

## TARTARATE-RESISTANT ACID PHOSPHATASE, OSTEOCALCIN AND N-TERMINAL PEPTIDE OF PROCOLLAGEN IN THE DIAGNOSTICS OF OSTEOPOROSIS

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**Summary:** The aim of our work was to determine whether serum levels of tartarate-resistant acid phosphatase (TRACP), osteocalcin (OC) and N-terminal peptide of procollagen (PINP) as markers of bone turnover could be useful in the diagnostics of osteoporosis and in distinguishing between osteoporosis and osteopenia. TRACP activity was estimated by the kinetic method, while OC and PINP concentrations were measured by the electrochemiluminescent procedure. Statistically significant differences between TRACP activity in patients affected by osteoporosis and both that in patients with osteopenia and the reference values were observed, while no changes in serum OC and PINP levels were recorded. Results suggest that determination of serum TRACP activity could improve the diagnostic potential of osteoporosis. It could be helpful in distinguishing between osteoporosis and osteopenia.

**Key words:** tartarate-resistant acid phosphatase, osteocalcin, N-terminal propeptide of type I procollagen, osteoporosis, osteopenia

### Introduction

Bone turnover (remodelling) represents a continuous process proceeding in three phases: resorption, formation and mineralization (1). This dynamic and equilibrated process provides specific architecture, volume and bone density, enabling bone tissue to respond to biomechanical and metabolic environmental requests (2). Bone mass achieved during youth is decisive for adulthood and old age, and a slow loss of bone mass in humans begins before 30 years of age (3). Disturbances in the equilibrium of formation of new and resorption of already formed bone tissue lead to the development of metabolic bone diseases.

Osteoporosis is one of the most frequent metabolic bone diseases. According to the definition of the World Health Organization (WHO), osteoporosis is

characterized by decreased mineral density, changes of microarchitecture and reduced biomechanical properties of bones that could lead to bone fractures and deformities (4). Numerous risk groups with metabolic bone diseases (aged individuals, patients on hemodialysis, immunosuppressive therapy, individuals with different chronic diseases that negatively influence bone metabolism, etc.) indicate that in the near future the number of patients with such pathology will be increasing, and even at present bone metabolic disturbances represent a significant problem from both medical and economical points of view.

Identification of individuals belonging to the group with a high risk of bone diseases, bone fractures or significant bone mass loss represents one of very important challenges in medicine (5). The development of osteoporosis depends on the interaction of different environmental (life style, diet, additional diseases and corresponding therapies, etc.) and genetic factors.

Diagnostics of bone metabolic diseases is faced with many problems due to the fact that bone meta-

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bolism, dynamics and structure are influenced by numerous factors that lead to different changes in interior bone structure, all having similar clinical outcome in the sense of decreased biomechanical bone characteristics and increased risk of bone fractures (1).

Histomorphometry of bioptic bone samples represents the most reliable diagnostic method, especially in differential diagnosis of metabolic bone diseases. However, this method is used more frequently for scientific research than in clinical practice. For routine diagnostics of osteoporosis, radiological examination of the skeleton, as well as changes in bone density estimated by densitometry, are usually applied. Since bone quality depends not only on bone density but also on geometrical structure, elasticity and interior microarchitecture, numerous recent studies were focused on the role new biochemical markers could play in the diagnostics of bone diseases. Priority is given to the estimates of disease etiology, identification of factors negatively affecting bone metabolism and keeping up with the influence of therapy (6). Nevertheless, opinions of authors involved in this topic differ very much, from those believing that biochemical markers are insufficiently specific parameters for the diagnosis of metabolic bone diseases, to those claiming that recently detected markers of bone turnover can significantly improve the diagnostic potential of these diseases (7–11). This led to a comparison of classical biochemical analyses (determination of calcium concentration in the blood serum and 24-h-urine, phosphorus, creatinine, alkaline phosphatase and numerous hormones) with markers of bone synthesis and disintegration (12).

