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Perspective Article

Hylonome

# Contribution of bone mineral density and bone turnover markers to the estimation of risk of osteoporotic fracture in postmenopausal women

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**Abstract**

In osteoporosis, the main cause for concern is the increase in the risk of fractures. The level of bone mineral density (BMD) measured by various techniques has been shown to be a strong predictor of fracture risk in postmenopausal women. However, half of patients with incident fractures have BMD value above the diagnostic threshold of osteoporosis defined as a T-score of -2.5 SD or more below the average value of young healthy women. Clearly there is a need for improvement in the identification of patients at risk for fracture. Several prospective studies have shown that an increased bone resorption evaluated by specific biochemical markers was associated with increased risk of the hip, spine and non-vertebral fractures independently of BMD. The use of bone markers in individual patients may be appropriate in some situations, especially in women who are not detected at risk by BMD measurements. For example, in the OFELY study including 668 postmenopausal women followed prospectively over 9 years, we found that among the 115 incident fractures, 54 (47%) actually occurred in non-osteoporotic women. Among these women, the combination of bone markers and history of previous fracture was highly predictive of fracture risk. Thus, bone markers may be used in the assessment of fracture risk in selected cases in which BMD and clinical risk factors are not enough to take a treatment decision. Advances in our knowledge of bone matrix biochemistry, most notably of post-translational modifications in type I collagen, may allow identification of biochemical markers that reflect changes in the material property of bone, which is an important determinant of bone strength. Preliminary *in vitro* studies indicate that the extent of post-translational modifications of collagen —which can be reflected *in vivo* by the measurement of the urinary ratio between native and isomerised type I collagen— play a role in determining the mechanical competence of cortical bone, independently of BMD. Further studies in osteoporosis should explore the changes in these biochemical parameters of bone matrix as they may represent a key component of bone quality.

**Keywords:** Bone Mineral Density, Bone Markers, Osteoporosis, Fracture, Type I Collagen

## Introduction

Osteoporosis is a systemic disease characterized by a low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in skeletal fragility and susceptibility to fracture[1]. This definition implies that the diagnosis can and probably should be performed before any fragility fracture has occurred, which is a real challenge for the clinician. The level of bone mass can be assessed with adequate precision by measuring bone mineral density (BMD) using dual X-ray absorptiometry (DXA). However this measurement does not capture all risk factors for fracture. Bone fragility also depends on the morphology, the architecture and remodeling of bone as well as on the quality (properties) of the bone matrix that cannot be readily assessed. It has been suggested that bone strength may be reflected, independently of BMD level, by ultrasonic measurements of bone and by measuring bone turnover using specific serum and urinary markers of bone formation and resorption. In addition, the risk of fracture is also influenced by muscle function, the propensity to fall and the ability to adapt to such falls and these extra-skeletal risk factors for

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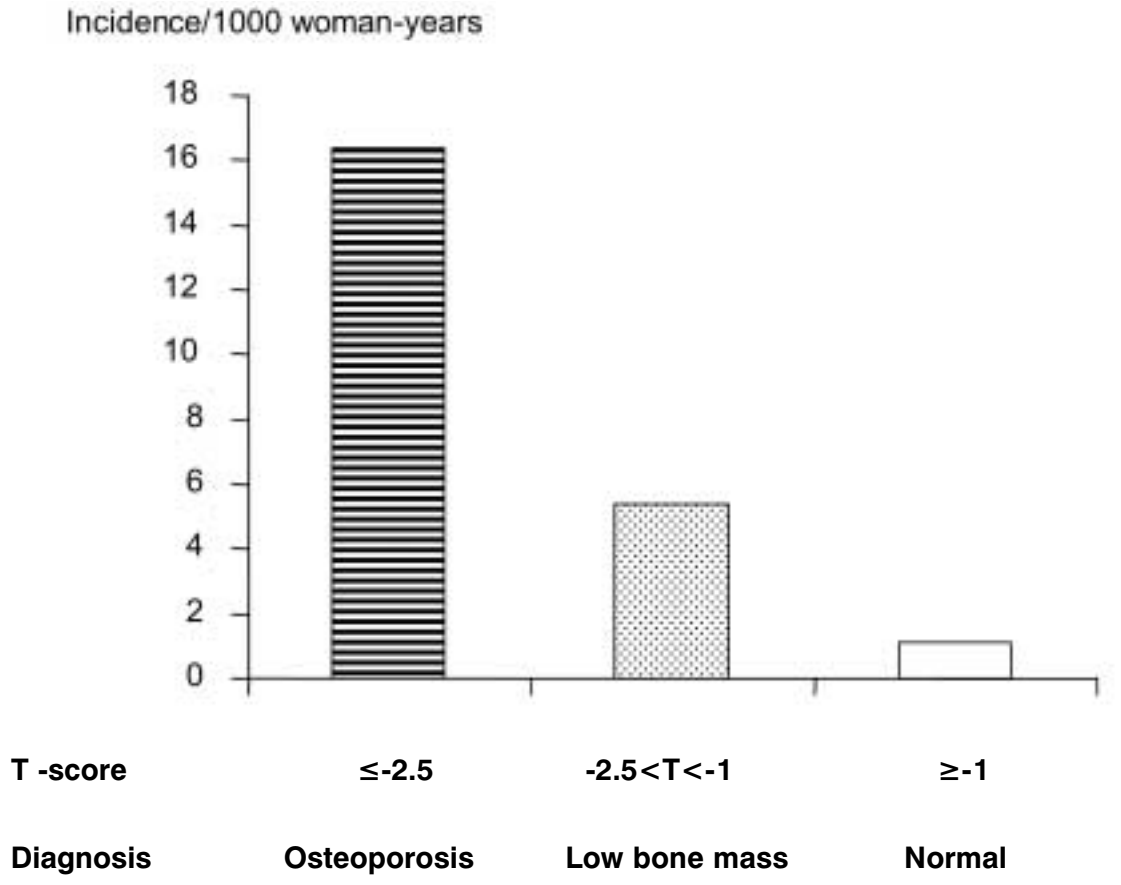


**Figure 1.** Hip fracture incidence per 1000 women-years assessed prospectively over 2 years of follow-up according to baseline level of femoral neck BMD in 7598 healthy women 75 years of age and older. The EPIDOS study. From Schott et al.[9] with permission.

fracture can be evaluated with simple questionnaire and clinical tests. In this paper we will review the relationships between BMD assessment, biochemical markers of bone turnover and fracture risk in untreated postmenopausal women.

