

**BONE MARKERS – THEIR NATURE AND CLINICAL USE**

## KOŠTANI MARKERI – PRIRODA I KLINIČKA UPOTREBA

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**Summary:** Bone remodeling units are the centerpiece of bone metabolism. They are fueled by a synchronized and well balanced interaction of osteoclasts and osteoblasts, the activity of which releases specific substances known as bone markers into the blood. Resorption markers result from osteoclastic activity, formation markers from osteoblastic activity, and turnover markers from both cell types. In clinical practice, bone markers are today widely used for monitoring of anti-resorptive therapy and patient compliance. There is strong evidence that they are also useful for risk assessment with respect to osteoporosis, here complementing established imaging methods. Other possible and partly not yet investigated indications include monitoring of side-effects of certain therapeutic drugs and oncology. In particular the combination of resorption and formation markers may open up a more differentiated insight into the metabolic situation of a patient's bone. The activity of osteoclasts and osteoblasts is triggered and modulated by numerous factors, some of which are of endocrine nature. Easily measurable in today's laboratory are for instance PTH, calcitonin and vitamin D. While calcitonin is not widely used in osteology, PTH and vitamin D define risk factors for an accelerated loss of bone and impaired mineralization of osteoid with the related diseases of osteoporosis, rickets and osteomalacia. Recent developments in lab diagnosis of bone diseases focus on rheumatic diseases like rheumatoid arthritis, where anti-CCP is a much more specific marker than the common rheuma factors.

**Keywords:** bone markers, bone metabolism, osteoporosis

**Introduction**

Bone consists of an inorganic and an organic phase. The inorganic phase is calcium phosphate doted with fluoride, a mineral otherwise known as apa-

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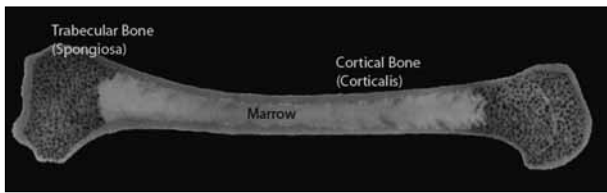
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**Kratak sadržaj:** Jedinice za koštano remodelovanje su središnja tačka metabolizma kostiju. Pokreće ih sinhronizovana i uravnotežena interakcija osteoklasta i osteoblasta, čija aktivnost izaziva otpuštanje u krv specifičnih supstanci poznatih pod imenom koštani markeri. Markeri resorpcije su rezultat aktivnosti osteoklasta, markeri formacije rezultat su aktivnosti osteoblasta, a markeri prometa mogu nastati od obe vrste ćelija. U kliničkoj praksi se koštani markeri danas koriste za praćenje toka antiresorptivne terapije i odgovora pacijenta na terapiju. Postoje i čvrsti dokazi o tome da su korisni za procenu rizika kad je reč o osteoporozu, gde su komplementarni ustanovljenim »imaging« metodama. Ostale moguće i donekle još nedovoljno istražene indikacije uključuju praćenje neželjenih efekata nekih lekova i onkologiju. Naročito kombinacija markera resorpcije i formacije može omogućiti bolje diferencirani uvid u metaboličko stanje kosti pacijenta. Aktivnosti osteoklasta i osteoblasta pokreću i modulišu brojni faktori, od kojih su neki endokrine prirode. U današnjim laboratorijama se lako mogu izmeriti npr. PTH, kalcitonin i vitamin D. Kalcitonin se ne primenjuje mnogo u osteologiji, ali PTH i vitamin D definišu faktore rizika za ubrzani gubitak koštane mase i narušenu mineralizaciju osteoida, uz srodne bolesti, osteoporozu, rahitis i osteomalaciju. Novija dostignuća iz oblasti laboratorijske dijagnostike fokusirana su na reumatske bolesti poput reumatoidnog artritisa, gde je anti-CCP mnogo specifičiji marker od običnih reumatskih faktora.

**Ključne reči:** koštani markeri, koštani metabolizam, osteoporoz

tite. The organic phase comprises mainly collagen 1, less osteocalcin and traces of other proteins (1).

