Special Article

Practical Experience in Some Aspects of Bone Markers

Narong Bunyaratavej MD*

** Department of Orthopaedic Surgery, Siriraj Hospital, Mahidol University, Bangkok, Thailand*

The biochemical bone markers are the firmly approved means for bone assessment with high accuracy for detecting the status of bone functions in real time. The values obtained from the bone markers are continuously fluctuating. The interpretation, thus, needs to be based on the controlled clinical criteria such as fasting blood, venous puncture required at 8:00-9:00, gender, age and information of previously medicated treatments. These characteristic values are compared with the normal ones derived by the healthy young adult persons. The bone markers are essentially requested when diagnosing the bone state. Both formation and resorption markers represent bone turnover. The low values of bone markers are the signs of being low bone turnover as well as the high values show high bone turnover. The bone markers strongly support the diagnosis of bone metabolism, bone diseases and the evaluation of medication and bone metastasis.

Keywords: *Bone marker*

J Med Assoc Thai 2009; 92 (Suppl 5): S116-20
Full text. e-Journal: *http://www.mat.or.th/journal*

There is a diversity of bone assessments. The roentgen film is generally used to identify the form of bone particularly to diagnose fracture. Bone densitometry shows the mineral parts of the bone called bone density. The function of bone cells can be detected by the remnant parts of bone texture such as collagen, bone proteins and bone cells' enzymes which are released to the circulation during the procedures of bone formation or resorption by osteoblast and osteoclast respectively. These can be indicated by antibody-antigen reaction although it is in a tiny amount (nanogram). This process can be successfully done by the biochemical bone markers.

The benefits of bone markers are their valuable interpretations of bone function such as drug response, efficacy of bone medicine. These interpretations enhance physicians' decisions for the continuation of the drug treatment in metabolic bone disease and for the selection of the suitable time for joint replacement of bone fixation.The joint replacement will be loosed if the bone is in the state of resorption. Moreover, the bone markers can detect the bone metastasis of some tumors. The nutritional status of patients can also be shown by the bone marker application. High bone resorption, for instance, can be interpreted that it is caused by habitual consumption of more than one liter of carbonated beverages per day.

Bone markers are categorized into two types: formation markers such as Procollagen type 1 nitrogenous peptide (PINP), Osteocalcin, Alkaline phosphatase; resorption ones such as BetacrossLap or CTx, Pyridinoline, etc. The osteocalcin is recognized as the bone formation marker, but it can also be the marker of resorption in some situations such as whenever the value of CTx is high, the state of bone resorption is implied. The high value of bone resorptive marker, or osteocalcin certainly represents the bone resorption or vice versa. The intact osteocalcin is unstable in the circulation that usually degrades into many fragments. The stable fragment is NMID osteocalcin containing 43 amino acids in its molecule that can be detected in either blood or urine. Thus, NMID is a well accepted representative of osteocalcin (Fig. 2).

The immature osteocalcin or under carboxylated osteocalcin which contains glutamic acid in 17th, 21st, and 24th residues of its molecule is also the marker of osteoblastic function (Fig. 1). The high level of this marker is recognized as the poor function of bone formation, or vitamin K2 deficiency. The high level of

Correspondence to: Bunyaratavej N, Department of Orthopedic Surgery, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: 0-2419-4545. E-mail: todrnarong @yahoo.com

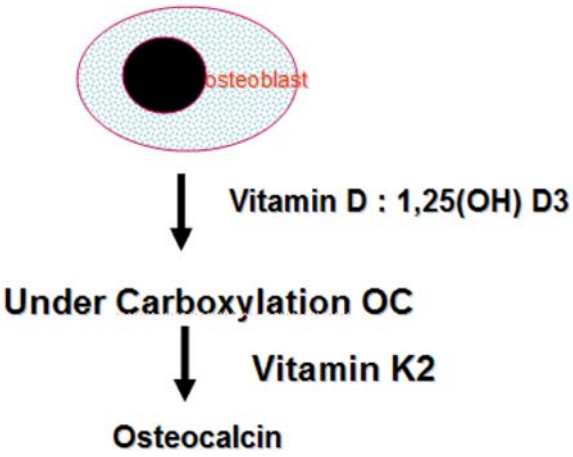

Fig. 1 The osteoblast synthesizes the osteocalcin molecule. At the first step, it needs vitamin D for the formation of immature osteocalcin, undercarboxylated osteocalcin (UcOC). This molecule will be changed into the mature osteocalcin by a cofactor, vitamin K2

