Prediction of bone loss in elderly women using bone turnover markers

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Abstract

Osteoporosis related fractures are common in old age and over 40 % of the women above 50 years of age are at a risk of developing a fragility fracture. Biochemical markers of bone turnover (BTMs) have proven to be of some value in fracture predictability. There is also a correlation between rate of decrease of areal bone mineral density (aBMD) and incident fractures.

In this series of studies, the correlation between BTMs and rate of bone loss (change of aBMD and ultrasound variables) over 5 years was investigated in a cohort of 75 year-old Swedish women. In addition, correlation of BTMs and bone metabolism, as assessed by scintigraphy, was tested in postmenopausal women. Finally, the effect of precision error on the longitudinal monitoring of change in aBMD was assessed in elderly women and in elderly men.

There was a strong correlation between all bone turnover markers and the results of scintigraphy (total skeletal uptake of 99mTc-labelled methylene diphosphonate), with no significant difference between bone formation markers and bone resorption markers. BTMs correlated to the 5-year rate of change of aBMD, especially in the legs and the total body, and 5 year change in speed of ultrasound. When serial measurements of BTMs were analysed, the mean value of measurements correlated more strongly to aBMD change than single measurements, and women with constantly high levels of BTMs had higher rates of bone loss. Precision error of aBMD measurement by dual-energy X-ray absorptiometry has an influence on the detection of individuals with aBMD change exceeding the least significant level. The calculated follow-up interval for detection of aBMD change beyond the least significant level in more than 50% of elderly individuals ranged from 332 years, and was dependent on the equipment used and the skeletal site tested.

These results indicate that BTMs are associated with future bone loss although the correlations may not be strong enough to predict bone loss at individual level. DXA also has some limitations when used in longitudinal follow up of elderly individuals. DXA is therefore of limited use in the longitudinal monitoring of bone loss. Further studies with novel bone turnover markers may improve the ability of BTMs to predict bone loss.

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Introduction

This oration is based on the following original publications, which are referred to in the text by Roman numbers (I-V):

I. Lenora J, Norrgren K, Thorsson O, Wollmer P, Obrant KJ, Ivaska KK. Bone turnover markers are correlated with total skeletal uptake of 99mTc-methylene diphosphonate (99mTc-MDP). *BMC Medical Physics.* 2009 Mar 30;**9**:3**. (12)**

II. Lenora J, Ivaska KK, Obrant KJ, Gerdhem P. Prediction of bone loss using biochemical markers of bone turnover. *Osteoporosis International.* 2007 Sep;**18**(9):1297-305. (9)

III. Lenora J, Gerdhem P, Obrant KJ, Ivaska KK. Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data. *Osteoporosis International*. 2009 Jul;**20**(7):1225-32. (10)

IV. Ivaska KK, Lenora J, Gerdhem P, Akesson K, Väänänen HK, Obrant KJ. Serial assessment of serum bone metabolism markers identifies women with the highest rate of bone loss and osteoporosis risk. *J Clin Endocrinol Metab*. 2008 Jul;**93**(7):2622-32. (11)

V. Lenora J, Åkesson K, GerdhemPEffect of precision on longitudinal follow-up of bone mineral density measurements in elderly women and men. *Journal of Clinical Densitometry* 2010 Oct-Dec;13(4):407-12. (13)

Bone is a living tissue that is continuously subjected to resorption and formation by coordinated action of osteoclasts and osteoblasts on the surface of trabecular bone and in the Haversian canals. In a health individual about 10 % of the skeleton is remodeled each year (1), allowing the skeleton to adjust its strength to mechanical stress and to repair any microdamage (2). Bone remodeling is also necessary for maintaining the metabolic function of the skeleton and calcium homeostasis (3). During the growth period in childhood and in adolescence bone formation predominates; increasing the bone size and strength until the maximum bone mass (peak bone mass) is reached in the 2nd or the 3rd decade of life (4). After reaching the peak bone mass, there is a state of equilibrium, when the rate of bone formation equals the rate of bone resorption. After the age of 40 years the bone resorption starts to predominate over formation. In women, bone resorption is accelerated in the first few years after the menopause due to estrogen deficiency (5). Postmenopausal women decrease the BMD or lose bone at a rate of 2-5% per year (5). Individuals who lose bone at a fast rate can develop osteoporosis and get fragility fractures at early ages.