## Bone mineral density measurements

Several cross-sectional studies have shown that bone mass —measured by a variety of techniques including single and dual photon absorptiometry, single or dual X-ray absorptimometry, quantitative computed tomography (QCT), radiography absorptiometry and radiogrammetry of the metacarpal— is significantly lower in osteoporotic patients than in controls. The strength of the association has been confirmed by several prospective studies showing that each decrease of 1 standard deviation of BMD measured by DXA is associated with an age-adjusted 50%-150% increase in the risk of osteoporotic fractures in postmenopausal women, mostly in short-term study[1,2]. The risk prediction bas been found across various age ranges (but is weaker after 80 years of age) in ambulatory and long-term care residents[4]. More recently, Melton et al.[5] analyzed the contribution of BMD to predict fracture risk over a median follow-up of 16 years (up to 21 years) in postmenopausal women. They found that the overall fracture risk including hip, vertebral and forearm fractures was best predicted by baseline femoral neck BMD, and that the predictive value was similar within the first decade and second decade of follow-up. Measurements of central (spine and hip) and peripheral (forearm, heel, finger) and total BMD with an adequate technique have similar value in predicting the risk of all osteoporotic fractures[6], as recently confirmed in the large prospective National Osteoporosis Risk Assessment (NORA) study[7]. It has also been shown that BMD estimated by digital X-ray radiogrammetry predicts hip, vertebral and wrist fracture in elderly women involved in the SOF study and follow-up for an average of 3.7 years, with similar predictive value as BMD of distal forearm, calcaneus and spine[8]. If the main concern is to detect the risk of hip fracture, measurement of hip BMD is preferable. Prediction of intertrochanteric fractures is improved by measuring the trochanteric area; prediction of femoral neck fractures is increased by measuring the femoral neck and further enhanced when measurement is limited to the upper half of the femoral neck[9,10]. The WHO definition of osteoporosis and low bone mass provides adequate cutoffs

| Formation | Resorption |
|---|---|
| | Serum/Plasma |
| | • Tartrate resistant acid phosphatase (TRAP, 5b isoenzyme) |
| • Total & **Bone-specific** Alkaline **Phosphatase (bone ALP)** | • N-terminal (S-NTX) and **C-terminal (S-CTX) crosslinking telopeptide of type I collagen** |
| • **Osteocalcin (OC)** | • C-terminal cross-linking telopeptide of type I collagen generated by MMPs (CTX-MMP) |
| • Type I collagen extension propeptides (PICP, **PINP**) | Urine |
| | • **Pyridinoline (PYD)** |
| | • **Deoxypyridinoline (DPD)** |
| | • U-**NTX** |
| | • U-**CTX** |
| | • Type I collagen helicoidal peptide 620-633 |
| | • Galactosyl-hydroxylysine |
| | • **Hydroxyproline (Hyp)** |

**Table 1.** Biochemical markers of bone turnover (abbreviations)

for identifying women at high risk of hip fracture, as shown in Figure 1[7]. Although highly precise, recent studies indicate that assessment of BMD by DXA *in vivo* is characterized by a significant inaccuracy that may exceed 20 to 50 % at relevant clinical sites such as the lumbar spine, especially in postmenopausal women with osteoporosis and in elderly patients[11-13]. This inaccuracy which cannot be avoided is related to the so called two-component limitation of DXA, while body is composed of three main tissues, i.e. bone, muscle and fat. This limitation is actually of clinical relevance as DXA may yield to artifactual lower T-scores in subjects of lower stature. Clearly BMD by DXA does not provide an accurate estimation of whole bone strength. Some of the limitation can be overcome by new generation of peripheral QCT (pQCT), which provides with good precision, separate assessment of trabecular and cortical bone density and geometry of bone. Indeed a recent study investigating the relationships between bone mineral assessment with DXA or pQCT of the distal radius and bone strength as measured *in vitro* by mechanical tests showed that the single best predictor of bone strength was pQCT[14]. In addition measures of cortical bone mineral content and bone geometry by pQCT improves significantly the prediction of bone strength. Thus pQCT may prove superior to DXA in the prediction of bone strength and fracture although large prospective studies will be necessary to confirm this hypothesis.

## Assessment of fracture risk by biochemical markers

### Hormones

Because trauma associated with hip fracture produces acute changes in hemodynamics and in a variety of hormones, hormonal measurements performed after fracture are difficult to interpret. Only data from prospective studies in which blood and urine sampling was performed prior to the fracture will be reviewed. Although vitamin D deficiency and age-related secondary hyperparathyroidism have been suggested to play a role in the skeletal fragility of elderly women, measurements of serum 25-hydroxyvitamin D, (25-OHD), 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D_3$) and serum parathyroid hormone (PTH) appear to have limited value in healthy women for the prediction of fracture. In the EPIDOS cohort, serum PTH and 25-OHD were not predictive of hip fracture risk[15]. These findings were confirmed in the Study of Osteoporotic Fractures (SOF) in women over 65 years of age showing no predictive value of serum PTH and 25-OHD for either hip or vertebral fractures[16]. In the same study women in the lowest quintile for serum 1,25-(OH)2 D (< 23 pg/ml) had a 2-fold increase in the risk of hip (but not of vertebral) fracture that was no longer significant after adjustment for calcaneal BMD[16]. In a younger population of

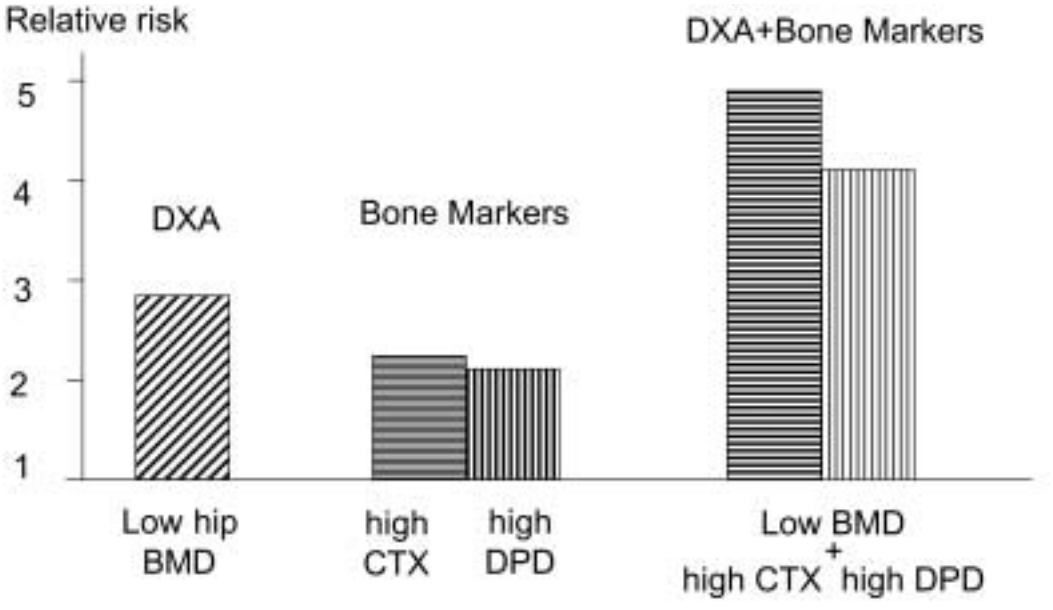


**Figure 2.** Combination of bone mineral density (BMD) at the hip assessed by dual X-ray absorptiometry (DXA) and of bone resorption to predict hip fracture risk in elderly women followed prospectively for 2 years: The Epidos study. Low BMD was defined according to the WHO guidelines, i.e. by a value lower than 2.5 SD below the young adult mean (T-score ≤ 2.5). High bone resorption was defined by urinary CTX or Free DPD values higher than the upper limit (mean + 2SD) of the premenopausal range. From Garnero et al.[15] with permission.