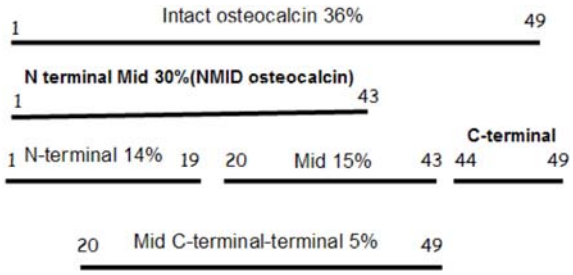

Fig. 2 The osteocalcin molecule consists of 49 amino acids called intact osteocalcin, but this molecule in the circulation will be disintegrated into fragments. The main fragment is N-terminal-Mid-osteocalcin or NMID osteocalcin which is a stable form. It is recognized as a representative of osteocalcin

under carboxylated osteocalcin is possibly related to the low bone mass in the elderly[6]. This molecule will be later changed into the mature form by vitamin K2.

Evaluation

In order to achieve effective interpretation, the following recommendations should be considered: 8-hour fasting, avoiding any kind of 3-12-month medication, venous blood puncture performed between 8:00-9:00 a.m., a pair test of both formation and resorption markers requested. If this recommended technique is not complied with, repetition of any actions is necessarily done at the same exact time when the previous actions had occurred before. The values of the second actions conducted in the same conditions are the baseline control; for example, the formation marker value is conducted in the third month; the resorption value needs to be done in the third month thereafter, neither before nor after.

The collected data are compared with the standard values when undergoing evaluation: 1) BetacrossLap 0.293-0.393 ng/ml in female[1], 0.359-0.464 ng/ml in male, and, 2) PINP 40.78-48.35 nanogram/ml in female[2], PINP 48.0-68-.6 nanogram /ml in male (Table 1, 2).

The interpreters inevitably answer the following questions:

a) Does the screening blood show the abnormal value after being compared with the standard values?

b) What are the values of bone formation and resorption and are both values in accord with the same direction, *i.e.* both values are either high or low?

c) If both values are low, is that bone function low turnover?

d) If both values are high, is that bone function high turnover?

e) If the formative markers are high and the others are low, does that mean the osteoclastic function is poor?

Table 1. The normal values of bone markers in the healthy, reproductive females[1]

	Mean*	95% CI
BetaXLaps** (n = 356)	0.310 ± 0.169	0.293-0.328
NMID** (n = 123)	16.460 ± 0.179	14.900-18.020
PINP*** (n = 109)	44.500 ± 19.920	40.780-48.350

* ng/ml

Table 2. The study of bone markers in the healthy, 20-40 year males

	Mean*	95% CI
BetaXLaps** (n = 53)	0.412 ± 0.191	0.359-0.464
NMID** (n = 53)	19.650 ± 9.100	17.170-22.190
PINP*** (n = 53)	58.100 ± 37.700	48.000-68.600

* ng/ml

f) If the resorption markers are high but the formation markers are low, does that mean the osteoblastic function is abnormal?

g) Are there any extra-osseous sources interfering the bone markers, *e.g.* skin diseases and tendon disease which are common factors?

The abnormal bone turnovers or values are recognized as malfunction of bone cells. The values of bone markers, practically, should be within the normal limits that are considered as good bone function (Fig. 4).

Comparison

The investigated values are compared to the normal values of young adult males or females such as a comparison between the menopausal women's values and the young adult females' bone markers.

Pair Test

Both bone formation and resorption markers should be applied together in order to compare the bone activities reflected in turnover. The low bone formation markers and the low resorption ones indicate the status of low bone turnover. In addition, the low bone turnover is a sign of the poor osteoblastic functions.

State of blood

The standard of interpretation requires the 8-hour fasting blood and the suitable time for venous puncture is between 8:00-9:00 a.m. According to the circadian rhythm of the bone markers, the observed time is advisedly between 5:00 and 8:00 a.m. as this duration shows the highest rate of change[3]. The levels of bone markers resulted from the fasting state do not fluctuated as much as the ones of the feeding state[4]. Thus, the state of fasting is very suitable for the examination and fasting blood is also qualified for the screening blood test.

Gender

Males and females are different in bone markers (Table 1, 2). The young adults manifest a high level of bone markers.

Age

The bone marker values are at the high peak in young children at the age of before 20[5]. One-third of the elderly women manifest the low bone turnover (Fig. 3).