The architecture of bone is complex. Basically, bones are hollow bodies. The relatively thin cortex is made of a hard, robust, but high density and brittle material, so-called cortical bone encompassing a cavity. Those regions of a bone that usually experience strong static or dynamic forces are reinforced by filling this cavity partly or completely with another type of calcified tissue named trabecular bone. Different from cortical bone, this is a porous material, which micro-

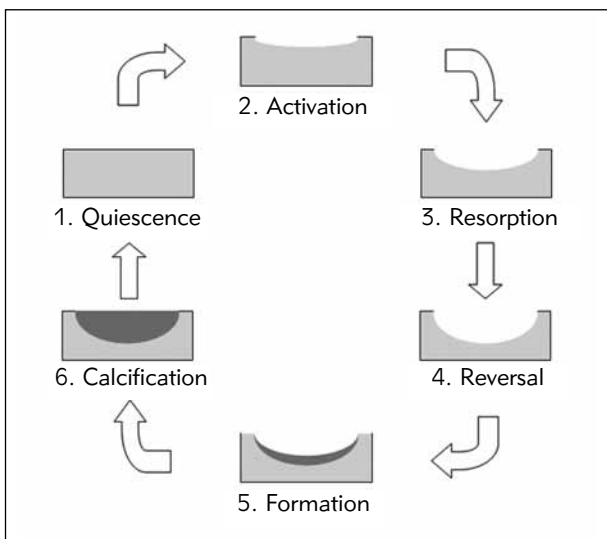


**Figure 1** Calcified tissues of bone.

scopically resembles a honeycomb structure. Thus, forces acting on a bone are distributed over a wide area, which reduces the resulting pressure to a tolerable strength. In humans, particular amounts of trabecular bone are found in the heads of the long bones, vertebral bodies, and the hip. Still empty cavities are filled with marrow, which can host the production site of blood and immune-competent cells.

Developing bones evolution has chosen the approach of discarding all material that is redundant, meaning not accepting or conducting any forces, while strain bearing regions are strengthened by including supporting material. Today's engineers copy this genial and enormously effective construction principal whenever parts have to be built that are equally light and sturdy. This so-called sandwich construction does not only find wide application in high tech segments like aircraft, space vehicles, and racing cars, but is also the basic idea behind something as common as corrugated card board.

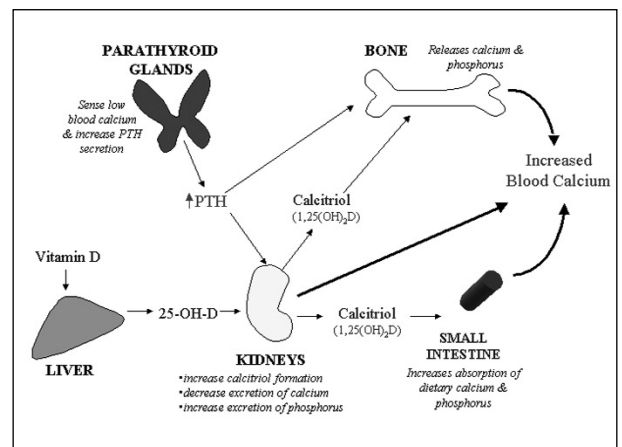
The main organic component of bone is collagen 1, not – as often thought erroneously – the marrow. Amongst the 28 different types of collagens known, collagen 1 is the most abundant one in the human body. It forms fibers which reside inside tiny channels pervading the apatite 1. Bone is metabolically highly active, as it is continuously resorbed and rebuilt. Though macroscopically appearing homogeneous, this process of remodeling is the collective result of myriads of indi-



**Figure 2** Bone remodeling cycle.

dual temporary processes taking place on the surface (cortical and trabecular) of bone under the control of specific cells: osteoclasts («bone destroyers»), osteoblasts («bone builders»), and osteocytes. In an individual bone remodeling unit these cells work together in a concerted and well synchronized cyclic way, the so-called bone remodeling cycle. Following a complex and still poorly understood mechanism, osteoclasts settle down in circumscribed areas on the surface of bone after a period of quiescence. Due to their morphology and their metabolic products they cauterize an indentation into the surface called lacuna. After this lacuna has reached a certain size (approx. 200  $\mu\text{m}$  in diameter and 50  $\mu\text{m}$  in depth) the osteoclasts stop their activity and another family of cells, the osteoblasts, settles down on the bottom of the indentation. They fill the lacuna with osteoid, which is a mixture of proteins, mainly consisting of immature (non-carboxylated) osteocalcin and precursors of collagen 1. After carboxylation of the osteocalcin and formation of the collagen 1, calcification of the osteoid takes place with the contribution of vitamin D and eventually the bone returns to its initial situation. The deposition of apatite starts in the vicinity of collagen 1 fibers and proceeds from there; mature osteocalcin is compulsory for this process (2). In the end, an onion ring like fine structure of apatite results with a central cavity that is filled with a collagen 1 fibre. In the same way as the insertion of reinforcement bars improves the properties of concrete, the collagen armed apatite is more elastic and ductile than the otherwise brittle and eventually fragile material.

