

CLINICAL VALUE OF THE BIOCHEMICAL MARKERS OF BONE REMODELING IN THE ASSESSMENT OF BONE METABOLIC DISEASES

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Summary: Bone markers have been useful research tools, with their clinical utility limited by their specific technical and analytical aspects and pre-analytical variability. Bone markers reflect different aspects of the quality of bone than BMD and, therefore, may add an independent, predictive value to the assessment of changes in bone mineral density and reductions in the risk of fracture. The decrease in bone marker levels is strongly related to the reduction in vertebral fracture risk through raloxifene, risedronate and alendronate. After anabolic therapy with teriparatide, early increases in bone formation markers are strong predictors of BMD responses. There are potential advantages of using markers for monitoring anti-osteoporosis treatment in the short term, in addition to the bone mineral measurements, to identify non-responders or non-compliance. The transition of biochemical bone markers into everyday clinical practice requires standardization of assays and quality control programs to reduce large inter-laboratory variations of data, defining criteria of a high bone turnover in terms of reference values, either young adult or age-matched, and better characterization of the markers across geographic areas and races and under various clinical conditions.

Key words: bone mineral density, bone quality, bone remodeling, fracture risk, monitoring, osteoporosis

Introduction

In clinical practice, low bone mineral density (BMD) and, therefore, scarcity of bone mineral is identified and quantified by dual energy X-ray absorptio-metry (DXA) (1). However, BMD predicts only 66–74% of variance of the bone strength (2), and many individuals with normal areal BMD suffer fractures (3). It is well recognized that other aspects of bone composition and structure may contribute, independently of BMD, to bone strength and the risk of fracture. Bone strength is determined by the bone mass, geometry and quality of bone (4).

Currently, the bone quality can be assessed *in vivo* by the measurement of bone turnover and of some aspects of the bone geometry and microarchitecture, both non-invasively using biochemical markers, and invasively using the bone biopsy histomorphometric analysis. The markers reflect whole-body turnover, but do not provide information about the remodeling balance in individual bone remodeling units. Bone turnover affects bone microarchitecture, matrix and mineral composition, mineralization and accumulation of microdamage in the bone. An elevated bone turnover reduces bone strength through the reduction of the bone mass and of the degree of mine-

ralization of the bone, and through its adverse effects on bone microarchitecture.

Markers and the imbalance in bone remodeling

After menopause, increased bone loss induced by estrogen deficiency results in an accelerated loss of predominantly cancellous bone, particularly on the endosteal surface of the bone. Estrogen deficiency increases osteoclast recruitment and the birth rate of new bone units that undergo a remodeling cycle (activation frequency), and extends the bone resorption phase (5) by reducing osteoclast apoptosis (6). The high-turnover phase and remodeling imbalance result in deep resorption cavities, trabecular plate perforation, wide separation and disconnection of trabeculae, and enlargement and coalescence of subendocortical spaces (7). The high remodeling rate and deep resorption cavities produce a loss of trabecular plates and their connectivity, which in turn produces a greater deficit in bone strength than does trabecular thinning. The rapid remodeling impairs isomerization and maturation of collagen (8) and reduces, mineralization of bone, as more densely mineralized bone is removed and replaced with younger, less densely mineralized

bone (9). The temporarily unfilled excavated resorption sites enable formation stress concentrators that predispose bone to microdamage. The microdamage in bone matrix (microcracks) severs canaliculi, which causes osteocytic apoptosis, with the location and extent of the damage defined by signals to lining cells. Bone loss caused by resorption cavities results in a large decrease in stiffness and in high strain peaks at the bottom of the cavities (10). Therefore, a reduction in the number and size of resorption cavities in antiresorptive drug treatment can result in large reductions in fracture risk, with small increases in bone mass.