Osteoporosis is a systemic skeletal disease characterized by low bone mass, micro architectural deterioration of bone tissue leading to increased risk of fragility fracture, most commonly affecting postmenopausal women and elderly men. After 50 years of age more than 40 % of women and 13% of

men in western countries are at a risk of developing a fragility fractures at any site during the rest of their life time (6). Osteoporosis is diagnosed by measuring bone mineral density (BMD) using dual energy X-ray absorptiometry (DXA) and defined as BMD value 2.5 standard deviations or more below the mean of young female adult population. It is important to identify individuals with osteoporosis and individuals with fast bone loss to take preventive measures to avoid fractures. Fast bone losers are detected using DXA, measuring BMD at least one to two years apart. It is expensive and consumes time during which the women lose bone further.

Bone turnover markers, (BTMs) or biochemical markers of bone turnover, are bone tissue proteins or their fragments, or enzymes released from bone cells during bone turnover. Proteins can be by-products of collagen formation or products of collagen degradation, or non-collagenous proteins such as osteocalcin or bone sialoprotein. Enzymes, such as bone-specific alkaline phosphatase and tartrate-resistant acid phosphatase 5b, can also be used as bone turnover markers. Bone turnover markers can be detected in serum or urine. Ideally, they should reflect only the activity of osteoblasts or osteoclasts. Bone turnover markers that are released predominantly during bone formation or resorption are known as bone formation or resorption markers, respectively (7). Formation and resorption are usually tightly coupled in time and space and therefore, any marker reflects the overall rate of bone turnover (8). Certain bone turnover markers may reflect different stages of formation and resorption but they cannot reflect disease-specific processes or for instance distinguish between the activities at cortical or trabecular bone (8).

When we consider countries like Sri Lanka, DXA is too expensive and are available only in few centers. Compared to DXA, assays of BTMs are less expensive and affordable to perform in laboratories with ELIZA and RIA facilities.

This study was conducted in Malmö University Hospital, Lund University, Swedenas a part of the Malmo Osteoporosis Prospective Risk Assessment (OPRA) study and the main objective was to study the possibility of predication of bone loss over five years using baseline levels of BTMs as well as a serial measurement of BTMs. The Malmo OPRA cohort included a population based sample 1604, 75 year old women who were randomly selected using the population register of the Malmo city of south Sweden (9-11). For the baseline investigation, 1,604 women were invited and 1,044 (65%) participated at baseline. *The women were invited for prospective follow-up visits after 1, 3 and 5 years. These women were assessed with DXA and QUS of calcaneus at baseline and after 5 year.* Eleven BTMs were measured at baseline, after 1 year, 3 years and 5 years using protocols published earlier (9-11). BTMs measured were Bone-specific alkaline phosphatase (S-Bone ALP) and four serum osteocalcin assays (serum intact osteocalcin (S-OC[1-49]), serum N-mid osteocalcin (S-Total OC (N-Mid®)), serum total osteocalcin (S-Total OC), serum total carboxylated osteocalcin (S-cOC) as bone formation markers; as bone resorption markers serum C-terminal cross-linking telopeptides of type I collagen (S-CTX-I), serum tartrate-resistant acid phosphatase 5b (S-TRACP5b) and Urinary deoxypyridinoline (U-DPD). In addition, three urinary osteocalcin assays were also performed (U-LongOC, U-TotalOC, U-MidOC). The women included into this analysis had not taken hormone replacement therapy or bisphosphonates during the study period and within two year prior to the study.
The effect of precision error of DXA measurements on the assessment of repeated bone densitometry in elderly women and men was also studied.

Paper I: Bone turnover markers are correlated with total skeletal uptake of ^{99m}Tc-methylene diphosphonate (99mTc-MDP) (12).

This study it was aimed to study whether bone turnover, as assessed by total skeletal uptake of Technetium 99-labelled methylene diphosphonate, correlate more to bone formation markers or to resorption markers.