Not all osteoblasts manage to escape from the calcifying protein matrix, but remain captured inside the apatite structure and are then transformed into osteocytes. These cells have a signaling function and indicate micro fractures or changing strain patterns triggering repair or remodeling mechanisms. Another osteoclast activation mechanism is the control of the necessary calcium concentration in blood; this mechanism is of an endocrine nature and starts with the release of PTH from the parathyroid glands in response to decreasing serum calcium levels. One func-

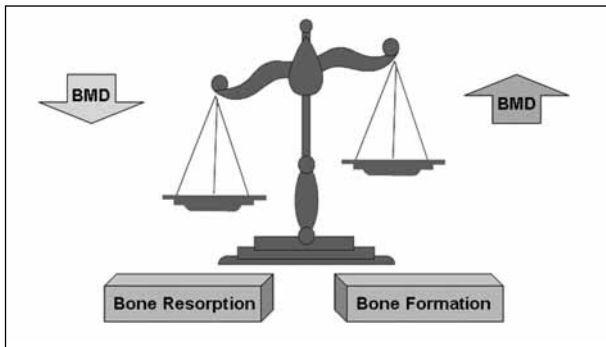


**Figure 3** Mechanism of PTH and vitamin D.

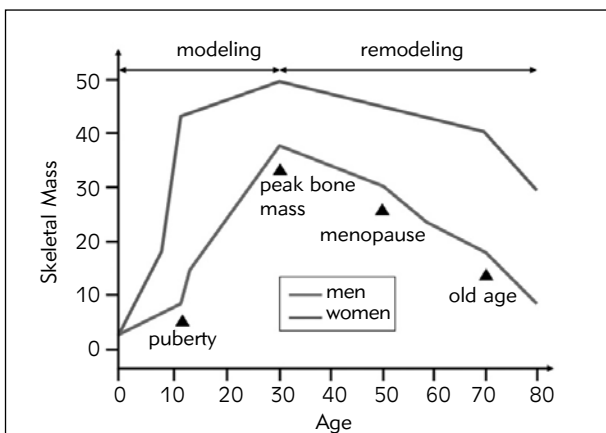
tion of PTH is the stimulation of osteoclasts, which as a result of their catabolic activity sets calcium free. Besides this bone quality hazardous mechanism, PTH primarily facilitates the resorption of calcium from food and the hydroxylation of 25-OH vitamin D to calcitriol further alleviating the intestinal calcium resorption; moreover, it reduces the renal loss of calcium.

It remains to be emphasized that formation can only follow a preceding resorption. In bone healthy individuals, no formation can occur independently. This synchronization is only impaired under pathological condition such as Paget's disease (3, 4).

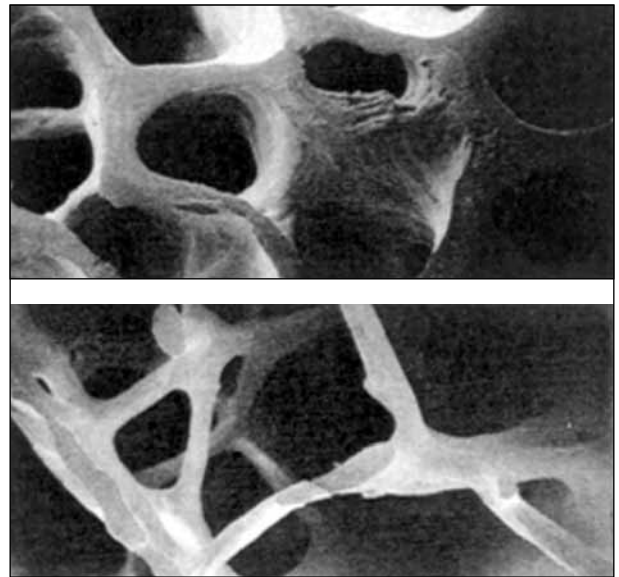
If osteoclastic and osteoblastic activity is balanced, macroscopically no change in individual bone mass will be observed. However, such a situation is only realized during a short period of human life, namely around 27–30 years of age when a peak bone mass and consequently a peak bone density is reached. Previously bone formation prevails, afterwards bone resorption, which leads to a gradual loss of bone density, both in men and women. Impairment of the bone remodeling cycle will inevitably lead to sickness such as Paget's disease, osteomalacia, or osteoporosis. On the other hand, the activity of the involved cells can be influenced therapeutically, opening possibilities to treat or to delay the development of certain bone diseases. Bone development discriminates



