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Biokemijski biljezi pregradnje kostiju – pregled

Biochemical markers of bone remodeling – review

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Sažetak

Danas je u upotrebi niz biokemijskih biljega pregradnje kostiju, uključujući biljege izgradnje i razgradnje kostiju. Oni pružaju klinički korisne dokaze normalnih i patoloških procesa, koji odražavaju aktivnosti koštanih stanica u skeletu. Biološki biljezi pregradnje kostiju mogu se koristiti za praćenje učinaka terapije kod bolesnika s nekom od bolesti kostiju te za moguće smanjenje potrebe čestog mjerenja gustoće koštane mase (denzitometrije).

Ključne riječi: biljezi izgradnje kostiju; biljezi razgradnje kostiju; osteoporoza

Abstract

A number of biochemical markers of bone remodeling which include bone formation and bone resorption markers are currently in use. They provide clinically useful evidence of the normal and pathological processes that reflect bone cell activities in the skeleton. Biomarkers of bone remodeling can be used to document the effects of therapeutic agents in some patients with bone diseases and possibly reduce the need for frequent bone density testing.

Key words: bone formation markers; bone resorption markers; osteoporosis

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Uvod

Usporedno s povećanim razumijevanjem biokemijskih procesa u kostima te izolacijom i karakterizacijom staničnih sastojaka skeletnog matriksa, povećava se i broj novih, biokemijskih potencijalnih biljega izgradnje i razgradnje kostiju. Općenito se dijele na:

a) enzimske biljege izgradnje (vezane uz aktivnost osteoblasta) i razgradnje kostiju (vezane uz aktivnost osteoklasta);
b) proteine koštanog matriksa i razgradne produkte organskoga skeletnog matriksa koji se otpuštaju u cirkulaciju za vrijeme izgradnje ili razgradnje kostiju; te
c) biljege anorganskoga koštanog matriksa (kalcij i fosfor, koji prije svega odražavaju homeostazu kalcija/fosfora i koji u ovom pregledu neće biti razmatrani).

Introduction

Parallel with better understanding of biochemical processes in bones and isolation and characterization of cellular components of skeletal matrix, the number of new potential biochemical markers of bone formation and resorption is increasing. Generally, markers are classified into the following groups:

a) enzyme activity markers of bone formation (connected with osteoblast activity) and of bone resorption (connected with osteoclast activity);
b) bone matrix proteins and resorption products of organic skeletal matrix, which are released into circulation during bone formation and resorption; and
c) inorganic skeletal matrix markers (calcium, phosphorus which, above all, reflect calcium-phosphorus homeostasis and which will not be considered in this review).

U tablicama 1. i 2. prikazani su do sada otkriveni biljezi izgradnje i razgradnje kostiju skupine a) i b) od kojih će neki biti podrobnije razmotreni. Opisano je njihovo tkivno podrijetlo, fiziološki uzorak u kojem se mjeri njihova koncentracija/aktivnost te trenutno raspoložive analitičke metode (1,2). U tekstu će se raspravljati o biljezima na čijoj se standardizaciji više radi, odnosno onima koji su u rezultatima dosadašnjih ispitivanja pokazali veću kliničku valjanost, te naglasiti nedostatke nekih, donedavno više korištenih biljega.

Koje je općenito značenje biokemijskih biljega koštane pregradnje?

Poznata temeljna prednost biokemijskih biljega u odnosu na mjerenje mineralnog sadržaja kosti i statičku histomorfometrijsku analizu biopsije kosti jest činjenica da njihovom uporabom dobivamo informaciju o statusu koštane pregradnje. Biokemijski su biljezi uz to i neinvazivna pretraga u usporedbi sa statičkom histomorfometrijskom analizom.

Informacija o dinamičkom stanju metabolizma kostiju mogla bi ranije ukazati na neke patološke promjene u kostima, odnosno na rizik nastanka nekih bolesti kostiju. Mjerenjem koncentracije/aktivnosti biokemijskih biljega moguće je nadalje dobiti bržu informaciju o terapijskom odgovoru u odnosu na mjerenje koštane mase. Značajne promjene biokemijskih biljega mogu se, naime, otkriti već nakon 1-3 mjeseca djelotvorne terapije, dok se promjena koštane mase može odgovarajuće procijeniti tek nakon 1. ili 2. godine (3,4). Potrebu za pronalaženjem specifičnih biokemijskih biljega naglašava i primjena novih, vrlo

Tables 1 and 2 show bone formation and resorption markers discovered so far for groups a) and b), some of which will be further discussed. Tables 1 and 2 also list the tissue origin of markers, their physiological sample which is measured for their concentration/activity, and currently available analytical methods (1,2). In this review we shall discuss the markers whose standardization is currently underway and which, according to past test results, have shown considerable clinical validity. We will also point out disadvantages of some of hitherto frequently used markers.

What is the general significance of biochemical markers of bone remodeling?

The familiar basic advantage of biochemical markers, compared with bone mineral densitometry and static histomorphometric analysis of bone biopsy, is that by using them we get the information about the status of bone remodeling. In addition, biochemical markers are non-invasive with regard to dynamic histomorphometric analysis of bone biopsy.

Information on bone remodeling status could be an early indicator of some pathological changes in bones or the risk of some bone disease. By measuring concentration/activity of biochemical markers, it is possible to gain information about therapeutic response faster than by measuring bone mass. Significant changes in biochemical markers can be discovered already after 1 to 3 months of effective therapy while bone mass changes can be adequately evaluated only after the 1st or rather the 2nd year (3,4). The need for specific biochemical markers is

Tablica 1. Biljezi izgradnje kostiju

Table 1. Bone formation markers

Marker	Tissue origin	Analytical sample	Analytical method
Total Alkaline Phosphatase (ALP); specific for bone formation only in patients with no liver or bile duct disease	bone, liver	serum	colorimetry
Bone alkaline phospatase (B-ALP); specific osteoblast product; some procedures show cross reactivity with ALP liver isoenzyme	bone	serum	colorimetry, electrophoresis, precipitation, IRMA, EIA
Osteocalcin (OC, BGP); specific osteoblast product; there are several reactive forms in blood; some can NASTATI during bone resorption	bone, trombocytes	serum	RIA, ELISA, IRMA, ECLIA
C-terminal propeptide of type I procollagen (PICP); specific proliferating osteoblast and fibroblast product	bone, skin, soft tissues	serum	RIA, ELISA
N-terminal propeptide of type I procollagen (PINP); specific proliferating osteoblast and fibroblast product; partially incorporated into skeletal matrix	bone, skin	serum	RIA, ELISA

IRMA – immunoradiometric assay; EIA – enzyme immunoassay; RIA – radio immuno assay; ELISA – enzyme-linked immunosorbent assay; ECLIA – electrochemiluminiscence immunoassay

Tablica 2. Biljezi razgradnje kostiju

Table 2. Bone resorption markers

Marker	Tissue origin	Analytical sample	Analytical method
Hydroxyproline, total and dialyzable (OH-Pro, OHP); specific for all fibrilar collagens and a part of collagen proteins, including Ciq and elastin; present in newly synthesized and mature collagen	bone, skin, cartilage, soft tissues	urine	colorimetry, HPLC
Pyridinoline (PYD, Pyr); high concentrations in cartilage and bone collagen: not present in skin; present only in mature collagen	bone, tendon, cartilage	urine	HPLC, ELISA
Deoxypyrindoline (DPD, d-Pyr); high concentrations only in bone collagen: not present in cartilage or in skin; present only in mature collagen	bone, dentine	urine	HPLC, ELISA
Cross-linked C-terminal telopeptide of type I collagen (ICTP); high proportion from bone collagen in type I collagen; can partly originate from newly synthesized collagen	bone, skin	serum	RIA
Cross-linked C-terminal telopeptide of type I collagen (fragments alpha-CTX, beta-CTX); in type I collagen; probably high proportion from bone collagen	all tissue containing type I collagen	urine, serum	ELISA, RIA, ECLIA
Cross-linked N-terminal telopeptide of type I collagen (fragments NTX); in type I collagen; big proportion from bone	all tissue containing type I collagen	urine (alpha/beta), serum (only beta)	ELISA, RIA, ICMA
Hydroxylysine-glycosides (Hyl-Glyc); collagens and collagen proteins; glucogalactosyl- hydroxilysine is highly represented in soft tissue collagens and C1q; galactosil-OHLys is highly represented in bone collagen	bone, skin, soft tissue, serum complement	urine	HPLC, ELISA
Bone sialoprotein (BSP); synthesized by active osteoblasts and lay in extracellular bone matrix; it seems to express osteoclast activity	bone, dentine, hypertrophic catrilage	serum	RIA, ELISA
Tartarat-resistant acid phosphatase (TR-ACP); osteoclasts, thrombocytes, erythrocytes	bone, blood	plasma/serum	colorimetry, RIA, ELISA
Free gamma carboxyglutamin acid (GLA); resulted from bone proteins (e.g. osteocalcin, matrix Gla protein) and from coagulation factor	blood, bone	serum/urine	HPLC

HPLC – high performance liquid chromatography; ELISA – enzyme-linked immunosorbent assay; RIA – radio immuno assay; ECLIA – electrochemiluminiscence immunoassay; ICMA – immunochemiluminometric assay

učinkovitih lijekova, koji snažno djeluju na metabolizam kostiju. Zajedno s mjerenjem koštane mase pokazuju se korisnima i u prognozi bolesti. Nedvojbeno je do sada pokazano da su neki od biljega ili kombinacije biljega, navedeni u tablicama 1. i 2. korisni u populacijskim i epidemiološkim ispitivanjima te praćenju učinka antiresorpcijske terapije. Međutim, s obzirom na neprestani razvoj novih, specifičnijih postupaka za njihovo mjerenje, konačna procjena njihove kliničke korisnosti u obradi bolesnika još je uvijek u tijeku. Preporuke su stoga i nadalje da se maksimalna pažnja posveti pitanju trebaju li se i kako uopće koristiti biljezi, te standardizaciji predanalitičkih i analitičkih postupaka mjerenja koštanih biljega.

also emphasized by the use of new, very efficient drugs which have very strong effect on bone metabolism. Together with bone mass measurements, they are also very useful in disease prognosis. It has been shown so far without doubt that some of the markers or marker combinations presented in tables are useful in population and epidemiological studies, as well as in monitoring of antiresorption therapy effect. However, given the continuous development of new, specific procedures for their measurement, the final assessment of their clinical usefulness in patient management is still underway. Therefore, it is still recommended to pay maximum attention to the issue of whether and how the markers are to be used and to standardization of preanalytical and analytical procedures of bone marker determination.

Na koji način treba procijeniti kliničku valjanost biljega?

