High prevalence of adynamic bone disease diagnosed by biochemical markers in a wide sample of the European CAPD population


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Abstract

Background. Adynamic bone disease (ABD) has been described in the current dialysis population to have an unexpectedly high prevalence. Moreover, it is clearly more prevalent in CAPD patients, compared to haemodialysis patients. Recently we demonstrated that both a low (≤27 U/l) level of bone alkaline phosphatase (BAP) as determined by an optimized agarose gel electrophoretic technique and a low (≤150 pg/ml) level of iPTH are good markers of ABD with sensitivities of 78.1% and 80.6% and specificities of 86.4% and 76.2% respectively.

Methods. In this study (n=212), the prevalence of ABD in the European CAPD population was evaluated by means of these biochemical markers. Clinical data on the patients included were recorded at the moment of blood sampling. In patients under CAPD treatment for longer than 9 months, we calculated an index of calcium exposure through PD fluid.

Results. In this population with a low exposure to aluminium, the prevalence of ABD as indicated by either a low level of BAP or PTH was 43%. The following risk factors could be identified: advanced age, shorter time on renal replacement therapy, male gender, and high calcium content of PD fluid. The index of calcium exposure was significantly higher in the patients with low BAP and low iPTH levels compared to those with either BAP >27 U/l or iPTH >150 pg/ml. The latter finding gives further support to the hypothesis that a high calcium load administered to renal failure patients may lead to ‘oversuppressed’ parathyroids in ABD. In a subgroup of patients with a high level of BAP associated with a low iPTH level a profile previously shown to be associated in the presence of aluminium overload, significantly higher serum aluminium levels were noted, suggesting that even in patients with low exposure to aluminium, this element still can affect bone metabolism.

Conclusion. A high prevalence of ABD—as diagnosed by biochemical markers—was observed in the European CAPD population. A number of risk factors could be put forward. The aetiology and pathogenesis of this type of renal osteodystrophy remain to be elucidated, but appear, however, to be multifactorial.

Key words: adynamic bone disease; bone alkaline phosphatase; CAPD; parathyroid hormone

Introduction

The bone histology described in patients with renal osteodystrophy (ROD) shows a wide spectrum of lesions, ranging from severe osteitis fibrosa, characterized by a high turnover, to adynamic bone disease (ABD), with very low turnover [1]. The gold standard for diagnosing these different types of ROD remains the histomorphometric analysis of a bone biopsy after double tetracycline labelling. Recently we demonstrated the good diagnostic performance of both bone alkaline phosphatase (BAP) and intact PTH (iPTH) in the non-invasive diagnosis of ABD [2]. Based on 103 bone biopsies including all types of renal osteodystrophy an optimal cut-off value for BAP and iPTH in the diagnosis of ABD was defined by ROC analysis. At a level ≤27 U/l BAP has a sensitivity of 78.1% and a specificity of 86.4% for diagnosing ABD, whereas for iPTH ≤150 pg/ml these figures are 80.6% and 76.2% respectively. Applying Bayes’ theorem, it was calculated that in the current haemodialysis population in which a prevalence of ABD up to 35% has been described, the positive and negative predictive values for the proposed cut-off levels are 75 and 88% for BAP and 65 and 88% for iPTH.

Recent data show that in the North American dialysis population the prevalence of ABD—based on histomorphometric analysis of bone biopsies—in recent years has increased up to 36% for haemodialysis and even 60% for CAPD patients [3]. Also in a Spanish study, Torres et al. [4] found an unexpectedly high prevalence of ABD in both haemodialysis and CAPD.
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patients, with a clear preponderance in the CAPD population.

The aetiology and pathogenesis of ABD remain largely unknown. Many authors have shown that aluminium is no longer the only culprit, since many cases of ABD have been described without any trace of aluminium in bone [3,5–7]. Patients with ABD have relatively low iPTH levels [3,5–10]. In this respect a possible role of vitamin-D-induced oversuppression of the parathyroid glands has been suggested [11]. In the largest series published so far on the prevalence of ABD [3], the effect of vitamin D was, however, not confirmed. Taking into account that ABD apparently is more prevalent in CAPD than in haemodialysis patients, some authors suggest that the maintenance of higher calcium levels in these patients induces a more effective suppression of the parathyroid gland [3]. In the same line of evidence Pei et al. [12] by multivariant analysis recently demonstrated the intake of CaCO$_3$ to be significantly associated with ABD.

