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Review article

BONE MARKERS AND OSTEOPOROSIS

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Summary: Diagnosis of a given disease is often the first step to a successful therapy. The use of biochemical markers of bone turnover in osteoporosis is becoming more important due to their capacity to give early information. Many of the new markers are proteins, peptides, or other large biomolecules, usually present at very low concentrations. Bone is a living, growing tissue that turns over at a rate of about 10% a year. It is lergely made up of collagen, that gives the bone its tensile strength and framework, and calcium phosphate, mineralized complex that hardens the framework. After age 24, bone resorption slowly begins to happen faster than bone formation. Bone loss is most rapid in women in the first few year after menopause but continues into the postmenopausal years. Loss although much slowly, also happens in men. In addition to bone porosity, the bone strength is determined by the trabecular microstructure in wich osteoclastic, and osteoblastic activities play an important role. Osteoporosis develops when bone resorption occurs too rapidly and bone formation fails to keep up. Risk factors for osteoporosis involves age, gender, ethnicity, use of certain drugs, excersize, smoking Vit D deficiency, Ca intake, sex hormones, alcohol intake etc. Mineralization markers are serum osteocalcin, bone alkaline phosphatase, serum prokollagen I extention peptides. Markers for the resorption of bone on the other hand are urine N-telopeptide crosslinks, urine deoxy-piridinoline, urine hydroxyproline, tartarate dependent acid phosphatase and Catepsin K. Biochemical markers of bone turnover should be used with BMD for diagnosis.

Key words: bone, osteoporosis, bone markers, calcium and osteoporosis

Introduction

Diagnosis of a given disease is often the first step to a successful therapy. Many diseases manifest themselves not only at the phenotypic but also at the biochemical level. Clinical analysis helps in the diagnosis of disease and the monitoring of therapy by following biochemical parameters such as the activity of certain blood enzymes or the presence of certain cell types. With the development of new technologies and the completion of major gene sequencing projects, biomarker research has entered into a new phase. In particular, the rapid profiling of complex biological samples such as serum or urine for the discovery of novel peptide or protein markers of disease has led to an explosion of data that need to be analysed and turned into knowledge. Biological samples often contain up to 30,000 different proteins. Many of the new markers are proteins, peptides, or other large biomolecules, usually present at very low concentrations. In the past, the search for biomarkers has mainly relied on traditional proteomic approaches, which have proven mostly futile, especially for biomarkers that are serum based. To find specific proteomic patterns that can distinguish healthy from diseased patients highly flexible, high-throughput antibody microarray platform are being developed.

Bone

Bone is a living, growing tissue that turns over at a rate of about 10% a year. It is largely made up of collagen, that gives the bone its tensile strength and framework, and calcium phosphate, mineralized complex that hardens the framework. This combination of collagen and calcium makes bone strong and yet flexible enough to bear weight and to withstand stres (1). More than 99% of the body's calcium is contained in the bones and teeth. The remaining 1% is found in the blood. Throughout ones lifetime, old bone is constantly being removed (resorption) and replaced by new bone (formation). During early childhood and in the teenage years, new bone is added faster than old

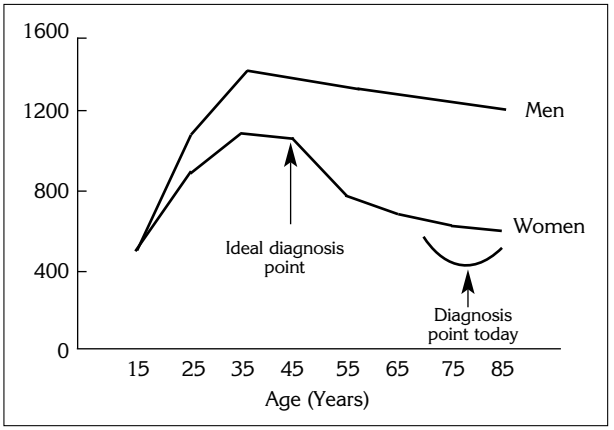

Figure 1. Age related changes in bone mass

bone is removed. As a result, bones become larger, heavier, and denser. Bone formation happens faster than bone resorption until you reach your peak bone mass (maximum bone density and strength), around age 24. After age 24, bone resorption slowly begins to happen faster than bone formation. Bone loss is most rapid in women in the first few years after menopause but continues into the postmenopausal years. Loss although much slowly, also happens in men (2).