healthy postmenopausal women from 50 to 89 years of age (OFELY study, mean age 64 yr.) we found no significant association between serum levels of 25-OH D and the risk fractures (40 % vertebral, 60% non spine fractures) whereas women in the highest quartile of PTH had a 1.8 fold increased risk[17].

Estrogen deficiency is believed to be one of the few major determinants of postmenopausal bone loss and skeletal fragility. However circulating 17beta-estradiol levels explain only a small proportion of inter-individual variability of BMD and bone loss[18] and most of cross-sectional studies have failed so far to show different serum 17beta-estradiol values in fracture cases and in controls. Cummings et al.[16] showed that women 65 years of age and older with undetectable serum estradiol (< 5 pg/ml) had a 2.5-fold increase in the risk of subsequent hip and vertebral fractures as compared with women with detectable serum estradiol, an association that remained significant after adjustment for age, weight, serum estrone, sex-hormone-binding globulin (SHBG) and calcaneal BMD. Such a finding was however not confirmed in another large prospective study (EPIDOS) in women 75 years of age and older in whom women with low estradiol levels were not at increased risk of hip fracture[19]. In contrast, we found that elderly women with serum levels in the highest quartile were moderately protected from fracture with a relative risk of 0.66 compared to the other women. However this protective effect disappeared after adjustment by body weight suggesting that the association between increased estradiol levels and decreased hip fracture risk is mainly mediated by body weight. The reasons for the discrepancies between the SOF and EPIDOS studies on serum estradiol data are unclear and could be due to differences in body weight (higher in the SOF study), but more probably to difference in age which was on average 8 years lower in the SOF study. It seems indeed reasonable to believe that the role of residual secretion of steroids is more likely to play a role in younger postmenopausal women. This hypothesis is supported by results obtained in younger postmenopausal women. In the OFELY study comprising women with a mean age of 64 years, serum estradiol levels in the lowest quartile (< 11 pg/ml) had a relative risk of all osteoporotic fractures of 2.2 compared to the other women, an association that remained significant after adjustment for age and body weight[19]. More recently, Melton et al. reported similar findings in postmenopausal women of mean age 68 years and found that the free estradiol index (as determined by the ratio of total estradiol on SHBG), but not total estradiol was predictive of overall fracture risk 20 years later, independently of age and BMD[5]. In the OFELY study, we also found that decreased levels of dehydroepiandrosterone sulfate (DHEAs) —which can be metabolized to both active androgens and 17beta-estradiol— were also associated with increased fracture risk independently of body weight and estradiol levels[19]. Thus, all together, these recent data suggest that the influence of the residual secretion of estrogen on the risk of fracture may decrease with advancing age. The increased risk associated with low estradiol levels in younger postmenopausal women does not seem to be mediated by increased bone resorption as in the OFELY study markers of bone resorption were predictive of fracture risk inde-

pendently of serum estradiol levels[19]. A potential mechanism could be that low estradiol levels are associated with increased osteocyte death[20], a common finding in elderly women with hip fracture[21]. SHBG binds 17beta-estradiol and thereby decreases its bioavailability. In the four above described studies a high serum SHBG was modestly, and not consistently across all fracture types, associated with an increased risk of osteoporotic fractures. In the SOF study women with both undetectable serum 17beta-estradiol and a high SHBG had a marked increase in hip and vertebral fractures with relative risks of 6.9 and 7.9, respectively[16].

## Biochemical markers of bone turnover

Bone remodeling is the result of two opposite activities, the production of new bone matrix by osteoblasts and the destruction of old bone by osteoclasts. The rates of bone production and destruction can be evaluated either by measuring predominantly osteoblastic or osteoclastic enzyme activities or by assaying bone matrix components released in the bloodstream and excreted in the urine (Table 1). These have been separated into markers of formation and resorption, but it should be kept in mind that in disease states where both events are coupled and change in the same direction such as osteoporosis, any marker will reflect the overall rate of bone turnover. Current bone markers cannot discriminate between turnover changes in a specific skeletal envelope, i.e., trabecular versus cortical, but reflect whole body net changes. Increasingly specific biochemical markers for bone remodeling have been identified in recent years[22]. At present, the most sensitive markers for bone formation are serum osteocalcin, bone alkaline phosphatase, and procollagen type I N-terminal propeptide (PINP). For the evaluation of bone resorption, immunological assays of pyridinium cross-links of collagen have superseded total pyridinoline assay by high performance liquid chromatography. Immunological assays are now available for pyridinoline and deoxypyridinoline in urine and for C-terminal and N-terminal type I collagen peptides (CTX and NTX, respectively) in serum or urine. Most of these assays are now available on automatic platforms with increased precision over manual assays and high throughout which allow convenient accurate measurements in large number of individuals.

## Biochemical markers and the rate of bone loss

Several cross-sectional studies indicate that bone turnover increases rapidly after menopause and this increase in both bone formation and bone resorption is sustained long after menopause, up to 40 years[23]. BMD measured at various skeletal sites correlated negatively with bone turnover assessed by various markers in postmenopausal women. We have shown that the correlation between bone markers and BMD becomes much stronger with advancing age, so that in women more than 30 years after menopause bone turnover accounts for 40 to 50% of the variance of bone mineral density of the whole skeleton[23]. These cross-sectional data suggest that a sustained increase of bone turnover in postmenopausal women induces a faster bone loss and therefore an increased risk of osteoporosis.

