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Biomarkers of bone turnover in diagnosis and therapy of osteoporosis

A consensus advice from an Austrian working group

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Biomarker des Knochenumbaus in Diagnose und Therapie der Osteoporose – Leitfaden einer österreichischen Arbeitsgruppe

Zusammenfassung

Ziel Sinnvoller Einsatz der Labordiagnostik zur Prävention, Diagnose, Therapie und Therapieüberwachung der Osteoporose.

Zielgruppe Ärztinnen und Ärzte für Allgemeinmedizin, Geriatrie, Gynäkologie, Urologie, Innere Medizin (besonders Endokrinologie und Stoffwechsel), Nephrologie, Med. und Chem. Labordiagnostik, Onkologie, Rheumatologie, Nuklearmedizin, Orthopädie, Pädiatrie, Rehabilitation und Physikalische Medizin, Radiologie, Sozialmedizin, Transplantationsmedizin, Unfallchirurgie, sowie Sozialversicherungsanstalten, Krankenanstalten, Selbsthilfegruppen.

Hintergrund Abklärung der Ätiologie von Knochenerkrankungen. Wachsendes Spektrum der Therapiemöglichkeiten von Knochenerkrankungen und der biochemischen Marker des Knochenstoffwechsels. Verbesserungen in der Beurteilung des Therapieerfolgs und bei der Überwachung der Compliance von Patienten. Forschungsperspektiven.

Grundlagen Wissenschaftliche Literatur, Leitlinien und Konsens-Gespräche.

Fazit Routine- und Spezial-Laboruntersuchungen sind für die Unterscheidung zwischen primärer und sekundärer Osteoporose und für die Wahl einer angemessenen Therapie wichtig. Biochemische Marker des Knochenumbaus sind ein zusätzliches Hilfsmittel bei der Abschätzung des individuellen Frakturrisikos. Mit diesen Markern kann ein Ansprechen auf eine knochenspezifische Therapie rascher erfasst werden als mit der Überwachung der Knochenmineraldichte, dies hilft auch die Compliance der Patienten zu verbessern.

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Eigenschaften, Präanalytik und Anwendung von ausgewählten Markern für Knochen- Resorption und Anbau und von Parametern, die den Knochenstoffwechsel regulieren, werden präsentiert.

Schlüsselwörter: Leitfaden, Biomarker, Knochenumbau, Osteoporose

Summary
Aim Reasonable application of laboratory parameters in prevention, diagnosis, treatment and therapy monitoring of osteoporosis.

Target groups Physicians from different specialist disciplines (general medicine, geriatrics, gynaecology, urology, internal medicine—especially endocrinology and metabolism, nephrology, laboratory medicine, rheumatology, nuclear medicine, orthopaedics, paediatrics, rehabilitation and physical medicine, radiology, social medicine, transplantation medicine, accident surgery), moreover social insurances, hospitals and self-help groups.

Background Evaluation of aetiology of bone disorders, widening of the therapeutic spectrum for diseases of bone and knowledge on biochemical markers of bone turnover. Improvements in judging the success of therapy and in monitoring the compliance of patients. Research perspectives.

Bases Scientific literature and guidelines, consensus meetings.

Résumé Basic and specialized laboratory investigations are important in differentiation between primary and secondary osteoporosis for an adequate therapy. Biochemical markers of bone turnover are an additional aid in evaluation of individual fracture risk. These markers identify responders to bone therapy faster than surveillance of bone mineral density, which helps to improve patient's compliance too. Characteristics, preanalytic precautions and applications are presented for selected markers of bone resorption and formation and for parameters regulating bone metabolism.

Keywords: Advice, Biomarkers, Bone turnover, Osteoporosis

Introduction

Once clinically relevant alterations of bone structures are detected by imaging techniques, laboratory investigations serve for further exploration. Discovery of the causes of osteoporosis is an important challenge for the medical laboratory. If reports are positive, targeted treatment may result from basal or expanded laboratory investigations.

Analyses of biochemical bone-markers serve for another purpose, namely for an estimation of the phenomena of the dynamic process of bone turnover. During life, bone metabolism differs in velocity and balance between resorption and formation. Understandably enough, a predominant resorption process together with an elevated rate of turnover will soon become clinically relevant. Thus, the most important prognostics are the estimations of the turnover rate and the net balance. Proving changes in bone turnover by specific therapeutic interventions is another assignment of biochemical bone markers [1, 2].

Osteoporosis

Definition, clinical aspects, epidemiology

Osteoporosis is a systemic skeletal disease, which was characterized by WHO by a decreased bone mineral density (BMD) and microarchitectural deterioration of bone tissue, resulting in an increased risk of fractures [3, 4]. Typical predilection sites for osteoporotic fractures are the distal forearm, vertebral bodies of thoracic and lumbar spine as well as the hip region.

Regarding recent epidemiologic and demographic data from the Western world, it can be assumed that about 46 % of women and 22 % of men will incur an osteoporotic fracture beyond 50 years of age (lifetime fracture risk) [5]. By 2050 the number of global hip fractures will increase to about 6 million per year [6], despite the fact that in some countries, including Austria, a clear levelling-off or even decrease in hip fracture incidence has been demonstrated [7, 8]. Osteoporotic fractures raise huge debits for the health budget as well as substantial bio-psycho-social burden for all persons concerned. Especially fractures of hip and vertebral bodies result in dramatic restraints in quality of life and are associated with an increased rate of mortality [9]. The dramatic increase in frequency of osteoporotic fractures raised the need for adequate methods to determine the individual risk of fractures in these context biochemical markers of bone turnover gained relevance.

Diagnosis of osteoporosis

In suspect bone loss, the five main pillars of the diagnostic exploration include detailed anamnesis and risk evaluation, clinical investigations, conventional X-ray of thoracic and lumbar spine, measurement of BMD and laboratory investigations.

Anamnesis and risk evaluation

An exact anamnesis is important for the diagnosis as well as for the estimation of the fracture risk, which is essential to derive accurate therapeutic measures. The main risk factors are summarized in Table 1 [10].