All the above controversies and dilemmas prompted us to study tartarate-resistant acid phosphatase (TRACP), osteocalcin (OC) and N-terminal peptide of procollagen (PINP) as biochemical markers of bone turnover in patients with osteoporosis and osteopenia.

Determination of catalytic TRACP activity in blood serum estimates osteoclastic activity, because it is secreted by cells during bone resorption (3). On the other hand, OC represents a specific product of osteoblasts and the major non-collagen protein of the bone matrix. This protein consists of 49 amino acids, three of which represent gamma-carboxy glutamic acids responsible for Ca-binding properties of OC (6). Also, osteocalcin is considered to be a sensitive and specific marker of bone synthesis, and it has been successfully applied in monitoring antiresorptive therapy of osteoporosis (13).

During the first step of the collagen type I synthesis, that makes 90% of the bone matrix, procollagens with bulky C- and N-terminal domains get secreted and released from the cells to be decomposed by protease action to propeptides PICP and PINP, and their presence in the circulation is taken as a measure of the active collagen synthesis (6). So, PINP concentration in the serum is proportional to the bone mineralization process and synthesis of bone matrix.

The aim of the present study was to determine whether the above biochemical markers of bone turnover could be significant in the diagnostics of osteoporosis, as well as in distinguishing this disease from osteopenia.

## Material and Methods

**Patients.** This study included 120 patients divided into two groups. The first group (n=60) consisted of patients (34 to 77 years old, average age 54.2 years; 50 women and 10 men) with diagnosed osteoporosis confirmed in the out-patient department of the Clinic for Orthopedics and Traumatology, Clinical Center, Banja Luka. According to the WHO standards, bone mineral density (BMD) served as a criterion for the diagnosis. The BMD of –2.3 in this group determined by the ultrasonic technique was compared with that of young and healthy population (control) using the T-score (standard deviation). According to the WHO standards, T values from –2.5 to –1 point to osteopenia, as found in the second group of patients (n=60; T values ranging from –0.4 to –1.7) consisting of 49 women and 11 men, average age 46.1 years, age range from 18 to 66 years.

**Determination of serum TRACP activity.** Catalytic TRACP activity in the blood sera was determined by the kinetic method, using BIOMERIEUX reagents (Germany) and a Mira Cobas plus analyzer (Germany).  $\alpha$ -Naphthyl phosphate dissolved in citrate buffered solution served as a substrate. After the incubation period, the amount of evolved  $\alpha$ -naphthol in the presence of a diazonium salt Fast Red TR was measured colorimetrically, within the reference range from 1.7 to 3.2 U/L.

**Measurement of OC and PINP levels.** Concentrations of serum OC and PINP were determined by the electroluminescent technique (ECL) using reagents produced by ROCHE (Germany) and an Elecsys 2010 analyzer (Germany) within the reference concentration range from 11 to 43 ng/mL, i.e. 20 to 100 ng/mL for OC and PINP, respectively.

## Results

As depicted in *Figure 1*, catalytic TRACP activities were  $5.49 \pm 1.63$  U/L and  $3.51 \pm 1.14$  U/L in the blood sera of patients with osteoporosis and those with osteopenia, respectively. The difference in the activity of this enzyme between these two groups was of a high statistical significance ( $p < 0.001$ ). Comparison of these results with the reference range of 1.7 to 3.2 U/L revealed statistically significant difference only in case of values found in examinees with osteoporosis ( $p < 0.001$ ), while that observed between TRACP activity in patients with osteopenia and the reference values was statistically insignificant.