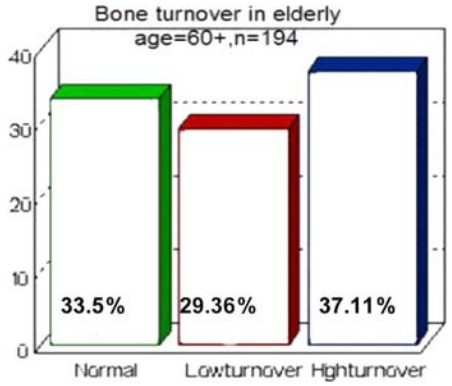

Fig. 3 The study of the 194 postmenopausal elderly women. The normal bone turnover is 33.5% while the low bone turnover is 29.36% and the high bone turnover is 37.11%

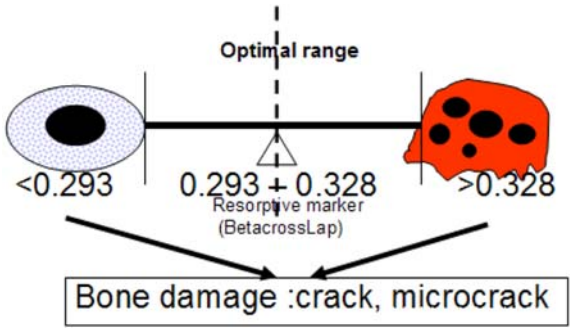

Fig. 4 The optimal range of CTx = 0.293-0.328 ng/ml if the value is lower than the optimal range or above this range. The bone will be deteriorated

Drug Monitoring

Antiresorptive therapy

The common antiresorptive agents are Bisphosphonate, Calcitonin, Estrogen, SERM etc. Bone markers of resorption are considered as the main parameter. Taking antiresorptive agents affecting CTx can lead to the decrease of resorption marker values. The more decrease of the bone markers really reflects the power of antiresorption. Thus, the sequential check-ups are necessarily done. The low or decreasing values of the resorptive markers mean the poor bone remodeling. This study shows the cut value of the bone formation markers at 0.01 ng/ml of CTx, the level of PINP and NMID are at 14.04 ng/ml and at 10.05 ng/ml subsequently. This phenomenon is considered as an indicator of the poor osteoblastic functions, *i.e.* the

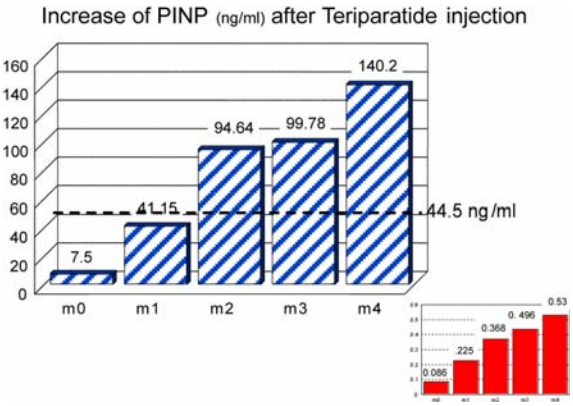

Fig. 5 Patterns of the mean of bone markers after the teriparatide injection in seventy cases of the post-menopausal women. The bone formation marker, PINP(Stripped bar) was increased as monthly as the bone resorption marker, CTx (dark column)

level of alkaline phosphatase is low while the under carboxylated osteocalcin (UcOC) is high.

Formative therapy

Some drugs, for example, vitamin K2, Parathyroid hormone (Teriparatide), acceptably, are the bone formative agents because they involve in the collagen formation, bone proteins synthesis, *e.g.* osteocalcin. The proper markers are PINP, PICP, NMID osteocalcin enhancing the evaluation of bone component synthesis. They are the early signs of osteoblastic function. However, the comparative values of these parameters in both formation and resorption need to be firstly adjusted to gain the same standard of results through calculating the CTx values derived by the second puncture. These results are compared with each value of PINP, PICP, NMID osteocalcin obtained by the first puncture considered the baseline data. The evaluated levels will indicate the actual bone formation or resorption (Fig. 5).

Suitable decisions whether the drug should be continued can be made when the drug therapy especially antiresorptive agents is carefully monitored. The prolongation of abnormal bone turnover will be harmful to the bone function. The bone markers greatly assure physicians when adjusting the optimal time for the duration of treatment.

The bone markers strongly support studying the dynamic change of bone, *i.e.* drastically quick responses in bone while the bone mass measurement shows a static status of bone.

