ICSI

Institute for Clinical Systems Improvement

Technology Assessment Report

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- physicians, nurses, and other health care professional and provider organizations;
- health plans, health systems, health care organizations, hospitals and integrated health care delivery systems;
- medical specialty and professional societies;
- researchers;
- federal, state and local government health care policy makers and specialists; and
- employee benefit managers.

This *ICSI Technology Assessment Report* should not be construed as medical advice or medical opinion related to any specific facts or circumstances. If you are not one of the expert audiences listed above you are urged to consult a health care professional regarding your own situation and any specific medical questions you may have. In addition, you should seek assistance from a health care professional in interpreting this *ICSI Technology Assessment Report* and applying it in your individual case.

This *ICSI Technology Assessment Report* is designed to assist clinicians by providing a scientific assessment, through review and analysis of medical literature, of the safety and efficacy of medical technologies and is not intended either to replace a clinician's judgment or to suggest that a particular technology is or should be a standard of medical care in any particular case. Standards of medical care are determined on the basis of all the facts and circumstances involved in a particular case and are subject to change as scientific knowledge and technology advance and practice patterns evolve.

Technology Assessment

Biochemical Markers for Bone Turnover in Osteoporosis

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Abstract

Description of Treatment/Procedure

Osteoporosis is a metabolic bone disease that is characterized by abnormalities in the amount and architectural arrangement of bone tissue which lead to impaired skeletal strength and an increased susceptibility to fractures. The decreased bone mass associated with osteoporosis is likely the result of an imbalance between bone resorption and bone formation. Biochemical markers of bone turnover are indicative of the overall bone metabolism and provide different information than that obtained from measurements of bone mineral density (BMD). In testing biochemical markers, it is important to consider that there is a high degree of variability associated with measurement of the markers (especially urinary markers).

Potential Uses

Potential uses for biochemical markers include to identify of slow and fast bone losers at menopause as an independent risk factor for future osteoporosis or fracture, to assist in making decisions about therapy, to monitor changes in bone turnover or to predict changes in BMD as a result of therapy, and to provide support for patient follow-through with recommended therapy.

Contraindications

Biochemical markers should not be used as a sole criteria for the diagnosis of osteoporosis.

Efficacy of Treatment/Procedure

In prospective studies of fractures in populations, resorption (but not formation) markers have been shown to predict risk of hip and non-spine fractures in elderly, white women. There are insufficient data on test performance characteristics in predicting fracture risk from biochemical marker data in individual patients. In untreated patients, the correlations between biochemical markers and BMD are typically r=0.40 or less indicating that the use of biochemical markers to predict bone mass is of limited value in that population. In follow-up of patients undergoing treatment for osteoporosis, there are only weak relationships between baseline marker levels and changes in BMD. Typically, no more than 23% of the variability in change in BMD is accounted for by changes in formation marker levels (≤13% for resorption markers) during the first months of treatment.

Committee Summary

With regard to the use of biochemical markers of bone turnover in osteoporosis, the ICSI Technology Assessment Committee finds the following:

1. The assessment of serum and urine biochemical markers of bone turnover is safe and minimally invasive.
2. While there is some population based evidence that increased values for biochemical markers are associated with increased fracture risk and that uncoupling of the bone formation/resorption mechanism is greater in fracture cases, it is not possible to predict an individual's fracture risk from biochemical marker measurements. A combination of bone mineral density and biochemical marker measurements may be of greater value but the data are inconclusive. (Conclusion Grade II)
3. Although population trends have been observed, biochemical markers do not have adequate sensitivity and specificity to predict osteoporosis in individual, untreated patients. The diagnosis of osteoporosis is based on a reduced BMD and/or the presence of fragility fractures. (Conclusion Grade II)
4. Several biochemical markers are responsive to various therapeutic options. However, there is no conclusive evidence that biochemical markers may be used to assist in selecting the type of therapy or to predict the amplitude of the BMD response for an individual patient. (Conclusion Grade II)
5. Although biochemical markers have the potential to be used to motivate individuals to maintain a therapy program, there are no studies of the use of biochemical markers for this purpose.

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ICSI Medical Brief

Biochemical Markers for Bone Turnover in Osteoporosis

Osteoporosis is a metabolic bone disease that leads to reduced skeletal strength and greater risk of fractures. Bone is a dynamic tissue and is continuously undergoing resorption and formation. It has been suggested that the decreased bone mass associated with osteoporosis is a result of an imbalance between bone resorption and formation.

There are biochemical markers, measured in serum or urine, of both bone resorption and bone formation. While testing for bone mineral density (BMD) indicates the amount of bone present at the time of the test (a static measure reflecting the impact of genetics, life-long exercise behavior, nutrition, and disease), biochemical markers reflect how fast the skeleton is remodeling at the time of the measurement. Potential advantages of using biochemical markers of bone turnover include more rapid detection of changes in bone turnover, minimal invasiveness, and no radiation exposure. Disadvantages include that the markers are poorly predictive of BMD, that the markers are not disease specific or site specific, and that the markers are present in tissues other than bone. Analytical and biological variability are also factors.