**Figure 4** Balance of resorption and formation.



**Figure 5** Age relation of BMD.



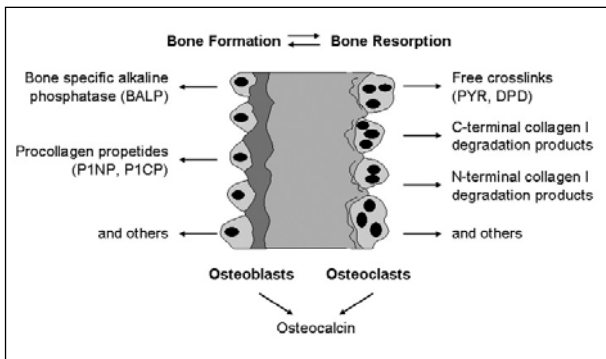
**Figure 6** Healthy and osteoporotic spongiosa.

against women long before the menopause. The peak bone mass attained in early adulthood is normally 30–50 % higher in men. The average woman therefore has to lose only about half as much bone as a man before becoming osteoporotic – and she is likely to lose it earlier in life.

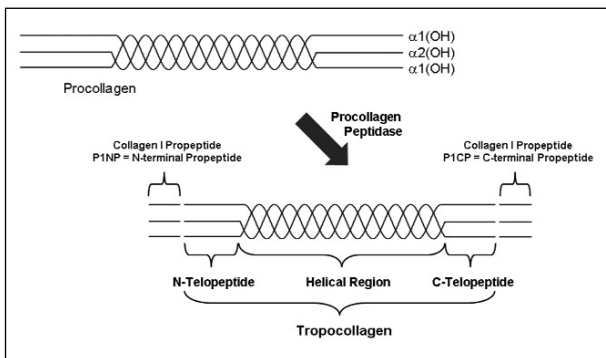
Bone metabolism leads to the fact that virtually each bone of the human skeleton is completely overdone and renewed after a certain period. For cortical bone this lasts approximately 6–8 years, for trabecular bone 4–6 years, respectively. Due to its vast surface, trabecular bone is metabolically more active than cortical bone. As a consequence, impairments of bone metabolism, such as causing certain types of osteoporosis, manifest themselves first in a deterioration of the spongiosa.

During bone formation and resorption characteristic metabolic and catabolic substances are produced and released into the blood, from where they are typically cleared via the kidneys. The concentration of such substances, which are then referred to as biochemical bone markers, can be measured in the clinical laboratory. Bone markers, which are exclusively produced during bone formation or resorption, are called formation markers or resorption markers, respectively. Substances that are released during resorption and formation are known as turnover markers. Nearly all modern bone markers are substances related to collagen 1 (5, 6).

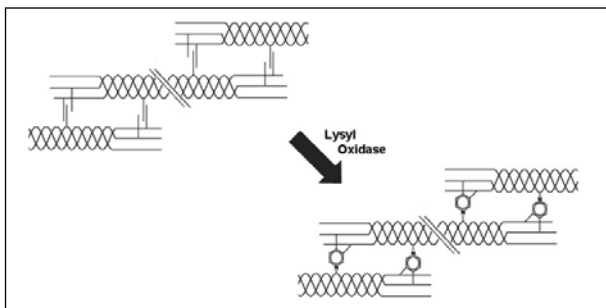
The biosynthesis of collagen 1 starts with alpha-1 and alpha-2 proteins, which are components of osteoid where they are present in a 1:2 ratio. In the next step, some of the lysine and prolin residues of these proteins are enzymatically hydroxylated to hydroxy-lysine and hydroxy-prolin, respectively. These modified alpha proteins organize themselves to a quaternary structure that includes a central triple-helical