Općenito, biokemičari i kliničari trebaju se upitati: *koji* biokemijski biljeg koštane pregradnje mjeriti, *kako* i *kada* ga mjeriti, *kako prikupiti uzorak* i *kako interpretirati dobiveni rezultat*. Važni kriteriji u kritičkoj prosudbi potrebe mjerenja nekog od biljega izgradnje ili razgradnje kostiju su:

- **Biološki čimbenici** (kao što su tkivna specifičnost, učinak promjene funkcije jetre ili bubrega na klirens biljega, biološki ritam biljega zbog standardizacije vremena uzimanja fiziološkog uzorka, imobilizacija i dr.);
- **Predanalitički čimbenici** (način pohranjivanja uzorka, odnosno vrijeme i temperatura, zamrzavanje i odmrzavanje uzorka, utjecaj antikoagulanta i dr.);
- **Analitička specifičnost i točnost** (mikroheterogenost biljega, kao npr. stupanj glikozilacije ALP, razgradivost biljega na više različitih fragmenata kao u slučaju osteokalcina, netočnost metoda zbog neusklađenih kalibracija, specifičnost protutijela i npr. inhibitori enzimskih aktivnosti);
- **Dijagnostička valjanost** (odnosno postoje li razlike između biljega s obzirom na njihovu dijagnostičku osjetljivost i specifičnost) (1,5).

U ovom kontekstu, pregled koštanih biljega koji slijedi treba čitatelju omogućiti procjenu moguće dijagnostičke vrijednosti pojedinih biljega koji su prema znanstvenostručnoj literaturi prošli djelomičnu evaluaciju [alkalna fosfataza (engl. *alkaline phosphatase*, ALP); koštana alkalna fosfataze (engl. *bone alkaline phosphatase*, B-ALP), osteokalcin (engl. *osteocalcin*, OC), C-terminalni propeptid prokolagena tipa I (engl. *carboxy-terminal type I procollagen propeptide*, PICP), piridinolin (engl. *pyridinoline*, PYD) i deoksipiridinolin (engl. *deoxypyridinoline*, DPD), C-terminalni telopeptid kolagena (engl. *C-telopeptide of type I collagen*, CTX) i N-terminalni telopeptid kolagena (engl. *N-telopeptide of type I collagen*, NTX)] te nedostataka i ograničenja pojedinih postupaka njihova mjerenja. Neki od gore navedenih čimbenika prosudbe koštanih biljega prikazani su u tablici 3. (1,2).

Biljezi izgradnje kostiju

Biljezi izgradnje kostiju su izravni ili neizravni produkti ili enzimi aktivnih osteoblasta, njihova koncentracija ili aktivnost mjeri se u serumu ili plazmi i općenito imaju umjereno izraženu biološku varijabilnost. Najčešće se mjeri aktivnost ukupne ALP, aktivnost ili masa B-ALP, te koncentracija OC i PICP.

Alkalna fosfataza (ALP)

Precizna fiziološka funkcija ALP još uvijek je nejasna, ali se pretpostavlja njena uloga u stvaranju osteoida i mineralizaciji kostiju. Fiziološki oblici kodirani su s 4 genska loku-

How to evaluate the clinical validity of markers?

Generally, biochemists and clinical specialists should ask themselves: *which* biochemical marker of bone remodeling to measure, *how and when* to measure it, *how to collect the sample and how to interpret the test result*. Important criteria in critical judgement on whether to measure some bone formation or bone resorption markers are the following:

- **Biological factors** (like tissue specificity, effect of change in liver or kidney function on marker clearance, biological rhythm of the marker due to standardization of physiological sampling time, immobilization, etc.):
- **Pre-analytical factors** (sample storage procedures, i.e. time and temperature, sample freezing and thawing, anticoagulant effect, etc.);
- **Analytical specificity and accuracy** (microheterogeneity of markers as, e.g., degree of ALP glycosylation, possibility of marker resorption into several different fragments as in case of OC, *bias* in methods due to non-harmonized calibrations, specificity of antibodies and of, e.g., enzyme activity inhibitors);
- **Diagnostic validity** (or the question of differences between markers considering their diagnostic sensitivity and specificity) (1,5).

In this context, the review of bone markers to follow should enable the reader to assess the possible diagnostic value of certain markers that have already been subjected to partial evaluation in scientific and professional literature [ALP (alkaline phosphatase), B-ALP (bone alkaline phosphatase), OC (osteocalcin), PICP (carboxy-terminal type I procollagen propeptide), PYD (pyridinoline), DPD (deoxypyridinoline), CTX (C-telopeptide of type I collagen) and NTX (N-telopeptide of type I collagen)], and to evaluate disadvantages and limitations of procedures applied to measure them. Some of the factors for judgement of bone markers listed above are shown in Table 3 (1,2).

Bone formation markers

Bone formation markers are direct or indirect products or enzymes of active osteoblasts, their concentration or activity is measured in serum or plasma, and generally they are characterized by moderately expressed biological variability. They most often include the measurement of total ALP activity, B-ALP activity or mass, and OC and procollagen type I propeptide concentrations.

Alkaline phosphatase (ALP)

Precise physiological function of ALP is still unclear, but it is assumed to play a role in osteoid formation and bone

Tablica 3. Predanalitičke i biološke značajke koštanih biljega

Table 3. Preanalytical and biological characteristics of bone markers

Marker	Sample stability	Influenced by	Diurnal rhythm
ALP	stable < -20 °C	liver function	not significant
B-ALP	stable < -20 °C	liver function	not significant
OC	non stable < -80 °C	kidney function	significant
PICP	stable < -20 °C	liver function	significant
PINP	stable < -20 °C	liver function	significant
OHP	stable < -20 °C	liver function nutrition/diet inflammation	significant
PYD	stable < -20 °C	liver function active arthritis	significant
DPD	stable < -20 °C	liver function	significant
ICTP	stable < -20 °C	liver function kidney function	significant
CTX	stable < -20 °C	liver function kidney function	significant
Hyl-Glyc	stable < -20 °C	liver function	significant
BSP	stable < -20 °C	liver function kidney function	significant
TR-ACP	non stable < -80 °C	hemolysis	not significant
GLA	?	nutrition (K vitamin) coagulation	not significant

ALP - Alkaline Phosphatase; B-ALP - Bone Alkaline Phosphatase; OC - Osteocalcin; PICP - C-terminal type I procollagen propeptide; PINP - N-terminal type I procollagen propeptide; OHP - Hydroxyproline; PYD - Pyridinoline; DPD - Deoxypyrindoline; ICTP - Cross-linked C-terminal telopeptide of type I collagen; CTX - Cross-linked C-terminal telopeptide of type I collagen; Hyl-Glyc - Hydroxylysine-glycosides; BSP - Bone sialoprotein; TR-ACP - Tartarat-resistant acid phosphatase; GLA - Free gamma carboxyglutamin acid

sa, tri tkivno specifična i jedan tkivno nespecifičan. Koštani i jetreni oblici ALP, koji su najzastupljeniji u serumu, nastaju posttranslacijskim modifikacijama, točnije različitim stupnjem glikozilacije tkivno nespecifičnog genskog produkta (različiti udio sijalinske kiseline i vezanih šećernih ostataka). Kao ekto-enzim tetramerna ALP je specifično, preko C-terminalnog glikan-fosfatidil sidra vezana na membrane osteoblasta. U cirkulaciji se nalazi uglavnom dimerna ALP, ali se mogu naći i tetramerni oblici vezani na membranu. Ispitivanjima *in vitro* ustanovljeno je nekoliko daljnjih oblika B-ALP koji nastaju djelovanjem proteaza te fosfolipaza C i D (1,2,6,7). U serumu odraslih zdravih osoba oko 50% ukupne aktivnosti ALP podrijetlom je iz jetre te oko 50% iz kostiju, dok u serumu djece i adolescenata prevladava B-ALP. Vrijednosti ukupne ALP veće su u muškaraca nego u žena, pretpostavlja se zbog suprimirajućeg učinka estrogena na metabolizam kostiju, a po-

mineralization. Physiological forms are coded by 4 genetic loci, three tissue-specific and one non-tissue-specific. Bone and liver forms of ALP, the most frequent in serum, are formed by post-translation modifications, or more precisely by different glycosylation stages of tissue-non-specific gene product (different ratio of sialic acid and attached sugar residues). Like ectoenzyme tetramer, ALP is specifically, through C-terminal glycan phosphatidyl anchor domain, attached to osteoblast membranes. In circulation, there is normally only one dimer ALP present, but other tetramer forms can be also found attached to the membrane. By *in vitro* examinations, some other forms of B-ALP were found that are the result of protease and phospholipase C and D activity (1,2,6,7).
In the serum of healthy adults, approximately 50% of total ALP activity originates from liver, and around 50% from bones, whereas B-ALP prevails in serum from children

većavaju se linearno s godinama u oba spola. Žene koje uzimaju kontraceptive imaju 13% manju aktivnost B-ALP. Nedostatak cinka i magnezija, koji je moguć kod parenteralne prehrane bez mikroelemenata, smanjuje aktivnost ALP u serumu. Ukupna aktivnost ALP u serumu stabilna je 7 dana na sobnoj temperaturi. Opetovano peterostruko zamrzavanje i odmrzavanje uzorka ne utječe na aktivnost B-ALP (7).

Postupci razlikovanja i mjerenja aktivnosti/koncentracije dva najzastupljenija izoenzimska oblika ALP (jetreni i koštani) koriste razlike fizikalno-kemijskih svojstava pojedinog izoblika (toplinska denaturacija, elektroforeza, precipitacija, selektivna inhibicija, HPLC i imunokemijski postupci).

Imunokemijskim postupcima moguće je mjeriti masene koncentracije B-ALP uz primjenu dva monoklonska protutijela prema B-ALP te aktivnosti nakon imunoadsorpcije na mikrotitarskim pločicama. Oba postupka pokazuju križnu reaktivnost od 14–20% s jetrenim oblikom ALP. Međutim, ustanovljeno je da u bolesnika s hepatobilijarnim bolestima, B-ALP ostaje unutar referentnog intervala ako ukupna aktivnost ALP u serumu ne prelazi gornju granicu referentnog intervala više od dva puta.

U *precipitacijskom postupku* ugljikohidratni (oligosaharidni) dio B-ALP, bogat N-acetilglukozaminom i N-acetil-neuraminskom kiselinom, precipitira s lektinima pšeničnih klica, dok jetrena ALP ne precipitira i mjeri se u supernatantu. Ovim postupkom dobivaju se lažno povećani rezultati kod bolesnika s hepatobilijarnim bolestima. Naime, bilijarna ALP može biti također precipitirana s lektinom (što se sprječava dodavanjem Tritona X100 u reagens), a zbog povećane propusnosti stanica jetre glikozilirani dio jetrenog oblika ALP može biti izmijenjen. S obzirom da priprema lektina može značajno varirati od bočice do bočice, B-ALP zapravo nije uvijek precipitirana u potpunosti pa je potrebna standardizacija ovog postupka prema referentnoj metodi. Odnos aktivnosti i mase B-ALP (procijenjen precipitacijom s lektinom i s IRMA), kao i aktivacijska energija (reakcija katalizirana s B-ALP) variraju ovisno o ispitanoj skupini bolesnika, vjerojatno opet uključujući različiti stupanj glikozilacije enzimske molekule.