Testing for biochemical markers of bone turnover could *potentially* be used to identify slow and fast bone losers at menopause (as an independent risk factor for osteoporosis or future fracture), to assist in making decisions about therapy, to monitor changes in bone formation or resorption and/or predict changes in BMD as a result of therapy, or to provide support for patient follow through with recommended therapy. Although the FDA has approved the use of biochemical markers to assess the response to treatment (especially with anti-resorptive agents), the markers are not routinely used because of large analytic and biologic variations. There are no clear guidelines on the use of biochemical markers in clinical practice and there is no data to support or refute the practice of using marker values to change dosages or monitor compliance with therapy. Biochemical markers *should not* be used as a sole criteria for the diagnosis of osteoporosis.

The National Osteoporosis Foundation recently recommended that future studies work to identify better measures of the relationship between bone resorption and formation. It was also recommended that there be more studies involving direct comparisons between different markers and more prospective studies with fracture as the outcome of interest. There is also a need for studies of populations other than white, postmenopausal women.

With regard to the use of biochemical markers of bone turnover in osteoporosis, the ICSI Technology Assessment Committee finds the following:

1. The assessment of serum and urine biochemical markers of bone turnover is safe and minimally invasive.
2. While there is some population based evidence that increased values for biochemical markers are associated with increased fracture risk and that uncoupling of the bone formation/resorption mechanism is greater in fracture cases, it is not possible to predict an individual's fracture risk from biochemical marker measurements. A combination of bone mineral density and biochemical marker measurements may be of greater value but the data are inconclusive.
3. Although population trends have been observed, biochemical markers do not have adequate sensitivity and specificity to predict osteoporosis in individual, untreated patients. The diagnosis of osteoporosis is based on a reduced BMD and/or the presence of fragility fractures.
4. Several biochemical markers are responsive to various therapeutic options. However, there is no conclusive evidence that biochemical markers may be used to assist in selecting the type of therapy or to predict the amplitude of the BMD response for an individual patient.
5. Although biochemical markers have the potential to be used to motivate individuals to maintain a therapy program, there are no studies of the use of biochemical markers for this purpose.

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Technology Assessment Report

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ICSI Technology Assessment Report Process

- A topic is selected by the Technology Assessment Committee based on expression of interest in that topic from the ICSI medical groups and HealthPartners.
- A work group of 4 to 6 physicians and other health care professionals who are experts in the topic area is assembled (with a formally designated leader).
- The literature search is completed using MEDLINE and PREMEDLINE; in addition, bibliographies of articles obtained from the literature search are examined to identify articles that may have been missed and work group members are asked to provide key references. The evidence is graded according to the system described in the reference section of the report.
- The ICSI staff person prepares a draft report.
- The work group meets to review the draft report and directs the ICSI staff person in revising the report.
- The work group leader presents the report to the ICSI Technology Assessment Committee. Committee members review the report to determine whether the conclusions are supported by the evidence cited. The Committee often requests revisions prior to approving the report for review and comment.
- The report is distributed to the ICSI Medical Groups for review and comment. Comments received are shared with the work group members and revisions to the report are made, if necessary.
- The Technology Assessment Committee reviews the comments and the work group response and makes the final decision regarding approval of the report for distribution.
- Reports are reviewed bi-annually and revised, if warranted.

Description of Technology/Procedure

Osteoporosis is a metabolic bone disease that is characterized by abnormalities in the amount and architectural arrangement of bone tissue which lead to impaired skeletal strength and an increased susceptibility to fractures (Consensus Development Conference, 1993). A World Health Organization study group recommended that osteoporosis be defined operationally in white women as bone mineral density levels more than 2.5 standard deviations below (T-score of –2.5) the young normal mean (Kanis, Melton, Christiansen, Johnston, & Khaltaev, 1994). There is no comparable definition for nonwhite women or for men.

It has been suggested that the decreased bone mass associated with osteoporosis is a result of an imbalance between bone resorption and bone formation within a bone remodeling unit. Bone remodeling units are focused sites on the surface of bone where bone metabolism occurs. Overall bone metabolism is the net effect of the many bone remodeling units and it has been suggested that another factor in osteoporosis is an increase in the activation frequency of these units. Although an increase in activation frequency can be due to estrogen deficiency, the cause of the resorption/formation imbalance is likely a combination of age-related factors (Christenson, 1997; Seibel et al., 1997; Delmas, 1998; Garnero & Delmas, 1998).

Biochemical markers of bone turnover are indicative of the overall bone metabolism (see text and table on pages 4 through 7 for information about specific markers). However, since the various substances tested for are all produced as a result of normal bone physiology, their presence in the blood or urine is not a direct result of a disease state (Caulfield, 1998). Although there are designated markers for bone formation and for bone resorption, there is no current method for combining these to produce an index representative of the bone remodeling imbalance. In addition, there is typically a 3 to 6 month lag period between resorption and formation at the resorption site. As a result of this delay, markers of bone resorption provide an earlier indication of changes in bone metabolism (Mallinak & Clemens, 1998). The markers used are either proteins or products derived from proteins and represent either enzymes derived from osteoblasts involved in bone formation, enzymes derived from osteoclasts involved in bone resorption, or constituents of the bone matrix (Russell, 1997; Seibel et al., 1977; Delmas, 1998; Garnero & Delmas, 1998). None of the markers relates directly to the signal for bone turnover (Caulfield, 1998).