The major abnormality in most cases of osteoporosis involves increased bone porosity in the trabecular bone (larger marrow spaces) and thinning of cortical bone. These decreases are modulated by the bone remodelling sequence. The bone loss will depend on the number of bone remodelling units called BMU's (Basic Multicellular Units or Bone Metabolic Units) and the amount of bone lost in each unit. The number of units depends upon the rate of origination of new BMU's and on their life-span; the amount of bone lost in each unit depends on the osteoblastic and osteoclastic activities. If each individual bone remodelling unit loses a small amount of bone, than an increase in number of units will result in increased bone loss. This situation is frequently referred to as »high bone turnover«, an ambiguous term. It is more specific to say »high bone formation rate with even higher bone resorption rate.« Bone loss can also be seen when bone formation rates are low, even though bone resorption rates are normal (3).

Factors that affect BMU origination rates

Origination is affected by mechanical loading, microdamage, or factors in the marrow. A host of hormones and cytokines which have attracted the attention of molecular biologists probably exert most of their influence at this step. These include PTH, 1,25 $(OH)_2$ vitamin D, interleukin 6 and 11, estrogens, and prostaglandins and growth factors. Some of these depend on estrogen, which is why menopause is associated with an increase in origination of BMUs.

Factors that affect osteoclastic activity

The osteoclastic activity can be enhanced by estrogen deficiency, acidosis, corticosteroids and vitamin D metabolites. Osteoclastic activity is reduced by calcitonin and bisphosphonates.

Factors that affect osteoblastic activity

With aging, the osteoblasts do not completely fill the resorbed spaces; this is probably caused by insufficient numbers of osteoblasts. Osteoblastic activity can be increased by growth factors such as TGF β, and depressed by corticosteroids, bilirubin and toxins such as aluminum (4).

Factors that affect mineralization

Once the osteoblasts have deposited a collagen matrix, it must be mineralized. This activity is also controlled by the osteoblasts. If mineralization is inhibited, patients will develop osteomalacia, which can mimic osteoporosis. This is seen with inadequate levels of calcium or phosphate, or with toxins, and is discussed later in this chapter. Treatment with bisphosphonates can result in increased mineralization (hardening of the bone). This will result in increased bone density even though the bone is still porous (5).

Bone structure and strength

In addition to bone porosity, the bone strength is determined by the trabecular microstructure. Perforations of individual trabecula occur when resorption cavities are too deep. This, too, is seen with estrogen deficiency. The remaining trabecula are not as well connected and are mechanically weaker. Microfracture healing is another aspect of bone strength that is not measured by bone density. Trabecula inside the bone may fracture and microcalluses are formed that resemble the calluses seen on x-rays of long bones after a »macro-fracture«. Osteoporotic bone is more susceptible to these fractures because the individual trabecula do not have as many reinforcing connections. The calluses may represent a method of repairing the bone and even connecting some of the trabecula. Bone which has lost the ability to form these calluses will be weaker. The age of the bone mineral crystals may also play a role in the strength of bone. This is an area that needs further research. Studies suggest that older bone is more brittle, and that one purpose of bone remodelling is to remove the old bone and replace it with newer, more elastic bone (5).

Osteoporosis

Osteoporosis, or porous bone, is a disease characterized by low bone mass and structural deteriora-

tion of bone tissue (6). This means patients may be more likely to get fractures of the hip, spine, and wrist. Osteoporosis develops when bone resorption occurs too rapidly and bone formation fails to keep up. It is more likely to develop if bones do not achieve their optimal mass during bone-building years. Men as well as women suffer from osteoporosis, a disease that can be prevented and treated. Men as well as women suffer from osteoporosis, a disease that can be prevented and treated. Osteoporosis is a major public health threat for 28 million Americans, 80% of whom are women. In the U.S. today, 10 million individuals already have osteoporosis and 18 million more have low bone mass, placing them at increased risk for this disease.

One out of every two women and one in eight men over 50 will have an osteoporosis-related fracture in their lifetime. More than 2 million American men suffer from osteoporosis, and millions more are at risk. Each year, 80,000 men suffer a hip fracture and one-third of these men die within a year. Osteoporosis can strike at any age. Osteoporosis is responsible for more than 1.5 million fractures annually, including 300,000 hip fractures, and approximately 700,000 vertebral fractures, 250,000 wrist fractures, and more than 300,000 fractures at other sites. Estimated national direct expenditures (hospitals and nursing homes) for osteoporosis and related fractures is $14 billion each year.