Postupak *sekvencijske denaturacije toplinom* koštanog izoenzima (56 °C) više se gotovo ne koristi.

Iako su *elektroforetski postupci* za razdvajanje izooblika ALP na različitim nosačima robusni i zahtijevaju iskustvo, zbog ostalih prednosti su i nadalje postupci izbora. Naime, primjenom elektroforetskog postupka moguće je otkriti i ostale izooblike ALP te uz glavni koštani oblik i njegove varijante (otkrivajući i oblik koji nosi sidro). Kada aktivnost B-ALP prelazi 50% ukupne aktivnosti enzima u serumu, potrebna je obrada uzorka s neuraminidazom koja uklanja sijalinsku kiselinu s B-ALP, ili aplikacija uzorka na drugi gel koji sadrži lektin (precipitira B-ALP). U meta-

and adolescents. Total ALP values are higher in men than in women presumably due to a down-regulating effect of estrogen on bone metabolism, and are linear increasing with age in both genders. In women using contraceptives, B-ALP shows 13% less activity. Zinc and magnesium deficiency, which may occur due to parenteral nutrition without microelements, decreases ALP activity in serum. Total ALP activity in serum remains stable for 7 days at room temperature. Repeated fivefold sample freezing and thawing do not influence B-ALP activity (7).

In activity/concentration differentiation and measurement procedures of two most frequently represented isoenzyme ALP forms (liver and bone), we use differences in physico-chemical characteristics of a certain isoform (thermal denaturation, electrophoresis, precipitation, selective inhibition, HPLC and immunochemical procedures).

By *immunochemical* procedures, it is possible to measure B-ALP mass concentrations using two monoclonal antibodies against B-ALP and activity after immunoadsorption on microtiter plates. Both procedures show cross reactivity of 14-20% with liver form of ALP. However, it has been found that B-ALP value remains within the reference interval in patients suffering from hepatobiliary diseases if total ALP activity in serum does not exceed more than twice the upper limit of the reference interval.

In *precipitation procedure*, carbohydrate (oligosaccharide) part of B-ALP, rich in N-acetylglucosamine and N-acetylneuraminic acid, precipitates with lectins from wheat seeds, whereas liver ALP does not precipitate and is measured in the supernatant. This method gives false increased test results in patients suffering from hepatobiliary diseases. Normally, biliary ALP can be precipitated with lectin (which is prevented by adding Triton X100 to the reagent) and, due to increased liver cell permeability, we can measure the glycosylated part of liver ALP. Considering the fact that lectin preparation from one bottle to another can vary significantly, B-ALP is in fact not always completely precipitated; therefore, this procedure should be standardized according to the standard reference method. B-ALP activity mass ratio (estimated by precipitation with lectin and IRMA), as well as activation energy (reaction catalyzed by B-ALP) vary depending on examined patient group and include probably different glycosylation stage of enzyme molecules.

Sequential heat-induced denaturation procedure of bone isoenzyme (56 °C) is nowadays almost abandoned.

Although *electrophoretic methods* for separation of ALP isoforms on different carriers are difficult and require experience, they are still in use due to other advantages. Actually, it is possible by usage of electrophoretic methods to discover also other ALP isoforms and main bone ALP form together with its variants (with concurrent detection of the anchor-carrying form). When B-ALP activity

boličkim bolestima kostiju primjenom HPLC izmjerene su tri vršne vrijednosti B-ALP (1,2,6,7).

U slučajevima kada je bolest jetre isključena, ukupna aktivnost ALP ima kliničku vrijednost, odnosno daje dobru informaciju o izgradnji kosti i broju aktivnih osteoblasta. Brojnim ispitivanjima (transplantacija koštane srži, karcinom prostate s metastazama u kostima, žene u postmenopauzi, praćenje antiresorpcijske terapije i dr.) ustanovljeno je da mjerenje B-ALP ima veću diskriminirajuću vrijednost od ukupne aktivnosti ALP, čime je naglašena viša dijagnostička specifičnost mjerenja B-ALP. Može se, dakle, zaključiti da mjerenje B-ALP bolje razlikuje između „normalnog" i „patološkog" stanja na gornjoj granici referentnog intervala, pa tako povećava dijagnostičku specifičnost za otkrivanje bolesti kostiju. Zbog križne reaktivnosti u imunokemijskim postupcima i njihove nepreciznosti u donjoj polovini referentnog intervala, elektroforeza ostaje zlatni standard za otkrivanje smanjene koncentracije/aktivnosti B-ALP i za potvrdu povećane B-ALP u slučajevima težih bolesti jetre (6,8,9,10).

Osteokalcin (OC)

Osteokalcin (engl. *osteocalcin*, OC) ili koštani Gla (engl. ***gl****utamic* ***a****cid*, Gla) protein glavni je nekolageni protein koštanog matriksa, koji primarno sintetitiziraju osteoblasti te odontoblasti i hipertrofični hondrociti. Neznatna količina OC može se osloboditi tijekom razgradnje kostiju, što se može izmjeriti nekim metodama, stoga bi ga se moglo nazvati i biljegom koštane pregradnje. Sadrži 49 aminokiselina (5,8 kDa) od kojih su tri gama-karboksi-glutaminske kiseline (postranslacijska, o vitaminu K ovisna enzimska karboksilacija) na pozicijama 17, 21 i 24, a odgovorne su za Ca-vezujuća svojstva ovog proteina. Točna uloga ovog proteina još uvijek nije sasvim jasna. Raspravlja se najviše o njegovoj ulozi u procesu mineralizacije kostiju, o ulozi glasnika za kalcitriol u razgradnji kostiju, te ulozi inhibitora leukocitne esteraze i aktivnosti faktora rasta. Poslije sinteze, koju značajno stimulira kalcitriol, otpušta se i ugrađuje u izvanstanični koštani matriks (>80%). Jedan dio (10-30%) novosintetiziranog OC otpušta se u cirkulaciju gdje se njegova koncentracija može mjeriti imunokemijski (odražava, dakle, sintezu OC u osteoblastima i izgradnju kosti). Ustanovljena je značajna heterogenost cirkulirajuće frakcije OC, budući da podliježe proteolitičkom cjepanju u jetri, bubrezima, plazmi, kao i u samim kostima. Dva su glavna mjesta enzimskog cijepanja „intaktne" molekule, a nastali poznati fragmenti u cirkulaciji su fragmenti 1-19, 20-43, 44-49, 1-43 i 20-49. U naizgled zdravih osoba cirkulirajuća frakcija „intaktnog" OC predstavlja samo 36% ukupnog imunoreaktivnog OC, N-terminalni/srednji regionalni fragment (1-43) 30%, a drugi fragmenti prisutni su u zanemarivim koncentracijama (1,2). Klinička su istraživanja pokazala da nedostatak vitamina K može dovesti do poremećaja karboksilacije OC, što rezultira

exceeds 50% of the total enzyme activity in serum, neuraminidase-treated sample is required (it removes sialinic acid from B-ALP) or sample application to other lectin containing gel (it precipitates B-ALP). In metabolic bone diseases, three B-ALP peaks were measured using HPLC (1,2,6,7).

In cases when liver disease is excluded, total ALP activity has a clinical significance and provides good information about bone formation and a number of active osteoblasts. Numerous examinations (bone marrow transplantation, prostate cancer with metastases in bones, women in postmenopause, antiresorption therapy monitoring, etc.) have shown that B-ALP measurements have more important discriminating value than the value of total ALP activity, pointing out the higher diagnostic specificity of B-ALP measurements. Therefore, it can be concluded that B-ALP measurement makes clearer difference between "normal" and "pathological" states with the upper reference range values, and thus increases diagnostic specificity in bone diseases. Due to cross-reactivity in immunochemical methods and their inaccuracy in the lower half of the reference interval, electrophoresis remains the gold standard for detecting decreased B-ALP concentration/activity and for confirmation of increased B-ALP in cases of severe liver diseases (6,8,9,10).

Osteocalcin (OC)

Osteocalcin or bone Gla-protein (**gl**utamic **a**cid) is the main non-collagen protein of bone matrix, which is primarily synthesized by osteoblasts and odontoblasts and hypertrophic chodrocytes. Slight quantity of OC may be released during bone resorption and measured by some methods, so it could also be called a bone remodeling marker. It contains 49 aminoacids (5.8 kDa) of which there are three gamma-carboxyl glutamic acids (post-translational, K vitamin dependent enzyme carboxylation) at positions 17, 21 and 24 and they are responsible for calcium-binding characteristics of this protein. The exact role of this protein still remains unclear. Its role in bone mineralization process is discussed, as well as its messenger role for calcitriol in bone resorption and its role as an inhibitor of leukocyte esterase and growth factor activity. After synthesis which is significantly stimulated by calcitriol, OC is secreted and incorporated in skeletal matrix (>80%). One part (10-30%) of newly synthesized OC is released into circulation where its concentration can be immunochemically measured (it reflects OC synthesis in osteoblasts and bone formation). The important heterogeneity of circulating OC fraction is determined due to the fact that it is proteolytically cleaved in liver, kidneys, plasma and bones. There are two main sites of enzymatic cleavage of "intact" molecules, and the resulting known fragments in circulation are: 1-19, 20-43, 44-49, 1-43 and 20-49. In seemingly healthy individuals, the circulating

nerazmjernim povećanjem koncentracije cirkulirajućeg oblika OC u cirkulaciji (11).

Budući da se vrlo brzo izlučuje kroz bubrege, poluživot cirkulirajućeg OC je oko 4-5 minuta (OC i njegovi fragmenti se nakupljaju i povećava im se koncentracija u serumu kada je promijenjena funkcija bubrega). Cirkulirajući OC pokazuje cirkadijalni ritam s najvećim vrijednostima noću i rano ujutro i najnižim tijekom prijepodneva, a razlike su do 50%. Žene koje uzimaju oralne kontraceptive imaju 24% niže vrijednosti cirkulirajućeg OC. S druge strane, ustanovljeno je da vrijednosti OC nisu pod utjecajem menstruacijskog ciklusa ili uzimanja kalcija s hranom. Zanimljivo je da je OC jedini biljeg izgradnje kostiju čije vrijednosti pokazuju značajno povećanje nakon dužeg boravka u krevetu, što je vjerojatno zbog oslobađanja OC inkorporiranog u kostima iz mjesta razgradnje. Također su samo vrijednosti OC, dakle ne B-ALP i PICP, povećane kod osoba sa značajnom fizičkom aktivnošću. U usporedbi s B-ALP, opisane su značajne analitičke i biološke varijacije OC. Vrijednosti OC više su kod djece nego kod odraslih osoba, posebno za vrijeme razdoblja intenzivnog rasta, više su kod muškaraca nego kod žena dok su kod žena značajno više za vrijeme menopauze (2,6,11). Koncentracija cirkulirajućeg OC u uzajamnoj je vezi s gustoćom koštane mase na vratu femura (13) te je nezavisan pretkazatelj prijeloma kuka kod populacije starijih žena (14).

