteodystrophy encompasses many different types of bone disease [1], of which ABD has gained recently great clinical and research interest. This low-turnover type of renal osteodystrophy is reported to present with an increasing prevalence all over the world [2–4].

At present histomorphometric analysis of a bone biopsy after double tetracycline labelling remains the gold standard for the diagnosis of the different types of renal osteodystrophy. Different biochemical markers such as osteocalcin and iPTH have been shown to be helpful tools to distinguish between low and high bone turnover. Superiority in diagnostic performance for a particular type of renal osteodystrophy in the individual patient has not, however, been demonstrated for any of them.

This study was designed in order to evaluate and compare three biochemical serum tests in the diagnosis of ABD to elaborate a non-invasive assessment and thus be able to avoid bone biopsy.

The main result of this diagnostic decision making assessment was that low levels of both BAP (⩽27 U/litre) and iPTH (⩽150 pg/ml) are good markers of ABD in the individual patient, as indicated by their respective sensitivity as well as specificity. Osteocalcin was shown to have a remarkably high sensitivity but at the expense of an unacceptable low specificity and thus a high proportion of false-positive patients. Combining two tests as a marker of ABD results in a gain of specificity, thus fewer false positives, only when both a low BAP and low iPTH are taken into account. Applying the sensitivities and specificities of BAP and iPTH—according to Bayes's theorema—to a population with a prevalence of ABD of 35%, we found a PPV of 75% for BAP, 65% for iPTH, and a NPV of 88% for both tests. This means that when the current haemodialysis population with a prevalence of ABD of 35% (as described by Sherrard *et al.* [3]) has to be screened for ABD, it can be concluded from our data that finding a low level of BAP in a patient means that this patient has a 75% chance of having the disease. On the other hand, if BAP is >27 U/l the chance of not having ABD is almost 90%. It is of note that both for BAP and iPTH none of the false positive patients had features of hyperparathyroidism on their bone biopsy in the population studied. They all had either normal bone histology or low turnover disease. Thus the finding of a low BAP excludes with a high degree of certainty (100% in this study population with 95% CI of 81.5–100%) the presence of hyperparathyroid bone disease, raising an absolute contraindication for any form of parathyroid-suppressive treatment in these patients.

Others [19] have reported lower diagnostic performances of low bone alkaline phosphatase but the method used (wheat germ lectin precipitation) is known to lose accuracy, particularly in the lower range [38]. In our laboratory an optimized agarose gel electrophoretic technique was developed [25]. With this assay it was shown that an accurate and reproducible quantitative measurement of the bone isoenzyme of alkaline phosphatase can be obtained [25], especially in the lower range, showing inter- and intra-assay CV's of 3 and 4% respectively in this low range. A well-trained technician can perform approximately 50 determinations a day. This assay was recently shown to be superior to an immunoradiometric test using a specific monoclonal antibody to quantify bone alkaline phosphatase activities [39]. Indeed, particularly in the low range of BAP, the cross-reactivity of the monoclonal antibody with the liver isoenzyme results in falsely high BAP determinations.

An additional advantage of BAP determination in renal failure patients is its high molecular weight (16 kDa) making its clearance independent of renal function. Moreover storage conditions for serum samples for alkaline phosphatase isoenzymes determination are less stringent (at 4°C for 48 h) compared to iPTH (−80°C). In most studies addressing the problem of good biochemical markers of bone disease in dialysis patients total alkaline phosphatase has always been discarded because of its lack of sensitivity (in the low range) or specificity (in the high range) [14,20,22]. Indeed, when TAP is determined, changes in the bone isoenzyme can easily be overlooked. This is particularly the problem in a dialysis population where a change in TAP is difficult to interpret, since many pathologies both of hepatic and osseous origin can contribute to the observed changes. Indeed, haemodialysis patients may have many reasons for an increased liver fraction of alkaline phosphatase: post-hepatitis cirrhosis, cardiac failure, haemochromatosis, liver metastasis, etc. When studying the alkaline phosphatase isoenzyme pattern monthly during 1 year in a group of 47 chronically dialysed patients, a raised level of the liver isoenzyme was detected, at least at three occasions in 16 patients