WHO issued a risk-score to assess 10 years of fracture risk. The FRAX® (fracture risk assessment) tool is based on individual patient models that integrate the risks associated with clinical risk factors with (or without) BMD at the femoral neck [11]. The FRAX® models have been developed from studying population-based cohorts from Europe, North America, Asia and Australia. In their most

Table 1. Risk factors for osteoporotic fractures [10]

– Low BMD	– Premature menopause
– Age	– Primary/secondary hypogonadism
– Female gender	– Primary/secondary amenorrhea
– Caucasian, Asiatic ethnicity	– Excessive alcohol abuse
– Loss in body height	– High turnover rate of bone (elevated bone marker levels)
– Low body weight	– Deficient vitamin D, diminished sun exposure
– Earlier fragility fracture	– Long immobilization
– Family history of hip fractures	– Low calcium intake
– Smoking	– Restrictions in ability to see
– Glucocorticoid therapy	– Neuromuscular diseases
– Rheumatoid arthritis	

Table 2. Definition of osteopenia and osteoporosis by T-Score, WHO 1994 [3]

	T-Score[a]
Normal	≥ – 1
Osteopenia	< – 1 to > – 2.5
Osteoporosis	≤ – 2.5
Manifest osteoporosis with fractures following inadequate trauma	≤ – 2.5

[a]Definition of *T*-Score: $(BMD_{pat} - BMD_{ref})/SD_{ref}$
BMD_{pat} actual BMD of patient, BMD_{ref} average BMD of healthy women < 30 years of age (reference population), SD_{ref} standard deviation of BMD of the reference population

sophisticated form, the FRAX® tool is computer-driven (questionnaire at http://www.shef.ac.uk/FRAX/). Simplified paper versions, based on the number of risk factors are also available. The FRAX® output is a 10-year probability of hip fractures and a 10-year probability of a major osteoporotic fracture (spine, forearm or shoulder fractures). The FRAX® tool is an important step towards individual case finding and a turn away from the *T*-score pragmatism (Table 2), which rated BMD results as a simple decision for diagnosis and therapy.

During diagnosis it is important to differentiate between primary osteoporosis and secondary generalized osteoporosis (Tables 3 and 4; Fig. 1), because this has a strong impact on therapeutic measures. Secondary osteoporosis may emerge from various disorders of the endocrine system or of the gastro-intestinal tract, from kidney insufficiency and other disorders [12–15]. Furthermore, loss of bone mineral content may be due to medications e.g. continuous cortisone therapy [16–23]. The differential diagnosis of metabolic osteoporosis needs a stepwise approach, corresponding to the severity of disease and including the diagnostic spectrum of various medical disciplines (Table 4).

Table 3. Osteologic evaluation

Assessment of risk	By anamnesis (see risk factors Table 1 and FRAX®-tool), clinical investigations and knowledge on previous fractures
Diagnosis	By BMD-measurement, skeletal X-ray and bone turnover markers
If BMD is reduced (*T-Score* <–1) *or other risk factors exist*	
"Basal laboratory"	Serum calcium, serum phosphate, alkaline phosphatise, creatinine, total protein, γ-glutamyl-transferase, thyroid stimulating hormone, protein-electrophoresis, CRP, haemogram, 25-hydroxy vitamin D[a], bone marker[b]
Further investigations for differential diagnosis: primary osteoporosis—secondary osteopathy (see Table 4: *underlying disease?*)	
"Special laboratory"	Calcium in 24 h urine or calcium/creatinine ratio (second void urine) parathyroid hormone, follicle stimulating hormone & estradiol (females), luteinizing hormone & testosterone (males), sex-hormone binding globuline, prolactin, cortisol
	Lactose intolerance testing, ant-tissue transglutamase-2 antibodies, vitamin B12, folic acid, homocystein, markers for bone resorption and formation

[a]Collection of blood: acute (immediately), but control desirably between January to April of next year
[b]Preferably a bone resorption marker

Table 4. Causes of secondary osteoporosis, metabolic osteo-pathy and other reasons for bone loss

Endocrinological	Hyperparathyroidism, hyperthyroidism, hypercortisolism, hyperprolactinemia, hypogonadism, pituitary insufficiency, diabetes mellitus type I
Inflammatory	Chronic inflammatory arthritis, M. Bechterew
Gastrointestinal	Hepatopathy, lactose intolerance, celiac disease, colitis ulcerosa, ileitis terminalis, postgastrectomy syndrome, exocrine pancreas insufficiency, chronic athropic gastritis
Pulmonal	Chronic obstructive pulmonary disease (COPD), asthma bronchiale
Renal	Chronic kidney insufficiency, alumina intoxication, hypophosphatemic osteomalacia
Myelogen	plasmocytoma, mastocytosis
Genetic	Cystic fibrosis, osteogenesis imperfecta, phenylketonuria, hereditary hypophosphatemia, hyperhomocystemia
Medicinal	Hormonal therapies: glucocorticoids, aromatase inhibitors, gonadotropin-releasing hormone agonists, thyroid replacement therapy; neurologic and psychiatric medications: antiepileptics, antidepressants, antipsychotics, lithium (?); mixed: heparine, vitamin K antagonists (?), proton pump inhibitors (long-term use), thiazolidinediones, cytotoxic drugs
Nutritional	Malabsorption, hypophosphatemic osteomalacia, anorexia nervosa, malnutrition (deficiency in vitamin D, vitamin C, vitamin K)
Other	Organ transplant, malignoma (especially breast cancer, prostate cancer), immobilization (bedriddenness, paresis)

(?) conflicting data are reported [18, 19 and 22, 23]

Clinical investigations

In manifest osteoporosis the painful degenerative skeletal changes emerge predominantly from the axial skeleton,