Longitudinal studies, which are necessary to avoid potential confounding factors, suffer however from methodological issues: indeed, when bone loss is assessed by annual measurement of BMD at the spine, hip or radius over 2-4 years, the amount of bone loss is of the same order of magnitude as the precision error of repeated measurements in a single individual, i.e., 3-4%. This technical limitation precludes a valid assessment of the relationship between the rate of bone turnover and the subsequent rate of bone loss in individual postmenopausal women, and probably explains some of the conflicting results that have been published and recently extensively reviewed[24]. Indeed, the association between baseline levels of bone markers and rate of bone loss is more consistent and stronger when bone loss is measured at a very precise site such as radius than when measured at the spine and hip[24]. When the precision error on the rate of bone loss is reduced by performing 9 measurements over 24 months, the correlation coefficient bone markers tend to improve from about -0.3 to -0.8[25]. In a cohort of 305 postmenopausal women aged 50-88 years (mean 64 years) who had annual radius BMD measurements over 4 years, we have investigated the predictive value of baseline values of bone turnover markers[26]. Serum and urinary C-terminal crosslinking telopeptide of type I collagen (CTX) and urinary N-terminal crosslinking telopeptide of type I collagen (NTX) for assessing bone resorption, and serum osteocalcin and procollagen type I N-terminal propeptide (PINP) for assessing bone formation, were found to be highly correlated with the rate of bone loss. In addition, in women within 5 years of menopause who had the highest rate of bone loss, correction of the observed correlation coefficients by errors on bone loss and bone marker measurements resulted in a marked increase in the predictive value of bone markers[24]. Women with baseline values of bone turnover above the premenopausal range had a rate of bone loss 4- to 6-fold higher than women with a low turnover. Ultimately, the weight of evidence will come from long-term studies. A retrospective study[27] performed over 13 years in women (mean age at baseline: 62 yr.) in whom calcaneal BMD loss was assessed by 8 measurements, showed that 1 standard deviation increase in new specific bone markers such as osteocalcin, bone specific alkaline phosphatase and free pyridinoline crosslinks was associated with a 2-fold increased risk of rapid bone loss defined as the upper tertile of rate of loss. There is currently only one prospective longitudinal long-term study in which the assessment of bone turnover with non-specific markers (serum alkaline phosphatase, fasting urinary calcium and hydroxyproline) at the time of menopause was correlated with the spontaneous rate of bone loss over the next 12 years. This study showed that early postmenopausal women classified as fast losers based on these markers had

| Reference | Study | Fracture type | Mean age (yr) | number of women: fracture /controls | Follow-up duration (yr) | Sampling conditions | Marker | odds-ratio of fracture |
|---|---|---|---|---|---|---|---|---|
| 42 | prospective Population-based | All | 69 | 43/285 | 5 | Non fasting morning serum | OC<br>PICP<br>CTX- MMP<br>PICP in w. 70-80 yr | NS<br>1.8 (P=0.015) for 1 SD increase<br>1.8 (p=0.04) for 1 SD decrease<br>2.4 (p=0.04) for 1 SD decrease |
| 17 | Population based nested-case control EPIDOS study | Hip | 82.5 | 109/292 | 1.8 | Non fasting serum<br><br>First morning void urine | OC<br>bone ALP<br>U-NTX<br>U-CTX<br>U-I-DPD | 1.8 (0.8-1.2) for 1 SD increase; 1.8 (0.6-1.8) for values above premenopausal range<br>0.9 (0.7-1.2) for 1 SD increase; 1.1 (0.7-1.7) for values above the premenopausal range<br>1.1 (0.9-1.4) for 1 SD increase; 1.4 (0.9-2.2) for values above premenopausal range<br>1.3 (1.0-1.6) for 1 SD increase; 2.2 (1.3-3.6) for values above premenopausal range<br>1.4 (1.1-1.7) for 1 SD increase; 1.9 (1.1-3.2) for values above premenopausal range |
| 17 | Population based OFELY study postmenopausal w. 50-69 yr | All | 67 | 55/380 | 5 | Fasting serum between 7.30+0.30 am<br><br>24 h urine sample | OC<br>bone ALP<br>PICP<br>PINP<br>U-NTX<br>U-CTX<br>S-CTX<br>U- I-DPD | 1.5 (0.8-2.7) for values above premenopausal range<br>1.9 (1.1-3.4) for values above premenopausal range<br>1.7 (1.7-2.5) for values above premenopausal range<br>1.6 (0.8-3.4) for values above premenopausal range<br>1.7 (0.9-3.6) for values above premenopausal range<br>2.3 (1.3-4.1) for values above premenopausal range<br>1.9 (1.1-3.6) for values above premenopausal range<br>1.8 (0.9-3.6) for values above premenopausal range |
| 34 | population based HOS study postmenopausal w. | All | 74 | 55/457 | 2.7 | Non fasting serum<br>spot urine | bone ALP<br>U-CTX | 1.53 (1.09-1.98) for 1 SD increase<br>1.54 (1.09-1.99) for 1 SD increase |
| 35 | Prospective population based Nested Case-control > 55 Yrs old Rotterdam study | Hip | 80.5 | 17/51 | not done | First morning void urine | HPLC total PYD<br>HPLC total PDPD<br>HPLC f-PYRD<br>HPLC f-DPD<br>I-DPD | 3.3 (1.3-9.6) for 1 SD increase<br>2.2 (0.9-6.0) for 1 SD increase<br>3.0 (1.2-7.2) for 1 SD increase<br>1.8 (0.9-4.1) for 1 SD increase<br>10.2 (1.4-74.6) for 1 SD increase |
| 40 | Population based nested-case control EPIDOS study | Hip | 82.4 | 212/638 | 3.3 | Non fasting serum<br>Morning: 7-11 am<br>Afternoon: 1-2 pm<br>First morning void urine | U-CTX<br>I-DPD<br>S-CTX: all samples<br>Morning samples<br>Afternoon samples | 1.7 (1.2-2.3) for values above premenopausal range<br>2.1 (1.5-2.3) for values above premenopausal range<br>1.2 (0.8-1.8) for values above premenopausal range<br>0.8 (0.4-1.9) for values above premenopausal range<br>1.9 (1.01-3.8) for values above premenopausal range |
| 5 | Population based cohort | All | 68 | 128/99 | 10.2 | serum and urine (sampling conditions unknown) | bovine assay for OC<br>total ALP<br>Urinary hydroxyproline | 0.92 (0.78-1.10) for 1 SD increase<br>1.06 (0.84-1.34) for 1 SD increase<br>0.95 (0.80-1.13) for 1 SD increase |
| 63 | Same cohort as (35) Rotterdam study | Hip | not done | 86/165 | 2.4 | first morning void urine | OC<br>bone ALP<br>HPLC total PYD<br>HPLC total DPD<br>HPLC f-PYD<br>HPLC f-DPD<br>f-D-PYD | 3.1 (1.0-9.2) for values below median<br>3.0 (0.8-2.8) for values below median<br>2.6 (0.9-3.6) for values above median<br>1.4 (0.5-4.0) for values above median<br>3.7 (1.2-11.3) for values above median<br>3.4 (1.1-10.6) for values above median<br>3.8 (1.3-11.8) for values above median |
| 41 | population based nested-case control SOF study | Hip and Vertebral | 72 | 158/300 | 4 | non-fasting serum | OC<br>bone ALP<br>S-CTX | 1.06 (0.85-1.14) hip Fx; 1.08 (0.86-1.36) Vert Fx; for 1 SD increase<br>1.09 (0.85-1.28) Hip Fx; 1.04 (0.84-1.29) Vert Fx; for 1 SD increase<br>1.07 (0.87-1.32) Hip; 1.07 (0.87-1.32) Vert Fx; for 1 SD increase |
| 64 | Same cohort as (35) Rotterdam study | All | ND | 207/200 | 3.8 | first morning void urine | I-DPD | 1.7 (1.1-2.6) for values in the two upper tertiles<br>4.8 (1.6-14.5) for values in the two upper tertiles<br>3.8 (1.1-13.7) for values in the two upper tertiles<br>1.1 (0.6-1.8) for values in the two upper tertiles |