Koncentracija imunoreaktivnog OC u biološkim tekućinama mjeri se imunokemijskim postupcima (RIA, ELISA, IRMA, ECLIA). Trenutno raspoloživi postupci mjerenja cirkulirajućeg OC razlikuju se s obzirom na princip mjerenja (kompeticijsko, imunometrijsko), izvor (poliklonska, monoklonska) i specifičnost protutijela (npr. za „intaktnu" molekulu ili različite fragmente), te izvor kalibratora (goveđi/humani). Različita specifičnost protutijela za fragmente OC u ovim postupcima rezultira velikom različitošću koncentracija imunoreaktivnog OC (ovo je posebno važno u bolestima kod kojih je zapaženo nakupljanje raznih fragmenata, kao što su kronične bolesti bubrega i Pagetova bolest). U ispitivanjima usporedivosti postupaka opažena je slaba korelacija. Najčešće se raspoloživim postupcima mjeri 1-43 fragment (N-terminal/MID fragment; dijelom ga mogu stvarati i aktivni osteoblasti), produkt proteolitičke razgradnje intaktnog OC. Premda se malo zna o funkciji ovog fragmenta, njegovo mjerenje dijelom uklanja problem predanalitičke nestabilnosti. Zabilježen je, naime, gubitak imunoreaktivnosti već nakon 1 sata stajanja uzorka na sobnoj temperaturi. Stoga je brza obrada uzorka nakon uzorkovanja neophodna za sve trenutno raspoložive postupke.

Uzorak u kojem se mjeri koncentracija OC u pravilu je serum, iako se za neke postupke može koristiti i plazma. Zbog dokazane nestabilnosti, odnosno djelovanja proteaza preporuča se uzorak odmah spremiti na led; može se dodati npr. inhibitor aprotinin, čime se stabilnost mo-

fraction of "intact" OC represents only 30% of total immunoreactive OC, N-terminal/middle regional fraction (1-43) 30%, and other fractions are present in very low concentrations (1,2). Clinical studies showed that vitamin K deficiency may lead to impairment in the carboxylation of OC, also resulting in a disproportionate increase in the undercarboxylated OC form in the circulation (11).

Because it is very quickly secreted through kidneys, the half-life of circulating OC is around 4-5 minutes (OC and its fragments accumulate and their concentration in serum increases when kidney function is changed). Circulating OC shows circadian rhythm with the highest values in the night and early in the morning and the lowest during early morning hours, with the differences reaching up to 50%. In women who take oral contraceptives, the circulating OC values are 24% lower. On the other hand, it has been established that OC values are not influenced by menstrual cycle or calcium consumption through nutrition. It is interesting that OC is the only bone formation marker whose values increase significantly after long-term bed rest, probably due to release of OC incorporated in bones from resorption sites. Also, only the values of OC, and not B-ALP or PICP, are increased in individuals with increased physical activity. In comparison to B-APL, significant analytical and biological OC variants have been described. OC values are higher in children than in adults, especially during the intensive growth period. They are also higher in men, and in women these values are considerably higher during menopause (2,6,12). The concentration of undercarboxylated OC correlates with bone mineral density at the femoral neck (13) and it is an independent predictor of hip fracture in a population of ambulatory elderly women (14).

Concentration of immunoreactive OC in human samples is measured by immunochemical methods (RIA, ELISA, IRMA, ECLIA). Current available methods for the determination of circulating OC differ according to measurement principle (competitive, immunometric), source (polyclonal, monoclonal), antibody specificity (e.g. for "intact" molecule or different fragments) and calibrator origin (bovine/human). Different antibody specificity for OC fragments in these methods results in great differences in immunoreactive OC concentrations (this is especially important in diseases characterized by the accumulation of different fragments as, e.g., chronic kidney disease and Paget's disease). Poor correlation is observed in method comparability studies. In most cases, these methods measure 1-43 fragment (N-terminal/MID fragment; partially it can be formed by an active osteoblast), a product of proteolytic resorption of intact OC. Although we know little about the function of this fragment, its partial measurement eliminates the problem of preanalytical instability. Actually, loss of immunoreactivity is recorded already after the sample is at room temperature for an hour. There-

že očuvati 5 sati na sobnoj temperaturi. Serum odvojen od stanica unutar 1. sata nakon uzorkovanja može se odmah zamrznuti, ali se opetovano odmrzavanje i zamrzavanje ne preporuča. Hemoliza može utjecati na mjerenje koncentracije OC zbog povećanog otpuštanja proteaza iz eritrocita (2,7,12). Antikoagulansi (ako se koristi krvna plazma) s oksalatima i fluoridima mogu smanjiti koncentraciju OC. Pretpostavlja se da razlog tome nije interferencija u imunokemijskom postupku, nego veća hemoliza koju uzrokuju ovi antikoagulansi u odnosu na druge. Nadalje je ustanovljeno da stabilnost OC ovisi izrazito o analitičkoj specifičnosti primijenjenog postupka. Imunometrijska mjerenja specifično mjere intaktni OC, koji je sklon brzoj proteolitičkoj razgradnji u serumu. Ovim postupcima izmjerene su niže vrijednosti (10%) OC 1-49 i OC 1-43 za vrijeme pohranjivanja 7 dana na +4°C.

Povećane vrijednosti OC opisane su u bolesnika s povećanom izgradnjom kostiju - hiperparatiroidizam, Pagetova bolest, značajna osteoporozna pregradnja, hipertiroidizam, bubrežna osteodistrofija, frakture i akromegalija. U žena u kasnijoj postmenopauzalnoj fazi isto dijagnostičko značenje ima mjerenje OC 1-49 i omjera OC 1-49/OC 1-43. Zanimljivo je da je u bolesnika s osteoporozom korelacija OC i B-ALP niska, dok u primarnom hiperparatiroidizmu koncentracije OC i B-ALP pokazuju usporedivu diskriminirajuću vrijednost (9). Kod bolesnika s tumorom i metastazama u kostima i onih s Pagetovom bolešću, mjerenje OC manje je važno od mjerenja B-ALP u smislu dijagnostičke osjetljivosti. Suprotno tome, mjerenje OC je značajnije od mjerenja B-ALP u praćenju bolesnika na kortikosteroidnoj terapiji. Kod bolesnika s kroničnim bolestima bubrega korisnost OC je znatno smanjena, jer je pod utjecajem funkcije bubrega. Smanjene vrijednosti OC nađene su u hipoparatiroidizmu, hipotiroidizmu, nedostatku hormona rasta, za vrijeme nadomjesne estrogenske terapije, te terapije s glukokortikoidima, bifosfonatima i kalcitoninom. U tablici 4. prikazana su klinička stanja povezana s promjenama B-ALP i OC u serumu. Za odgovarajuće tumačenje podataka koncentracije OC važno je temelji li se primijenjen mjerni postupak ili ne na reakciji s fragmentima OC otpuštenima iz koštanog matriksa za vrijeme razgradnje. Samo u slučaju izostanka reakcije s navedenim fragmentima opravdano je tražiti opetovano mjerenje biljega izgradnje kostiju (OC 1-43 je fragment koji se vjerojatno ne otpušta iz kosti za vrijeme razgradnje, već samo iz novosintetiziranog OC). Mjerenje specifičnog fragmenta OC obećava budući da su određivanja intaktne molekule podložnija predanalitičkoj nestabilnosti.

Propeptidi prokolagena tipa I (PINP, PICP)

Kolagen tipa I je glavni protein koštanog matriksa (> 90% sadržaja matriksa) te u manjoj mjeri kože, dentina, tetiva, korneje i brojnih drugih tkiva. Sintetizira se u osteoblastima u obliku prethodničke molekule prokolagena I kofore, fast sample processing after sampling is considered to be essential for all currently available methods.

The sample for OC concentration measurement is normally serum, although for some methods plasma can also be used. Due to confirmed instability and protease action, it is recommended to put the sample immediately on ice, e.g. inhibitor aprotinine can be added, thus preserving stability for five hours at room temperature. Serum, separated from cells within the first hour after sampling, can be immediately frozen. However, repeated freezing and thawing are not recommended. Hemolysis can affect OC concentration measurement due to increased release of proteases from erythrocytes (2,7,12). Anticoagulants (the case of blood plasma use) with oxalates and fluorides can decrease OC concentration. It is assumed that the reason is not interference in immunochemical method, but increased higher hemolysis caused by these anticoagulants compared with the others. Furthermore, it has been concluded that OC stability depends extremely on analytical specificity of the applied method. Immunometric measurements specifically measure intact OC which tends to accelerate proteolytical resorption in serum. By these methods, lower values (10%) of OC 1-49 and OC 1-43 were found during 7 day storage at +4 °C.

Elevated OC values are described in patients suffering from increased bone formation – hyperparathyroidism, Paget's disease, significant remodeling due to osteoporosis, hyperthyroidism, renal osteodystrophy, fractures and acromegaly. In women in later post-menopausal period, measurements of OC 1-49 and of OC1-49/OC 1-43 ratio have the same diagnostical significance. It is interesting that in patients suffering from osteoporosis OC and B-ALP correlation is low, while in primary hyperparathyroidism OC and B-ALP concentration show comparable discriminating value (9). In patients having bone tumor and metastases and those with Paget's disease, OC measurements are less important than B-ALP measurements regarding diagnostic sensitivity. On the contrary, OC determination is more important than B-ALP measurement in monitoring patients under corticosteroid therapy. In patients with chronic kidney diseases, the usefulness of OC is significantly decreased because it is influenced by renal function. Decreased OC values were found in hyperparathyroidism, hypothyroidism, growth hormone deficiency, during estrogen substitution therapy and therapy with glucocorticoid, biphosphonate and calcitonin. Table 4 shows clinical conditions connected to changes in B-ALP and OC in serum. For appropriate data interpretation of OC concentration, it is essential whether the applied measurement method is based on reaction of OC fragments released from bone matrix during resorption. Only in case of no reaction with these fragments is it justified to measure bone formation markers repeatedly (OC 1-43 is a fragment that is probably not released from the

Tablica 4. Klinička stanja povezana s promjenama vrijednosti B-ALP i OC u serumu

Table 4. Clinical conditions connected to changes in B-ALP and OC values in serum

Condition	B-ALP	OC
Increase		
Paget's disease	+++	++
Primary hyperparathyreoidism	++	++
Osteomalacy and rickets	++	+
Chronical kidney diseases, with hemodialysis	+ ++	++ ++
Osteoporosis	+	+
Metastatic carcinoma	++	++
Hyperthyriodism	+	+
Chronic liver diseases	+	+
Familial hyperphosphatasemia	+	
Chusing's sydrome	+	+
Gaucher's disease	+	+
Decrease		
Hypothyroidism	-	-
Familial hypophosphatasia	-	

(+ to +++) = relative increase; (-) decrease

ja sadrži N- i C-terminalnu trimernu produženu domenu [dva identična polipeptidna lanca (alfa1 I) i jedan (alfa2 I)] poznatu kao propeptid (PINP i PICP). Karakteristike dva navedena propeptida prikazane su u tablici 5. (1).