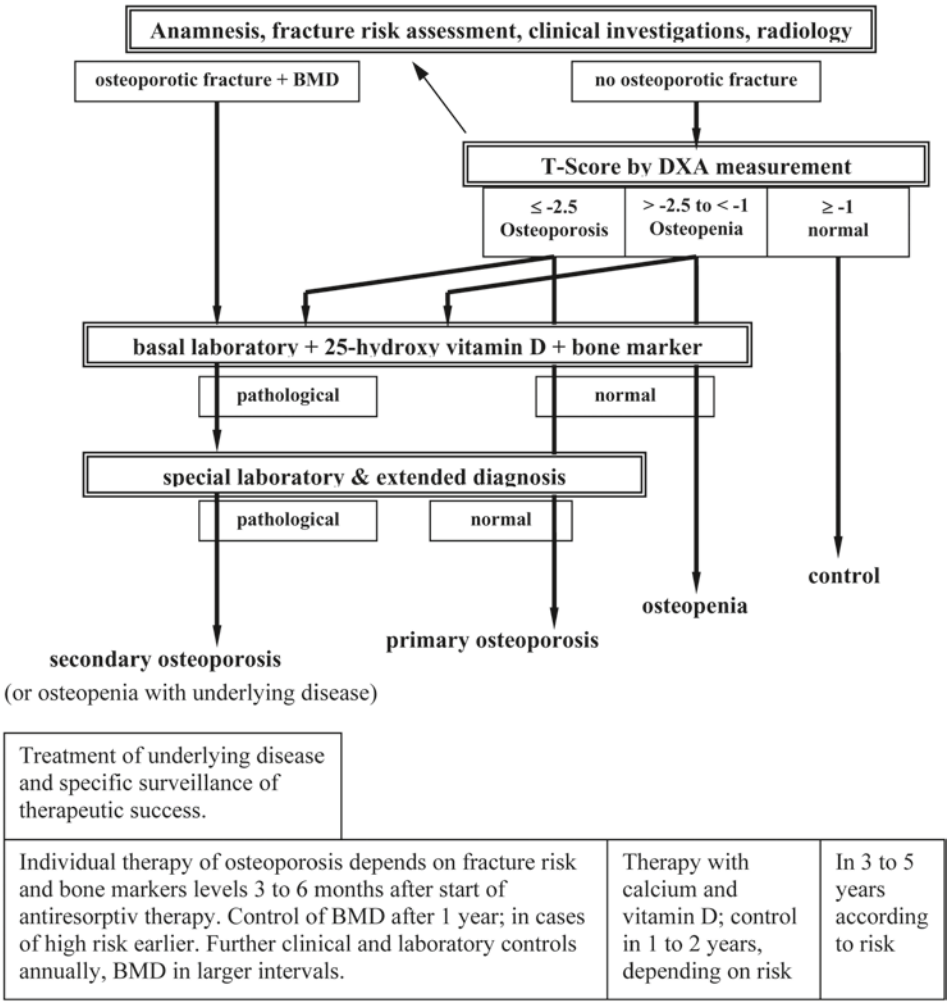

Fig. 1 Stepwise diagnostic approach of suspicion of bone loss, taking into account the laboratory and general advices on therapeutic interventions

because of altered geometry of the spine. Characteristics are increased kyphosis of the thoracic spine, protruded abdomen, narrowing or diminished crista-costa space and in most cases loss in body height.

Conventional X-ray

X-ray investigations of thoracic and lumbar spine are essential, because about 25–30 % of all osteoporotic fractures, including vertebrate body fractures, occur in cases of non-osteoporotic lowered BMD [3].

BMD

Gold-standard is the dual-energy X-ray absorptiometry (DXA). BMD is expressed in g/cm^2, thus it is not a density in the common physical dimension, but in fact it considers the bone diameter, which has a determining influence on bone fragility [24, 25]. BMD measurements are important to estimate the individual fracture risk (see above and references [26–28]) and to differentiate between minor and severe forms of bone loss (Table 2).

Laboratory

Investigations of routine parameters ("basal laboratory") are useful when differentiating between primary and secondary metabolic osteoporosis. Underlying diseases can be detected by additional laboratory analyses which may also serve to secure a diagnosis and to provide pretherapeutic starting values for therapy monitoring (Table 3).

A chronic deficiency in vitamin D results in an impaired bone mineralization. Less severe cases of deficiency or insufficiency cause a hypocalcemic trend by decreased intestinal absorption of calcium, which in turn increase the concentration of the parathyroid hormone (PTH), a stimulant of bone resorption involved in the maintenance of calcium homeostasis. Long-lasting secondary hyperparathyroidism contributes to changes in bone remodelling and osteoporosis in the elderly [29, 30]. An overview on other detrimental consequences of vitamin D deficiency [28, 30] is not a topic of this paper. Vitamin D status is controlled by measurement of 25-hydroxy vitamin D (25OHD); but findings on its association to BMD are controversial [31–34]. However, supplementation with vitamin D and calcium prevented seasonal

bone loss during winter [35]. A meta-analysis showed, that sufficient supplementation with vitamin D reduced the risk of hip and nonvertebral fractures [36]. For these reasons we strongly recommend that one include 25OHD analysis in the "basal laboratory". Similarly, a recent guideline recommends screening for vitamin D deficiency in individuals at risk and advises dosages for oral vitamin D supplementation [37].

Biochemical bone turnover markers are circulating components of bone metabolism, reflecting formation and resorption processes. The biochemical markers are slightly elevated in healthy postmenopausal women due to their deficiency of estrogens and there is an increase in cases of enhanced bone turnover in this group as well. Biochemical markers indicate changes in bone metabolism early, before alterations are reflected by BMD or X-ray of hip and vertebrae [38–40]. Supplementary to BMD measurements they provide information on fracture risk [41]. Therefore, we recommend including at least a bone resorption marker in the "basal laboratory". Increased levels should be interpreted as a matter of clinical judgement, either as an individual risk factor or as an additional argument to recommend hormone replacement therapy to menopausal women with serious climacteric symptoms, especially with osteopenia. For primary osteoporosis, the bone resorption marker concentration represents the baseline level for individualized therapy according to fracture risk evaluation.

Genetic markers may assess a population based on risk, because of the high genetic disposition of osteoporosis. However, genetic markers are subjected mostly to rare cases (e.g. osteogenesis imperfecta) or scientific questions. The first genetic marker described in 1994 is an association of vitamin D receptor genotypes with low BMD [42]. A genetic assay for a disposition to lactose-intolerance is clinically relevant because of dietary implications [43, 44]. A recent meta-analysis suggested that the collagen type I α1 (COLIA1) Sp1 polymorphism initially described in 1996 may be associated with osteoporotic fracture in postmenopausal women [45].

Résumé (see Fig. 1) *Evidence on individual fracture risk is derived from anamnesis, clinical investigations, conventional X-ray and BMD measurements—however, often without knowledge of a possible underlying disease. Frequently, a "basal laboratory" is not sufficient to explain the aetiology of osteoporosis, because of the abundance of differential diagnoses. Extended analyses of laboratory parameters including hormones may be necessary to confirm suspicion. Biochemical markers of bone metabolism and vitamin D status provide additional information on fracture risk. Treatment depends on the underlying disease and the severity of the bone impairment.*

Biochemical markers of bone turnover

Clinical relevance

Prognosis of loss in bone mass

Longitudinal studies have shown two characteristic groups of untreated postmenopausal women: women with high bone turnover lose significantly more BMD than women with normal or low bone turnover [46].