Other risk factors described for ABD include diabetic nephropathy as underlying kidney disease [3,12–14], advanced age [3,9,14,15], and shorter time on dialysis treatment [3].

The purpose of this study was (i) to evaluate the prevalence of ABD in a wide sample of the European CAPD population as diagnosed by validated biochemical markers of osteoblast (BAP) and parathyroid (iPTH) function; and (ii) to examine the relationship between the prevalence of ABD in this particular population and previously described possible risk factors. In order to address these questions 212 CAPD patients from 11 European centres were examined.

Subjects and methods

Patients

Ninety-one CAPD-performing centres in 10 European countries were contacted by mail for their participation. We finally included 212 stable ambulatory patients in 11 centres in six countries (Belgium, 5; The Netherlands, 1; United Kingdom, 2; France, 1; Germany, 1; Spain, 1).

At the time a blood sample was taken a questionnaire on clinical patient data was completed. The mean age in our patient population was 57.1 ± 15.5 years (range 21–89 years), 50.2% of the patients were male. The mean ages in male and female patients were of 58.3 ± 15.2 years, and 55.9 ± 15.7 years respectively and did not differ statistically ($P = 0.264$). The current medication consisted of calcium-containing phosphate binders in 66.4% of the cases (53.5% CaCO$_3$, 13.8% Ca-acetate), of Al(OH)$_3$ in 7.8% and of vitamin D analogues in 30.4% (21.2% as 1α2-hydroxycholecalciferol and 9.2% as 1,25-dihydroxycholecalciferol). At the time of sampling the calcium content of the PD fluid was 1.75 mmol/l and 56.7% of the patients versus 1.25 mmol/l in the remaining 43.3%. The time that a patient had been on his or her current treatment for longer than 9 months ($n = 130$) the mean calcium concentration of the PD fluid per month was calculated as one-ninth of the area under curve of the calcium concentration of the PD fluid versus time curve for the last 9 months before sampling. In 25% of the patients at least one episode of hypercalcaemia was recorded the year prior to inclusion in the study. In 3.3% a parathyroidectomy and in 8.4% a previous renal transplantation episode was reported.

The renal diagnoses according to the EDTA code were chronic glomerulonephritis 24.6%, chronic pyelonephritis 19.4%, analgesic nephropathy 2.9%, polycystic kidney disease 4.3%, diabetic nephropathy 16.4%, renal vascular disease 10.1%, other diseases 10.7%, unknown 11.6%.

Biochemical determinations

In each patient a 10-ml blood sample was collected in the morning, before administration of the first PD-fluid bag. Blood samples were taken in aluminium-free syringes and centrifuged, approximately 2 h after sampling. The serum was transferred to three dry aluminium-free test tubes (a,b,c) for the determination of (a) iPTH, (b) total alkaline phosphatase (TAP) and isoenzymes by electrophoresis, and (c) aluminium. All serum samples were stored immediately at −80 °C and forwarded on dry ice to our laboratory.

Intact iPTH was measured using the Nichols’ IRMA kit (Nichols Institute, San Juan Capistrano, CA). With this kit the normal values for patients with normal renal function are between 10 and 65 pg/ml.

Total alkaline phosphatase was determined with Baker Biochemicals according to the recommendations of the SSCC at 25 °C with a Hitachi 705. With this method the normal range varies between 63 and 166 U/l. The bone isoenzyme was determined by a method combining electrophoresis in agarose gel (Isopal Beckman, Brea, CA) and preincubation of the sample with a polyclonal antiplacental–intestinal alkaline phosphatase antibody [16]. This pretreatment retards the migration of isoenzymes of placental and intestinal origin and thus allows for better identification and quantification of the bone and liver fractions. If the bone fraction appears to account for more than 50% of the total alkaline phosphatase, sample pretreatment with neuraminidase is applied to allow a complete separation of the liver and bone fractions in order to obtain an accurate determination of the bone isoenzyme. Quantification of the fractions is performed by scanning the gel using a computerized densitometer (Appraise, Beckman Inst., Brea, CA). This method has been shown to have an intra-assay CV of 2% and an interassay CV of 7% for BAP values within the reference range [16]. 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 [17].