Prokolagen je stoga 50% duža molekula od konačnog proteina, a njegova funkcija je sprječavanje prijevremene agregacije molekule kolagena u fibrile unutar stanice. Prije sazrijevanja kolagenskih fibrila, ovi tzv. C- i N-propeptidi cijepaju se s prokolagena tipa I specifičnim izvanstaničnim tkivnim endopeptidazama. C-terminalni propeptid prokolagena tipa I (PICP) je glikoprotein koji sadrži dva polipeptidna lanca (alfa1 I) od 246 i jedan polipeptidni la-

bone during resorption, but only from a newly synthesized OC). The measurement of specific OC fragment is more promising since intact molecule determinations are subject to preanalytic instability.

Type I propeptid procollagen (PINP, PICP)

Type I collagen is the main protein of bone matrix (>90% matrix content) and to a lesser extent of skin, dentine, tendon, cornea and other tissues. It is synthesized in osteoblasts as a precursor of procollagen I that contains N- and C-terminal trimeric extended domain [two identical polypeptide chains (alpha1 I) and one (alpha2 I)] known

Tablica 5. Usporedba propeptida prokolagena tipa I

Table 5. Comparison of propeptide type I procollagen

	PINP	PICP
Localization	aminoterminal	carboxytermal
Molecular mass (kDa)	70	115
Form	extended	globular
Chemical nature	phosphorylated	glycoprotein, contians mannose-rich oligosaccharides
Crosslinked bonds	non-covalent	disulphide

nac (alfa2 I) od 247 aminokiselinskih ostataka, s među- i unutarstaničnim disulfidnim vezama (115 kDa) (1,6,12,15). Ugljikohidratna komponenta C-terminalnog propeptida sadrži ostatke N-acetilglukozamina i manoze. PICP se metabolizira preko manoza-6-P receptora na endotelnim stanicama jetre (poluživot 6-8 minuta), a PINP pomoću receptora čistača. Svaka disfunkcija jetre može rezultirati promjenom jetrenog klirensa PICP, odnosno povećanim koncentracijama u cirkulaciji. Suprotno tome, djelovanje proupalnih citokina, koji reguliraju endocitozu preko sinusoidalnih stanica, može rezultirati smanjenim vrijednostima PICP. Opisan je također značajan genetski utjecaj na vrijednosti PICP, zatim diurnalni ritam s amplitudom od 20% (veće vrijednosti noću i niže u popodnevnim satima) i relativna stabilnost u uzorku (15 dana na +4 ˚C, nekoliko mjeseci na -20 ºC). PICP nije, dakle, ugrađen u koštani matriks, ali se otpušta u cirkulaciju gdje se može odrediti raznim imunokemijskim postupcima (RIA, ELISA). Smatra se da je stvaranje PICP u drugim tkivima puno sporije, pa se podrazumijeva da malo pridonosi cirkulirajućem *poolu*, iako ovaj podatak nije sasvim jasan.

U primarnom hiperparatiroidizmu su vrijednosti PICP unutar granica vrijednosti zdravih osoba (dok su npr. vrijednosti ALP i OC povećane). Kod bolesnika s osteomalacijom koji su primali vitamin D zabilježene su povećane vrijednosti kao i nakon paratireoidektomije. Smanjene vrijednosti PICP zabilježene su u bolesnika s *osteogenesis imperfecta* te u osoba na glukokortikoidnoj terapiji (1,2). Općenito, temeljem ispitivanja na raznim kliničkim modelima čini se da je PICP manje osjetljiv i specifičan od B-ALP i OC zbog relativne nespecifičnosti za kost i različitog klirensa.

Biljezi razgradnje kostiju

Osim TR-ACP (engl. *tartrate-resistant acid phosphatase*, tartarat rezistentna kisela fosfataza), biljezi razgradnje su razgradni produkti koštanog kolagena. Kako se izlučuju mokraćom, donedavno su se i određivali uglavnom u mokraći, uzorku uz koji je vezana značajna varijabilnost rezultata. Stoga je glavni znanstveni i komercijalni interes usmjeren na postavljanje i procjenu postupaka za njihovo mjerenje u serumu.

Poprečne veze kolagena

Serijom intra- i intermolekularnih kovalentnih veza (poprečnih veza), između terminalnoga nehelikalnog dijela jedne molekule kolagena i helikalnog dijela druge molekule kolagena, u koštanom se matriksu stabilizira molekula zrelog kolagena tipa I. Poprečne veze, u obliku 3-hidroksipiridinskog prstena, nastaju deaminacijom ε-aminoskupine lizina ili hidroksilizina, uz katalitičko djelovanje enzima lizil-oksidaze. Dvije su nereducibilne poprečne veze identificirane u mokraći ljudi: deoksipiridinolin (en-

as propeptide (PINP and PICP). Characteristics of the two mentioned propeptides are shown in Table 5 (1).

Procollagen is, therefore, 50% longer molecule than the final protein, its function is to prevent a precocious aggregation of collagen molecules in fibrils inside the cell. Before collagen fibril maturation, these so-called C- and N-propeptides are cleaved from procollagen type I by specific extracellular tissue endopeptidases. C-terminal propeptide type I procollagen (PICP) is a glycoprotein that contains two polypeptid chains (alpha1 I) of 246 and one polypeptide chain (alpha2 I) of 247 amino acid residues, with intra- and intercellular disulphide bonds (115 kDa) (1,6,12,15). Carbohydrate component of C-terminal propeptide contains N-acetylglucosamine and mannose residues**.** PICP is metabolized through mannose-6-P receptor on liver endothelial cells (half-life of 6-8 minutes) and PINP through scavenger receptor. Every liver dysfunction might result in variation of PICP liver clearance, or elevated concentration in circulation. In contrast, proinflammatory cytokine action, which regulates endocytosis through sinusoidal cells, can result in low PICP values. Significant genetic influence on PICP values is also described, as well as diurnal rhythm with amplitude of 20% (higher values during the night and lower in the afternoon) and relative sample stability (15 days at +4°C, several months at -20°C). PICP is therefore not incorporated in bone matrix but is released into circulation, where it can be measured by different immunochemical methods (RIA, ELISA). It is considered that the process of PICP formation in other tissues lasts longer; therefore, it is thought that it contributes a little to a circulating pool, although this information is not completely clear.

In primary hyperparathyroidism, PICP values are within reference value limits in healthy individuals (while, e.g., ALP and OC values are elevated). In patients suffering from osteomalacy on D vitamin supplementation, elevated values were recorded also after paraidectomy. Lowered PICP values were found in patients with *osteogenesis imperfecta* and in individuals receiving glucocorticoid therapy (1,2). Generally, through examinations on different clinical models, it appears that PICP is less sensitive and specific than B-ALP and OC due to relative bone nonspecificity and different metabolic clearance.

Bone resorption markers

Aside from TR-ACP (*tartrate-resistant acid phosphatase*), bone resorption markers are resorption products of bone collagen. As they are secreted in urine, they were mostly measured in urine until recently; however, samples had significant test result variability. Therefore, the main scientific and commercial interest has been focused on setting up and evaluating methods for their measuring in serum.

gl. *deoxypyridinoline*, DPD) koji nastaje reakcijom pokrajnjih lanaca dvije molekule hidroksilizina i jedne molekule lizina, te piridinolin (engl. *pyridinoline,* PYD) koji nastaje reakcijom pokrajnjih lanaca tri molekule hidroksilizina (oba spoja posjeduju prirodnu imunogenost i fluorescenciju) (2,6,7,12,16). DPD je nađen većinom u kostima, manje u dentinu, dok je PYD lokaliziran u kolagenskim fibrilama kosti i hrskavice te u manjoj mjeri u drugim tkivima (tetive, ligamenti, stjenke krvnih žila). Budući da kost ima najintenzivniju pregradnju, smatra se da je najvažniji izvor DPD i PYD. Kada se matriks kolagena proteolitički razgrađuje, obje vrste poprečnih veza otpuštaju se u cirkulaciju, a budući da su male molekulske mase, izlučuju se mokraćom gdje im se može mjeriti koncentracija. Oba tipa poprečnih veza izlučuju se kao slobodni (40%) i peptidno vezani (60%) aminokiselinski derivati. Opisani su neki biološki čimbenici koji mogu utjecati na vrijednosti DPD i PYD: cirkadijalni ritam naznačava maksimalne vrijednosti između 5 i 8 sati, a minimalne između 17 i 20 sati; menopauza, osteopenija ili duži boravak u krevetu nemaju utjecaja na vrijednosti; praćenjem u periodu od 15 mjeseci zabilježena je intraindividualna varijabilnost između 20 i 30%; kod muškaraca je visok linearni odnos između starosne dobi i izlučivanja ovih spojeva mokraćom, dok kod žena tako jasna ovisnost nije zabilježena; za vrijeme trudnoće povećava se vrijednost oko 91% od prvog trimestra do poroda. DPD se smatra specifičnijim biljegom razgradnje, budući da nastaje za vrijeme sazrijevanja kolagena (ne biosinteze, dakle pojavljuje se samo kao razgradni produkt zrelog matriksa), ne metabolizira se prije izlučivanja u mokraći. Njegov je glavni izvor kost i ne apsorbira se iz hrane (1,2,16,17).

Metode za mjerenje koncentracije DPD i PYD su HPLC i fluorometrijsko kvantificiranje te imunokemijski postupci. S ELISA tehnikom je moguće specifično mjeriti slobodni, nevezani i na peptid vezani oblik DPD. Tako dobivene vrijednosti DPD i PYD pokazuju visoku korelaciju (R > 0,95) s vrijednostima dobivenim s HPLC, metodom koja se smatra zlatnim standardom. Kao uzorak preporuča se druga jutarnja mokraća (između 8 i 10 sati) poslije 12-satnog posta. Izlaganjem uzorka UV svjetlu dolazi do brze razgradnje ($t_{1/2}$ < 30 sekundi) obje poprečne veze, dok normalno dnevno svjetlo ne pokazuje tako izrazit učinak. Stabilnost biljega na -20 °C je 10-20 godina. Nije zabilježen niti značajniji pad vrijednosti ako se uzorci spreme na temperaturu nižu od 20 °C u periodu od 6 tjedana. Opetovano zamrzavanje i odmrzavanje uzorka ne utječe na vrijednosti DPD i PYD. U tablici 6. prikazane su prednosti i nedostatci poprečnih veza kolagena kao biljega razgradnje kostiju.