For this study 22 healthy post-menopausal women (aged 5280 years) were recruited (12). Bone scintigraphy procedure) was performed after injecting intravenous dose of 520 (517 15) MBq of ^{99m}Tc-MDP (Medronate®, Amersham International) at 09.00 h. Whole body imaging was performed directly (3 minutes) after injection and 5 hours after injection using a double-headed gamma camera system (Siemens Multispect 2) Total skeletal uptake (TSU) of ^{99m}Tc-MDP was calculated using 3 minutes images 5 hour images, excluding the urinary bladder and the soft tissue uptake as described by Brenner *et*

al 14). Blood and urine samples were collected at the same time for assessment of nine bone turnover markers.

There was a significant correlation between all bone turnover markers, with r-values from 0.52 ($p = 0.013$) to 0.90 ($p < 0.001$). The two bone resorption markers had numerically higher correlations (S-TRACP5b: r = 0.90; and S-CTX-I: r = 0.80) than the bone formation markers (S-Total OC: r = 0.72; and S-Bone ALP: r = 0.66), but the differences were not statistically significant. There was no correlation between the TSU of ^{99m}Tc-MDP and age, weight, body mass index or total body BMD (12).

Prediction of bone loss using bone turnover markers. Papers II, III and IV were aimed to study this objective.

Paper II, *Prediction of bone loss using biochemical markers of bone turnover.*601 women who had attended both the baseline and the 5-year DXA measurements were included for this analysis (9).

Significant associations ($p < 0.01$) in the aBMD change of the leg region (derived from the total body measurement) were found for four different S-OCs (standardized regression coefficient -$ß_{std}$ = 0.20 to -0.22), U-DPD ($ß_{std}$ = -0.19), S-TRACP5b ($ß_{std}$ = -0.19), S-CTX-I ($ß_{std}$ = -0.21), two of the three U-OC/crea ($ß_{std}$=-0.16).

After adjustment for baseline total body BMC, associations were found for all S-OC:s ($ß_{std}$=-0.11 to -0.15), two of the three U-OC:s ($ß_{std}$ = -0.14 to -0.16) and aBMD change at the total hip, and for three of the four S-OC:s ($ß_{std}$= -0.14 to -0.15), S-TRACP5b ($ß_{std}$ = -0.11), two of the three U-OC:s ($ß_{std}$ = -0.14 to -0.15) and aBMD rate of change at the femoral neck.

Paper III, *Bone turnover markers are correlated with quantitative ultrasound of the calcaneus: 5-year longitudinal data.*506 women who had attended both the baseline and the 5-year QUS measurements were included for this analysis (10). When the correlations between the baseline bone markers and 5-year prospective changes in QUS were evaluated, bone turnover markers (S-OCs, S-CTX-I, S-TRACP 5b) showed statistically significant but week correlations with SoS ($ß_{std}$ = -0.09 [$p < 0.05$] to -0.17 [$p < 0.001$]). BUA did not show a significant correlation with BTMs (10).

Paper IV, *Serial assessment of serum bone turnover markers identifies women with the highest rate of bone loss and osteoporosis risk* (11). 573 women were included from OPRA cohort.They attended both the baseline and the 5-year DXA measurements, and had given serum and/or urine samples at baseline and at the 1-, 3- and 5-year follow-ups.

Baseline BTMs showed a weak correlation with change in total body aBMD, but the association was more pronounced when we used the average of two measurements of each marker (standardised regression coefficient from ($ß_{std}$ = 0.12 to 0.23, $p < 0.01$). Adding a third and a fourth measurement further strengthened the correlation ($ß_{std}$ of up to -0.30, $p < 0.001$). Changes in BTMs did not correlate to bone loss as strongly as the average values. Women with constantly high turnover lost significantly more bone at total body (-2.6%) than women with intermediate (-1.6%) or low turnover (-0.2%, p for trend < 0.001). They also had greater bone loss at the hip (-8.3%, -6.0% and -5.1%, respectively; $p = 0.01$). Results were similar in the subgroup of women with osteopenia (11).