Women with high bone turnover present with elevated concentrations of bone turnover markers in their blood and urine compared with low bone turnover women [47]. In prospective studies on postmenopausal women elevated levels of resorption markers at the beginning were associated with a significant loss of bone mass of the lumbar spine after 1 year compared with low initial marker levels [48]. In a long-term study the decrease of BMD was measured in the fore arm. Women with high bone turnover incurred a 2–6 fold of higher loss in bone mass compared with women with low bone turnover [39]. Thus bone markers are suitable to identify female patients with fast bone loss.

Pre-therapeutic bone turnover markers and fracture risk

Bone turnover markers, especially resorption markers, are an independent predictor for fracture risk, additive to BMD, because elevated turnover markers correlate with elevated fracture risk in osteoporosis. This may be explained by disruptions of trabecula at high turnover rates without detection by densitometry [47, 49]. Three prospective studies, namely EPIDOS [38], Rotterdam [50] and OFELY [40], showed that high resorption markers predict an elevated risk of vertebral fractures, fractures of the femur neck and other peripheral fractures in postmenopausal women. Concentrations exceeding the upper limit of premenopausal women were associated with a two fold elevated fracture risk [51].

Resorption markers reflect future loss in bone mass and increase the predictive value of BMD in an additive fashion (Fig. 2) [38, 47, 52]. Inconsistent prospective studies on the value of formation markers for prognosis of fractions exist, however a correlation of formation marker levels with fracture risk is still a matter of debate [52].

Planning of therapy considering bone turnover markers

In planning a therapy one must consider, that all medications will have side effects, individual tolerance and possible interferences with other drugs or contraindications because of comorbidity. Fortunately, several bone-specific medications exist with different mechanisms of action.

Antiresorptiva like bisphosphonates are more efficient in osteoporotic patients with fast bone turnover (elevated marker levels) than in cases of low turnover. Patients with

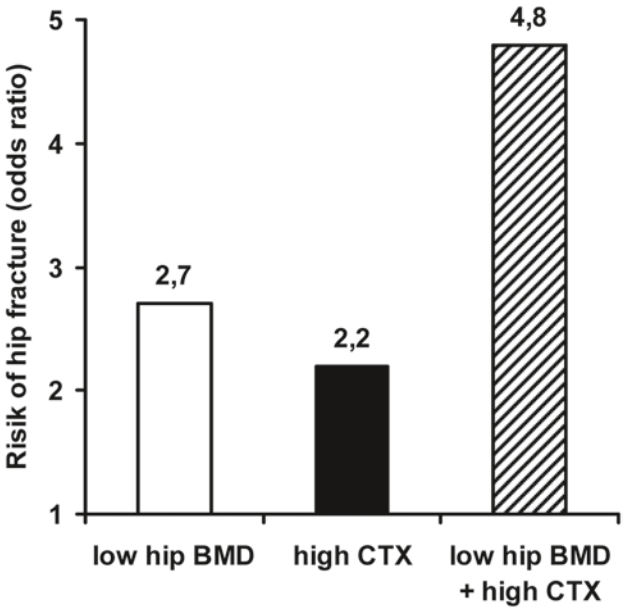

Fig. 2 Combined assessment of BMD and bone resorption rate to predict hip fracture risk in the elderly. Low BMD was defined by a *T* score ≤2.5. High bone resorption was defined by CTX above the upper limit (>mean+2SD) of the premenopausal range. Low BMD and elevated bone resorption marker are independent risk factors for hip fracture. Modified from Garnero P et al. [38], with permission.

high levels of circulating formation markers benefit by this therapy in a reduction of fracture rate [53]. Compared with bisphosphonates, the selective estrogen receptor modulator Raloxifen exhibits less severe side effects, even in long-term treatment [54]. The MORE study demonstrated its efficacy not only in prevention of fractures but also in the therapy of postmenopausal osteoporosis [55], which is characterized by pretherapeutically elevated bone marker levels. A new antiresorptive therapy makes use of an antibody (Denosumab), which inhibits a factor necessary for the development and activity of osteoclasts [56]. In advanced osteoporosis refractive to antiresorptive therapy, because of new fractures or sustained low BMD and high bone markers, another strategy makes use of the anabolic effect of intermittent PTH. A daily application of the recombinant PTH fragment Teriparatide causes improvements of bone microarchitecture, BMD and reduces fracture risk [57, 58]. Antiresorptive as well as anabolic actions on bone are claimed for strontium-ranelate; treatment with this drug leads to an increase of BMD and reduction of fracture risk [59].

Surveillance of therapy with bone turnover markers

Bone turnover markers are suitable to monitor therapy of osteoporosis. Especially at the beginning of therapy their rating surpassed BMD measurements because of faster change [60]. During antiresorptive therapy resorption markers decline about 30–70 % compared with initial values within 3–6 months, reaching a plateau thereafter [61]. Similarly, the formation markers decrease, but the decrease is less pronounced and it occurs somewhat later. A connection was observed between the extent of the marker decline and the reduction of the fracture risk [62]. As a rule, changes in BMD can be mostly observed after 1 year but the response to antiresorptive therapy by markers can be observed much earlier [60]. With antiresorptive therapy, bone turnover markers should be measured before; at 3–6 and 12 months [63]. Thereafter, yearly controls are adequate. An insufficient drop in marker concentrations indicates failure in therapy or flawed compliance of the patient [1].

A fast increase of formation markers occurs within 1 month of anabolic PTH therapy and a plateau is reached within 3–12 months. This is in line with the initial anabolic action of pulsatile PTH. In contrast, the resorption marker's increase is delayed for several months indicating subsequent osteoclast activation and start up of bone remodelling [64]. The increase in formation markers 1 month after starting a therapy with the PTH fragment Teriparatide correlated well with an improvement of bone structures as confirmed by biopsies [65]. With anabolic therapy bone turnover markers should be measured before starting and at 1–3 and 12 months.