Physical stress increases bone mass, but immobilization leads to bone loss. Obesity is associated with higher bone mass. Insufficient dietary intake of calcium, phosphorus, and vitamin D are associated with age-related bone loss. Late menarche and early menopause, alcohol use, and cigarette smoking may decrease bone mass. Blacks and Hispanics have > bone mass than whites and Asians, and men have > bone mass than women. Osteoporosis occurs in postmenopausal women (and men with testosterone deficiency) and is related to the loss of gonadal function. Estrogen loss leads to elevated levels of IL-6 which may stimulate osteoclast precursors in trabecular bone and increase bone resorption. Osteoporosis is also associated with normal aging and a progressive decline in osteoblasts. Fractures of cortical bone are more common. Genetic factors play an important role in the development of osteoporosis. Family history of fractures in postmenopausal women is a predictor of osteoporosis. Correlation between abnormal receptors for vitamin D and osteoporosis in multiple generations. May be other genetic abnormalities that explain the expression of an osteoporosis phenotype.

Risk Factors

Certain factors are linked to the development of osteoporosis or contribute to an individual's likelihood of developing the disease. These are called »risk factors«. Many people with osteoporosis have several of these risk factors, but others who develop osteoporosis have no identified risk factors. There are some risk factors that you cannot change, and others that you can (6).

Risk factors you cannot change

Gender – Your chances of developing osteoporosis are greater if you are a woman. Women have less bone tissue and lose bone more rapidly than men because of the changes involved in menopause.

Age the older you are, the greater your risk of osteoporosis. Your bones become less dense and weaker as you age.

Ethnicity – Caucasian and Asian women are at highest risk. African-American and Latino women have a lower but significant risk.

Family history – Susceptibility to fracture may be, in part, hereditary. People whose parents have a history of fractures also seem to have reduced bone mass and may be at risk for fractures.

Risk factors you can change

Sex hormones: abnormal absence of menstrual periods (amenorrhea), low estrogen level (menopause), and low testosterone level in men.

Anorexia.

A lifetime diet low in calcium and vitamin D.

Use of certain medications, such as glucocorticoids or some anticonvulsants.

An inactive lifestyle or extended bed rest.

Cigarette smoking.

Excessive use of alcohol.

To reach optimal peak bone mass and continue building new bone tissue as you get older, there are several factors you should consider (7).

Calcium. An inadequate supply of calcium over the lifetime is thought to play a significant role in contributing to the development of osteoporosis. Many published studies show that low calcium intakes appear to be associated with low bone mass, rapid bone loss, and high fracture rates. National nutrition surveys have shown that many people consume less than half the amount of calcium recommended to build and maintain healthy bones. Good sources of calcium include low fat dairy products, such as milk, yogurt, cheese and ice cream; dark green, leafy vegetables, such as broccoli, collard greens, bok choy and spinach; sardines and salmon with bones; tofu; almonds; and foods fortified with calcium, such as orange juice, cereals and breads. Depending upon how much calcium you get each day from food, you may need to take a calcium supplement.

Calcium needs change during one's lifetime. The body's demand for calcium is greater during child-

Table I Daily calcium intakes as mg/day recomended by National Academy of Sciences (1997) and National Institutes of Health

National Academy of Sciences (1997)		National Institutes of Health (1994)	
Ages			
Birth – 6 months	210	Birth-6 months	400
6 months – 1 year	270	6 months – 1 year	600
1–3	500	1–10	800–1200
4–8	800	11–24	1200–1500
9–13	1300	25–50 (women & men)	1000
14–18	1300	51–64 (women on ERT & men)	1000
19–30	1000	51+ (women not on ERT)	1500
31–50	1000	65 or older	1500
51–70	1200		
70 or older	1200		
Pregnant or lactating	1000	Pregnant or lactating	1200–1500
14–18	1300		
19–50			

hood and adolescence, when the skeleton is growing rapidly, and during pregnancy and breastfeeding. Postmenopausal women and older men also need to consume more calcium. This may be caused by inadequate amounts of vitamin D, which is necessary for intestinal absorption of calcium. Also, as you age, your body becomes less efficient at absorbing calcium and other nutrients. Older adults also are more likely to have chronic medical problems and to use medications that may impair calcium absorption.