The accelerated phase gradually weakens over 4–8 years into a subsequent slow phase of postmenopausal bone loss (11). A number of other age-related factors contribute to this slow bone loss, such as secondary hyperparathyroidism (12) caused by age-related decrease in the ability to adapt to a lower calcium intake by increasing intestinal calcium absorption (13), decrease in renal calcium conservation (14), and impaired vitamin D metabolism (15). In aging adults, impaired osteoblast recruitment and function contribute to the bone loss. The number of osteoblasts recruited to erosion surfaces is reduced, as well as their functional capacity, resulting in a decreased rate of bone formation (16, 17). The activity of osteoblasts and their replicative life span decrease with the loss of estrogen (18) and with age (19). Consequently, wall thickness (the depth of bone structural units on bone surfaces after the completion of bone formation) decreases in women after menopause, indicating that each erosion cavity is refilled with a smaller than normal volume of bone (20). Lower rates of bone formation – assessed by serum osteocalcin, bone alkaline phosphatase or procollagen type I propeptides levels – are often seen in conjunction with either normal or accelerated rates of bone resorption (21).

A long-term imbalance in bone metabolism, particularly decreased bone formation and increased bone resorption, may result in increased bone fragility. However, other factors, such as age, medication, immobilization and the fracture itself, strongly influence bone metabolism and need to be considered when interpreting biochemical data in individual patients.

The current interest in bone markers has been stimulated by the research into osteoporosis; namely, by the failure of bone mineral density (BMD) measurement in monitoring the efficacy of treatments of osteoporosis in individual patients. The validated markers include markers of bone resorption, either type I collagen degradation products such as C- and N-telopeptide of type I collagen (CTX and NTX, respectively) in serum or urine, or serum osteoclastic acid phosphatase (5b ACP), and markers of bone formation (serum concentrations of bone alkaline phosphatase (bone ALP), osteocalcin (OC) or type I collagen synthesis products (the amino- and carboxyl-terminal extension peptides of the procollagen type I propeptides, PINP and PICP, respectively). A review of the basic biochemistry of most of the currently used

bone markers can be found elsewhere (22, 23). Every marker provides information on a different aspect of bone metabolism. Moreover, some of these markers (such as serum OC or urinary hydroxyproline) may reflect, at least to a certain degree, both bone formation and bone resorption. Except for the bone ALP and type 5b ACP, the markers are present in tissues other than bone and may therefore be influenced by non-skeletal processes as well. This review will emphasize that bone markers reflect different aspects of the quality of bone than BMD does, and therefore should not be used as a surrogate or substitute for BMD in assessing the extent and rate of bone loss. Instead, in addition to bone densitometry, bone markers may add an independent, predictive value to the assessment of fracture risk and in monitoring anti-osteoporosis treatment help to rapidly identify non-responders to therapy, or non-compliance, and to predict response to treatments in terms of decreasing the risk of fractures.

Changes in the biochemical markers of bone turnover reflect alterations in skeletal metabolism independently of the underlying cause. Moreover, clinical use of bone markers in the treatment of individual patients depends on biochemical and technical limitations of the markers, namely, on their preanalytical variability.

Clinical utility of bone markers

Serum and urine markers of bone turnover have been proven helpful in evaluating the physiology and the pathophysiology of bone metabolism, and in elucidating the pathogenesis of bone disease in deficiencies or excesses of hormones, immobilization, systemic inflammation or malignancy, or effects of bone-specific drugs such as glucocorticoids, diuretics, and immunosuppressants. Also, several serum and urine markers have been used as intermediate end-points in all major studies of therapies of metabolic and neoplastic bone diseases.

In clinical practice, markers of the bone turnover have been employed in differential diagnosis and the management of metabolic bone disease, such as postmenopausal osteoporosis, Paget's disease of bone, osteomalacia/rickets, or hyperparathyroidism. The bone markers reflect the whole body rates of bone resorption and bone formation and are likely to reflect changes in the number of bone remodeling sites in postmenopausal women. Therefore, none of the biochemical markers of bone turnover has proven useful as a diagnostic test to discriminate between healthy, osteopenic, and osteoporotic populations in a primary diagnostic set-up. Most population-based studies have indicated that biochemical markers of bone metabolism do not provide sufficient diagnostic information to distinguish individuals with normal from those with low bone mass. The bone markers, even when combined with anthropometric measures, offer little practical information for estimating BMD

levels in individual women (24). Thus, *the markers cannot be used as a surrogate of the BMD measurement to make a diagnosis of osteoporosis in individual patients.*