Serum OC level was higher in the group of patients with osteoporosis in relation to the group with

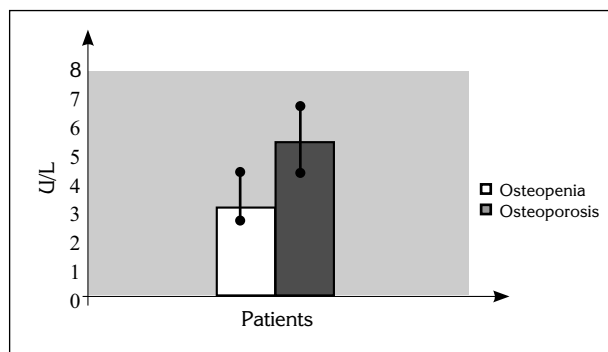


Figure 1 Catalytic activity of tartarate-resistant acid phosphatase in the blood sera of patients with osteoporosis and osteopenia

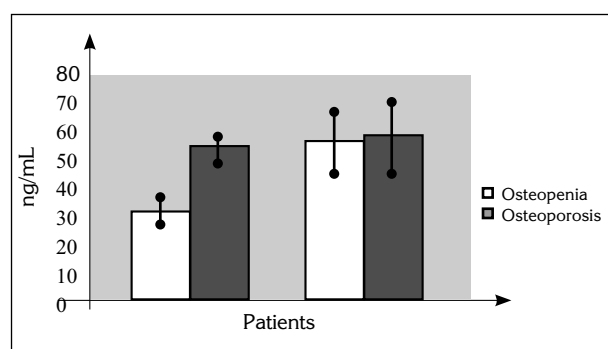


Figure 2 Serum levels of osteocalcin (left) and N-terminal procollagen peptide (right) in patients with osteoporosis and osteopenia

osteopenia ( $32.07 \pm 6.24$  ng/mL vs.  $29.26 \pm 3.65$  ng/mL) and the difference was statistically significant ( $p < 0.01$ ). However, no statistically significant differences were observed between either of the two groups and the corresponding reference values (Figure 2).

The data on the content of PINP in blood sera are presented in Figure 2. It can be seen that the PINP levels were  $58.60 \pm 12.85$  and  $55.9 \pm 11.3$  ng/mL in patients with osteoporosis and osteopenia, respectively. The difference between these two groups was not statistically significant, and neither was that one observed between either of the groups and the corresponding reference values.

## Discussion

Bone densitometry does not distinguish osteoporosis from osteomalacia or other bone diseases characterized by decreased bone density. Besides, this approach does not provide data on the kind of bone metabolism disturbances necessary for the prescription of the most adequate therapy (1). Different disturbances in bone tissue turnover can result in metabolic bone diseases and, related to that, disbalance between resorption and bone formation or defects in bone mineralization (14). These can be conditions characterized either by an increased rate of bone resorption that is dominant in relation to bone

formation (active osteoporosis), or decreased bone formation (inactive osteoporosis) (1).

In the present study, the T-score value, generally accepted as the WHO criterion for osteoporosis, was applied. Since the etiology of osteoporosis was not taken into consideration, while the examined groups of patients were not sex-matched, this work did not provide data that could be useful in choosing the most adequate therapy.

The estimate of a possible risk for bone fractures correlates with the loss of bone mass, but it is difficult to distinguish between osteopenia and osteoporosis, taking into account the upper limit of the T-score of  $-2.5$ . Numerous prevention measures related to osteoporosis development became quite known to the public (15), but it seems that osteopenia remained somewhat neglected in this regard. It is of great importance, therefore, to improve the diagnostics of osteoporosis and osteopenia using reliable biochemical parameters (16).

With respect to bone diseases, biochemical markers represent the molecules directly connected to both the structure and function of bone tissue. The fact that changes in either the concentration or activity of these biochemical markers are reflecting dynamic status of bone metabolism is taken as advantageous. There are three groups of biochemical markers of bone turnover. The first group, that includes markers related to calcium and phosphorus homeostasis, has been used for a long time in laboratory diagnostics. The second group of biochemical markers consists of enzymes reflecting the activity of osteoblasts and osteoclasts, while recently identified markers of bone turnover, subdivided into markers of bone formation and bone disintegration, belong to the third group. During the choice of variables determined throughout the present work, this classification of bone biochemical markers was taken into account. So, OC as a marker of bone formation, PINP representing an indicator of mineralization process and bone matrix formation, and catalytic TRACP activity as a measure of osteoclastic activity were selected.