**Figure 7** Biochemical bone markers.

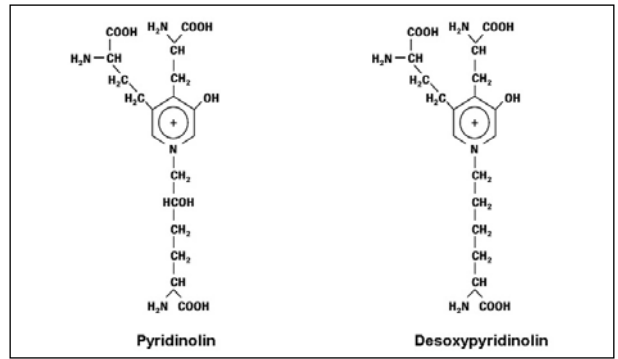


**Figure 8** Procollagen to tropocollagen.

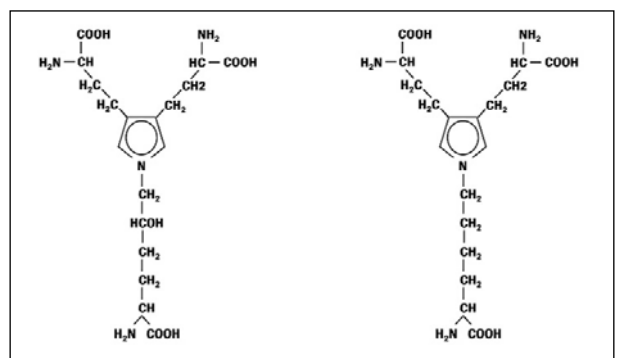


**Figure 9** Tropocollagen to collagen.

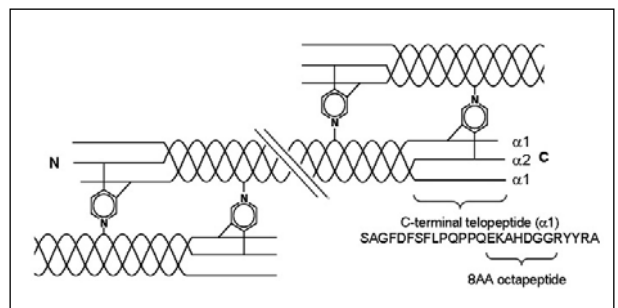
domain with terminal untwisted strands known as procollagen 1. Under the influence of procollagen peptidase short peptides are cleaved off from the terminal strands of procollagen 1, leaving tropocollagen. Depending on the terminus these peptides come from they are called Collagen 1 N-terminal propeptide (P1NP) and Collagen 1 C-terminal propeptide (P1CP). These peptides are only produced during collagen 1 synthesis, making them definitive bone formation markers (7, 8). Tropocollagen still includes the triple-helical domain with shortened terminal untwisted strands named telopeptides. These tropocollagen units arrange themselves in a side by side manner allowing their side chains – in particular lysine, prolin, hydroxy-lysine and hydroxy-prolin – to overlap. Now enzymes induce an oxidative condensation reaction between overlapping



**Figure 10** Pyridinolines.



**Figure 11** Ehrlich's chromogens.



**Figure 12** 8AA octapeptide.

side chains leading eventually to a covalently cross linked polymeric fibrous structure – collagen 1.

In the ready made collagen 1 the tropocollagen units are polymerized by cross links of a heterogeneous nature, most of which are derived from pyridine or pyrrol.

Osteoclastic activity includes the release of proteases which are capable of destroying the collagen 1 structure. A variety of decomposition products is formed and released into the blood, where they are further broken down and finally excreted in urine. Amongst these products are the pyridine (pyridinolin, desoxypyridinolin) and pyrrol (Ehrlich's chromogens) derivatives that are part of the structures cross linking the tropocollagen units. These low molecular substances are readily eliminated in urine. In particular pyridinolin (PYR) and des-

oxypyridinolin (DPD), also known as Crosslinks, are well known markers of bone resorption.

Another group of collagen decomposition products has gained attention over the last years: Fragments with telopeptides including specific epitopes, as there are beta-collagen 1 C-terminal cross linked telopeptides (beta-CTx), beta-Crosslaps, collagen 1 N-terminal cross linked telopeptides (NTx), and C terminal telopeptide of type 1 collagen (ITCP).

Beta-CTx and beta-Crosslaps assays recognize fragments of collagen 1 that have the beta-isomerized 8AA-octapeptide (EKAHD-beta-GGR) which builds an epitope located on C-terminal telopeptides. Often the terms beta-CTx and beta-Crosslaps are used synonymously. However, there is a small, test format dependent difference: Crosslaps comprises fragments that contain at least one 8AA peptide 6,7; beta-CTx comprises fragments that contain at least two 8AA-peptides. The terms Crosslinks and Crosslaps are often confused: Crosslinks is not the same as Crosslaps, both are very different analytes.

Osteocalcin is possibly released during resorption and formation and should therefore be considered a turnover marker.