**Table 2.** Relationships between markers of bone turnover and the risk of osteoporotic fracture in prospective studies.

lost 50% more bone 12 years later than those diagnosed as slow losers[28].

In summary, with the current performance of bone marker and bone loss measurements, it appears that a single measurement of biochemical markers of bone turnover can not predict the absolute rate of bone loss in an individual woman. However, clearly increased levels of bone markers in postmenopausal women can be regarded as a risk factor for rapid bone loss in the subsequent years.

## Markers of bone turnover and fracture risk

With the emergence of effective —but rather expensive— treatments, it is essential to detect those women at higher risk of fracture. As discussed above, several prospective studies have clearly demonstrated a strong association between BMD measurements and the risk of hip, spine and forearm fractures. However, half of patients with incident hip fractures have baseline BMD assessed by DXA above the diagnostic threshold of osteoporosis defined as a T-score of -2.5 SD or more below the average value of young healthy women. Clearly there is a need for improvement in the identification of patients at risk for fracture. In addition to age, several other risk factors have been shown to contribute to fracture probability independently of BMD in postmenopausal women, including a family history of hip fracture, prior fragility fractures, low body mass index in some studies, although these data have been generated mainly in elderly populations and for the prediction of hip fracture risk. More recently, we analyzed clinical risk factors for all fragility fracture in younger postmenopausal women (mean age 59 yr.) followed for 5 years. In this study we identified six BMD independent risk factors which included older age, past falls, lower grip strength, maternal history of fracture, low physical activity and personal history of fractures[29]. As suggested by the studies reviewed below, high values of biochemical markers of bone turnover may also play a role and be part of the strategy for improving risk assessment.

Several retrospective studies have compared bone marker levels in patients with osteoporotic fractures and in controls that we recently reviewed[30]. However bone turnover can change after a fracture because of immobilization, because of callus formation and/or because of the frequent regional activation of bone turnover[31]. To overcome these limitations, relationships between bone turnover markers and fracture have been investigated when samples were taken within a few hours after the hip fracture[32] or several years after when the primary remodeling period of high activity has passed[30]. Although most of these retrospective studies suggested bone resorption is increased and bone formation is decreased in fracture cases compared to age-matched controls, we cannot exclude that a proportion of these changes of bone turnover may be the result of acute changes in body fluid and hormone levels related to the trauma. Thus, it seems difficult from retrospective studies to determine whether differences in bone turnover levels are related to the underlying rate of bone turnover leading to fracture, or to changes of bone turnover occurring after the fracture.

Relating baseline bone turnover levels with the subsequent risk of osteoporotic fractures is the valid methodology to assess their clinical utility. The previously mentioned study[28] was extended to a 15-year follow-up of 182 women during which 23 women experienced a peripheral fracture and 25 had one or more spinal fractures[33]. The fracture group had a significantly lower BMD than the group without fracture and a higher initial 3 yr. rate of bone loss after menopause. Interestingly, bone mass and the rate of bone loss predisposed to fracture to the same extent, with odds ratios of about 2. Women with both initial low bone mass and an increased rate of bone loss had a 3-fold increase in the risk of fracture compared with the whole population. This study suggests that initial peak bone mass and postmenopausal rate of bone loss are both important determinants of osteoporosis, although a predictive value of the rate of hip bone loss and overall fracture was not confirmed in a recently published 20 yr. follow-up study[5]. More recently prospective studies have looked at the relationships between the measurement of biochemical markers of bone formation and bone resorption measured before the fracture has occurred and the subsequent risk fractures[30].

## Markers of bone formation

Prospective studies investigating the relationships between bone formation markers and fracture risk have yielded conflicting results. In the large multicentric cohort of elderly women in France (EPIDOS), no significant relationships was found between levels of serum osteocalcin and bone alkaline phosphatase and the risk of hip fracture occurring during a 2 year follow-up[15]. In contrast in two prospective studies performed in younger healthy postmenopausal women [OFELY and Hawai Osteoporosis Study (HOS)], a significant positive association between increased levels of bone alkaline phosphatase and the risk of vertebral and non vertebral fracture was observed[17,34]. The differences between the studies may be related to the type of fracture but more probably to the duration of follow-up which was of 22 months in the EPIDOS study and of 5 years in the OFELY study. More recently, we re-assessed the OFELY data[17] after a median of 9 years of follow-up in which we recorded 158 incident fractures in 116 women including 50 vertebral and 108 non-vertebral fractures. Over this long follow-up period we also found a significant association between increased baseline levels of serum osteocalcin, bone alkaline phosphatase and PINP and the risk of fractures (unpublished data). Melton et al.[5] however could not find any significant relationship between total alkaline phosphatase levels and serum osteocalcin and the risk of fracture which occurred within the following 20 years. However these markers which were measured 20 years ago were not the most specific ones and this may have resulted in decreased power to detect a significant association. As discussed above, high levels of

| Collagen parameters | Bone Mechanical properties (r values adjusted for BMD by QCT) | | |
|---|---|---|---|
| | Stiffness (Mpa) | Ultimate strengh (Mpa) | Ultimate strain (%) |
| Collagen (%) | 0.25 | 0.26* | - 0.27 |
| Intermediate Immature crosslinks | | | |
| DHLNL (mol/mol Col.) | - 0.09 | 0.01 | 0.14 |
| HLNL (mol/mol Col.) | - 0.11 | - 0.09 | -0.09 |
| DHLNL/HLNL | - 0.12 | 0.00 | 0.19 |
| Mature crosslinks | | | |
| PYD (mol/mol Col.) | 0.09 | 0.25 | 0.37** |
| DPD (mol/mol Col.) | - 0.20 | -0.06 | 0.39** |
| PYD/DPD | 0.47*** | 0.40** | - 0.16 |

* P <0.05, ** p<0.01, *** p<0.001

**Table 3.** Collagen intermolecular crosslinks as BMD independent predictors of human vertebral cancellous bone strength.

bone formation markers are associated with a greater bone loss[26]. Thus, if the increased risk of fracture is mediated partly through a more rapid bone loss[28], a follow-up of several years may be necessary to detect the association.