Reference

1. Bunyaratavej N, Kittimanon N, Boonthitikul S. Study of the level of biochemical bone markers: NMID osteocalcin and bone resorptive marker (Beta CTx) in Thai women. J Med Assoc Thai 2001; 84 (Suppl 2): S560-5.
2. Bunyaratavej N, Kittimanon N. Study of procollagen type 1 nitrogenous propeptidees (PINP) in reproductive female. J Med Assoc Thai 2005; 88 (Supple 5): S27-8.
3. Fraser WD, Anderson M, Chesters C, Durham B, Ahmad AM, Chattington P, et al. Circadian rhythm studies of serum bone resorption markers: implications for optimal sample timing and clinical utility. In: Eastell R, Baumann M, Hoyle NR, Wieczorek L, editors. Bone markers: biochemical and clinical perspectives. London: Martin Dunitz; 2001: 107-18.
4. Blumsohn A, Delmas PD, Greenspan SL, McClung MF, Ravn P, Rosen CJ, et al. Discussion paper C. In: Eastell R, Baumann M, Hoyle NR, Wieczorek L, editors. Bone markers: biochemical and clinical perspectives. London: Martin Dunitz; 2001: 187-96.
5. Henry YM, Eastell R Biochemical markers of bone turnover: age, gender and race as a sources of biological variability. In: Eastell R, Baumann M, Hoyle NR, Wieczorek L, editors. Bone markers: biochemical and clinical perspectives. London: Martin Dunitz; 2001: 95-106.
6. Soontrapa S, Soontrapa S, Bunyaratavej N. Serum concentration of undercarboxylated osteocalcin and the risk of osteoporosis in Thai elderly women. J Med Assoc Thai 2005; 88 (Suppl 5): S29-32.

ประสบการณ์การใช้โบนมาร์คเกอร์

ณรงค์ บุณยะรัตเวช

โบนมาร์คเกอร์ ได้นำมาใช้ทางด้านบริการที่ รพ ศิริราช ใน ปี 2542 ทำให้สะดวกในการรักษาและทราบถึงการเปลี่ยนแปลงกระดูกได้ดียิ่งขึ้น เพราะสามารถทราบการแปลี่ยนแปลงกระดูกล่วงหน้าได้เร็วกว่าการตรวจกระดูกโดยวิธีอื่น ๆ โบนมาร์คเกอร์ที่ใช้ในปัจจุบันมี 2 ชนิดคือ ใช้วัดการสร้างกระดูก ได้แก่ NMID osteocalcin, PINP อีกชนิดใช้วัดการเปลี่ยนแปลงในการสลายตัวกระดูก ได้แก่ C-telopeptide (CTx) การแปลผลจำเป็นต้องทำตามขั้นตอน เนื่องจากค่ามาตราฐานที่ศึกษาในคนไทย ได้จากการตรวจเลือดในประชากรหนุ่มสาวที่ไม่มีโรคใด ๆ โดยเลือดที่ตรวจต้องได้จากงดอาหารอย่างน้อย 8 ชั่วโมง ไม่ได้รับประทานยารักษาใด ๆ มาก่อนไม่น้อยกว่า 3 เดือน เก็บตัวอย่าง 3 ซีซี ในช่วงเวลา 8.00-9.00 นาฬิกาใส่ในหลอดแก้วที่มี สารกันเลือดแข็งตัว K2 EDTA5.4 mg ตัวอย่างที่เก็บควรเก็บรักษาในที่เย็นและส่งตรวจภายใน 30 นาที การตรวจต้องตรวจด้วยโบนมาร์คเกอร์ทั้งสองประเภท ค่าที่วัดได้นำไปเปรียบเทียบกับค่าปกติของบุคคลวัยเจริญพันธุ์ โดยให้เข้ากับเพศเดียวกันของค่าปกติ ถ้าได้ค่าสูงกว่าทั้ง bone formation และ bone resorption markers แสดงว่ากระดูกอยู่ในสภาพ high bone turnover และในทางกลับกัน ถ้าค่าต่ำทั้งคู่ กระดูกก็อยู่ในสภาพ low bone turnover สองสภาพนี้ถ้าปล่อยให้เกิดนานจะมีผลเสียต่อกระดูกก่อนได้รับยารักษากระดูก ผู้ป่วยควรได้รับการตรวจโดยโบนมาร์คเกอร์เพื่อดูสภาพกระดูกว่าเป็นอย่างไร และเก็บเป็นค่าไว้เปรียบเทียบหลังการรักษาในระยะเวลาหนึ่งเพื่อบอกถึงผลการรักษา ในคนสูงอายุมีถึง 33% ที่มีค่าโบนมาร์คเกอร์ปกติ แต่หากได้รับยาบางประเภทจะให้โทษมากกว่าคุณ