Serum aluminium was determined by graphite furnace atomic absorption spectrophotometry [18].

Statistical analysis

The results were analysed with SPSS 6.0 for Windows. A logistic regression, using the ‘forward stepwise’ method model, was constructed that included both binomial and continuous covariates for the prediction of either low BAP (i.e. $< 27 U/l$) or low iPTH (i.e. $< 150 pg/ml$). A covariate was considered to be predictive for a low level of the marker when its estimated coefficient was significantly different from 0 based on the Wald statistic ($P < 0.05$).

For the comparison of the mean of two groups, Student’s $t$-test was used with the appropriate correction for the inequality of variances. Variables that were not normally distributed, as detected by Kolmogorow–Smirnov goodness
of fit test were compared using the Mann-Whitney U test. For comparison of more than two groups, Kruskal-Wallis ANOVA was used with Mann-Whitney U test for post hoc testing. Categorical data were compared by the chi-square test. In view of the fact that the sample selection of this cross-sectional study is by random, the results can be analysed either using a cohort or a case-control strategy [19]. We preferred the cohort approach since it allowed us to calculate true relative risks which are more readily applicable in clinical differential diagnostic thinking where the exposure to a risk factor is known, than odds ratios. These relative risks were calculated as the ratio of 2 prevalence rates, namely the prevalence of the event in the cohort that was exposed to the risk factor and the prevalence rate in the cohort that was not exposed. The confidence interval of the risk estimate was constructed after logarithmic transformation [20].

Results

The overall prevalence of ABD as diagnosed by a low (≤27 U/l) level of BAP in European CAPD patients is 42.9%. A comparable prevalence of 43.3% is observed by using a low level of iPTH (≤150 pg/ml) as marker. As is also shown in Figure 1, these prevalences differ substantially from centre to centre. Moreover, it is clear from this Figure that patients with a low level of BAP not always have a low level of iPTH and vice versa. The prevalences of concordant and discordant patients for the two markers are shown in Table 1.

For the prediction of a low BAP level, logistic regression analysis identified four significant independ-

<table>
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<th>BAP (U/l)</th>
<th>iPTH (pg/ml)</th>
<th>% of patients (n)</th>
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<tr>
<td>≤27</td>
<td>≤150</td>
<td>26.5% (54)</td>
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<tr>
<td>≤27</td>
<td>&gt;150</td>
<td>16.2% (33)</td>
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<tr>
<td>&gt;27</td>
<td>≤150</td>
<td>17.2% (35)</td>
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<tr>
<td>&gt;27</td>
<td>&gt;150</td>
<td>40.2% (82)</td>
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n, absolute numbers of patients.

Table 1. Prevalences (%) of different combinations of the results (either below or not below the cut-off level) of both markers (BAP and iPTH) in 204 patients in whom both markers were available.

ent covariates: the calcium content of the PD fluid, the total time on renal replacement therapy, age, and gender of the patient. The following factors did not possess a significant predictive value in the diagnosis of a low BAP level: time on peritoneal dialysis, time on previous haemodialysis, duration of former functioning renal transplant, intake of Al(OH)₃, intake of calcium-containing phosphate binders, intake of vitamin D analogues, simultaneous intake of calcium-containing phosphate binders and vitamin D analogues, serum aluminium level, number of episodes of hypercalcaemia, centre, status postparathyroidectomy, diabetic nephropathy. Further analysis of the four predictive covariates showed that the prevalence of a low BAP level is significantly (*P* = 0.0002) higher in the patient group dialysed with a high (1.75 mmol) calcium content of the PD fluid: 53.8% versus 46.2% in the low calcium group. Also the proportion of patients with a low level of BAP was significantly

![Graph showing percentage of patients with low BAP and iPTH levels](image)
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Exploring the patients in whom a low level of BAP was not accompanied by a low iPTH level and vice versa, we observed that in 'low BAP-high iPTH' patients a wide range of high iPTH level was reached (up to 1400 pg/ml). Also in 'low iPTH, high BAP' patients BAP levels up to 160 U/l were measured. The factors shown to be predictive for either a low level of BAP or iPTH were found not to be associated significantly with either of the discordant groups.