Bolesnici s neliječenim primarnim hiperparatiroidizmom pokazuju značajno povećane koncentracije poprečnih veza kolagena, koje koreliraju s ukupnom aktivnošću ALP i koncentracijom PTH. Bolesnici s tumorom sa i bez me-

Collagen cross-links (PYD, DPD)

With a series of intra- and intermolecular covalent bonds (cross-links) between terminal nonhelical portion of the molecule and helical portion of a neighboring collagen molecule, a molecule of mature collagen type I is stabilized in skeletal matrix. Cross-links, as a 3-hydroxypyridinium ring, are formed by deamination of lysine or hydroxylysine epsilon-amino group with catalytic action of enzyme lysyloxydase. There are two nonreducible cross-links identified in human urine: deoxypyridinoline (DPD) which is formed by reaction of side-chains of two hydroxylysine molecules and one lysine molecule, and pyridinoline (PYD) which is formed by reaction of side-chains of three hydroxylysine molecules (both compounds have inborn immunogenetics and fluorescence) (2,6,7,12,16). DPD is found mostly in bones, not so much in dentine, while PYD is located in bone collagen fibrils and cartilage and to a lesser extent in other tissues (tendons, ligaments, blood vessel walls). Since the bones have the most intensive remodeling, they are considered as the most important PDP and PYD source. When collagen matrix is proteolytically degraded, both cross-links are released into circulation and, due to their small mass, they are secreted by urine where they can be measured. Both types of cross-links are secreted as free (40%) and peptide-linked (60%) amino acid derivatives. Some biological factors that can influence DPD and PYD values have been described: circadian rhythm indicates maximum values between 5 and 8 a.m. and minimum between 5 and 8 p.m.; menopause, osteopenia or long-term bed rest do not affect the values; after a 15-month monitoring period, intraindividual variability between 20 and 30% has been recorded: in men the linear ratio between age and secretion of these compounds through urine is high, while such clear dependence has not been recorded in women; during pregnancy the values are elevated for about 91% comparing the first and the last trimester. DDP is considered a specific resorption marker because it is formed during collagen maturation (not during biosynthesis and therefore it appears only as a resorption product of the mature matrix), and it does not metabolize before secretion into urine. The main source of DPD is bone, and it is not absorbed from food (1,2,16,17).

DPD and DYP concentration measurement can be performed using HPLC, fluorometric quantification and immunochemical methods. With ELISA, we can specifically measure free, non-linked and peptide-linked DPD form. Thus attained DPD and DYP values show high correlation (R > 0.95) with the values measured using HPLC, a method considered a–gold standard. The second morning urine (between 8 and 10 a.m.) after a 12-hour fast is the recommended sample. Exposing the sample to the UV light causes fast resorption (half-life < 30 seconds) of both cross-links, while normal daily light does not show so

Tablica 6. Prednosti i nedostatci poprečnih veza kolagena

Table 6. Advantages and disadvantages of collagen cross-links

Advantages	Disadvantages
Good correlation with bone resorption	Current available determination methods in serum are still undergoing extended clinical evaluation
Bone resorption indicators are released only from mature extracellular collagen	Great biological variability in urine
Are not metabolised	High analytical variability with one method
Not used again in collagen synthesis	There is no adequate reference material
Not under food influence (DPD)	Standards usually contain biological material
	There is no standard scheme for quality assurance
	Published data usage is problematic

tastaza u kostima mogu se razlikovati istodobnim mjerenjem B-ALP i imunoreaktivnog PYD s točnošću od 0,89 (analiza ROC). Bolesnici s nedostatkom vitamina D pokazuju trostruko povećanje koncentracije poprečnih veza kolagena. Usporedba vrijednosti PYD i DPD između premenopauzalnih zdravih žena i postmenopauzalnih (osteopeničnih) žena pokazuje povećanje izlučivanja poprečnih veza kolagena mokraćom i do 105%. Izlučivanje PYD mokraćom povećano je kod 40% bolesnika s Pagetovom bolešću, dok je npr. ukupna aktivnost ALP unutar referentnog intervala. Povećane vrijednosti PYD u serumu ustanovljene su i kod bolesnika s bubrežnom osteodistrofijom te koreliraju s histomorfometrijskim pokazateljima razgradnje kostiju.

Hidroksiprolin (OHP)

S obzirom na karakteristike novijih specifičnijih biljega razgradnje kostiju, mjerenje tradicionalno korištenoga biokemijskog biljega hidroksiprolina (engl. *hidroxyproline*, OHP) ne preporučuje se zbog sljedećih nedostataka: OHP je nađen i u kolagenima drugih tkiva, u C1q, elastinu i acetilkolinesterazama; nije specifičan biljeg razgradnje budući da se otpušta i za vrijeme izvanstaničnog metabolizma novosintetiziranog (pro)kolagena; 90% OHP oksidacijom se metabolizira u jetri; izlučivanje OHP u mokraći izrazito je ovisno sadržaju kolagena u prehrani.

Telopeptidi kolagena tipa I (CTX, NTX)

Teorijska osnova za mjerenje telopeptidnih regija kolagena, umjesto poprečnih veza kolagena je činjenica da poprečno vezanje uvijek uključuje specifičnu domenu molekule, tzv. C- ili N-terminalni telopeptid. Kad se kolagen tipa I razgrađuje osteoklastima, N- i C-terminalni telopeptidni fragmenti, još uvijek pričvršćeni poprečnim vezama na helikalni fragment susjedne molekule, otpuštaju se u cirkulaciju i uklanjaju kroz bubrege (1,6,7,18,19). Pri tome se stvara višak razgradnih telopeptidnih produkata, budući da se cijepanje polipeptidnih lanaca može dogoditi na nekoliko mjesta unutar telopeptida. Telopeptidi mogu ili ne moraju biti poprečno vezani, može postojati nekoli-

strong effect. Marker stability at -20°C is 10-20 years. No significant value decline is recorded if samples are stored at temperature <20°C during a period of 6 weeks. Repeated sample freezing and thawing do not affect DPD and DYP values. Table 6 shows advantages and disadvantages of collagen cross-links as bone resorption markers.

Patients with non-treated primary hyperparathyroidism show significantly elevated cross-link values which correlate with total ALP activity and PTH concentration. Tumor patients with and without metastases in bones can be differentiated by simultaneous B-ALP and immunoreactive PYD measurements with 0.89 accuracy (ROC analysis). Patients with D vitamin deficiency show triple increase in cross-links; PYD and DPD value comparison between premenopausal healthy women and postmenopausal (osteopenic) women shows increase in urinal cross-link secretion by up to 105%; urinary PYD is increased in 40% of patients suffering from Paget's disease, while, e.g., total ALP activity lies within the reference interval; elevated PYD values in serum have been determined also in patients with renal osteodystrophy and correlate with histomorphometric indicators of bone resorption.

Hidroxyproline (OHP)

Considering characteristics of newer, more specific bone resorption markers, it is not recommended to measure traditionally used biochemical hidroxyproline (OHP) marker because of the following disadvantages: OHP is also recorded in collagens of other tissues, in C1q, elastin and acetylcholinesterases; it is not a specific resorption marker because it is also released during extracellular metabolism of newly synthesized (pro)collagen; 90% of OHP is metabolized by oxidation in liver; OHP secretion in urine is extremely dependent on collagen content in nutrition.

Telopeptides of type I collagens (CTX, NTX)

Theoretical basis for measuring telopeptide collagen regions, instead of collagen cross-links, is the fact that cross-links always involve specific molecular domain, the so-called C- or N-terminal telopeptide. During osteocla-

ko tipova poprečnog povezivanja, jedan ili više telopeptidnih lanaca može biti promijenjen beta-izomerizacijom, a i telopeptidi mogu biti već poprečno povezani na helikalnu kolagensku regiju. Za neke od razgradnih produkata postavljeni su i mjerni postupci. Postoji imunokemijski postupak za mjerenje C-terminalnog telopeptida kolagena (engl. *C-telopeptide of type I collagen*, CTX) u mokraći, točnije razgradnog produkta C-telopeptida koji koristi monoklonska protutijela protiv sintetskog oktapeptida koji sadrži poprečno vezano mjesto (Glu-Lys-Ala-His-beta-Asp-Gly-Gly-Arg), nazvano beta-CTX ili beta *CrossLaps*).

Koncentracije u mokraći povećane su u više od 1/3 žena u ranoj postmenopauzi. Nakon hormonske nadomjesne terapije vrijednosti se značajno smanjuju (do 61%). Moguće je i mjerenje neizomeriziranog oktapeptida u mokraći (alfa-CTX) te istodobno mjerenje alfa/beta CTX, kao indeksa pregradnje kostiju (indeks je povećan u Pagetovoj bolesti, a smanjen nakon terapije s bifosfonatima). Druga metoda je imunokemijski postupak za mjerenje CTX u serumu koji koristi monoklonska protutijela specifična za izomerizirani oblik sekvence (EKAHD-beta-GGR) iz alfa-1 lanca humanog kolagena tipa I (2,6,7). No, lipemičan serum može interferirati u ovom postupku, pohranjivanje uzorka na sobnoj ili temperaturi od +4 °C prate smanjene vrijednosti do 13%, a opetovano zamrzavanje i odmrzavanje rezultira smanjenom koncentracijom (10%). Prema dosadašnjim ispitivanjima koncentracije CTX pokazuju visoku specifičnost (100%) i osjetljivost (83,8%) u praćenju odgovora na antiresorpcijsku terapiju (nakon 6 mjeseci više od 92% žena na antiresorpcijskoj terapiji). Vrijednosti CTX odgovarajuće odražavaju lošu prognozu u multiplom mijelomu, a povezane su s težim radiografskim nalazima kod bolesnika s reumatoidnim artritisom. Visoke vrijednosti CTX povezane su s niskom koštanom masom kod bolesnika sa Crohnovom bolešću te pokazuju visoku učinkovitost u dijagnozi metastaza u kostima. Komercijalno je raspoloživ i mjerni postupak za N-telopeptidne fragmente kolagena (engl. *N-telopeptide of type I collagen*, NTX). Protutijela prepoznaju konformacijski epitop poprečno vezanog α_2-N-telopeptida s određenom sekvencom (QYDGKGVG), koja je produkt osteoklastne proteolize. Opisan je cirkadijalni ritam vrijednosti, a zanimljivo je povećanje vrijednosti u zdravih žena za vrijeme folikularnog perioda i pad za vrijeme lutealne faze. Kod žena se vrijednosti povećavaju s godinama i veće su nego vrijednosti u odgovarajućoj dobnoj skupini muškaraca. Dosadašnji rezultati pokazuju da bi ovaj biljeg mogao biti značajan u procjeni razgradnje kostiju budući da izlučivanje N-terminalnog telopeptida mokraćom kod djece odražava razinu rasta, vrijednosti su značajno povećane kod bolesnica s postmenopauzalnom osteoporozom, vrijednosti izlučivanja adekvatno odražavaju supresiju pregradnje kostiju pomoću estrogenske nadomjesne terapije, kod