## Markers of bone resorption

More consistent data have been obtained on the relationship between increased levels of bone resorption markers and fracture risk. Four prospective studies (Rotterdam, EPIDOS, OFELY and HOS) found that bone resorption assessed by urinary or serum CTX or urinary free deoxypyridinoline above the premenopausal range were consistently associated with about a two fold higher risk of hip, vertebral and non-hip and non-vertebral fractures over follow-up periods ranging from 1.8 to 5 yr.[15,17,34,35] (Table 2). It remains however to be investigated whether bone resorption markers would predict fracture risk over periods which exceed 5 years. Because serum CTX levels are markedly affected by food intake[36,37], an effect which is likely to be mediated by gastro-intestinal hormones[38], it should be measured on fasting morning samples to reduce the variability of the measurement[39]. This technical limitation probably explains the lack of significant predictive value of serum CTX levels measured on non-fasting morning serum samples both in the EPIDOS[40] and SOF[41] studies. In these studies, the odds ratio were not modified after adjusting for potential confounding factors such as mobility status and were only marginally decreased after adjusting for BMD measured by DXA[30]. Thus, the combination of BMD and bone turnover measurement allows the identification of a subgroup of elderly women at much higher risk of hip fracture than those identified by each test alone (Figure 2). Increased bone resorption was associated with increased fracture risk only for values which exceed a threshold, especially when defined as levels above the upper limit of the premenopausal range, indicating that bone resorption becomes deleterious for bone strength only when it exceeds a normal physiological threshold which appears to be the upper range of premenopausal women. These data also suggest that increased resorption could lead to increased skeletal fragility through two independent mechanisms. First, a prolonged increase of bone turnover will lead after several years to a lower BMD as discussed previously, which is a major determinant of reduced bone strength. Second, increased bone resorption above the upper limit of the normal range may induce microarchitectural deterioration of bone tissue such as perforation of trabeculae, a major component of bone strength.

## Post-translational modifications of bone matrix proteins

Type I collagen molecules, the main organic component of bone matrix, undergo enzymatic and non-enzymatic post-translational modifications and some of them may be of clinical relevance for the investigation of metabolic bone diseases including osteoporosis. Among the enzymatic modifi-

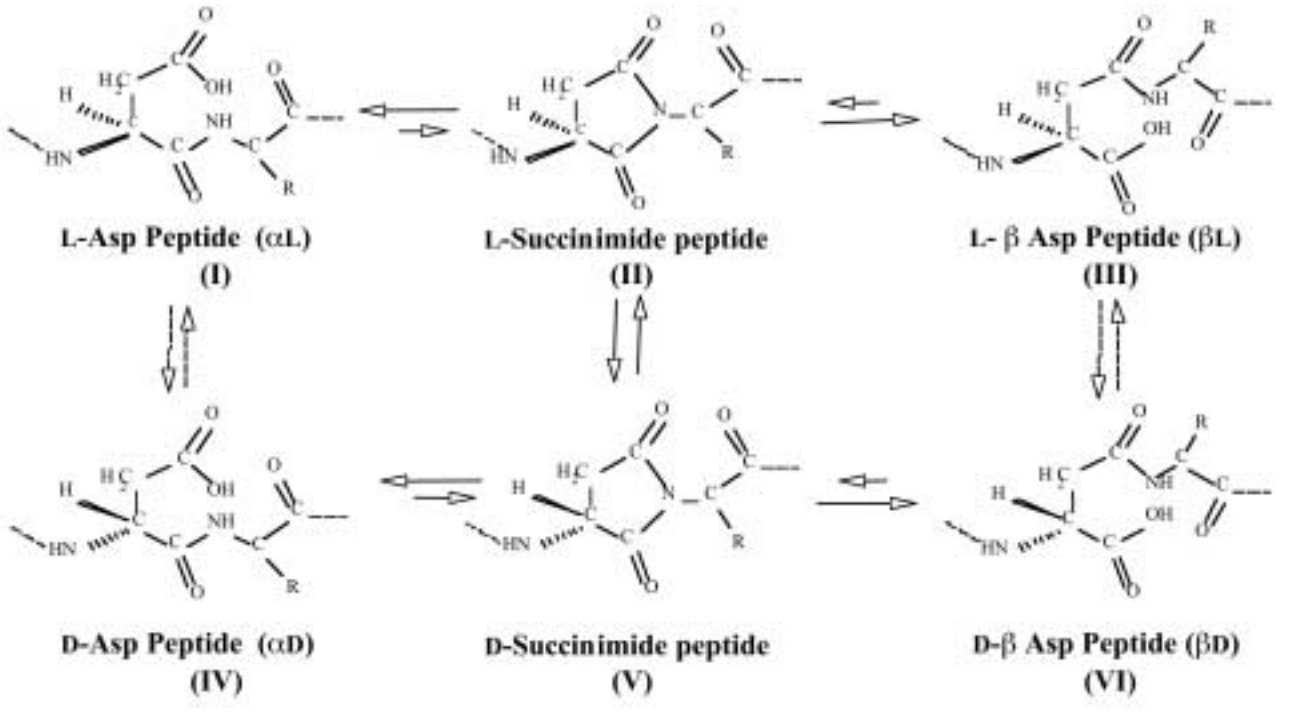


**Figure 3.** Racemization and Isomerization of type I collagen C-telopeptides. An attack by a peptide backbone nitrogen on the side chain carbonyl group of an adjacent aspartyl residue can result in the formation of a succinimide ring (I→II). The succinimide ring is prone to hydrolysis and racemization producing peptides and b-aspartyl peptides in both the D and L configurations. Racemization is thought to proceed primarily through the succinimide pathway (II→V), but other pathways as direct proton abstraction (I↔IV and III↔VI) may also contribute to the formation of D-aspartyl. Throughout the figure, the peptide backbone is shown as a bold line. The 4 types of C-telopeptides are present in bone matrix; the native form alpha L) and 3 age-related forms; an isomerized β-L), a racemized alpha D) and an isomerized/racemized β-D) form). With increasing age of type I collagen molecules, the proportion of b- isomerized and D racemized form within bone matrix increases. Degradation products of these 4 CTX forms of type I collagen can be measured in urine independently by immunoassays using specific conformational monoclonal antibodies. From Cloos & Fledelius[48] with permission.

cations, biochemical studies performed on human bone specimens have shown that an over-hydroxylation of lysine residues, an over-glycosylation of hydroxylysine and a reduction in the concentration of non-reducible crosslinks can be associated with reduced bone strength[42-45]. In human vertebral specimens, Banse et al.[46] recently analyzed the content of immature [hydroxylysinorleucine (HLNL) and dihydroxylysinorleucine (DHLNL)] and mature crosslinks (PYD, DPD and pyrolle). They showed that the ratio of PYD/DPD was significantly associated with the compressive biomechanical properties of the vertebrae independently of BMD, suggesting that type I collagen crosslinks may be a determinant of bone strength (Table 3). Non-enzymatic modifications of collagen could also play a role in the mechanical properties of bone tissue. For example, Wang et al.[47] showed that the pentosidine concentration of human femoral bone —an index of non-enzymatic advanced glycation end products— increases with age and higher levels are associated with decreased bone strength. This suggests that non-enzymatic glycation of collagen could lead to alterations of the biomechanical properties of bone that may ultimately result in increased skeletal fragility.