A biochemical assessment of bone turnover produces different information than that obtained from measurements of bone mineral density (BMD). It has been suggested that these two measures may be complementary in helping to predict the risk of future fracture (Eyre, 1997, Delmas, 1998; Garnero & Delmas, 1998). Bone mineral density, typically measured with dual-energy x-ray absorptiometry (DXA), provides an indication of how much bone is present. It is a "static" measurement reflecting the impact of genetics, life-long exercise behavior, nutrition, and disease on the status of the bone at the time of measurement (Caulfield, 1998). DXA is considered to be the only acceptable non-invasive method for establishing a diagnosis of metabolic bone disease (Demers, 1997). Biochemical markers reflect how fast the skeleton is remodeling; they are an indication of the bone activity at the time of the measurement (Eyre, 1997; Caulfield, 1998). With biochemical markers, it is possible to detect changes in bone turnover within 3 months whereas with radiographic methods, it would take at least 12 to 24 months to be able to observe changes (Christenson, 1997; Demers, 1997; Rosalki, 1998). Although the gold standard in the evaluation of biochemical markers has frequently been BMD, the ability to predict the risk for subsequent osteoporotic fracture would have the greatest clinical value (Delmas, 1998). It is unclear whether short-term measures of bone turnover can predict long-term fracture risk. Extreme values may be real, statistical outliers, or a reflection of the dynamic nature of bone. A change in marker level may reflect a true change in the parameter or regression to the mean.

In addition to the ability to more rapidly detect changes in bone turnover, the advantages of using biochemical markers include that they are minimally invasive, reflective of the turnover of the entire skeleton, involve no radiation exposure, and allow repeated evaluation (Eastell & Blumsohn, 1997; Rosalki, 1998). The disadvantages include that the analysis of biochemical markers provides no information about the work of individual cells, that there is no indication of mineralization, and that the markers are not disease specific (Eastell & Blumsohn, 1997; Seibel et al., 1997). Although bone resorption is higher in patients with overt osteoporosis, there is overlap in the marker values between those with osteoporosis and age-matched controls. In addition, biochemical markers are poorly predictive of bone mineral density (Eastell & Blumsohn, 1997).

Several markers of bone formation and bone resorption have been identified (see Table). In testing for these markers, there are a number of factors that should be considered (see also Risks and Limitations section). Although the markers are classified as markers of either formation or resorption, some may reflect both processes. In addition, nearly all of the markers are present in tissue other than bone and therefore may be influenced by non-skeletal processes (Seibel et al., 1997). There are also differences depending on whether the marker is being tested in serum or urine. Serum markers have been reported to vary by less than 10% within a given individual while urinary markers may vary by up to 30% (Rosen & Tenenhouse, 1998). The observed variability is a combination of biologic variability (non-uniform rates of bone turnover, variable excretion of cross-links, time of day that the sample is measured, and season of the year) as well as variability in the assay for a given marker. The protocol for specimen collection and processing (including use of the same reference clinical laboratory) should be replicated exactly when follow-up measurements are made taking into consideration that protocols used in well-designed trials may not be feasible in a clinical setting. To address concerns about variability in the measures, it has been suggested that a least significant change (LSC) be used to determine whether a change is significant, that age- and sex- specific references ranges be established under defined conditions, and that the ratio of day-to-day variability to the width of the reference range (the index of individuality) be used to compare the performance of different markers (Seibel et al., 1997). Selection of a marker or markers to test for should be based on the purpose of the testing and the performance of those markers (e.g., analytical and biological variance).

Bone Formation

Biochemical markers of bone formation are typically tested in serum. Serum is faster to collect than a 24-hour urine collection and does not require a correction for renal function (Edelson & Kleerekoper, 1998).

Serum total alkaline phosphatase (ALP) activity is the most commonly used marker of bone formation but it lacks sensitivity and specificity in patients with osteoporosis. Alkaline phosphatase is an enzyme synthesized by osteoblasts and is believed to be involved in mineralization (Seibel et al., 1997). Assays that isolate the bone isoenzyme have resulted in a more sensitive and specific marker for the detection of increased bone turnover (Eyre, 1997; Russell, 1997; Seibel et al., 1997; Garnero & Delmas, 1998). An increase in serum bone alkaline phosphatase (BSAP) reflects an increased release from osteoblasts. However, this increase may be due to increased bone formation, as a result of osteomalacia, or as a result of decreased clearance as a consequence of liver disease (Seibel et al., 1997).

Osteocalcin (OC), which is also referred to as bone gla-protein (BGP) is a small noncollagenous protein specific for bone tissue and dentine. Its exact function is unknown. It is synthesized by osteoblasts and incorporated into the extracellular matrix of bone. In the process, a small amount is released into the circulation where it can be measured by immunoassay. Osteocalcin is cleared quickly by the kidney. Levels of circulating osteocalcin vary with a difference of approximately 15% between the high and low points. As a product of osteoblasts, increased levels of osteocalcin reflect bone formation

(Seibel et al., 1997; Garnero & Delmas, 1998). There are several commercial assays available. It is difficult to compare results from studies using different assays since the different antibodies used may recognize different portions and fragments of the osteocalcin molecule thereby giving different results (Russell, 1997; Seibel et al., 1997).

Undercarboxylated osteocalcin (ucOC) is a form of OC that is increased when there is a impairment of the carboxylation process. This increase has been linked to a vitamin K deficiency (Vergnaud, Garnero, Meunier, Bréart, Kamihagi, & Delmas, 1997).