Hopes, that a combination therapy with antiresorptive and anabolic agents has benefits, were forced to be abandoned for the first time. Indeed, the positive effect of PTH on BMD was blunted by the bisphosphonate alendronate [66]. This was reflected by biochemical markers of bone turnover (Fig. 3). Alendronate quickly reduced bone resorption and bone formation soon after. Overall, the rate of turnover was reduced within 1 year. Monotherapy with PTH resulted in an enhanced turnover rate by raising bone formation and bone resorption later on as well. Combined therapy led to a slight reduction of the turnover rate compared with the baseline.

Therapy with strontium ranelate stimulates bone formation and hinders resorption. Changes in bone markers are significant, but small. Therefore monitoring strontium-ranelate therapy by markers seems useless [59].

Estimating the significance of marker changes by therapy is essential for an assessment of surveillance data. This can by calculated by the "least significant change":

$$\mathrm{LSC} = 1.96 * \sqrt{2} * \sqrt{\left[\mathrm{CVa}^2 + \mathrm{CVi}^2\right]}$$

$$= 2.77 * \sqrt{\left[\mathrm{CVi}^2 + \mathrm{CVa}^2\right]}$$

LSC	least significant change
√	square root
CVa	analytic coefficient of variation
CVi	individual (within-subject) coefficient of variation

If change of marker level (**a**ctual—**p**revious), which is expressed as an absolute percentage from the mean of the **a**ctual and **p**revious levels (% $|(\mathbf{a}-\mathbf{p})|*2/(\mathbf{a}+\mathbf{p})$), exceeds the least significant change, a significant difference can be assumed [67]. Least significant change is about 20–50 % depending on the marker (Table 5), mea-

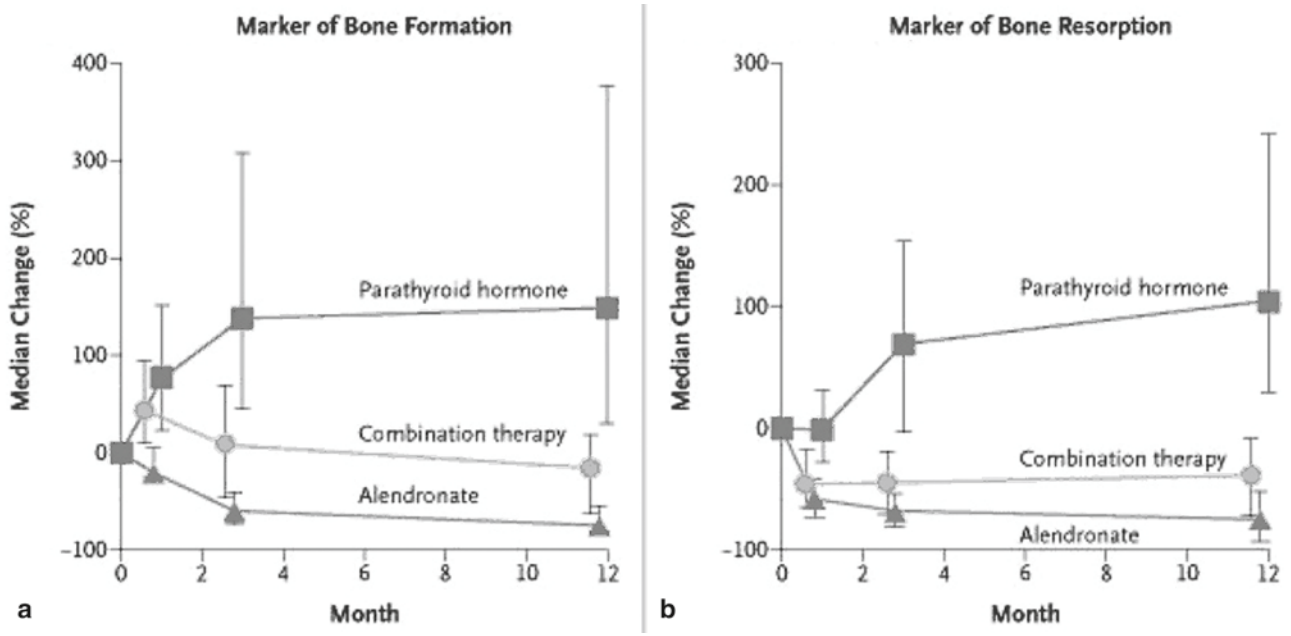

Fig. 3 Median percent changes in the serum concentrations of biochemical markers of bone formation (panel **a**: N-terminal propeptide, of type 1 collagen) and bone resorption (panel **b**: C-terminal telopeptide of type 1 collagen) during antiresorptive therapy with bisphosphonate (Alendronate), anabolic therapy with intermittent parathyroid hormone and combination therapy, respectively. Bars represent the interquartile ranges. Differences between all groups at 12 months were significant ($p < 0.001$). (Reproduced from Black et al. [66] with permission)

Table 5. Approximate least significant change (LSC) of bone remodelling markers [68]

			LSC
Bone turnover marker	Resorption marker	ß-CTX	40-50%
		TRAP 5b	~20%
	Formation marker	OC	30-40%
		P1NP	30-40%
		BALP	~20%

ß-CTX	ß CrossLaps (C-Telopeptid)
TRAP 5b	bone specific tartrate resistant acid phosphatase
OC	osteocalcin
P1NP	total N-terminal procollagen of type I procollagen
BALP	bone specific alkaline phosphatase

ning that changes according to this magnitude prove to have a therapeutic effect [47, 68].

Differential diagnosis

As an important aspect in differential diagnosis, elevated bone turnover markers can also be observed in patients with bone metastases. Especially in carcinomas of the prostate and breast the prevalence of bone metastases is about 70 %. Due to changes of bone turnover by metastatic spread to bones, bone metastases may be detected early by bone turnover markers; moreover, antiresorptive therapy normalized bone marker levels and improved survival [69–77].

Résumé *The monitoring of therapy in osteoporosis and also recently in metastatic bone diseases is the most established application of bone turnover markers. Reaction to therapy is registered rapidly in comparison to BMD measurements. We suggest control of bone markers before, at 3–6, 12 months and thereafter at biannual or annual intervals of antiresorptive therapy (with anabolic therapy controls that should be before and at 1–3 and 12 months), especially in patients with high fracture risk. The compliance of the patient can be judged and adherence to therapy is improved by marker investigations. Knowledge on least significant change is important for an interpretation of the time course of marker changes.*

Selected markers of bone turnover

The following markers were tested in large clinical studies and intervention trials. Their usage is in centres specialized on bone metabolism and results from their analytical and clinical practicability and from their availability. However, there is no recommendation for products from specific companies.