Paper V, *Effect of precision on longitudinal follow-up of bone mineral density measurements in elderly women and men* (13).

For this analysis, 691 women were included (13). These women had a baseline and 5-year follow-up DXA measurements available. In addition, 211 men from the Malmö part of the MrOs study who attended DXA measurements at baseline and at the 5 year follow-up were included. The MrOs study is (An international multi-centre study on risk factors for osteoporosis and fracture in elderly men).Precision error (in g/cm^2) for Lunar DPX-L in women ranged from 0.010 at TB to 0.028 at TH. Precision error using Lunar Prodigy for women ranged from 0.009 at TB and TH to 0.039 at LS). Precision error using Lunar Prodigy for men ranged from 0.007 at TB to 0.031 at LS.

Mean change in aBMD (in g/cm^2) per year in women was ranged from -0.003 (0.007) at TB to -0.011 (0.016) at TH. Corresponding results in men were -0.003 (0.006) at TB, to -0.006 (0.009) at TH.

The number of individuals with 5-year aBMD change at TB that exceeded the LSC was 244 women (38.6%) and 73 men (35.6%). The corresponding

results at TH were 265 women (41.4%) and 78 men (38.6%); at LS the numbers were 303 women (45.0%) and 51 men (24.6%).

Monitoring time interval (i.e. LSC/median rate of change in aBMD) for both populations was 8 years (for TH aBMD) and 13 years (for LS aBMD). Based on Prodigy precision data, the monitoring time intervals for women were 3 and 32 years for TH and LS, respectively (13).

Discussion

To the best of my knowledge, this study is the largest study in elderly women to assess the ability to predict bone loss over several years. The design of the OPRA study has several advantages: it has a well-defined population with a high attendance rate, a long follow-up, and the use of novel and established bone turnover markers.

The overall aim of the work described in this study was to improve the prevention of fragility fractures in the future. There are numerous risk factors for fragility fracture. Bone mineral density is one of the most important risk factors that are potentially modifiable. For diagnostic purposes, a diagnostic threshold is used for bone density test results, below which the term osteoporosis is used. However, a large proportion of individuals who sustain a fragility fracture are not osteoporotic (4, 23, 24). Bone density test results only reveal the current situation and do not show the ongoing bone turnover; thus, they do not provide information on future changes in bone density.

There are several reasons for the development and use of bone turnover markers. The work in this study illustrates efforts to find ways of predicting future bone loss by the measurement of bone turnover markers (**Paper II and III**), of how to improve this assessment (**Paper IV**), and to investigate whether some markers are more specific than others (**Paper IIV**). Since the time required to assess bone density changes with bone density equipment is very long (**Paper V**), it seems unreasonable to follow up compliance and effect of anti-osteoporotic medication by repeated bone density measurements.

Currently, bone turnover markers are being used extensively in research applications and also being tested as tools for the management of metabolic bone diseases such as osteoporosis and Paget's disease in clinical practice, because these markers are non-invasive and relatively inexpensive. Monitoring of the efficacy of bone-active drugs is currently the most promising clinical application of bone turnover markers, because of the possibility of detecting a change in the levels of bone turnover markers within a few weeks of treatment (15-18). Some markers, particularly resorption markers such as S-TRACP5b, S-CTX-I, U-CTX-I, U-NTX-I and U-DPD, and some bone formation markers such as S-bone ALP and S-OC, have shown some degree of fracture predictability in different populations(7), but the prediction is not strong enough to use in individual patients. The fracture predictability afforded by bone turnover markers is weaker than the predictability afforded by DXA (19), but it is somewhat inconsistent between studies (20-22).

A high rate of bone turnover is associated with a high rate of bone loss and osteoporosis (23-24). Early detection of individuals who are at high risk of developing osteoporosis could be important for clinical decision-making. In particular, individuals with osteopenia and individuals with a high rate of bone loss may need more careful follow-up.