Despite the fact that the median serum aluminium level was only 5 µg/l (range 1–202), and that only 11 (5.1%) patients had a serum level of ≥30 µg/l, which is considered to be an index of potential aluminium toxicity [21], it was demonstrated, by Kruskal–Wallis ANOVA, that the serum aluminium levels significantly differed between the four patient groups as defined by the con- or discordance of their two markers. By Mann–Whitney U post hoc testing, it could be demonstrated that the 'high BAP, low iPTH' group of patients possessed any significant predictive value. The effect of a high calcium content of the PD fluid on the higher prevalence of a low level of iPTH was at the limit of significance by $\chi^2$ testing ($P=0.05$). Mean age of low iPTH patients was significantly ($P=0.004$) higher (60.5 ± 13.6 years) than that of high iPTH patients (54.5 ± 16.4 years). Relative risks and their 95% confidence intervals for both factors are shown in Figure 2.

When analysing the subpopulation of the patients longer than 9 months on CAPD treatment, the mean calcium concentration of the PD fluid per month was shown also by logistic regression analysis to be a significant risk factor both for a low BAP and low iPTH level. Mean calcium concentration of PD fluid per month was shown to be significantly ($P=0.01$) higher in patients with either BAP≤27 U/l or iPTH≤150 pg/ml.

In view of the small modification by post-translational glycosylation of the tissue unspecific alkaline phosphatase, which is of liver, bone, and kidney origin, the electrophoretic separation of its isoenzymes requires additional procedures to obtain their accurate separation and quantification. By pretreatment of the serum with polyclonal antiplacental–intestinal antibodies before its application on agarose gel we succeeded in developing an accurate and reproducible methodology [16]. This method was also shown to be equally effective in the lower range of BAP; in contrast to

**Discussion**

![Fig. 2. Relative risk (and 95% CI) for low level of BAP (≤27 U/l) or iPTH (≤150 pg/ml) as calculated for the covariates that were identified by logistic regression analysis.](image-url)
Recently we demonstrated both BAP $\leq 27$ U/l, as determined by our improved agarose electrophoretic technique and iPTH $\leq 150$ pg/ml to be good biochemical markers of ABD as indicated by their sensitivity of 78.1 and 80.6% and specificity of 86.4 and 76.2% respectively [2]. These diagnostic indices were assessed in a multicentre haemodialysis population including patients from outside Europe and patients showing aluminium-related bone disease. The population under study here differs from the previous one in three respects. First, aluminium exposure appeared to be very limited. Only 7.8% of the patients included were taking aluminium-containing phosphate binders, and both the mean serum aluminium level, and the number of patients reaching the potentially toxic level of 30 $\mu$g/l [21] were low.

As we could show in our previous paper [2], however, aluminium overload decreases the diagnostic performance of low BAP. Indeed, aluminium overload may lead to false negative results of BAP with respect to the diagnosis of ABD. Hence the lack of exposure to aluminium in the present study will reasonably increase the sensitivity of BAP. Second, as was recently shown by Torres et al. [4], the bone response to PTH is similar in CAPD and haemodialysis, and thus the value of PTH as a marker of a bone disease can be expected to be comparable. Third, taking into account Torres' data on the prevalence of ABD in CAPD, which is diagnosed by bone biopsies is 48%, and applying Bayes' theorem, it can be calculated that both for BAP and PTH the positive predictive value will be even higher than the figures published in our previous report, being 85 and 78% for a prevalence of ABD of 35%; a value based on Sherrard's data [3].