Racemization and beta-isomerization of the Aspartate (D) residue of the $^{1209}$AHDGGR$^{1214}$ sequence (CTX) of the C-telopeptide of type I collagen are other non-enzymatic post-translational modifications more recently investigated[48] (Figure 3). Histological studies have shown a decreased degree of type I collagen isomerization within the woven pagetic bone, a tissue characterized by increased fragility[49]. Alterations of the degree of bone type I collagen isomerization can be detected *in vivo* by the differential measurement of native (alpha) and isomerized (beta) CTX fragments in urine[48-51]. In the OFELY prospective study, we found that an increased urinary ratio between native and beta isomerized CTX was significantly associated with increased fracture risk independently of both the level of hip BMD and of bone turnover rate measured by serum bone ALP[51] (Table 4). These data suggest that a decreased degree of type I collagen isomerization could be associated with alterations of bone strength properties, an hypothesis that requires to be confirmed by studies correlating the degree of type I collagen modifications with mechanical properties of bone specimens.

Osteocalcin is a non-collagenous protein of bone matrix which contains three residues of γ-carboxyglutamic acid

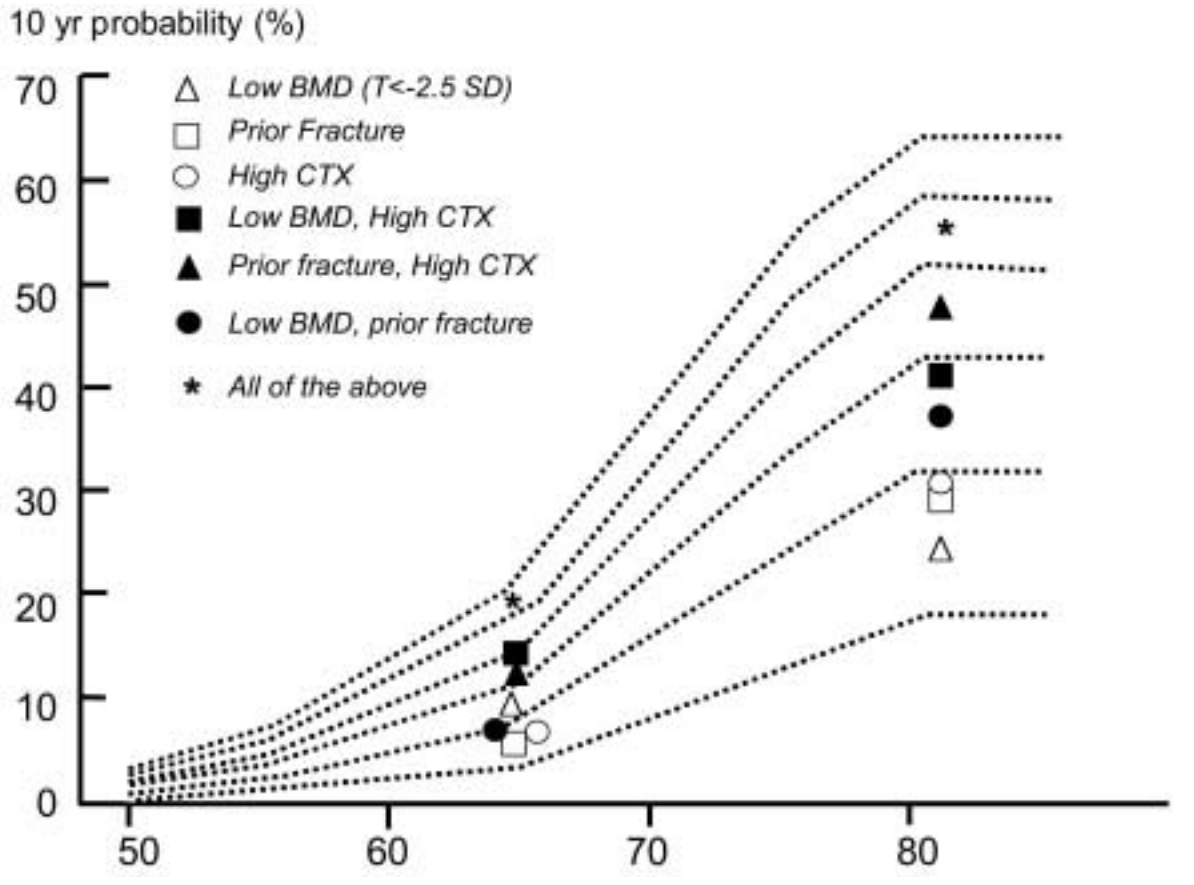


**Figure 4.** Combination of clinical risk factors, bone mineral density and bone turnover measurements to identify women with the highest risk of fracture. The figure shows the ten-year probability of hip fracture according to age and relative risk. The symbols show the effect of risk factors on fracture probability derived from women aged 65 years (OFELY study) and 80 years (EPIDOS study). The data from the OFELY study are derived from information of all fractures. Low hip BMD was defined as values at 2.5 SD or below the mean of young adults. High urinary CTX corresponds to values above the upper limit of premenopausal women (mean+2SD). From Johnell et al.[60] with permission.

(GLA), a vitamin K-dependent amino acid. It was postulated that impaired γ-carboxylation of osteocalcin could be an index of both vitamin D and vitamin K deficiency in elderly populations. Vitamin K status may be involved in the maintenance of skeletal integrity. Vitamin K2 treatment in postmenopausal women with osteoporosis decreases serum levels of undercarboxylated osteocalcin (ucOC), increases spine BMD and reduces the risk of fragility fractures[52]. In two prospective studies performed in a cohort of elderly institutionalized women followed for 3 years[53,54] and in a population of healthy elderly women (EPIDOS study)[55], levels of ucOC above the premenopausal range was associated with a two to three fold increase in the risk of hip fracture, although total osteocalcin was not predictive. Like for markers of bone resorption, the prediction was still significant after adjusting for hip BMD. More recently, it was reported that a decreased ratio between carboxylated and total osteocalcin- which is an index of increased ucOC- was also associated with increased fracture risk in elderly women living at home[56]. The mechanisms relating increased undercarboxylation of osteocalcin and fracture risk is unclear. Serum ucOC[57] and the ratio between carboxylated and total osteocalcin[58], but not total osteocalcin, have been found to be associated more strongly with ultrasonic transmitted velocity (which has been suggested to reflect in part changes in bone structure) at the os calcis and tibia than with BMD[57], suggesting that poor quality may explain the fracture risk associated with undercarboxylated osteocalcin possibly as a result of inadequate vitamin K status.