Collagen is an essential element in the integrity and strength of bone matrix and therefore an assessment of collagen synthesis should provide information about bone formation (Russell, 1997). Procollagen type I molecules are secreted by osteoblastic cells and subsequently there is a cleavage of the extension peptides (amino-terminal or PINP and carboxy-terminal or PICP) prior to the formation of mature type I collagen molecules. Both peptides are detected by immunoassay. The assay for PICP has been found to lack sensitivity. A recent assay to detect only an intact form of PINP has shown greater sensitivity for detecting bone turnover following menopause and for evaluating the efficacy of estrogen and bisphosphonate therapy (Garnero & Delmas, 1998).

Bone Resorption

Markers of bone resorption provide an earlier indication of changes in bone metabolism following initiation of therapy. As a result, they may be more useful to clinicians (Pedersen, Ravn, & Bonde, 1998).

Hydroxyproline (Hyp) is found in collagen and, given that much of the collagen is found in bone, urinary hydroxyproline levels are viewed as a marker of bone resorption. However, hydroxyproline is also found in other proteins (besides bone collagen) and dietary sources of collagen can influence the levels present in the urine, limiting its value as a marker of bone resorption (Eyre, 1997; Russell, 1997; Garnero & Delmas, 1998). Testing of early morning fasting urine samples is recommended if this marker is to be used (Russell, 1997). As with urinary calcium, low levels of hydroxyproline are unlikely in cases of increased bone turnover (Christenson, 1997).

TABLE. Biochemical Markers of Bone Turnover

Marker (Abbreviation)	Commercial Assay Available	Serum or Urine	Biologic (Intra-individual) Coefficient of Variation (%)*	Analytic (Intra-assay) Coefficient of Variation (%)*	Analytic (Inter-assay) Coefficient of Variation (%)*
Bone Formation					
Total Alkaline Phosphatase (ALP)	Yes	Serum	7.0 (Range 5-11)		2.2
Bone Specific Alkaline Phosphatase (BSAP)	Yes	Serum	8.0 (Range 7-9)	2.6-18.7	3.2-10.0
Osteocalcin (OC) [or bone Gla protein (BGP)]	Yes	Serum	13.0 (Range 7-27)	3.7-12.0	6.0-12.0
Undercarboxylated Osteocalcin (ucOC)	No	Serum		9.0	11.4

Carboxyterminal Propeptide of Type I Procollagen (PICP)	Yes	Serum	9.0 (Range 9-10)	3.0-10.0	4.2-10.0
Aminoterminal Propeptide of Type I Procollagen (PINP)	Yes	Serum	8.0	2.2	
Bone Resorption					
Hydroxyproline (Hyp)	Yes	Urine	35.0 (Range 18-53)	2.7-10.0	4.8-13.0
Pyridinoline (Pyr)	Yes	Urine	17.0 (Range 10-26)	5.7-10.4	10.0-13.3
Deoxypyridinoline (dPyr)	Yes	Urine	26.0 (Range 12-63)	4.1-15.0	4.2-15.0
Carboxyterminal Crosslinked Telopeptide of Type I Collagen (ICTP)	No	Serum	10.0 (Range 9-10)	5.0-7.9	7.0-11.8
Aminoterminal Crosslinked Telopeptide of Type I Collagen (NTx)	Yes	Urine or Serum	22.0 (Urine) (Range 16-33)	7.6-9.6 (Urine)	4.0-8.0 (Urine)
Type I C-Telopeptide Breakdown Products (CTx)	No	Urine or Serum	48.0 (Urine)	6.4-9.0 (Urine); 4.7-4.9 (Serum)	12 (Urine); 5.4-7.9 (Serum)
Tartrate-resistant Acid Phosphatase (TRAP)	Yes	Serum	5.4	4.4	
Urinary Calcium (Ca)	Yes	Urine	32.6-40.1	2.5	
Urinary Hydroxylysine Glycosides (GHYL)	No	Urine		3.0	3.0

*Coefficient of variation data compiled from Beck Jensen et al. (1997a); Bouman et al. (1995); Braga de Castro Machado et al. (1999); Dressner-Pollak et al. (1996); Garnero et al. (1994); Guerrero et al. (1996); Hannon et al. (1998); Kress et al. (1999); Miura et al. (1995); Raisz et al. (1996); Riis et al. (1995); Rosenquist et al. (1998); Looker et al. (2000). NOTE: the coefficient of variation values reported may not be generalizable to other settings/populations.

Pyridinoline (Pyr) and deoxypyridinoline (dPyr) are two non-reducible pyridinium cross-linking amino acids present in the mature form of collagen. Cross-links are added during the final stages of collagen synthesis and are the first substances to be broken down during

bone resorption (Rosen & Tenenhouse, 1998). They are released into the serum with bone resorption and excreted in the urine (in either free or peptide-bound forms). dPyr is more specific than Pyr as a marker of bone degradation (Eyre, 1997; Rosalki, 1998), however neither is found exclusively in bone and therefore their specificity to bone metabolism must be considered. The urinary marker displays a circadian rhythm with peak excretion in the morning and minimal excretion in the afternoon. Given that the difference between the peaks may reach 100%, the timing of urine collection should be standardized. Overall, there is greater variation in assessment of urinary markers than in assessment of serum markers (Eyre, 1997; Russell, 1997; Garnero & Delmas, 1998).