It is necessary to consider that immunoassays for markers of bone turnover from different manufacturers may give different results, but typically the concentrations that are measured are very well correlated. This is due to the epitope specificity of antibodies, the test format, and of course standardization. At present there are no inter-

national standards defined, though an IFCC committee has been created to address these questions focused on a number of representative markers for bone formation and bone resorption, and to make reference materials available. A suggested nomenclature of bone markers has been published in 2000 [2].

Résumé *In follow-up controls with biochemical markers of bone turnover a change in methods (possibly by changing the laboratory) should be avoided.*

Formation markers	Resorption markers
Are direct or indirect products of activated osteoblasts. These markers are formed during different phases of the lifecycles of osteoblasts, representing different aspects of bone formation. Therefore dynamics of markers may differ	Are direct or indirect products of activated osteoclasts. The crosslinks of collagen molecules within the bone matrix lead to special structures, which are used in analytics
Total N-terminal propeptide of type I procollagen (P1NP)	C-terminal crosslinked telopeptide of type I collagen (β-CrossLaps, CTX)
Bone-specific alkaline phosphatase (BALP) Osteocalcin (OC)	Tartrat-resistant acid phosphatase type 5b (TRAP 5b)

Preanalytics

Preanalytic variability of biochemical markers of bone turnover includes both biological variation of individuals tested and inconsistencies due to sample collection and handling [67]. Biological variation can be divided broadly into two categories [2]: (a) Uncontrollable sources enclose age (compared with adult levels, markers are high during childhood and puberty and elevated during menopause), gender, ethnicity, fractures, pregnancy and lactation [78], physical exercise [79, 80] and various other influences including diseases and medications (see Table 4). (b) Controllable sources are adherence to therapy (hormone replacement, antiresorptive or anabolic bone therapy) and mainly seasonal and circardian variations of bone markers, insufficient storage of specimens and analytic imprecision [67].

Measuring bone markers in serum or plasma samples is well established in routine laboratories because manual analyses from urine of e.g. bone resorption markers (like deoxypyridinoline or N-terminal collagen fragments) are more prone to analytical errors and are more expensive. Although desirable, a 24-h collection did not force through, because of a lack of compliance of patients and reliability of collection. As an alternative, the results from the second void urine have to be related to creatinine excretion. But mostly an additional blood sample is demanded for the analyses of formation marker and other laboratory parameters [81].

Distinct circadian rhythm is a frequent observation with most of these markers [82, 83]. It is strongly recommended, that especially the blood collection for CTX be taken from overnight fasting patients between 7 and 9 a.m., to reduce individual oscillations [84]. In addition, the collagen resorption markers decrease within minutes in respond to a raise of glucose [85]. Therefore, only the drinking of water or unsweetened tea or coffee is allowed prior to blood collection. If it is doubtful whether the patient indeed had fasted especially for reporting collagen resorption marker concentrations, glucose and triglycerides may be measured additionally. Thus, preanalytic cautions are similar to that of lipid-analyses.

Blood samples should be collected before dialysis from dialysis patients with a loss of renal elimination and circadian rhythm. One must acknowledge that the reference values from bone turnover markers, which are cleared via the kidney, are unusable in kidney insufficiency. For these patients a monitoring of BALP and TRAP 5b is in favour. These markers are cleared via the liver and not the kidney [2, 63, 86] while in liver diseases osteocalcin, P1NP and CTX are in favour.

Recent bone fractures or surgical intervention on bones limit the usage of bone turnover markers, because they increase due to the remodelling processes. Depending on the site and size of fractures, marker levels might return to initial values up to 6–12 months after the event [87–90].

Résumé *For a correct estimation of bone turnover, blood should be collected between 7 and 9 a.m. The patient has to fast overnight and must not drink sweetened beverages prior to blood collection.*

Concerning the route of clearance, appropriate bone markers have to be selected for dialysis patients and for patients with severe liver diseases. Blood has to be collected prior to dialysis.

CAVE: Reference values are unusable in kidney insufficiency. For these patients BALP and TRAP 5b are in favour, because they are cleared via the liver and not the kidney.

CAVE: Bone markers may be elevated up to a year following fractures.

Characteristics of formation markers

Bone-specific alkaline phosphatase (BALP) [86, 91, 92] Alkaline phosphatases belong to a family of ubiquitous, membrane-associated enzymes with a molecular weight of about 140 kDa. The different isoforms are coded by one gene, but differ in degree of glycosylation and sialylation. Then most abundant are isoforms from the liver and bone. BALP, a tetrameric glycoprotein is anchored in the plasma membrane of osteoblasts and is released as a dimer into circulation by phospholipase cleavage during bone formation [90].

Analytical methods are enzyme electrophoresis, lectin binding, selective inhibition of other isoforms by heat or urea, immunoassays and by immuno-extraction with subsequent measurement of enzyme activity. Relevant cross-reactions to the liver isoform are possible with some methods. Because of the different analytical procedures and units, the reference values differ extremely. BALP slightly increases during the luteal phase and is

elevated in Paget's disease, hyperthyroidism, primary hyperparathyroidism, acromegaly and bone metastases.

Sample material: Serum, heparin plasma.

Stability: Up to 48 h at room temperature, 1 week at 4 °C, 1 year at – 70 °C.

Interferences: Haemolysis, lipidemia, incorrect high in liver disease due to cross-reaction of antibodies with the liver enzyme.

Clearance: Predominantly by the liver.

Total N-terminal propeptide of type I procollagen (P1NP) [86, 92–97] The bone matrix is composed of about 90 % of type I collagen, which is synthesized by osteoblasts. Sinews, skin and cartilage are other sources of type I collagen. The monomer procollagen chains are secreted and drilled to helical triplets extracellularly. Thereby the terminal procollagen regions are eliminated into circulation. These fragments (N-terminal and C-terminal, molecular weight 100 kDa and 35 kDa, respectively) represent the newly synthesis of type I collagen and are early markers of osteoblast activity. P1NP concentrations show a wide dynamic range and markedly rise during puberty. P1NP exhibits low circadian variation. In circulation P1NP is heterogeneous, where the trimeric form partly dissociates at 37 °C, which may have an impact on several assays. P1NP is well suited for the monitoring of anabolic bone therapy, because of a high dynamic range.