Results pointed to the significance of the serum TRACP activity in recognizing disturbances in the bone metabolism. Statistically significant differences in the catalytic activity of this enzyme between the two groups of examinees, as well as in relation to the reference range, support and confirm the data of Topić et al. (3), while being in disaccord with the report of Petro (6). The results demonstrating significantly higher TRACP activity in the sera of patients with osteoporosis in relation to reference values are of utmost importance, in spite of the opinion of some authors that determination of TRACP activity is a very expensive method for everyday practice. However, the specificity of this enzyme is still in question. Recent reports of Halleen et al. (17) and Nenonen et al. (18) pointed to TRACP 5b as a specific and sensitive marker of bone resorption because its activity was

significantly increased in osteoporosis, but also in osteopenia. This suggests that the determination of total TRACP catalytic activity and of its isoenzyme forms in the cases of decreasing bone mass could help to better understand this topic.

In our opinion, significant difference in the catalytic activity of TRACP between the groups with osteoporosis and osteopenia observed in the present study is very important, especially having in mind the limitations of the T-score analysis. The analysis of individual TRACP values in the group with osteopenia showed that some 2/3 of examinees had values above the upper limit of the reference range. This result could be taken into account during the diagnostics of osteopenia.

Opposite to the data of Stavropoulou et al. (13) obtained on experimental animals, no statistically significant differences were observed when the OC concentrations of either group were compared with the corresponding reference range. On the other hand, our results agree well with the data of Topić et al. (3) who found increased osteocalcin levels in 30% of patients with primary osteoporosis. However, in the present study, statistically significant differences in the OC level between the group with osteoporosis and that with osteopenia were observed ( $p < 0.01$ ), pointing to the differences in bone turnover in these two groups of examinees.

No statistically significant differences were recorded between the PINP level of either of the examined groups and reference range. This is in accordance with previous clinical studies showing a regular interrelationship of PICP, as well as of PINP and OC, considered to be less sensitive markers for the estimation of bone turnover. It should also be mentioned that although originating predominantly from bone tissue, type I collagen originates from some other tissues as well.

Analysis of all results on the PINP concentration in patients with osteoporosis and those with osteopenia showed that they were significantly under the lower limit of the reference range, i.e. under 20 ng/mL. This strongly suggests that a new reference range of our own which would help in the correct interpretation of results should be introduced.

On the basis of results obtained throughout the present study, it can be concluded that, together with other biochemical markers of bone turnover, determination of catalytic TRACP activity in the blood sera can improve not only the diagnostic potential of osteoporosis, but also its distinction from osteopenia. At the same time, serum concentrations of either OC or PINP do not represent reliable parameters for distinguishing between these two bone diseases.

## TARTARAT-REZISTENTNA KISELA FOSFATAZA, OSTEOKALCIN I N-TERMINALNI PEPTID PROKOLAGENA U DIJAGNOSTIKOVANJU OSTEOPOROZE

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*Kratak sadržaj:* Cilj rada je bio da se utvrdi da li tartarat-rezistentna kiselina fosfataza (TRACP), osteokalcin (OC) i N-terminalni peptid prokolagena (PINP) kao serumski markeri koštane pregradnje mogu pomoći u dijagnostikovanju osteoporoze i razlikovanju ovog oboljenja od osteopenije. Katalitička aktivnost TRACP mjerena je kinetičkom metodom, dok su koncentracije OC i PINP određivane elektrohemiluminiscentnim postupkom. Uočena je statistički značajna razlika između katalitičke aktivnosti TRACP u ispitanika sa osteoporozom u odnosu na referentne vrijednosti, kao i u odnosu na vrijednosti dobijene u ispitanika sa osteopenijom. Međutim, koncentracije OC i PINP u serumima pacijenata sa osteoporozom nisu bile statistički značajno izmijenjene. Ovi rezultati sugerišu da određivanje aktivnosti TRACP u serumu može da unaprijedi ne samo dijagnostički potencijal osteoporoze, već i razlikovanje ovog oboljenja od stanja osteopenije.