Collagen cross-linking occurs at regions of the collagen molecule known as telopeptides. Both the carboxyterminal (C) telopeptide (ICTP) and aminoterminal (N) telopeptide (NTx) can be measured (Rosalki, 1998). A urinary-specific sequence for a breakdown product of the C-telopeptide has also been measured (CTx). A serum test for NTx was approved by the Food and Drug Administration in February, 1999 (United HealthSystem Consortium, 1999).

Tartrate-resistant acid phosphatase (TRAP) is produced by osteoclasts and is thought to be active in bone matrix degradation (Eyre, 1997; Garnero & Delmas, 1998). Plasma TRAP is increased during conditions in which bone turnover is increased. Although the value in assessing the overall plasma TRAP activity is limited by several factors, an immunoassay using antibodies specific to the bone isoenzymes of TRAP should provide a better assessment of osteoclast activity in osteoporosis (Eyre, 1997; Russell, 1997; Garnero & Delmas, 1998).

Fasting urinary calcium (Ca) (corrected by creatinine [Cr] excretion) is the least expensive marker of bone resorption but lacks sensitivity. The fasting value reflects both the release of calcium during resorption and the management of calcium by the kidney. Calcium levels are also influenced by excesses in hormones. Perhaps most useful is the fact that low urinary calcium levels are unlikely in patients with high bone turnover except when the skeleton is poorly mineralized (Christenson, 1997; Garnero & Delmas, 1998).

Although much less abundant than hydroxyproline, hydroxylysine is another potential marker of bone resorption (specifically collagen degradation). Testing for galactosylhydroxylysine (GHYL), one component of hydroxylysine, may provide a more sensitive assay (Eyre, 1997; Russell, 1997; Garnero & Delmas, 1998).

Potential Uses

Potential uses for biochemical markers of bone turnover include the following (Demers, 1997; Eastell & Blumsohn, 1997; Eyre, 1997; Seibel et al., 1997; Garnero & Delmas, 1998; Hough, 1998; Rosalki, 1998):

a. to identify potential slow and fast bone losers at menopause as an independent risk factor (at that time) for osteoporosis or future fracture;

b. to assist in making decisions about therapy (i.e., identifying those with greater potential for efficacy of therapy, selecting between anti-resorptive therapy and bone formation-stimulation therapy, determining the optimal dose);

c. to monitor changes in bone formation or resorption and/or to predict changes in bone mineral density as a result of therapy;

d. to provide support for patient follow through with recommended therapy.

FDA approval was given for the use of biochemical markers to assess the response to treatment (especially anti-resorptive agents) (Seibel et al., 1997). However, biochemical markers are not routinely used because large analytic and biologic variations make it difficult to differentiate between those with and without fracture risk (Dessauer, 1997). Potential use has also been limited by confusion about which marker to use, cost (who will

pay for the analysis), and lack of clear guidelines on the use of biochemical markers in clinical practice. The greatest needs (with respect to osteoporosis) are to be able to identify patients at greatest risk of fracture (i.e., those with the most rapid rate of bone loss) and to monitor the effects of a specific treatment for an individual patient (Russell, 1997). This goal implies that if a treatment fails, there are alternative treatments to use. Furthermore, it has been suggested (Kleerekoper & Alvioli, 1996; Greenspan, Parker, Ferguson, Rosen, Maitland-Ramsey, & Karpf, 1998) that biochemical markers may be use to provide feedback to patients that will encourage them to comply with expensive, somewhat unpleasant, treatment protocols. Looker et al. (2000) chose not to address the question of using biochemical markers for the purpose of increasing or monitoring compliance because there was no data to support or refute the practice.

Contraindications

Biochemical markers should not be a used as a sole criteria for the diagnosis of osteoporosis.

Efficacy of Treatment or Procedure

As defined in the Potential Uses section (above), assessment of biochemical markers may be done for one of several reasons. The following sections will address the use of biochemical markers to predict fracture risk, to monitor bone loss, and to assess the effects of treatment. Refer to the table on pages 5 and 6 for an explanation of the marker abbreviations.

Biochemical Markers as an Independent Risk Factor for Future Fracture

The evidence, to date, suggests that bone resorption may be increased and bone formation decreased prior to hip fractures. It has been suggested that biochemical markers may provide an independent predictor of fracture risk since high bone turnover may disrupt the trabecular network (a condition that would not be reflected in a bone density measurement) (Seibel et al., 1997). However, there are limited prospective data, typically a single baseline marker level has been obtained (rather than a change in marker level over the study period), the relationship between fracture risk and markers of bone turnover has not been the primary focus of the studies, and the studies have included primarily elderly, white women. In prospective studies of fractures in populations (see Appendix A), formation markers, including OC (hip fractures) (Garnero et al., 1996) and BSAP (hip and vertebral fractures) (Garnero et al., 1996; Ross et al., 2000) have not been found to predict fracture risk. The exception is the relationship between PICP and non-spine fractures observed by Åkesson et al. (1995). Resorption markers have shown greater promise in predicting fracture risk in elderly women. Specifically, CTx and dPyr were found to predict risk of hip fracture (Garnero et al., 1996) and ICTP was found to predict risk of non-spine fracture (Åkesson et al.,1995). NTx was not found to predict hip fracture risk (Garnero et al., 1996) nor was CTx found to predict vertebral fracture risk (Ross et al., 2000). There is insufficient data on the value of combining markers of bone turnover and BMD measures for the purpose of predicting fracture risk. Only one study, Garnero et al. (1996) provided sensitivity and specificity data necessary to determine the suitability of predicting fracture risk in individual patients. These studies, along with several studies that compared markers of bone turnover in patients with and without fractures, are described in the following paragraphs.