Sample material: EDTA plasma preferred, serum or heparin plasma.

Stability: Twenty-four hours at room temperature, 5 days at 4–8 °C, 6 months at –20 °C. No influence of five freezing-thawing cycles.

Interferences: Liver diseases (significant higher levels in liver cirrhosis).

Clearance: Liver and kidney.

Osteocalcin [86, 98–107] Osteocalcin, the most abundant noncollagenous protein of bone, is synthesized solely by osteoblasts. The synthesis is regulated by 1,25 dihydroxy vitamin D. Osteocalcin is formed from 49 amino acids, the carboxylation of three N-terminal glutamine residues depends on vitamin K and is critical for the high affinity of osteocalcin to hydroxylapatite. The majority of osteocalcin is integrated into the bone matrix, but 20–30 % arrives in circulation. Osteocalcin represents a late marker of osteoblast activity and is formed during differentiation subsequent to BALP and type I collagen.

Osteocalcin circulates in a heterogeneous and partly fragmented manner. In vitamin K deficiency a part of osteocalcin is imperfectly carboxylated, which increases the risk of hip fractures. Undercarboxylated osteocalcin can be measured with special assays and is involved in glucose haemostasis, insulin control and probably androgen formation. Apparently, there is an endocrine regulation of energy metabolism by the skeleton.

During bone resorption short osteocalcin fragments are liberated, however osteocalcin (1–34) and/or osteocalcin (1–49) predominantly reflect bone formation.

Sample material: EDTA-plasma preferred, serum or heparin plasma.

Stability: The data are inconsistent because of the epitope specificity of immunoassays. In practical use, direct immunoassays which recognize the mid-fragment osteocalcin (1–43) seem advantageous. Proteolytic cleavage of the 6 C-terminal amino acids is fast but individually different. Preanalytics are more sensitive when using intact osteocalcin (1–49) assays.

Stability N-Mid osteocalcin: EDTA plasma 2 days at room temperature, 3 days at 4 °C, 3 months at –20 °C. Serum or heparin plasma: 8 h at room temperature. Avoid haemolysis, freeze only once.

Interferences: Liver diseases, chronic kidney insufficiency.

Clearance: Mainly by kidney, less by liver.

Characteristics of resorption markers

C-terminal cross-linked telopeptide of type I collagen (CTX, Crosslaps) [86, 108–113] The proteolytic degradation of bone matrix forms a variety of type I collagen fragments. The structures of these cleavage products are more or less bone-specific. This results from the primary structure of protein chains and the characteristic modifications during collagen fibril formation, maturation and ageing of the matrix. Optimal bone specificity is known for the peptide bound crosslink structures of the N- or C-terminal teleopeptide regions (NTX, CTX) of type I collagen. A β-isomer of C-telopeptide is formed spontaneously by slow isomerization of a peptide-bond; analysis of "β-CrossLaps" is specific for elderly tissue—and therefore for bone and its proteolytic resorption. CTX is well suited for monitoring the efficacy of antiresorptive drugs including the different bisphosphonates.

Sample material: EDTA-plasma preferred, serum or heparin plasma, CAVE preanalytics!

Stability: EDTA-plasma 24 h at room temperature. Three months at –20 °C. Avoid haemolysis, freeze only once.

Interferences: Liver diseases, chronic kidney insufficiency.

Clearance: Mainly by kidney, less by liver.

Tatrate-resistant-acid phosphatase Type 5b (TRAP 5b) [86, 114–117] TRAP is expressed by osteoclasts, macrophages and dendritic cells. The isoenzyme TRAP 5b lacks sialic acid and works at a higher pH optimum than isoenzyme 5a. TRAP 5b levels resemble the number of activated osteoclasts. Kathepsin K activates TRAP 5b by elimination of a propeptide domain. The enzyme is formed from two subunits, which are connected by two disulfide bonds. Although its high protein-tyrosin-phospatase activity is known, the substrate has not been identified yet. Another function of the enzyme may be the formation of free oxygen radicals which supplement the degradation of the organic bone matrix.

In circulation about 90 % of TRAP is fragmented. Analysis is done by immune-extraction with TRAP 5b-specific antibodies and measurement of bound enzyme activity at pH 6.1. Alternatively, the enzyme activity can be measured by inhibition of TRAP 5a by tartrate and heparin.

Serum TRAP 5b activity is less influenced by kidney function than the resorption markers derived from type I collagen. Therefore, main indications of TRAP 5b are patients with reduced kidney function, such as dialysis patients.

Sample material: Only serum, transport should be done in dry ice.

Stability: Serum should be obtained within 4 h after blood collection. Samples have to be stored at –20 °C or below. Transport in dry-ice. Freeze only once.

Interferences: Haemolysis. Formation of TRAP 5b complexes with alpha-2-macroglobulin might reduce the measured concentration.

Clearance: Liver.

Some selected parameters which regulate bone metabolism

Parathyroid hormone (PTH) *Time of sample collection:* In the morning, optimal before 9 a.m., the patient must fast overnight. As an exception, blood is sampled from dialysis patients prior to dialysis for convenience, but before the long dialysis interval. Although this results in slightly higher PTH and phosphate, the dangers of elevated phosphate and PTH may be reflected more sensitively.

Selection of assay: The test results should be compatible with the recommendations given in the guideline of National Kidney Foundation [118, 119]. The test should correlate well with the PTH (1–84) assays.

Sample material: EDTA-plasma preferred, serum or heparin plasma.

CAVE: Serum is unusable in pancreatitis due to a fast degradation of PTH!

Stability: EDTA plasma 24 h at room temperature, 72 h at 4° C, 12 month at –20 °C.

Serum 4 h at room temperature, 24 h at 4 °C, 6 month at – 20 °C.

Interferences: Inadequately filled EDTA tubes may cause a pH shift, which may cause false low PTH in certain assays.

25-hydroxy vitamin D (25OHD, "calcidiol") *Time of sample collection:* In the morning, the patient must fast overnight. Analyses of 25OHD should be carried out every 5 years starting with the age of 50. Samples should be collected between January and April. During this timeframe the 25OHD levels are lower and those of PTH are higher [120].

Sample material: Serum preferred, plasma.

Stability: Avoid direct sunlight; shipping is possible without refrigeration for 48 h.