st-mediated resorption of type I collagen N- and C-terminal telopeptide fragments, still attached with cross-links to the helical fragment of neighboring molecule, these are released into circulation and eliminated by kidney filtration (1,6,7,18,19). Thereby is created an excess of telopeptide resorption products due to the following reasons: cleavage of polypeptide chains can occur at several sites in a telopeptide which can or does not have to be cross-linked, there can be several types of cross-linking, one or more telopeptide chains can be altered by beta-isomerization, and telopeptides can be cross-linked to helical collagen region. For some of these resorption products, measurement methods are available. There is an immunochemical method for measurement of C-terminal telopeptide in urine or, more precisely C-telopeptide resorption product that uses monoclonal antibodies against synthetic octapeptide that has a cross-linked site (Glu-Lys-Ala-His-beta-Asp-Gly-Gly-Arg) called beta-CTX or beta *CrossLaps*).

Urinary concentrations are elevated in more than 1/3 of early postmenopausal women. After hormone replacement therapy, values decrease significantly (up to 61%). It is possible to measure nonisomerized urine octapeptide (alpha-CTX) and simultaneously to measure alpha/beta CTX as a bone remodeling index (the index is increased in Paget's disease, and decreased after biphosphonate therapy). Another method is the immunochemical method for CTX measurement in serum that uses monoclonal antibodies specific for isomerized sequence (EKAHD-beta-GGR) from alpha-1 chain of human type I collagen (2,6,7). However, lipemic serum can interfere with this method, the samples stored at room temperature or at +4 °C have lowered values by up to 13%, and repeated freezing gives results with lower concentration (10%). According to present studies, CTX values show high specificity (100%) and sensitivity (83.8%) in monitoring the response of anti-resorption therapy (after 6 months over 92% women on anti-resorption therapy). CTX concentrations adequately express bad prognosis in multiple myeloma, they are connected with more severe radiography test results in patients suffering from rheumatoid arthritis. High CTX values are related to low bone mass in patients suffering from Crohn's disease and show high efficacy in diagnosis of bone metastases. Methods for telopeptide N-fragments (NTX) are commercially available. The antibodies recognize conformational epitope of cross-linked alpha-2-N-telopeptide with a certain sequence (QYDGKGVG) which is a product of osteoclastic proteolysis. Circadian rhythm is described, and it is interesting to find that there is an increased concentration during the follicular period which declines during the luteal phase in women. Also, concentrations increase with age and are higher than those in the respective male age group. Present results show that this marker could be significant in bone resor-

osoba koje uzimaju bifosfonate vrijednosti mokraćnog izlučivanja N-terminalnog telopeptida odražavaju razgradnju kostiju specifičnije nego poprečne veze kolagena.

Tartarat-rezistentna kisela fosfataza (TR-ACP)

Kisele fosfataze su lizosomski enzimi raznih tkiva (trombociti, eritrociti, kost, prostata), koji hidroliziraju fosfomonoestere kod niske vrijednosti pH. U plazmi je otkriveno 5 izoenzimskih oblika enzima koji se razlikuju prema tkivnom i kromosomskom podrijetlu, kao i po molekularnoj masi te elektroforetskoj pokretljivosti. Prema elektoforetskoj pokretljivosti klasificiraju se kao izoenzimi 1-5, a prema osjetljivosti na inhibiciju s L(+)-tartaratom klasificiraju se dodatno na tartarat-osjetljive i tartarat-rezistentne oblike (6,11,20,21,22). Mjerenje ukupne aktivnosti TR-ACP u serumu kao biljega razgradnje kostiju ima dosta nedostataka (relativno mala aktivnost, prisutnost inhibitora, nestabilnost kod alkalnog pH, interferencija hemolize), stoga se ne preporuča za postavljanje dijagnoze.

TR-ACP tipa 5 stvaraju makrofagi (TR-ACP 5a; aktivnost povećana u Gaucherovoj bolesti i leukemiji vlasastih stanica) i osteoklasti (TR-ACP 5b; nema sijalinskih ostataka; aktivnost je povećana u bolestima kostiju, posebice osteopetrozi). Promjene TR-ACP tipa 5b u biti odražavaju broj aktivnih osteoklasta.

Većinom postupaka za mjerenje aktivnosti TR-ACP nije moguće razlikovati osteoklastni (izoforma 5b) oblik enzima od drugih oblika (izoforma 5a) koji se nalaze u plazmi. Nedavno postavljena dva postupka mjerenja TR-ACP 5b u fazi su evaluacije. To su kinetički postupak mjerenja u kojem se koristi inhibicija TR-ACP 5b s fluoridom, a TR-ACP 5a s heparinom te imunokemijski postupak koji koristi monoklonsko protutitijelo za TR-ACP 5b.

Aktivnost TR-ACP povećana je kod bolesnika s različitim oboljenjima (Tablica 7.), poglavito kod metastaza u kostima. Smatra se manje vrijednim biljegom razgradnje kostiju, posebno u praćenju bolesnika s Pagetovom bolešću koji su na terapiji bifosfonatima.

Mjerenje ukupne aktivnosti TR-ACP kao biljega razgradnje kostiju trenutno se ne preporuča zbog relativno male aktivnosti enzima i prisutnosti inhibitora u serumu, nestabilnosti pri alkalnijem pH, činjenice da je L(+) tartarat kompetitivni inhibitor i jake interferencije hemolize.

Hidroksilizin-glikozidi (Hyl-Glyc)

Galaktozil-hidroksilizin (GHL) i glukozil-galaktozil-hidroksilizin (GGHL) nastaju iz lizina za vrijeme postranslacijske faze sinteze kolagena, a otpuštaju se u cirkulaciju za vrijeme razgradnje kolagena. GHL je relativno specifičniji za razgradnju koštanog matriksa nego GGHL, a nedvojbeno više specifičan od npr. OHP. Stvarna prednost Hyl-Glyc kao biljega razgradnje je u tome što se ovi oblici ne metaboliziraju i nisu pod utjecajem čimbenika prehrane. U mokraći se mogu mjeriti metodom HPLC. Poželjno je pos-

ption assessment because N-terminal telopeptide in the urine of children displays growth rate, its concentration is significantly elevated in (female) patients with postmenopausal osteoporosis, indicating an adequate suppression of bone remodeling through estrogen supplementary therapy. In individuals taking biphosphates, the urinary excretion of N-terminal telopeptide is a better indicator of bone remodeling than collagen cross-links.

Tartarate-resistant acid phosphatase (TR-ACP)

Acid phosphatases are lysosomal enzymes of different tissue origin (platelets, erythrocytes, bone, prostate) that hydrolyzes phospho-monoesters at low pH value. Five isoenzymatic forms of enzymes have been found in plasma that differ regarding tissue and chromosomal origin, as well as molecular mass and electrophoretic mobility. By electrophoretic mobility, they are classified as isoenzymes 1-5, and according to sensitivity or inhibition with L(+)- tartarate; they are furthermore classified by tartarate-sensitive and tartarate-resistant forms (6,11,20,21,22). Total TR-ACP activity measurement in serum, classified as a bone resorption marker, has many disadvantages (relatively low activity, presence of inhibitors, instability by alkaline pH, interference by hemolysis) and it is, therefore, not recommended for diagnosis.

Type 5 TR-ACP is formed by macrophages (TR-ACP 5a; activity enhanced in Gaucher's disease and hairy cell leukemia) and osteoclasts (TR-ACP 5b; non sialyl residues; increased activity in bone diseases, especially in osteoporosis). Type 5b TR-ACP changes reflect a number of active osteoclasts.

The majority of TR-ACP activity measurement methods cannot distinguish between osteoclast enzyme form (isoform 5b) and other forms in plasma. Two recently set up measurement methods are being evaluated. These are kinetic measurement procedure where TR-ACP inhibition with fluoride and TR-ACP 5a with heparin is used, and immunechemical method that uses monoclonal antibody for TR-ACP 5b.

TR-ACP activity is increased in patients with different diseases, as shown in Table 7, especially in bone metastases. It is also considered as a second class bone resorption marker, especially in monitoring patients suffering from Paget's disease who received biphosphonate therapy.

Total activity measurement of TR-ACP as a bone resorption marker is currently not recommendable given the low enzyme activity and inhibitor presence in serum, instability at alkaline pH, the fact that L(+) tartarate is a competitive inhibitor, and strong interferential hemolysis.

Hydroxylysine-glycosides (Hyl-Glyc)

Galactosyl hydroxylysine (GHL) and glucosyl-galactosyl-hydroxylysine (GGHL) both originate from lysine during posttranslatory phase of collagen synthesis and are re-

Tablica 7. Klinička stanja povezana s promjenama aktivnosti TR-ACP

Table 7. Clinical conditions associated with changes in TR-ACP activity

Condition	TR-ACP
Increase	
Metastasis carcinoma	+++
Osteomalacy	++
Paget's disease	+
Primary hyperparathyreoidism	++
Osteoporosis	+
Hyperthyreoidism	+
Multiple myeloma	+
Gaucher's disease	++
Hairy cell leukaemia	++
Decrease	
Hypothyroidism	-

(+ to ++) = relative increase; (-) decrease

taviti prikladan imunokemijski postupak i svakako načiniti konačnu evaluaciju kliničke vrijednosti ovog biljega (1,6,12).

Ostali proteini koštanog matriksa

Manji je broj literaturnih podataka o dva proteina matriksa koji, kako se čini, imaju određeni potencijal kao biljezi pregradnje kosti.

Osteonektin je sekrecijski Ca-vezujući glikoprotein koji je nađen u raznim stanicama, uključujući osteoblaste, endotelne stanice i fibroblaste. Prisutan je u aktivnim osteoblastima i mladim osteocitima (ali ne i u mirnim/tihim osteocitima) pa se smatra pogodnim biljegom diferenciranja osteogenih stanica kosti, ukazujući na izgradnju kostiju. Međutim, budući da je prisutan i u većem broju vezivnih tkiva te u trombocitima, smanjena mu je uloga kao cirkulirajućeg biljega.