Markers in the prediction of the rate of bone loss in postmenopausal women

Relationships between the biochemical markers of bone turnover and the rate of bone loss in women after menopause have been appropriately investigated in several prospective studies. They indicate that individuals with high rates of bone resorption lose bone faster than subjects with a normal or low bone turnover. Prospective studies are limited by the precision error of repeated measurements of both the markers and BMD, and by differential rates of bone loss between various skeletal sites. Moreover, it is not clear whether the bone loss at various sites is consistent over time. In one study, serum levels of markers were relatively stable over 4 years in older postmenopausal women (25). However, 20–30% of 268 healthy untreated postmenopausal women classified as having a high bone turnover at the baseline on the basis of serum OC, CTX or both markers were differently classified by the same method 4 years later. Moreover, cyclic long-term variations in the markers have been reported (26). Thus, the decision on treatment should not be based solely on a single bone marker measurement. Consequently, the maximum available information obtained by a marker or a panel of markers explained 20% to 40% of the variance in the BMD change. Several other studies have demonstrated more modest correlations and/or failed to find a significant association (27). A variable production of sex hormone precursors and individual response to estrogen deficiency are some of the possible causes for an increased inter-individual as well as long-term variability of bone loss. Thus, the available data do not indicate that measuring the individual serum and urine markers of bone turnover can accurately predict the rate of bone loss at the spine and hip over a 3-year period.

Markers in the prediction of fracture risk in untreated postmenopausal women

As indicated above, bone markers do not account entirely for BMD and rates of its changes, because the bone mineral and bone markers reflect different aspects of the quality of bone. This has been clearly documented by a reanalysis of a randomized clinical trial of transdermal estrogen and placebo therapy in postmenopausal women with osteoporosis. The authors used the computer generated, three dimensional graphic plots relating the observed vertebral fracture rate to lumbar spine BMD and to bone turnover assessed directly by tetracycline-based histomorphometry of iliac biopsy samples (28). In this study, two vertebral fracture peaks in the placebo-treated women were compared: one was associated with high bone turnover and one with low baseline lumbar spine BMD.

After 1 year of estrogen treatment, the fracture peak associated with high bone turnover was eliminated whereas the peak associated with the low BMD was maintained, presumably because the increase in BMD induced by treatment was modest.

The hypothesis that modulation of both BMD and bone turnover may lead to a change in fracture risk (28) is supported by prospective studies showing that the baseline level of the markers of bone resorption can predict fracture risk independently of BMD. Concordant results have been obtained in 3 prospective studies (EPIDOS, Rotterdam and OFELY), indicating that increased levels of S-CTX, and U-CTX and free DPD are associated with increased risk of hip, vertebral and non-hip and non-vertebral fractures over follow-up periods ranging from 1.8 to 5 yrs. (29). Similar results were obtained in 693 patients treated with risedronate (5 mg daily) or placebo (30). Baseline CTX and NTX in urine were related to the incidence of vertebral fracture over 1 or 3 years, while only NTX levels were related to non-vertebral fracture incidence over 3 years.

Biochemical markers can predict fractures, and in particular clinical vertebral fractures, in elderly women (31). Ten bone turnover markers, including two novel assays for OC fragments, were used to predict clinical fracture in 1040 randomly recruited 75-year-old women. Over an average of 4.6 years (range 3–6.5) of follow-up, 178 of the women sustained at least one fracture, mainly vertebral (49 women) and hip (41 women) fractures. When women with markers at the highest quartile were compared with others, serum CTX, 5b ACP and urinary OC predicted clinical vertebral fracture (odds ratio of 1.94, 2.28, and 2.71, respectively, all $P < 0.05$). However, bone markers were not able to predict hip fracture, confirming that in the elderly, hip fracture is determined mainly by the risk of falls.

The studies indicated that high resorption markers contribute significantly to the prediction of risk of fracture obtained from a low BMD and/or prevalent fracture. However, the overlap of fracture risk between subgroups of patients indicates that, in clinical practice, a single measurement at the baseline of only one bone resorption marker may not be used to predict risk of fracture in an individual patient. The place of biochemical markers of bone resorption in the assessment of fracture risk is likely to be in the combination with other important risk factors including low BMD, personal and maternal history of fracture and low body weight. Risk of clinical fractures increases with age in elderly women with both normal and increased marker levels, but the increase is twice higher in women with elevated markers.