Vitamin D. Vitamin D plays an important role in calcium absorption and in bone health. It is synthesized in the skin through exposure to sunlight. While many people are able to obtain enough vitamin D naturally, studies show that vitamin D production decreases in the elderly, in people who are housebound, and during the winter. These individuals may require vitamin D supplementation to ensure a daily intake of between 400 to 800 IU of vitamin D. Massive doses are not recommended.

Exercise. Like muscle, bone is living tissue that responds to exercise by becoming stronger. The best exercise for your bones is weight-bearing exercise, that forces you to work against gravity. These exercises include walking, hiking, jogging, stair-climbing, weight training, tennis, and dancing.

Smoking. Smoking is bad for your bones as well as for your heart and lungs. Women who smoke have lower levels of estrogen compared to nonsmokers and frequently go through menopause earlier. Postmenopausal women who smoke may require higher doses of hormone replacement therapy and may have more side effects. Smokers also may absorb less calcium from their diets.

Alcohol. Regular consumption of 2 to 3 ounces a day of alcohol may be damaging to the skeleton, even in young women and men. Those who drink heavily are more prone to bone loss and fractures, both because of poor nutrition as well as increased risk of falling.

Medications that cause bone loss. The long-term use of glucocorticoids (medications prescribed for a wide range of diseases, including arthritis, asthma, Crohn's disease, lupus, and other diseases of the lungs, kidneys, and liver) can lead to a loss of bone density and fractures. Other forms of drug therapy that can cause bone loss include long-term treatment with certain antiseizure drugs, such as phenytoin (Dilantin©) and barbiturates; gonadotropin releasing hormone (GnRH) analogs used to treat endometriosis; excessive use of aluminum-containing antacids; certain cancer treatments; and excessive thyroid hormone. It is important to discuss the use of these drugs with your physician, and not to stop or alter your medication dose on your own.

Osteoporosis is often called the »silent disease« because bone loss occurs without symptoms. People may not know that they have osteoporosis until their bones become so weak that a sudden strain, bump, or fall causes a hip fracture or a vertebra to collapse. Collapsed vertebra may initially be felt or seen in the form of severe back pain, loss of height, or spinal deformities such as kyphosis, or severely stooped posture (8).

Detection. Following a comprehensive medical assessment, your doctor may recommend that you have your bone mass measured. Bone mineral density (BMD) tests measure bone density in the spine, wrist, and/or hip (the most common sites of fractures due to osteoporosis), while others measure bone in the heel or hand. These tests are painless, noninvasive, and safe.

Bone Markers

Mineralization markers are serum osteocalcin, bone alkaline phosphatase and serum prokollogen I extention peptides. Markers for the resorption of bone on the other hand are urine N telopeptide crosslinks, urine deoxy-piridinoline, urine hydroxyproline, tartarate dependent acid phosphatase and catepsin K. Biochemical markers of bone turnovershould be used with BMD for diagnosis (9, 10). They measure formation and resorption of boneand can be used for moniterization of drug therapy. Biochemical markers also measure the complience and response of the patient giving early information about the status of the patient (11).

Recently, new specific markers of bone turnover with increased specificity and sensitivity were developed. These include:

a) assays for bone formation as serum bone specific alkaline phosphatase, oseocalcine and carboxy-terminal propeptide of type I collagen (the specific collagen type of bone).