Clearly these findings open new perspectives for the clinical use of bone markers, not only to measure quantitative changes of bone turnover, but also to assess changes of bone quality, an important determinant of bone strength.

## Combined assessment of fracture risk

Cummings et al.[6] looked at the value of combining a variety of clinical risk factors obtained by questionnaire and physical examination with BMD of the calcaneus to predict the risk of hip fracture in women over 65 years of age. The incidence of hip fracture ranged from 1.1 per 1000 woman-years among women with no more than two risk factors and

| Urinary CTX ratio at baseline | Relative Risk* (95% CI) of fracture for values in the upper quartile | |
|---|---|---|
| | All Fractures | Non-vertebral fractures only |
| αL/βL | | |
| Unadjusted | 2.0 (1.2-3.5) | 2.5 (1.3-4.6) |
| Adjusted for bone ALP | 1.8 (1.1-3.2) | 2.2 (1.1-4.2) |
| Adjusted for femoral neck BMD | 1.8 (1.03-3.1) | 2.2 (1.2-4.0) |
| Adjusted for bone ALP + femoral neck BMD | 1.7 (0.95-2.9) | 2.0 (1.04-3.8) |
| αL/αD | | |
| Unadjusted | 1.8 (1.02-3.2) | 1.9 (1.01-3.7) |
| Adjusted for bone ALP | 1.6 (0.92-2.9) | 1.7 (0.87-3.3) |
| Adjusted for femoral neck BMD | 1.7 (0.97-2.9) | 1.8 (0.95-3.5) |
| Adjusted for bone ALP + femoral neck BMD | 1.6 (0.89-2.8) | 1.7 (0.86-3.2) |

* Adjusted for age, presence of prevalent fracture, and physical activity

**Table 4.** Increased urinary CTX ratio as an indipendent predictor of the risk of osteoporotic fractures. Four hundrend and eight women participating in the OFELY study were followed prospectively during 6.8 years. 55 non-vertebral fractures and 16 incident vertebral fractures were recorded. The table shows the relative risks of fracture for women with baseline levels of αL/βL CTX and αL/αD CTX in the upper quartile.

calcaneal BMD in the upper tertile, to 27 per 1000 woman-years among those with five or more risk factors and BMD in the lowest tertile.

In the OFELY prospective cohort we have shown that combination of the strongest single clinical risk factor (history of fracture after the age of 45 years), with a low hip BMD and high levels of bone resorption assessed by urinary CTX improve the predictive value of a single test with relative risk of fracture increasing from 1.8-2.8 to 5.8[17]. Similarly, in a nested case-control analysis of the EPIDOS study, we have compared the ability of history of fracture after the age of 45 years, hip BMD, heel broadband ultrasound attenuation and urinary CTX to predict the risk of hip fracture and we investigated whether a combination of these parameters could improve the predictive value[59] (Figure 4). The outcome of these combinations depends on whether the goal is to improve the sensitivity or the specificity and therefore depends on the treatment strategy. If the strategy is to increase the sensitivity, i.e. to detect as many at-risk patients as possible and to avoid false negatives, combining different tests is no more effective than lowering the diagnostic threshold of a single test. Conversely, combining two tests with adequate cutoffs is useful to increase the specificity without decreasing the sensitivity obtained with a single test – a strategy to target a subset of high-risk patients. For example, the combination of urinary CTX with either hip BMD or heel BUA increases the specificity by 10% with sensitivity similar to hip BMD or heel BUA alone. Such a combined diagnostic approach might be more cost effective than BMD measurement alone, as it results in a lower number of patients to be treated to avoid one hip fracture. If DXA or ultrasound is not available, we found that the combination of a high bone resorption marker and a positive history of any type of fracture gave a predictive value similar to that obtained with BMD or heel BUA alone[59]. As recently discussed by Johnell et al.[60], the use of odds-ratio is not ideal for clinical decision making, since the risk may decrease or remain stable with age whereas absolute risk increases. Thus calculating absolute risk such as 10 year probabilities – which depend on knowledge of the fracture and death hazards – is probably more appropriate. Based on the probability of hip fracture in the Swedish population and on the data from the EPIDOS and OFELY studies, it was found that combining urinary CTX with BMD or history of previous fracture, results in a 10 year probability of hip fracture that was about 70 to 100% higher than that associated with low BMD[60] alone with a similar pattern for the prediction of all fractures in younger postmenopausal women[60] (Figure 4).

Thus, clearly the use of multiple risk factors such as BMD, biochemical markers and other important risk factors such as personal history of previous fracture is likely to perform better than the use of BMD alone. It remains to be investigated what is the optimal model and follow-up duration , as the predictive value of some factors, such as bone turnover markers, may decrease with time.

## Conclusion

Osteoporosis can be identified early during the course of the disease before the occurrence of major fragility fractures such as vertebral and hip fractures using diagnostic tests with appropriate prognostic value. With the emergence of effective —but rather expensive— treatments, it is essential to detect those women at higher risk of fracture.

Because the pathogenesis of fragility fractures is multifactorial, including not only the level of BMD but also bone architecture and bone matrix quality, bone turnover, fall-related factors and muscle function, a global diagnostic approach is probably desirable. There is some evidence that such an integrated strategy using major clinical risk factors (e.g., history of fractures, low body weight), measurement of BMD by DXA and measurement of bone resorption by urinary or serum biochemical markers and potentially post-translation modifications of type I collagen or other bone matrix could be useful to detect high risk patients. Based on the present evidence, bone resorption markers measured in serum, first or second morning void urine appear the most adequate to predict fracture risk in postmenopausal women, although careful collection procedure should be applied i.e. after an overnight fast. We have recently found that on average levels of serum bone formation and bone resorption remain stable over 4 years in postmenopausal women and that the majority of women classified at high bone turnover on one occasion remain at high bone turnover 4 years later. However for women with intermediate levels of bone turnover, classification should be confirmed by a second measurement. The combinations of diagnostic tests should be validated in other prospective studies of postmenopausal women and operational thresholds should be developed and adapted to treatment strategies. In addition, the value of bone turnover markers to predict fracture risk in other populations such as men and patients with steroid-induced osteoporosis should be explored in large and long-term studies.

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