[40]. Furthermore, in haemodialyzed patients, the asialoglycoprotein intestinal alkaline phosphatase is frequently increased, associated or not with cirrhosis [41]. These changes can even occur in the absence of an increased TAP level, particularly when there is a concomitant decrease in the bone fraction. Undoubtedly, differentiation of the isoenzymes of alkaline phosphatase is of interest in a dialysis population [42]. In the present study, when comparing a low level of TAP ($\leqslant$123 U/litre) with a low level of BAP ($\leqslant$27 U/litre) as markers of ABD, a performance comparable to that of BAP and iPTH was found. These results should be interpretated cautiously however. Indeed, patients with severe liver problems were excluded from this study. Furthermore, we do find a lower sensitivity and thus higher number of false negative patients for TAP in comparison to BAP. The increased number of false negative patients was due to an increased liver and or intestinal isoenzyme in these patients.

Finally, iPTH suffers conceptually from the fact that it mainly reflects the parathyroid gland function and as such is not a marker of what is happening in bone. Alkaline phosphatase, which is an ectoenzyme of the osteoblast, was recently shown to play a crucial role in the mineralization process [43], and in this regard to reflect a specific activity of the osteoblast.

On the other hand, osteocalcin, a secretion product of the osteoblast, was shown to have good sensitivity but poor specificity. This marker has been shown by others [23,44] to discriminate well between high and low turnover types of renal osteodystrophy. Both groups however found the osteocalcin levels in their renal failure patients to be considerably higher than levels found in patients with normal renal function, both in high- and low-turnover renal osteodystrophy. Osteocalcin is indeed mainly dependent on renal function for its clearance [14]. Also the contribution of osteocalcin fragments has been shown to be a confounding factor in the determination of osteocalcin levels in renal failure patients [46]. From this it can be expected that osteocalcin is not a good biochemical marker for bone disease in renal failure patients, especially when the lower range of the determination is important such as in low bone turnover.

Recently, iPTH as assayed by a two-site immunoradiometric method was described to be relatively low in ABD patients [2,3,7,8,12–15]. The strikingly higher sensitivities and specificities of iPTH in these studies [14,15] in comparison with our findings can be attributed to the lower number of patients included. Also, in contrast with these studies, in our study all types of renal osteodystrophy were considered, including aluminium-related bone diseases and osteomalacia. Indeed, when analysing the false negative results in our series (BAP $>$27 U/l in patients with ABD) we could demonstrate evidence of aluminium overload in five of these seven cases. This raises the idea that in ABD, which is ascribed to different aetiologies, some subgroups such as aluminium-induced ABD, may display less osteoblastic functional impairment at the cellular level compared to others. The low level of iPTH in all of these false-negative patients is in accordance with this evidence for Al overload, which is known to directly suppress the parathyroid glands [47], and illustrates the discrepancies which may arise between markers of osteoblastic and parathyroid cell function.

On the other hand, two confounding factors giving rise to false-positive results (i.e. BAP $\leqslant$27 U/l in patients with no ABD) could be identified. First, the age of these patients is significantly higher than in the true negatives, suggesting that the cut-off level for detecting ABD in the elderly may be somewhat lower. Secondly, the mean serum ferritin level in the false positive patients is higher compared to the truly positive patients, adding evidence to earlier data [10,11] suggesting that iron overload is implicated in osteoblastic function.

Finally, when trying to analyse false-positive and false-negative results, one has to take into account the cross-sectional character of this study: some of the 'false' results could be only a reflection of the transitions the bone metabolism in renal patients undergoes from high to normal or low turnover and *vice versa*. Moreover, the shortcomings of quantitative bone histomorphometry are well documented [48]. In the literature, different histological criteria are described for diagnosing the different types of renal osteodystrophy e.g. the use of osteoid width instead of osteoid area, different cut-off levels for normal range of bone formation rate, etc. This bias on the gold standard used in our study may of course elicit some false-positive or -negative results, but any diagnostic decision making assessment will always have to cope with 'weaknesses' of its respective gold standard.

In conclusion, a level of BAP $\leqslant$27 U/l as assayed by the method described by Van Hoof *et al.* [25] was shown by diagnostic decision making assessment to have a performance comparable to iPTH, and superior to osteocalcin in the diagnosis of ABD in the individual patient. The relatively easy and cheap methodology, the closer physiopathological relation with osteoblastic function, are additional advantages of this non-invasive assessment of ABD. Moreover, like iPTH it is an excellent test to exclude hyperparathyroid bone disease. This promising marker remains to be evaluated in other populations such as CAPD patients.

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