Whether the bone formation marker concentrations are related to fracture risk remains unclear. Prospective studies relating levels of bone formation markers to risk of fracture have yielded conflicting results. A decrease, no difference or an increase of bone formation markers, have all been reported to be associated with increased fracture risk (29). A dissociation

between increased concentration of bone ALP except/and osteocalcin and reduced spinal bone formation of bone formation measured using the direct functional imaging technique of ^{18}F -fluoride positron emission tomography in women with osteoporosis (32) indicate a need for a better non-invasive assessment of the function of osteoblasts and osteocytes.

Markers in the prediction of fracture risk in treated postmenopausal women

Earlier studies on postmenopausal osteoporosis have suggested that the therapeutic efficacy of anti-resorption therapies may be influenced by the level of pre-treatment bone turnover. However, these studies have used BMD as the primary end-point. To test the hypothesis that this association holds true for incident fractures, a post hoc analysis was made of a subset of the risedronate trials, using the urinary excretion of deoxypyridinoline as an index of pre-treatment bone resorption. The efficacy of risedronate in reducing incident vertebral fractures in women with postmenopausal osteoporosis was largely independent of pre-treatment bone resorption rates. However, increases in lumbar spine BMD were greater in women with higher pre-treatment bone resorption rates.

The ultimate goal of any anti-osteoporosis treatment is to decrease the risk of fractures. As noted above, a decrease in fracture risk with anti-resorption therapy for osteoporosis is the consequence of a decrease in bone remodeling and an increase in BMD. The reduction of vertebral and non-vertebral fractures in women on antiresorption therapies is only partially explained by increases in BMD (33, 34). In this respect, the bone markers were used to study the relationship between change in the bone turnover and vertebral fracture risk during raloxifene therapy using 3-year data from the MORE trial, where one-third of the 7705 randomized women underwent measurement of bone markers at the baseline and after 6 and 12 months participation (35). The prediction of the vertebral fracture risk was examined using changes in BMD and two markers. Logistic regression models were constructed using 1-year percentage change in BMD, bone markers and relevant baseline demographics to predict the risk of vertebral fracture with raloxifene at 3 years. The mean percentage decrease in serum OC, bone ALP and the increase in femoral neck BMD were -28.2, -29.0, and 1.9%, respectively, after 1 year. The signal-to-noise ratio was best for serum OC. Prevalent vertebral fracture status ($P < 0.0001$), baseline lumbar spine BMD ($P < 0.0001$), and years since menopause ($P < 0.0005$) were independent predictors of fracture risk. Changes in femoral neck BMD and OC significantly predicted effects of raloxifene on fracture risk as compared with the placebo. Change in serum OC was significantly related to future risk of vertebral fracture ($P = 0.01$), also after adjustments for baseline vertebral fracture status and BMD. Contrary, the change in femoral neck BMD after 12 and 24 months was not related to fracture risk in any of the analyses (36).

To provide more data on the relationship between short-term changes in the biochemical markers and the risk of non-spinal fracture among aminobisphosphonate-treated women, the relationship between 1-year percentage change in markers after alendronate or placebo treatment and the subsequent risk of hip, non-spine and spine fracture among 6186 post-menopausal women enrolled in the Fracture Intervention Trial was examined (37). Each 1 SD reduction in the 1-year change in bone ALP was associated with fewer spine fractures (odds ratio of 0.74, 95% CI 0.63, 0.87), non-spine fractures (0.89; CI 0.78, 1.00) and hip fractures (0.61; CI 0.46, 0.78). The associations between 1-year change in serum PINP and CTX and fracture risk were of a similar magnitude, but did not reach significance. A reduction of 30% or more in bone ALP occurred in 56% of alendronate-treated women. These women had a lower risk of non-spinal (0.72; CI 0.55, 0.92) and hip fractures (0.26; CI 0.08, 0.83) compared to those with reductions of less than 30%. Greater reductions in bone turnover with alendronate therapy are associated with fewer hip, non-vertebral and vertebral fractures.