The relationship between fracture occurrence and bone turnover was assessed by Åkesson et al. (1995). A total of 328 women ages 40, 50, 60, 70, and 80 years old participated in the study. The sample of 328 represented 68% of a group of 481 women who were randomly selected from the population for inclusion in the study. Bone mineral content of the forearm was measured with single photon absorptiometry. OC, PICP, and ICTP were

assessed from serum samples. All fractures, including those in childhood and adolescence were recorded with special attention to fractures in the 6 years prior to the study. They excluded minor fractures of the hand or foot. A follow-up study of fractures was done 5 years after the initial enrollment. At the time of enrollment, 127 fractures had occurred in 87 women. In the immediate 6 years prior to enrollment, 37 women experienced one or more fractures. In the 5 year follow-up period, 43 women experienced 52 fractures (including 12 hip fractures, 15 vertebral fractures, 8 radius fractures, and 6 pelvic fractures). In both the retrospective and prospective data, the majority of fractures were found in the 70- and 80-year old age groups. OC and ICTP were positively correlated with age ($r=0.36$ and $r=0.44$, respectively with both $p<0.001$) and negatively correlated with BMC ($r=-0.35$ and $r=-0.31$, respectively with both $p<0.001$). PICP was only weakly correlated with both age ($r=0.13$, $p<0.05$) and BMC ($r=-0.08$, NS). In the 50 year old group, 24 women were premenopausal and 39 were postmenopausal. Levels of OC ($p=0.03$), PICP ($p=0.02$), and ICTP (non-significant) were higher in the postmenopausal subgroup. Using the retrospective fracture data, women with at least one fracture in the 6 years prior to the study had significantly lower levels of OC than those without fracture ($p=0.006$). Among 70 and 80 year olds, the PICP level was also significantly lower in those who had sustained a fracture ($p=0.046$). Using the prospective data, overall there were no significant differences in levels of any of the markers among those who were to sustain a fracture compared to those with no fracture. In the 70- and 80- year old groups, PICP was lower in those who were to sustain a fracture ($p=0.042$). Women with a previous fracture were more likely to suffer a new fracture ($p=0.004$).

Garnero et al. (1996) studied elderly women who were part of the EPIDOS cohort (a prospective study of the risk factors for hip fracture performed in five French cities). Included in the cohort were 7,598 healthy volunteers age 75 years and older recruited from population-based listings. All were ambulatory at the time of enrollment with 90% living independently. Baseline testing included gait speed, femoral neck BMD (with DXA), and collection of serum and urine samples from which serum OC, serum BSAP, urinary NTx, urinary CTx, urinary dPyr, and serum Ca, creatinine (Cr), albumin, and ALP activity could be determined. During the follow-up period (a mean of 22 months), 126 women sustained a hip fracture after a moderate trauma (an osteoporotic fracture). Since it was not possible to measure all of the biochemical markers for all of the subjects, a nested case-control analysis was done matching (on the basis of age and time of recruitment) each patient with three controls. After excluding patients (both cases and controls) with primary hyperparathyroidism, on chronic hemodialysis, or taking substances known to influence calcium metabolism, there were 109 with hip fracture and 292 controls. A premenopausal control group of 144 women ages 35 to 55 years was also studied.

Baseline comparisons between fracture cases and controls indicated that femoral neck BMD and gait speed were significantly lower in the hip fracture cases. Levels of all of the markers of bone turnover were higher in the elderly women (both groups) than in the premenopausal group. CTx ($p=0.02$) and dPyr ($p=0.005$) were higher for the fracture cases than for the age-matched controls. Odds ratios associated with a one standard deviation increase in CTx or dPyr were 1.3 ($p=0.05$) and 1.4 ($p<0.05$), respectively. The odds ratio for a one standard deviation decrease in BMD was 1.7 ($p<0.05$). When the women were divided into groups based on quartiles of CTx or CTx levels expressed as a number of standard deviations from the premenopausal mean, the odds ratios were significant only for the groups with the highest bone resorption rate. Odds ratios (representing increased risk of hip fracture) were close to 2.0 for these subgroups. Although gait speed was a significant predictor of fracture risk, after adjustment for gait speed, CTx and dPyr levels above the upper limit of premenopausal controls remained significantly associated with an increased risk of hip fracture (OR=2.1 and 1.9, respectively; both $p<0.05$). Similar values were observed after adjustment for femoral neck BMD (OR=2.0 and 1.7; both $p=0.05$). For women with low BMD and high CTx or high dPyr, the odds ratios for the risk of hip fracture were 4.8 and 4.1, respectively. All markers of bone turnover were

significantly correlated with BMD (r=-0.15 to -0.26, p<0.005). The results indicate that bone turnover rate remains high in elderly women and that markers of bone resorption (but not bone formation) are associated with increased risk of hip fracture independent of the BMD level. The authors suggested measuring both BMD and bone resorption.