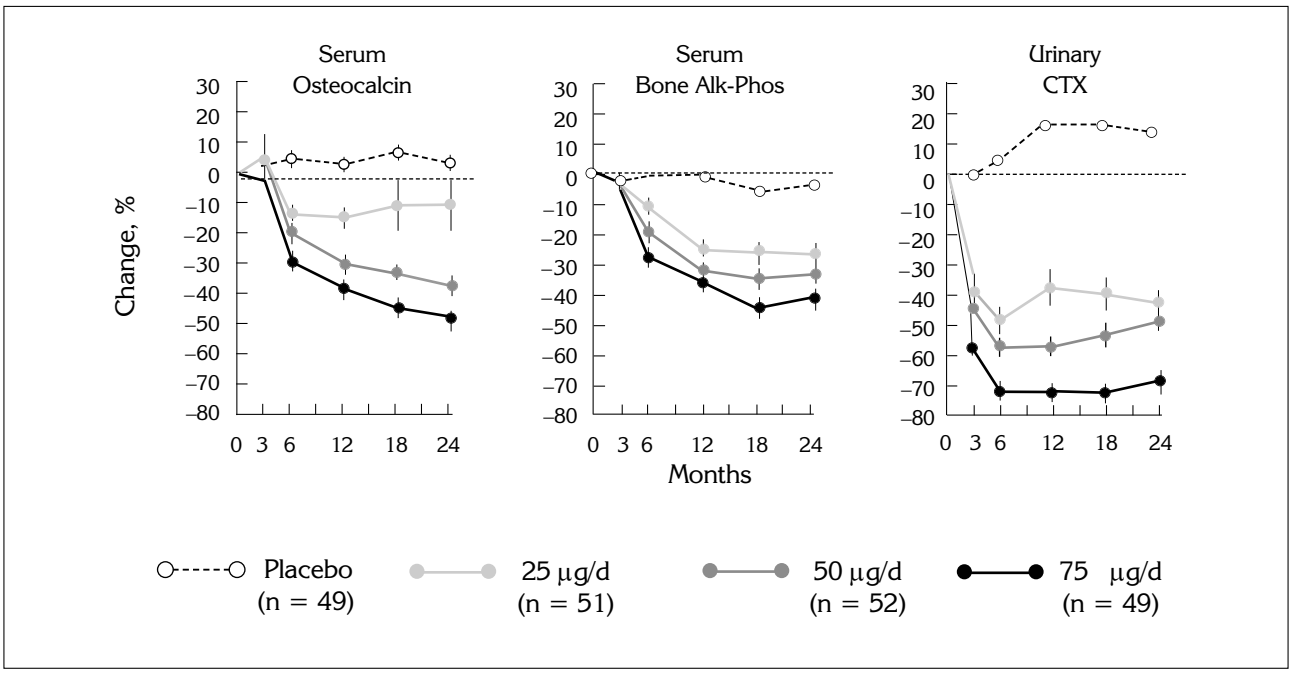

Figure 2. The use of biochemical markers of bone turnover in osteoporosis. Bisphospohonate treatment

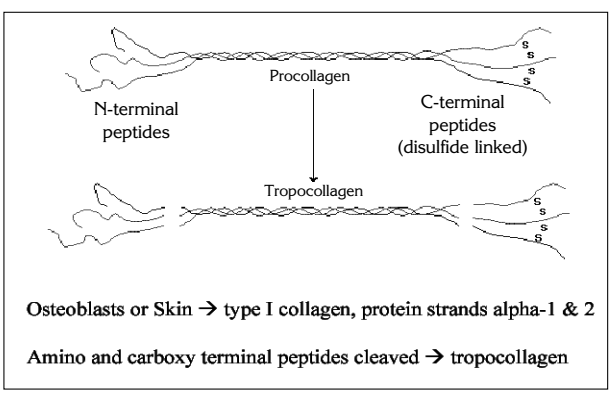

Figure 3. Procollagen and tropocollagen conversion. The peptides removed are shown

b) assays of bone resoprtion that mainly measure the urinary excretion of cross-links of the extracellular matrix of bone, both as free amino acids and the peptide bound from as: pyridinolline, free pyridinoline, deoxypyridinoline, collagen type I cross-linked N-telopeptide and c-telopeptide as well as serum levels of the type I cross-linked c-telopeptide. (12)

Changes in most biochemical markers reflect modulation of bone turnover in various metabolic skeletal diseases including postmenopausal osteoporosis. Most markers discriminate well between normal women and patients with postmenopausal osteoporosis. However, the long-term variability, over periods of months, ranges between 15 to almost 30 percent for the various markers.

Although biochemical markers cannot quantify bone mass, it predicts the rate of bone loss, especially if markers of bone resorption and formation are being used together. There is emerging evidence that increased rates of bone turnover as measured by the biochemical markers can predict fracture risk independent of bone density. There are observations that show an increase response to antiresorptive treatment in patients with higher turnover rates. Treatment with bisphosphonates caused a marked decrease in the biochemical parameters of bone turnover with resorption markers decreasing eartier than formation markers. The decrease in bone turnover was associated with an increase in bone mineral density. A highly significant negative correlation was found between changes in bone markers at 3 months and percent changes in bone mineral density after 24 months of treatment (13).