*Ključne riječi:* tartarat-rezistentna kiselina fosfataza, osteokalcin, N-terminalni peptid prokolagena, osteoporoza, osteopenija

## References

1. Krpan D. Patogeneza i diferencijalna dijagnoza metaboličkih koštanih bolesti. Osteoporoza 15. simpozij Hrvatskog društva medicinskih biohemičara, Zagreb, Hrvatska, 2004.
2. Bonjour JP, Tsang RC. Nutrition and Bone Development. Lippincott-Raven, 1999; 19–243.
3. Topić E, Primorac D, Janković S. Medicinsko-biokemijska dijagnostika u kliničkoj praksi. Medicinska Naklada, Zagreb, Hrvatska, 2004.
4. Beastall GH. Evidence-based osteoporosis. Jug Med Biohem 2004; 23: 213–19.
5. Toman Lj. Genetika osteoporoze. Osteoporoza 15. simpozij Hrvatskog društva medicinskih biohemičara, Zagreb, Hrvatska, 2004.
6. Petro D. Biokemijski biljezi u dijagnostici metaboličkih koštanih bolesti. Osteoporoza 15. simpozij Hrvatskog društva medicinskih biohemičara, Zagreb, Hrvatska, 2004.
7. Risteli J. Biomarkers of Bone Formation. EMBODY, Cambridge, UK, 2000.
8. Robins SP. Biochemical Markers of Bone Resorption. EMBODY, Cambridge, UK, 2000.
9. Emerk K. Bone markers and osteoporosis. Jug Med Biohem 2004; 23: 221–8.
10. Majkić-Singh N. The role of biochemical markers in the detection of bone metabolism and diseases. Jug Med Biohem 1997; 16: 185–196.
11. Majkić-Singh N. The biochemical markers of osteoporosis. Scientific Review. Serbian Scientific Society, Series: Medical and Biological Science, 2000; 27: 57–74.
12. Garnero P. Clinical Uses of Bone Markers in Osteoporosis. EMBODY, Cambridge, UK, 2000.
13. Stavropoulou A, Christopoulou GE, Anastassopoulos G, Panteliou SD, Lyritis GP, Spiliotis BE, et al. Alteration in serum leptin correlates with alterations in serum N-telopeptide of collagen type I and serum osteocalcin during the progression of osteoporosis in ovariectomized rats. Clin Chem Lab Med 2005; 43 (12): 1359–65.
14. Juppner H. Role of parathyroid hormone-related peptide in skeletal development. In: Bonjour JP, Tsang RC. Nutrition and Bone Development. Lippincott-Raven, 1999.
15. Hodgson S, ed. Mayo Clinic o osteoporozi. Medicinska Naklada, Zagreb, Hrvatska, 2005.
16. Kleerekoper M. Use of bone markers in the management of women with low bone density. Meno Med 2002; 10: 5–9.
17. Halleen JM, Alatalo SL, Janckila AJ. Serum tartarate-resistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. J Bone Miner Res 2000; 15 (1 Suppl): 524S.
18. Nenonen A, Cheng S, Ivaska KK, Alatalo SL, Lehtimäki T, Schmidt-Gayk H, et al. Serum TRACP 5b is a useful marker for monitoring alendronate treatment: Comparison with other markers of bone turnover. J Bone Miner Res 2005; 20 (8): 1804–12.

*Received: June 15, 2006*

*Accepted: August 12, 2006*