Interferences (*depend on the assay in use*)*:* Lipemia, cross-reactions with hydroxylated metabolites of vitamin D_3, *Intoxication* with dihydrotachysterol (AT10®, Tachystin®).

Analytical methods: Immunoassays, protein binding analyses, HPLC and HPLC-mass-spectrometry. A co-measurement of 25OHD3 (metabolite of cholecalciferol) and 25OHD2 (metabolite of ergocalciferol) is desirable, although ergocalciferol is not available from Austrian pharmacies, but may be purchased via the internet. 25OHD assays do not measure 1,25-dihydroxy vitamin D (calcitriol, Rocaltrol®, Bocatriol®), or α-calcidiol (Bondiol®, Doss®, Etalpha "Leo"®)

Résumé *The optimal 25OHD concentration for the bone and the ability to avoid a variety of diseases was defined by a consensus conference of experts to be 30–40 µg/l (75–100 nmol/l) [33, 121]*

Recently another expert group (Institute of Medicine) recommended at least 20 ng/ml (50 nmol/l) 25OHD, but less than 50 µg/l (125 nmol/l) to avoid adverse effects [122].

Recommendations for monitoring a therapy and check for an optimal vitamin D substitution

Parameter	Frequency	Target
Bone resorption marker	Before, 3–6 months after start of antiresorptive therapy. Yearly controls to check compliance	Significantly lowered or near low limit of reference range
Bone formation marker	Basal and after 1, (if no response: 3–6), 12, 18 month of anabolic therapy	Significantly increased or near upper reference range
25OHD	At least once in winter during substitution	20–40 µg/l (50–100 nmol/l)
PTH	At least once in winter	Middle reference range

Synopsis

Remarkable features of biochemical markers of bone turnover and a proposal of their use are summarized in Table 6 as a shortcut.

Conclusion

A detailed anamnesis in combination of an evaluation of individual risk factors e.g. by the FRAX® tool of WHO may raise suspicion of clinically relevant bone loss, which would be confirmed by clinical investigations, conventional X-ray and BMD measurements. Due to its strong impact on therapeutic measures, it is important to differentiate between primary and secondary osteoporosis which is achieved by a "basal laboratory" and by extended laboratory investigations, if necessary. 25OHD and bone turnover markers support the assessment of fracture risk and the markers enable the estimation of the bone turnover rate. An underlying disease must be treated appropriately and the therapeutic success must be proved by disease-specific parameters.

Table 6. Synopsis: Bone metabolism: what we have to consider

Diagnosis/suspected diagnosis	Parameter to be analysed
Bone resorption: follow-up of antiresorptive therapy	*CTX*
	Before and soonest 3 months after the start of antiresorptive therapy
Menopause	Estradiol (<20 ng/l)
Male osteoporosis	(Bioavailable) testosterone
Metastasizing tumours (breast-, prostate-, lung cancer)	P1NP, TRAP 5b, PTH-related peptide
Bone formation: follow-up of anabolic therapy	*Osteocalcin, P1NP, BALP*
	Before and after 1, (3, 6), 12, 18 months
	↓ Due to glucocorticoid therapy
Vitamin-D-deficiency	*25OHD,* avoid <20–30 µg/L (50–75 nmol/l)
	Ca, ALP, CREA, (PTH)
	Special laboratory: anti-tissue transglutaminase-2 antibodies, anti-deamidated gliadin antibodies
Ca^{++} supply ↓ by lactose-intolerance	Molecular biological test, H_2 respiratory test
Primary/secondary *hyperparathyroidism* (HPT)	*PTH*
	Serum Ca and 24 h urine Ca, phosphate; *25OHD;* bone turnover marker
Hyper- oder hypo*calziuria*	*Ca/CREA, 24 h urine Ca;* (ionised) *serum Ca*
	Total protein: CAVE dysproteinemia: protein binding ↑ (ionised Ca ⊥), PTH (additional parameter see HPT)
Hypo- oder hyper*phosphatemia*	*Phosphate + Ca+ALP+CREA,* (PTH)
Kidney insufficiency (*chronic dialysis*)	*CREA, Ca, phosphate, BALP, total protein*
	PTH, 25OHD
	Bone turnover ↑ TRAP 5b↑
Hyperthyroidism	*TSH, fT4, fT3, ionisied Ca*
	TSH ↓ and fT4, fT3 ↑: activity of osteoclasts↑, differentiation of osteoblasts ↓ bone resorption↑: *CTX*↑
Liver disease	*AST (GOT), ALT (GPT), γGT, Ca, phosphate*
	Hepatobiliary disease: BALP↑ useless, therefore *osteocalcin*
	CAVE: additional risk factors for osteoporosis such as hypogonadism, low BMI, excessive alcohol abuse, vitamin D-deficieny
Morbus Paget	*BALP, P1NP, CTX* ↑
Glucocorticoid therapy	*CTX* ↑, *P1NP & osteocalcin* ↓↓

Ca calcium, *ALP* alkaline phosphatase, *CREA* creatinine, *AST* aspartate-aminotransferase, *ALT* alanine-aminotransferase, *γGT* γ-glutamyltransferase, *BMI* body mass index

Bone treatment depends on fracture risk assessment and the severity of the impairment. Clearly, high-risk patients must be treated and monitored more efficiently compared to patients of low risk. Today a plurality of bone-specific medications exists with different mechanisms of action. The drugs are dosed daily or as a depot and operate in antiresorptive and/or anabolic fashion. Understandably, the therapy must be optimized to the patient's individual responsiveness. Monitoring drug treatment of osteoporosis is an established application of bone turnover markers, because markers allow an early judgement of therapeutic success or failure within months compared to late decisions obtained from BMD reports. This has an important economical aspect. Taking a costly daily medication and detecting its failure about 1–2 years later by BMD examination will waste health budget, frustrate the patient, diminish his confidence in the prescribing physician and the compliance for a further therapy. Osteoporosis therapy monitoring by bone turnover markers in patients with high fracture risk most likely improve economic perspectives, not only by the enhanced efficacy in the early detection of a response, but also by the well-documented adherence of patients to the prescriptions and a better relationship with the physician. By a reasonable application of bone turnover markers in the monitoring of bone therapy, BMD will not lose relevance in the surveillance of bone disease, but its importance can be restricted to controls in larger time intervals, which may also help to save costs.

Conflict of interest

Authors declare that there is no conflict of interest.

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