Issues to be discussed: the usefulness of biochemical markers, in practice, for the selection of patients to be treated, to monitor efficacy and predict response to treatment with antiresorptive drugs.

Recent technological developments have heightened the interest in biochemical markers of bone metabolism, with particular focus on bone resorption markers. Assays for bone resorption provide clinical utility by establishing a patient's level of bone resorption and monitoring effect of antiresorptive therapy. It is important, however, that the test be specific to bone resorption and practical to run.

Published clinical studies have demonstrated that NTx provides the most responsive and specific indicator of the bone resorption process of all currently known markers (7–9). These include the collagen degradation products, pyridinoline and deoxypyridinoline, measured in total by high performance liquid chromatography or as the free amino acids by immunoassay.

The Analytes

Cross-linked N-telopeptides (OSTEOMARK NTx) OSTEOMARK measures cross-linked N-telopeptides (NTx). NTx is generated during osteoclastic activity on bone collagen. (4, 5) NTx is specific to type I collagen, which forms 90% of the organic matrix of bone. The unique feature of NTx recognized by the OSTEOMARK monoclonal antibody is the collagen alpha-2 chain N-telopeptide, a favored site of cross-linking in bone collagen.Osteoclasts (cells that resorb bone) liberate NTx. Because other type I collagen sources such as skin are not actively degraded by osteoclasts, their breakdown does not contribute to the urinary pool of NTx. This is confirmed by the marked responsiveness of OSTEOMARK to the effects of bone specific antiresorptive therapies (2, 5, 6).

Total deoxypyridinoline measured by high performance liquid chromatography (HPLC) measures both the peptide bound and free fractions of deoxypyridinoline.

Total deoxypyridinoline and pyridinoline are two forms of matur cross-linking amino acids found in the collagen of many tissues. Deoxypyridinoline is the less abundant structure, but in bone the ratio of deoxypyridinoline to pyridinoline is uniquely high. Deoxypyridinoline is also present in ligament, vascular tissue, and muscle (10–12). Although bone appears to be a primary source of the deoxypyridinoline excreted in urine, other tissues must contribute. The relative contribution from these tissues is yet to be quantified. About 60% of the deoxypyridinoline in urine is in the form of small peptides and 40% as free amino acids. Total deoxypyridinoline can be measured in urine by HPLC after acid hydrolysis, a procedure inconvenient for routine clinical use.

Free deoxypyridinoline (Pyrilinks®-D) measures only the free fraction of deoxypyridinoline. While it is unclear which tissue site is responsible for generating free deoxypyridinoline from collagen degradation products in the body. Osteoclasts do not appear to be responsible (9). Metabolism in the liver and kidney is likely. A lack of suppression in urinary levels of free deoxypyridinoline in response to bisphosphonate therapy provides clinical confirmation that osteoclasts do not generate free deoxypyridinoline (9). Bisphosphonates specifically target activeosteoclasts, inhibiting bone resorption. The measurement of free deoxypyridinoline in urine can be performed by HPLC or immunoassay.

It is very important to differentiate between the measurements of total and free deoxypyridinoline, as the two are not equally responsive to antiresorptive therapy. Clinical studies on the effects of bisphosphonate therapy to demonstrate the impact of antiresorptive therapy on bone, a bone resorption marker should show a significant suppression from baseline measurements. This decrease must be greater than the normal variability in the excretion of the analyte to be useful in monitoring individual patients.

In recent studies, Garnero (9) et. al. compared the response of NT (OSTEOMARK), total deoxypyridinoline (HPLC), and free deoxypyridinoline (Pyrilinks©-D) to the bisphosphonate pamidronate. Pamidronate was administered intravenously for three days to patients with established osteoporosis and patients with Paget's disease of bone. A significant decrease as a result of therapy was seen for NTx and total deoxypyridinoline, but not for free deoxypyridinoline.

Because bisphosphonates are highly selective in suppressing osteoclastic resorption of bone, these studies confirm the high specificity of urinary NTx, the lower specificity of total deoxypyridinoline, and the lack of specificity of free deoxypyridinoline to bone resorption (14,15).