Vergnaud et al. (1997) reported on an additional marker tested in the women in the EPIDOS cohort, serum ucOC. Two assays were used to measure ucOC, an indirect assay based on affinity for hydroxyapatite (HAP) and a direct enzyme-linked immunosorbent assay (ELISA). Baseline levels of total OC and ucOC (measured by either assay) did not differ between those who would subsequently experience a hip fracture and those who would not. The ratio of ucOC (measured by HAP) to total OC was higher (p=0.04) in patients who would later experience a fracture. For women with levels of ucOC in the highest quartile of values for the control patients, increased levels of ucOC (measured by ELISA) were associated with an increased risk of hip fracture (OR=2.0; 95%CI 1.2-3.2). The odds ratio for ucOC measured by HAP was not significantly greater than 1.0. After adjustment for the correlation between ucOC and bone mass, the odds ratio (ELISA method) remained significant (OR=1.8, 95%CI 1.1-3.0). Women with both high levels of ucOC (either assay) and low femoral neck BMD were at higher risk (p<0.05) of hip fracture (OR=5.5 for the ELISA assay and OR=4.4 for the HAP assay). It was concluded that increased levels of ucOC, but not total OC, predict hip fracture in elderly women independent of bone mass.

Ross et al. (2000) measured baseline BSAP, CTx, and calcaneus BMD in 512 community-dwelling postmenopausal women (part of the Hawaii Osteoporosis Study). The mean age of the women at baseline was 69 years. Spine and non-spine fractures that occurred after baseline were noted over an average of 2.7 years. At least one osteoporotic fracture occurred in 55 (10.7%) of the women; there were 33 vertebral fractures and 25 non-spine fractures. Baseline BSAP and CTx were higher and baseline BMD was lower among those who experienced a fracture compare to those who did not (all p<0.007). In a univariate analysis, BSAP (OR=1.53), CTx (OR=1.54), and BMD (OR=1.61) were significantly associated with new fractures (both spine and non-spine). In a multivariate analysis, only BSAP and BMD were significant predictors (p=0.017 and p=0.002, respectively). In a hierarchical regression analysis, BMD was entered first and found to be significantly associated with new fractures (p=0.0003). BSAP was entered into the model next and was found to significantly contribute to the prediction (p=0.0009). High bone turnover was significantly associated with an increased risk of osteoporotic fractures in post-menopausal women.

Several retrospective studies have also identified biochemical markers significantly associated with fractures. In studies of this type, the potential for recall bias exists and it is difficult to assess whether the fracture caused the change in bone turnover level or whether the change in bone turnover level contributed to the fracture. Melton, Khosla, Atkinson, O'Fallon, and Riggs (1997) found that only Pyr was significantly associated with fracture history among 213 postmenopausal women (45 with prior osteoporotic fractures). OC, BSAP, PICP, dPyr, and NTx were also assessed. Takahashi, Kushida, Hoshino, Ohishi, and Inoue (1997) observed higher levels of resorption markers (ICTP, Pyr, dPyr) in women who had experienced either hip or vertebral fractures (compared to post-menopausal women without fractures, p<0.01). There was no difference in the formation markers (ALP, OC, PICP). However, there was a greater uncoupling of the balance between formation and resorption in the fracture patients than in the non-fracture patients. In the study presented by LoCascio et al. (1999), only GHYL was significantly higher (p<0.001) in the fracture group than the non-fracture group. All of the women were post-menopausal with osteoporosis. Hyp, NTx, dPyr, and ALP were also measured.

Monitoring of Bone Loss and Identification of Potential Fast and Slow Bone Losers (Untreated Patients)

There is no consensus on whether biochemical markers can be useful in identifying fast bone losers. It is unclear whether those who are initially classified as fast losers will remain fast losers; it appears that an individual's rate of bone loss can vary from day to day. The timing of the sample with respect to menopause must be considered in interpreting the results (Rosso et al., 1996; Garnero et al., 1999). In addition, it must be recognized that the levels of the different markers are a reflection of the total skeleton bone turnover and not just turnover at a particular site of interest. Although significant correlations have been reported between BMD (initial levels and/or changes in BMD) and biochemical markers in population studies (Rosso et al., 1995; Miura et al., 1995; Dresner-Pollak et al., 1996; Ross & Knowlton, 1998; Garnero, 1999), the values are typically low (r=0.40 or less). There are also studies that have failed to find a correlation between BMD and markers of bone turnover (Keen et al., 1996; Bauer et al., 1999). The use of biochemical markers to predict change in bone mass has been reported to be of limited value for individual, untreated patients (Bauer et al., 1999; Garnero et al., 1999; Looker et al., 2000).