Biochemical bone markers do not and cannot substitute the well known imaging methods such as DXA and CT, which are today widely used for measuring bone mineral density (BMD); rather they add an additional dimension to the diagnostic repertoire. While imaging methods are ideal to determine the current status of bone, they are very sluggish to indicate changes of it. It may take several years until changes of BMD exceed the noise of the method. Biochemical bone markers, on the other hand, are not very suitable to estimate the current BMD, but they indicate how this will change in future. They reflect readily, within weeks, changes of the metabolic situation of bone as they may occur in response to a therapy or in consequence of pathological processes.

An important indication for the determination of bone markers is the management of osteoporosis patients. This multistage process can start with a predisposition analysis, followed by risk assessment, diagnosis, and monitoring. Predisposition analysis uses tests to find alterations of genes known to be related to bone health, such as the gene coding for the vitamin D receptor. Obviously, this is not the field of biochemical bone markers.

However, for assessment of the risk to develop osteoporosis, there is strong evidence that bone markers give valuable information. As a result of the OFELY-study, Delmas reports that the loss of BMD in arm bones was significantly higher in those women, who at the beginning of the study presented with high bone resorption markers (9). Although this is not yet a common use of bone markers, algorithms as the one shown in Figure 13 were already suggested, where imaging data are supplemented in an ideal way by biochemical markers. A specialist in the field has once

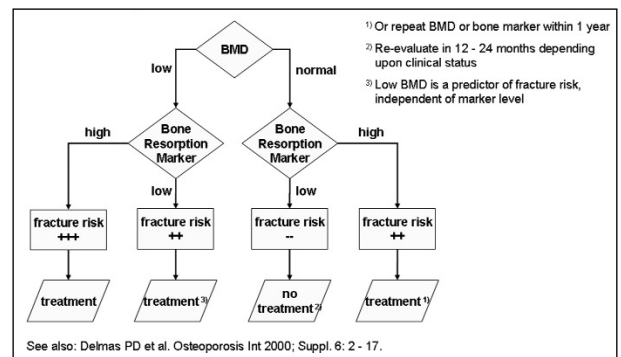


Figure 13 Possible algorithm.

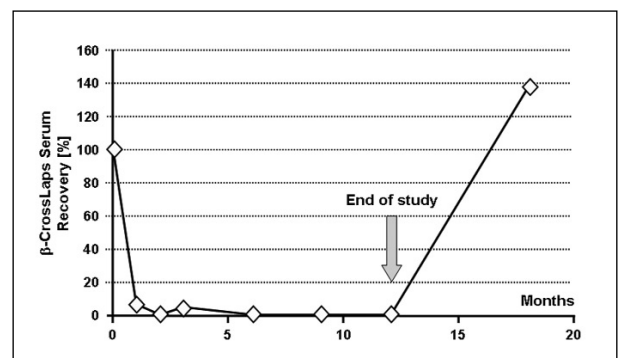


Figure 14 CTx under effective bisphosphonate therapy.

expressed the opinion that: «...when bone density is not quite low and you are not sure whether the patient should receive therapy, bone markers will help you to make the decision...».

The diagnosis of osteoporosis, meaning the measurement of the actual bone density and the collection of morphological data, is and will also in future be the domain of imaging methods.

The field where bone markers really show their potential is monitoring of patients, possibly under therapy (10–13), (14–16). Already 1–2 months after a change in therapy the bone markers clearly indicate whether a patient responds or not. Antiresorptive therapy with hormones or bisphosphonates will reduce the osteoclastic activity and hence lead to a drop in resorption markers. Due to the synchronization of osteoclasts and osteoblasts, formation and turn over markers will also decrease (12, 17). Anabolic therapies may have different patterns. Delmas has shown for CTx that under antiresorptive therapy a decrease of more than 35% has to be achieved to be significant (17), a value that is usually reached easily. If patient compliance is poor or drug resistance is developing, bone markers will instantly increase giving a clear signal to the clinician. Another bone specialist once said: «...There are a lot of patients who do not respond to medication and you risk to put them on medication for a whole year unless you monitor them with bone markers...».

Urine markers were valuable tools before the advent of serum markers. Today, most bone markers can be determined in blood, reducing the biological variance due to the sample material enormously. CTx and Crosslaps have a circadian rhythm if the patient is not fasting (18–21) (19–22).

Although modern bone markers today are widely used as routine diagnostics, their potential is by far not yet exploited. Especially the joint assessment of formation and resorption markers may help to answer questions that are still pending. Other indications that appear worthwhile to be investigated are for instance tumor diseases and arthritis of different etiologies.

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