Koštani sijaloprotein (engl. *Bone Sialoprotein*, BSP) je fosforilirani glikoprotein, značajno postranslacijski modificiran, a sintetiziraju ga osteoblasti i odontoblasti. Suprotno drugim fosforiliranim glikoproteinima koštanog matriksa (osteonektin), BSP je relativno ograničen na kost. Stimulira stvaranje hidroksiapatita *in vitro*, a pretpostavlja se da djeluje i kao stanična adhezijska molekula omogućujući stanicama (osteoklastima) da se pričvrste na izvanstanični matriks. Za sada su raspoloživi samo preliminarni rezultati o procjeni vrijednosti ovog biljega koštane pregradnje (ELISA) kod bolesnika s ranim reumatoidnim artritisom (povećana vrijednost u serumu) kao i u sinovijalnoj

leased into circulation during collagen resorption. GHL is relatively more specific for bone matrix resorption than GGHL, and without doubt more specific than, e.g., OHP. The real advantage of Hyl-Glyc as a resorption marker is the fact that these forms are not metabolized and are not affected by nutrition factors. In urine, they can be measured by HPLC. It is recommended to set up an appropriate immunochemical method and to make final evaluation of this marker for clinical significance (1,6,12).

Other bone matrix proteins

There is a small number of published scientific data about two matrix protein that seem to have certain potential as bone resorption markers.

Osteonectine is secreted as Ca-binding glycoprotein found in different cells, including osteoblasts, endothelial cells and fibroblasts. It is present in active osteoblasts and young osteocytes (but not in inactive osteocytes) and therefore it is considered suitable as a marker for differentiation of osteogenetic bone cells indicating bone formation. However, due to its presence in the majority of connective tissues and in platelets, its role as a circulating marker is diminished.

Bone Sialoprotein (BSP) is a phosphorylated glycoprotein, significantly posttranslationally transformed and synthesized by osteoblasts and ondoblasts. In contrast to other bone matrix phosphorylated glycoproteins (osteonectin), BSP is relatively limited to the bone. It stimulates *in vitro* formation of hydroxyapatite and is assumed to act as a

tekućini bolesnika s reumatoidnim artritisom kod kojih je uznapredovao poremećaj funkcije zgloba (nađene su veće vrijednosti nego u bolesnika s očuvanom strukturom zgloba). Opažena je također povećana koncentracija cirkulirajuće frakcije u postmenopauzalnih žena u usporedbi s premenopauzalnim referentnim vrijednostima (1). Kao dobar pokazatelj mogućih fraktura i koštane mase spominje se i katepsin K, koji je cisteinska proteaza, važna u procesu razgradnje kostiju (23,24,25).

Na temelju literaturnog pregleda može se zaključiti:

Mjerenje aktivnosti **B-ALP** ima prednost pred mjerenjem ukupne aktivnosti ALP zbog veće dijagnostičke osjetljivosti i specifičnosti. Imunokemijski postupci za mjerenje B-ALP pokazuju križnu reaktivnost (14-20%) s jetrenom ALP. Međutim, to ne umanjuje kliničku korisnost ovog mjerenja, osim kod bolesnika s teškim oblicima bolesti jetre.

OC je protein specifičan za kost, ali pokazuje brojne nedostatke u smislu nestabilnosti u uzorku i neusklađenosti rezultata dobivenih raznim mjernim postupcima. Međutim, u nekim situacijama (kortikosteroidna osteopenija, odsutnost razorene strukture kosti), OC može poslužiti kao osjetljivi biljeg koštane pregradnje.

Izlučivanje poprečnih veza kolagena mokraćom djelomično je evaluirano u analitičkom smislu i u smislu kliničke korisnosti na temelju čega se smatra da ovaj biljeg može zamijeniti mjerenje OHP kao postupak izbora za procjenu razgradnje kosti.

Ostali **razgradni produkti telopeptida kolagena tipa I** (NTX, CTX) mjere se kao pokazatelji razgradnje kostiju, ali još uvijek zahtijevaju detaljnu evaluaciju, posebno u smislu njihova izvankoštanog klirensa i mogućih drugih izvora izvan kosti. Njihova klinička korisnost varira ovisno o skupini bolesnika koja se ispituje.

Mjerenje aktivnosti **TR-ACP** ne preporučuje se za upotrebu.

U tablici 8. sažeto je prikazana dosadašnja prosudba kliničke korisnosti biljega pregradnje kostiju.

Za izbor biljega pregradnje kostiju važna je mogućnost međulaboratorijske usporedivosti rezultata i dobra definicija biljega koji će se koristiti. Bitno je uspostaviti referentne intervale, standardizirati mjerne postupke, odrediti točan koncept kvalitete osiguranja, pokušati smanjiti individualnu varijabilnost mjerenjem biljega u serumu, a ne u mokraći, automatizirati mjerne postupke odgovarajućom kalibracijom, te ustanoviti stabilnost ključnih reagensa.

S obzirom na zastupljenost osteoporoze u široj populaciji te vrste primijenjene antiresorpcijske terapije, IOF (engl. *International Osteoporosis Foundation*) 2000. godine objavila je preporuke o korištenju vrste (biljezi izgradnje kostiju B-ALP, OC i PINP u serumu; biljezi razgradnje kostiju NTX u mokraći, CTX u serumu i mokraći, DPD u mokraći) i broja koštanih biljega, uzorkovanju i intervalima mjerenja

cell adhesion molecule, enabling cells (osteoclasts) to attach to extracellular matrix. Currently there are only available preliminary test results on evaluation of this bone remodeling marker (ELISA) in patients suffering from the early stage of rheumatoid arthritis (elevated values in serum), as well as in synovial fluid of patients suffering from rheumatoid arthritis with progressed joint function disorder (higher values were found than in patients with preserved joint structure). Elevated concentration of circulating fractions in postmenopausal women is also noticed in comparison to reference values in premenopausal period (1). As a good indicator of possible fracture and bone mass, cathepsin K has also been mentioned, which is a cysteine protease important in the bone resorption process (23,24,25).

Based on the published scientific data review, the following can be concluded:

B-ALP activity measurement has an advantage compared to the measurement of total ALP activity because of greater diagnostic sensitivity and specificity. Immunochemical methods for B-ALP measurement show cross reactivity (14-20%) with liver ALP. However, this does not diminish clinical efficacy of these measurements, except in patients with severe liver diseases.

OC is a bone specific protein but it shows numerous disadvantages like instability in the sample and differences in test results due to different measurement methods. However, in some diagnostic situations (corticosteroid osteopenia, lack of destroyed bone structure) OC can be used as a sensitive bone remodeling marker.

The urinary excretion of collagen cross-links is partly evaluated analytically but, based on its clinical efficacy; it is considered that this marker can replace OHP measurement as the method of choice for bone remodeling evaluation.

Other **telopeptide type I collagen resorption products** (NTX, CTX) are measured as bone resorption indices; however, they demand further evaluation, especially of their extraosseous clearance and other possible extraosseous sources. Their clinical efficacy varies depending on which patient group is examined.

TR-ACP activity measurement is not recommended for clinical use.

Table 8 summarizes the evaluated clinical efficacy of bone remodeling markers.

In order to select a bone remodeling marker, it is important to do inter-laboratory comparisons and to define the characteristics of the bone marker to be used; to establish reference intervals, to standardize measurement methods, to establish a good quality assurance program, to try to diminish individual variability by measuring markers in serum and not in urine, to use automated measurement methods with appropriate calibration, and to test the stability of reagents.

Tablica 8. Kritička prosudba kliničke korisnosti biljega pregradnje kostiju

Table 8. Critical evaluation of clinical efficacy of bone remodeling markers

Marker	Sample	Very useful	Useful	Not useful
B-ALP	S	X		
OC	S/P		X	
PICP	S			X
Cross-links	U	X		
N-terminal telopeptide type I collagen	U		X	
CrossLaps	U		X	
C-terminal telopeptide type I collagen	S			X
TR-ACP	S/P			X

S (serum); P (plasma); U (urine)
B-ALP - Bone Alkaline Phosphatase; OC - Osteocalcin; PICP - C-terminal type I procollagen propeptide; TR-ACP - Tartarat-resistant acid phosphatase

koncentracije biljega za vrijeme terapije te graničnim vrijednostima s obzirom na procjenu rizika nastanka prijeloma i moguće malignosti. Preporuka je koristiti jedan koštani biljeg ili jedan biljeg izgradnje i jedan biljeg razgradnje kostiju, pri čemu se prednost daje mjerenju biljega u serumu. Krv treba uzeti natašte prije 9 sati; ako se biljeg mjeri u mokraći, preporuka je koristiti prvi ili drugi jutarnji uzorak mokraće. Preporuča se mjerenje biljega izgradnje kostiju prije i nakon 6 mjeseci terapije, a biljega razgradnje kostiju prije i nakon 3 ili 6 mjeseci terapije (25).

Zaključak

Zaključno treba naglasiti da je i nadalje neosporna potreba dugotrajnih longitudinalnih ispitivanja biokemijskih biljega pregradnje kostiju na velikom broju ispitanika uz korelaciju s referentnim postupkom mjerenja koštane mase. Potrebna će nadalje biti racionalizacija korištenja biljega ili kombinacije biljega za odgovarajuću bolest kostiju kako bi se uklonili oni s niskom kliničkom korisnošću. Najveći izazov za skoru budućnost je dakako kombinacija genetičkih i biokemijskih biljega u procjeni rizika osteoporoze te ostalih bolesti kostiju.

Considering the frequency of osteoporosis cases in a wider population and different types of antiresorption therapy, *International Osteoporosis Foundation* (IOF) published in 2000 some guidelines to use on various types (bone formation markers B-ALP, OC and PINP in serum; bone resorption markers NTX in urine, CTX in serum and urine, DPD in urine) and number of bone markers, sampling and measurement intervals of bone marker concentrations during therapy periods, and limiting values considering risk assessment for fracture and possible malignancy stages. These guidelines recommend the use of one bone marker or one bone formation and one bone resorption marker, with the priority given to the measurement of a marker in serum. Blood should be collected after an overnight fast, before 9 a.m.; if a marker is measured in urine, it is recommended to use 1st or 2nd morning urine sample. It is recommended to measure bone formation markers before and 6 months after therapy, and bone resorption markers before and 3 or 6 months after therapy (25).

Conclusion

To summarize, there is a need for long-lasting longitudinal studies of biochemical bone remodeling markers on a great number of individuals with correlation to reference procedures of bone mass measurement. Furthermore, rationalization in marker usage or their combination will be essential in order to eliminate those with low clinical value. Indeed, the greatest challenge of the future is the combination of genetic and biochemical markers in risk assessment of osteoporosis and other bone diseases.

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