A similar hypothesis was tested in 693 patients treated with risedronate (5 mg daily) or placebo for 3 years (30). Baseline CTX and NTX in urine were related to the incidence of vertebral fracture over 1 or 3 years, while only NTX levels were related to non-vertebral fracture incidence over 3 years. The reductions in CTX (median 60%) and NTX (51%) at 3–6 months with risedronate therapy were significantly ($P < 0.05$) associated with the reduction in vertebral fracture risk (75% over 1 year and 50% over 3 years). The changes in NTX and CTX explained for 49% and 55%, of risedronate effect respectively in reducing the risk of vertebral fractures in the first year and 67% and 66%, respectively, over 3 years, compared with placebo. However, there was little further improvement in fracture benefit observed below a decrease of 55–60% for CTX and 35–40% for NTX, suggesting a threshold of reduced bone resorption and vertebral fracture risk reduction. Similarly, a threshold of change in BMD and reduced vertebral fracture risk reduction was observed in the ibandronate clinical trial (38).

Early reductions in biochemical markers of the bone turnover with anti-resorption therapies correlate negatively with subsequent increases in BMD. To test the hypothesis that early changes in bone turnover predict either the BMD response or fracture risk reduction with anabolic therapy, serum concentrations of three bone formation markers (bone ALP, PINP and PICP) and urinary concentrations of two bone resorption markers were assessed for population subset in daily teriparatide (human PTH 1–34) clinical trial. The baseline bone turnover status as indicated by NTX and PINP correlated positively and significantly with BMD response ($r = 0.40$ and 0.41 respectively, $P < 0.05$). Increases in PICP at 1 month and PINP at 3 months correlated best with increases in lumbar spine BMD at 18 months ($r = 0.65$ and 0.62

respectively, $P < 0.05$), and were of the highest sensitivity (59% and 69% respectively) and predictive value (93% and 88% respectively). However, there were no correlations between changes in the markers and fracture risk during teriparatide treatment because the study was limited by the small number of fractures in the biochemical marker cohort.

Monitoring treatment with antiresorptive drugs in individual patients

Measurement of BMD by DXA as a surrogate indicator of the treatment efficacy has been widely used in clinical trials of antiresorptive therapies that have demonstrated a reduction in fracture risk after 2–4 years. In clinical practice, however, it is important to detect within the first year after initiation of treatment whether the chosen therapy is effective in an individual patient. In this respect, given a short-term precision error of 1 to 1.5% of BMD measurement at the spine and hip, the individual change must be greater than 3 to 5% to be seen as significant. Such a change is seen only at the lumbar spine in just a fraction of patients on antiresorption treatments. Therefore, within the first year of the therapy, DXA does not allow the identification of all responders to alendronate or risendronate, raloxifene or nasal calcitonin.

Whereas BMD measurements are not fully expressed until 3 years after the initiation of therapy, the most effective antiresorptive treatments induce a decrease in the bone turnover that reaches a plateau within a few weeks or months, depending on the potency and route of administration of the drug and on the marker. Hormone replacement therapy, as well as treatment with raloxifene and oral alendronate, induce a rapid decrease of the bone resorption markers within 3 to 6 months, and bone formation markers within 6 to 12 months. The plateau achieved using raloxifene is usually within ± 1 SD of the premenopausal mean reference concentration for markers of resorption and formation (39, 40). The concentrations of markers observed after 3 years of treatment with the oral alendronate usually remain in about half of the patients below the lowest concentration of the premenopausal reference range for the markers of resorption (CTX) and formation (PINP) (41, 42). The concentrations of markers observed after 3 years of treatment with the oral risendronate are on average in the lowest tertile of the premenopausal reference range for the markers of resorption and formation (30). The bone markers in patients treated with nasal calcitonin are on average at the upper level of the premenopausal range (43).

The baseline bone turnover does not appear to be a useful parameter for predicting the individual response to therapy. However, the decrease of bone turnover markers under antiresorptive therapy, usually expressed in a percentage of the initial value, is correlated to the increase in BMD. The major studies of antiresorptive therapies, using bone markers as intermediate end-points, have demonstrated that mark-