Using low BAP as well as low iPTH we found in the present study a prevalence of ABD of $\pm 43\%$ in 212 European CAPD patients. This prevalence is considerably lower than the 60% diagnosed histomorphometrically by Sherrard et al. [3] in a North-American CAPD population ($n = 142$). In this study, however, it is difficult to eliminate aluminium as a contributing factor to this high prevalence, since only 49 of the 128 patients with ABD had no measurable bone surface aluminium. Meanwhile it has become clear that aluminium can also evolve in the absence of aluminium overload. In the Spanish study of Torres et al. [4] the prevalence of ABD in 32 CAPD patients is closer to ours: 48%. This still rather high prevalence of ABD was present despite a very low bone surface aluminium staining (mean of 0.4%). As already argued before, also in our patients under study, aluminium will not have been a major cause of ABD.

Discarding aluminium as a causative factor in the development of ABD, which was described earlier by different groups [3,5–7], it appeared recently that other risk factors have to be taken into account also. Diabetic patients have been described to be more prone to this type of bone disease than non-diabetics [3,12–14]. This was not confirmed in our study; here the relative low number of diabetics ($n = 23$), displayed a prevalence of a low level of BAP and iPTH comparable to the overall prevalence (data not shown). Also suppression of bone turnover has been described in patients returning into dialysis after transplantation and in parathyroidectomized patients. In the present study, none of these risk factors reached a significant relationship with the presence of a low level of either BAP or iPTH. It should be noted, however, that the number of this type of patients was low. On the other hand, older age, which was also described earlier [3,9,14] to be associated with the occurrence of ABD, was also in our study a risk factor for the prevalence of low levels of BAP and iPTH.

The strikingly higher prevalence of ABD in CAPD vs haemodialysis patients as shown both by Sherrard et al. [3] and Torres et al. [4] has led to the hypothesis that CAPD is a risk factor for ABD through the exposure of the patient to a higher calcium load resulting in oversuppression of the parathyroid glands [3]. In the same line of evidence Pei et al. [12] and Hercz et al. [7] point towards the increasing prevalence of ABD since the previously widespread used Al(OH)$_3$ was replaced by calcium-containing phosphate binders and thus higher oral calcium loads are administered to the patients. Also, oversuppression of the parathyroid glands with vitamin D analogues has been implicated in the development of ABD [11]. In our study, however, no significant relationship between the prevalence of low levels of either BAP or iPTH and the intake of calcium-containing phosphate binders or vitamin D analogues was apparent. The data collected by our questionnaire, however, did not allow for exact calculations of cumulative doses of medications. We could only correlate the presence of a low level of either marker with the fact of a patient was ‘on’ or ‘off’ a medication at the time of blood sampling. On the other hand, a high calcium content (1.75 mmol/l) of the PD fluid, which is also an indicator of the calcium load administered to the patients, was strongly predictive of the presence of a low level of BAP. The high calcium content of the PD fluid was also predictive for the presence of a low level of iPTH but less than for low BAP. Since most nephrologists nowadays are well aware of the risk of ABD in patients with low iPTH levels, a bias may consist in the sense that the calcium content of the PD fluid is lowered by the treating physicians when confronted with decreasing iPTH levels in their patients [23]. This bias, however, does not interfere with our BAP results, since BAP determination is not yet routinely performed. Indeed, in the group of patients with iPTH $\leq 150$ pg/ml relatively more (36%) patients were found on a PD fluid with low calcium content, than in the patients group with BAP $\leq 27$ U/l (28%).

In addition to the current calcium concentration of the PD fluid which is only indicative of the calcium load administered, we also calculated the ‘mean calcium concentration of PD fluid per month’ for the last 9 months of treatment prior to inclusion in the study,
in order to obtain a more accurate estimate of the calcium load administered to the patient. In the subpopulation of patients who had been on CAPD treatment for more than 9 months \((n=130)\), it was demonstrated that both low BAP and low iPTH patients had significantly higher calcium exposures, adding evidence to the above-mentioned hypothesis of CAPD patients being more at risk to oversuppression of the parathyroid glands through exposure to higher calcium loads.