Variability in Measuring Bone Markers

Signal-to-noise ratio becomes an importatnt concept in measuring bone markers. Least significant change (LSC) is defined because 30–171% increases are observed for collagen crosslinks in postmenopause vs premenopause and 20–80 % decrease

Table II Good laboratory practise in using bone markers for diagnosis and monitorisation of osteoporosis

Recommendations
Never rely on the result of a single test
When ordering serial testing, be certain to order every test from the same laboratory using the same assay kit
Be certain that the disease marker selected for monitoring recurrence was elevated in the patient prior to surgery
Consider the half-life of the disease marker when interpreting the test result
Consider how the disease marker is removed or metabolized from the blood circulation
Consider ordering multiple markers to improve both the sensitivity and the specificity for diagnosis
Order the nonspecific markers only for cost-saving and for their high sensitivity
Be aware of the possibility of a hook effect
Be aware of the presence of ectopic tissue markers

on antiresorptive therapy at 3–6 months. LSC at 90% confidence is clinically appropiate. The question then is can serum assay be an improvement (16).

One has to keep in mind that while with BMD 2 years are necessary to see LSC bone markers give results in 3–6 months even tough the variation is high.

Sources of biological variations for bone markers are menapause, seasonal changes (as a result of secondary hyperparathyroidsm due to lack of vit. D), circadien rythm (highest 2–8 a.m. lowest 01–11 p.m.) (15). Factors such as age, gender, diet, etc. also contribute to variations between individuals and populations. Another source of biological variation for bone marker evaluation is the variation of urine creatinine during the day by 20%. Therefore baseline and follow-up tests are neede to draw correct conclusions. Changes >30% are significant althuogh ther are large overlap in normal and osteoporotic women. Because of biological variation difference two measurements have to vary e.g. 54% for urine CTx and 108% for Gal-OH-Lys to be significant (17–19). Serum markers exhibit lower long-term variability than most urine markers.

In summary, biochemical markers of bone turnover, particular markers reflecting processes related to bone resorption, appear to be helpful in the non-invasive assessment of bone involvement in patients with osteoporosis and related disease. With regard to the diagnosis, the spontaneous follow-up, the therapeutic monitoring and the prognosis of patients with osteoporosis, biochemical markers of bone turnover seem to provide useful additional information to estab-

KOŠTANI MARKERI I OSTEOPORPOZA

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Kratak sadržaj: Dijagnoza datog oboljenja je često prvi stupanj za uspešnu terapiju. Primena biohemijskih markera koštanog prometa u osteoporozi postaje sve važnija zbog toga što mogu da pruže ranu informaciju. Novi markeri su proteini, peptidi ili druge biomolekule, koji se obično nalaze u niskim koncentracijama. Kost je živo tkivo koje raste i ima promet od oko 10% godišnje. Uglavnom se sastoji iz kolagena, koji kostima daje čvrstinu i oblik, i kalcijum fosfata koji kao mineralna komponenta daje kostima težinu. Posle 24 godine života, resorpcija kosti se održava brže od samog formiranja kosti. Gubitak kosti je mnogo brži kod žena u prvim godinama nakon menopauze i nastavlja se u periodu posmenopauze. Gubitak kosti se odvija i kod muškaraca, mada mnogo sporije. U vezi sa poroznošću kostiju, jačina kosti je određena trabekularnom mikrostrukturom u kojoj osteoklastna i ostoblastna aktivnost imaju veoma značajnu ulogu. Osteoporoza se razvija kad dođe do resorpcije kosti i kad se prevaziđe proces formiranja kosti. Faktori rizika za osteoporozu su godine, pol, etničko poreklo, primena nekih lekova, vežbanje, pušenje, deficit vitamina D, unošenje Ca, polni hormoni, unošenje alkohola itd. Markeri mineralizacije su serumski osteokalcin, koštana alkalna fosfataza, serumski prokolagen I itd. Markeri resorpcije kosti su N-telopeptid, deoksi-piridinolin i hidroksiprolin u urinu, tartarat zavisna kisela fosfataza i katepsin K. Biohemijske markere koštanog prometa treba koristiti sa BMD za postavljanje dijagnoze osteoporoze.

Ključne reči: kost, osteoporoza, koštani markeri, kalcijum i osteoporoza

lished invasive and imaging techniques.

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