In **Paper II** and **III**, baseline bone turnover markers, in particular S-OCs, U-DPD/crea, S-TRACP5b, S-CTX-I, U-LongOC/cea and U-MidOC/crea could be correlated to rate of change of aBMD in the legs. To some degree, there were correlations with rate of change of aBMD in the arms, in the total body, in part of the body, in the total hip and in the femoral neck. None of the markers were found to be correlated to rate of change of aBMD at the lumbar spine; nor did S-Bone ALP and U-TotalOC/crea show any correlation with rate of change of aBMD. When the correlation between bone turnover markers and 5-year change of QUS variables was examined, all markers except S-Bone ALP showed correlations with changes in SoS, while none of the markers showed any correlation with changes in BUA **(Paper III)**. When the mean of serial measurement of bone turnover markers was used instead of baseline measurement, the correlations became stronger as the number of samples used increased, and the women with constantly elevated levels of bone turnover markers had a significantly higher rate of bone loss **(Paper IV)**.

In general, the correlation between bone turnover markers and the change in aBMD was not strong.

The strongest correlation coefficients were 0.22 when the baseline levels were used, and they were 0.32 when the mean of four serial measurements was used. None of the markers proved to be superior to the others. Bone formation and resorption markers had almost similar magnitudes of correlations. This could be due to the tight coupling of bone formation and resorption. This idea is supported by the results of **Paper I**, in which no difference between bone formation markers and resorption markers in TSU of ^{99m}Tc-MDP was found. Bone turnover markers are released from the whole skeleton. This may be the reason for higher correlations with bone turnover markers at large skeletal sites including the total body, the partial body and the legs, than smaller sites such as the femoral neck and the lumbar spine (**Paper II and IV**).

Many other factors also affect the clinical usefulness of bone turnover markers. Pre-analytical conditions affecting bone turnover markers such as age, gender, menopausal state, ethnicity and recent fracture are not controllable, whereas other factors such as the effect of food intake, physical activity and circadian rhythm can be controlled (25). The OPRA study was designed to control for factors such as age, gender, ethnicity and menstrual status. Samples were taken in the morning in the non-fasting state, which could have affected the results, mainly the S-CTX-I levels(26). Many other factors such as time of the day, recent fracture and level of physical activity may have an effect on bone turnover markers. The study design was deliberately not changed during the study period, and all samples were collected in the same manner to make comparisons possible within the cohort.

Bone density has a smaller annual change or response to anti-resorptive and anabolic treatment compared to the response of bone turnover markers. Precision has an effect on the shortest follow-up interval between repeated scans. In the population-based cohorts in **Paper V**, several years were needed to detect a significant change between measurements. The estimated monitoring time intervals (i.e. least significant change/median rate of change in aBMD) were between 3 and 32 years, depending on the site of measurement and the equipment used. Only when a high degree of bone loss is expected may a shorter follow-up time be useful. Thus, DXA has shortcomings in detecting rapid losers and individuals with a high risk of developing osteoporosis.

Single measurements of bone turnover markers and follow-up measurements of DXA both have limitations in their ability to detect individuals with rapid bone loss. Serial assessments of bone turnover markers can substantially improve the ability to find individuals with increased loss of bone density. Whether or not intervals shorter than one year could be used to improve the predictive ability of bone turnover markers remains to be evaluated.

Conclusions

There is a correlation between levels of bone turnover markers and the rate of bone loss in elderly women, with varying degrees of correlation coefficients at different skeletal regions. In general, bone turnover markers correlate better with change in aBMD particularly at large skeletal sites, such as the total body, and weight-bearing sites such as the legs, than with aBMD change at specific clinically important relatively smaller regions such as the femoral neck and the total hip. Correlations between bone turnover markers and rate of bone loss become stronger when serial measurements of bone turnover markers are used. The individuals with constantly high levels of bone turnover markers have higher change in aBMD. However, these correlations may not be strong enough to be predictive of bone loss at the level of the individual patients. DXA is used to monitor change in aBMD to aid in treatment decisions. However, long durations of follow-up are needed to detect aBMD changes in elderly women and men that exceed the least significant change. DXA is therefore of limited use in the longitudinal monitoring of bone loss. Therefore there is a need of further studies to develop new bone turnover markers with higher predictive value.

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