The gender of the patients appeared also a determining factor for the prevalence of a low level of BAP, the male gender being more affected. We are not aware of literature data confirming this finding. BAP, being a marker of osteoblastic function, might, however, be systematically higher in female dialysis patients due to their postmenopausal (be it premature or not) state, since the cessation of ovarian function is known to induce a marked increase in bone turnover [24] and increased levels both of BAP and osteocalcin have been described in postmenopausal osteoporotic patients with normal renal function [25]. Moreover, in their recent bone biopsy study Gerakis et al. [26], also found higher indices of bone formation in women than in men, despite similar levels of iPTH. They also suggest the lack of the antiresorptive effect of oestrogen in postmenopausal women as a possible explanation.

Patients with BAP \(\leq 27\) U/l were significantly shorter on renal replacement therapy. Pei et al. [12] also found in their multivariant analysis of risk factors for the different types of renal osteodystrophy a significantly shorter duration of dialysis in ABD patients. In the paper describing the first cases with non-aluminium-associated ABD Morinie`re et al. [5] also described shorter time on dialysis of the ABD patients. The reason(s) for this phenomenon remain(s) unclear, though the observation of Torres et al. [4] that the bone response to PTH in renal failure increases after dialysis (haemo- or peritoneal) is initiated might offer an explanation: the dialysis treatment might remove toxins responsible for the impaired bone response to PTH.

Finally, despite the fact that both a low level of BAP \(\leq 27\) U/l and iPTH \(\leq 150\) pg/ml by themselves have good diagnostic performances in the detection of ABD we were struck by the substantial number of patients with either ‘low BAP, high iPTH’ or ‘high BAP, low iPTH’. Also in our assessment study on the diagnostic value of these markers [2] a comparable percentage of discrepant cases \((25.6\% \text{ in total})\) was encountered. In case of the ‘high BAP, low PTH’ a significant association could be demonstrated between this profile and the histological and/or chemical evidence of aluminium overload (unpublished data). The low PTH level in these patients is in accordance with the known direct suppressive effect of aluminium on the parathyroid glands [27]. The reason(s) for the relatively high BAP levels in these aluminium-overloaded patients are less clear. Experimental data regarding the effect of aluminium on osteoblast activity are indeed conflicting [28]. However, Lieberherr et al. [29] demonstrated a stimulatory effect of low concentrations of aluminium on osteoblast alkaline phosphatase activity. Our finding in the CAPD population under study of higher mean serum aluminium levels (though not frankly toxic) in the group of patients with the ‘high BAP, low PTH’ profile is in line with the idea of a specific effect of aluminium, even at low levels of exposure, on osteoblast activity.

Regarding the patients displaying the other type of discordant markers, no definite conclusions can be drawn. However, due to the different sensitivities and specificities of both markers some discrepant cases are likely. Moreover, one has to take into account that both markers reflect the functional state of two different cell types: the osteoblasts and the parathyroid cells. The exact pathophysiological link between these two organs in ABD (is hypoparathyroidism cause or consequence of ABD?) has not been elucidated so far, neither are there prospective data available showing which of these markers responds first to changes of treatment such as calcium loading or vitamin D. The findings of Goodman et al. [30] on the development of histologically proven ABD after calcitriol treatment in patients with persistent high iPTH levels is, however, indicative of the discrepant behaviour that may arise between these two organs. Due to the cross-sectional character of our study, it can be argued that in some patients a change in bone turnover (e.g. due to a recent change of medication) may be already apparent in one marker but not yet in the other. Also our different results regarding risk factors for either low BAP or low PTH are in agreement with this line of thought. However, whereas many reasons can be put forward trying to explain the discrepant cases, no data are available regarding the exact value of these findings in the diagnosis of ABD. Also in our previous study on the evaluation of these markers the number of discrepant profiles encountered were too low to allow for significant conclusions.

The value of either marker (BAP or iPTH) by itself is well defined. Despite the fact that these markers do not reflect the gold standard for 100\%, they may provide us with a good estimate of the prevalence of ABD. In this study, this prevalence was shown to be relatively high, confirming earlier biopsy-based data in a smaller but, with regard to the low level of aluminium exposure, analogous European CAPD population [4].

Moreover, we could show that besides the known risk factors of advanced age and shorter time on dialysis, male gender also appears to be a significant risk factor for this type of renal osteodystrophy, as well as a high calcium load administered through the dialysate.

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