ers may be used to monitor the BMD response to HRT (39), tibolone (44), alendronate (45), risendronate (30) and calcitonin (46). A marked decrease of the markers was associated with a subsequent positive BMD response, while non-responders showed little or no changes in the markers. It should be noted, however, that in these studies the results from placebo and treatment groups were pooled in order to produce a high correlation. Even then changes from baseline at month 12 in serum OC and CTX, and urinary CTX and NTX, explained for 40 to 60% of variance in 2 years response in spinal BMD (45). This approach enables us to identify non-responders, i.e. patients who will fail to demonstrate a significant increase of BMD after 2 years of treatment, rather than to assess changes of BMD in individual patients. The optimal threshold of the bone marker change associated with a positive BMD can be defined using a receiver operating characteristics analysis, or by using logistic regression models. The cut-off values can be obtained with a pre-specified sensitivity or specificity that provides adequate predictive value of the subsequent 2 year BMD response in a single patient (24). The cut-off values, expressed in percentage change from baseline, are approximately 20 % lower for alendronate than for HRT. In women treated with alendronate, urinary CTX and NTX predicted a change in spine BMD greater than 0%, with a positive predictive value (70–80%) and a high specificity (68–80%) (47). The recommended cut-off values of markers established for defining responders/non-responders were tested only with estrogens and alendronate treatments, and should be validated in other cohorts using the same therapeutic regimens.

It is not known if a patient whose both bone resorption and bone formation markers were brought to the premenopausal reference range (i.e. HRT, raloxifene, risendronate) would benefit more from the anti-resorptive therapy than a patient with the markers significantly below the premenopausal reference range (i.e., alendronate) (41, 42). This question cannot be answered by the BMD measurement. Morphometric vertebral and non-vertebral fracture risk was similar between women treated with alendronate for 10 years and those treated for 5 years (49). In patients receiving risendronate for 6–7 years there was no change in the rate of new vertebral fractures (50). Moreover, direct investigations of antifracture efficacy using long-term placebo controlled clinical trials require large study populations and raise ethical issues relating to withholding of the effective medication in placebo controlled trials.

The antiresorptive drugs differ in beneficial effects on tissues other than bone, acceptable risk of long-term side effects, and also in effects on bone quality. These include effects of suppression of bone remodeling on the degree of synthesis of bone organic matrix and homogeneity of bone mineralization (51), elasticity of bone (52), and anecdotal reports of hypo- or adynamic bone disease (53) and osteoche-

monocrosis of the jaw (54) in patients undergoing long-term treatment with aminobisphosphonates. Also, an increased microdamage accumulation occurs in older patients, those with low BMD and those with prevalent fractures and these associations occur

only within the alendronate-treated population and are not evident in untreated patients (55). In clinical practice, the effect on bone quality in long-term over-suppression of bone remodeling in individual patients is likely to be predicted using the bone markers (55).

KLINIČKA VREDNOST BIOHEMIJSKIH MARKERA ZA REMODELIRANJE KOSTIJU I ISPITIVANJU BOLESTI KOŠTANOG METABOLIZMA

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Kratak sadržaj: Koštani markeri su veoma korisno dijagnostičko sredstvo, mada je njihova klinička upotreba ograničena specifičnim tehničkim i analitičkim aspektima, kao i njihovom pre-analitičkom varijabilnošću. Koštani markeri, za razliku od mineralne gustine kosti, ukazuju na posebne aspekte kvaliteta kostiju, te stoga nude zasebnu i prognostičku perspektivu u ispitivanju promena mineralne gustine kosti i smanjivanju rizika od fraktura. Smanjenje nivoa koštanih markera usko je povezano sa smanjenjem rizika od frakture vratnih pršljenova usled korišćenja raloksifena, rizedronata i alendronata. Posle anaboličke terapije teriparatidom, ubrzano formiranje koštanih markera je jasan pokazatelj reakcije gustine minerala proteina. Postoje brojne mogućnosti za korišćenje ovih markera u kratkoročnom praćenju toka lečenja osteoporoze, pored merenja mineralne koštane gustine, kako bi se otkrili pacijenti koji ne reaguju na terapiju. Upotreba koštanih markera u svakodnevnoj kliničkoj praksi zahteva standardizaciju merenja i programa kontrole kvaliteta kako bi se smanjile varijacije u podacima iz različitih laboratorija, definisali kriterijumi za ubrzani metabolizam kostiju u pogledu referentnih vrednosti, i postiglo preciznije određivanje markera u pogledu geografskih oblasti i rasa, kao i u različitim klimatskim uslovima.

Ključne reči: mineralna gustina, kvalitet kostiju, remodeliranje kostiju, rizik od fraktura, praćenje toka lečenja, osteoporoza

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