Eastell, Robins, Colwell, Assiri, Riggs, Russell (1993) evaluated the use biochemical markers for the purpose of identifying patients with above-normal resorption rates. Two groups of subjects were identified: 67 postmenopausal women (ages 50-79 years with lumbar spine BMD [measured with dual-photon absorptiometry and spine radiographs] within the age-specific normal range) and 63 postmenopausal women with vertebral fractures (ages 53-74 years with BMD at or below the fracture threshold). The fracture patients had never been treated with fluoride or bisphosphonates, had not taken estrogen during the previous 6 months, and had not taken calcium during the previous 3 months. Serum OC and urinary dPyr, Pyr, and Hyp were measured. The urinary markers were expressed in a ratio with Cr. Free crosslinks of dPyr, Pyr, and glycosylated Pyr (also divided by urinary Cr) were also determined. All of the markers of bone turnover were higher in the women with vertebral fractures ($p<0.01$) with the exception of glycosylated Pyr/Cr. Urinary Cr excretion was similar for both groups. To evaluate heterogeneity of bone turnover, variances were calculated. The serum OC and free crosslink excretion variances were similar for patients with osteoporosis and normal postmenopausal women. The variances for urinary dPyr/Cr ($p<0.05$), urinary Pyr/Cr ($p<0.001$), and urinary Hyp/Cr ($p<0.001$) were all greater in the osteoporotic patients. Z-scores indicated greater increases in the markers of bone resorption (dPyr, Pyr, and Hyp) than in the marker of bone formation (OC). Of the resorption markers, the greatest increase was in Pyr and the least increase was in Hyp. The uncoupling indices (the z-score for the marker of resorption minus the z-score for OC) were 0.45, 0.83, and 0.18 for dPyr, Pyr, and Hyp, respectively.

Using a longitudinal study design, Rosso et al. (1995) sought to better define the changes in BMD and markers of bone turnover in the immediate postmenopausal period. The subjects were 45 healthy women who were 3 years or less from their last menstrual period. They excluded women receiving medications for the prevention or treatment of osteoporosis. Urinary Cr, Ca, and Hyp and serum ALP and OC were determined. BMD was assessed at an ultradistal radial site using dual photon densitometry. The measurements were repeated in 29 patients at a mean of 30 months since cessation of ovarian function and in all subjects at the final evaluation (40 months after menopause). At the initial evaluation, the values for each of the biomarkers were significantly higher (all $p<0.004$) than those for a fertile age-matched population. At the time of the final testing, the mean z scores for serum OC were significantly higher than for any of the other markers. Only OC ($p<0.001$) and BMD ($p<0.038$) differed significantly from the initial values. The correlation between initial and final BMD was significant ($r=0.91$, $p<0.001$), however, there was no correlation between individual rates of bone loss and initial bone mass ($r=-0.1$). There was a weak inverse correlation ($r=-0.298$, $p<0.046$) between initial serum ALP and percent yearly bone loss.

Miura et al. (1995) assessed bone turnover in pre- and post- menopausal women and examined whether bone loss or BMD could be predicted by baseline bone turnover measures. The subjects were 51 premenopausal women (ages 28-59 years) and 30 post-menopausal women (ages 42-59 years). The median period after menopause (in the postmenopausal group) was 2.5 years but the group included 7 women who underwent menopause during the study period. Lumbar spine BMD was assessed with DXA annually for 3 consecutive years (4 measures). Pyr, dPyr, and free-dPyr were measured from urine samples and expressed as a ratio to urinary Cr excretion. Serum OC, PICP, and ALP were also assessed. The BMD of the premenopausal women was found to decrease by a mean of 1.3% in 3 years (not significant) while that of the postmenopausal women decreased by a mean of 5.6% ($p<0.05$). Of the biochemical markers tested, only PICP showed a significant correlation with the decreased BMD level ($r=0.48$; $p<0.05$). dPyr ($r=-0.66$; $p<0.001$), PICP ($r=-0.64$; $p<0.001$), Pyr ($r=-0.60$; $p<0.05$), and ALP ($r=-0.43$; $p<0.05$) were all significantly correlated with the initial measure of BMD. Combinations of several markers (in multiple regression models) improved the correlations with the highest value observed when PICP, ALP, and dPyr were included ($r=0.84$; $p<0.0001$). For the estimation of bone loss, only a model with dPyr, OC, and PICP was significant ($r=0.76$; $p<0.01$).

In an attempt to determine whether baseline markers of bone turnover could predict a high fracture risk group, Keen, Nguyen, Sobnack, Perry, Thompson, and Spector (1996) studied a group of 141 women ages 45 to 62 years (all within 5 years of menopause and not taking HRT or other medications known to affect bone metabolism). From serum samples, OC, ALP, estradiol, estrone and estrone sulphate, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulphate (DHEAS), and testosterone were measured. From urine samples, Ca, Hyp, Pyr, dPyr, estrone glucuronide, and Cr were determined. BMD of the lumbar spine and hip was assessed at baseline, 12, 24, and 48 months. At baseline, there was no significant correlation between BMD and any of the demographic measures. BMD at the spine was correlated with BMD at the hip ($r=0.41$, $p<0.001$). Baseline BMD was not correlated with any of the individual biochemical markers. The mean annual percentage change in BMD when the initial BMD was included in the slope calculation was –1.41% per year for the lumbar spine and –0.86% per year for the femoral neck. The correlation between the loss rates at the two sites was not significant ($r=0.13$) and there were no significant correlations between annual percentage change in BMD at the spine or hip and any of the biochemical markers (either individually or in combination). The sensitivity and specificity of the biochemical and hormonal assays for predicting bone loss was poor at both the spine and the hip (sensitivity 33-58%, specificity 50-72%).

A subset of participants (n=295) from the Study of Osteoporotic Fractures (SOF) with hip BMD tested at baseline and a mean of 3.8 years later and baseline biochemical marker data (OC, BSAP, NTx, CTx, dPyr, Pyr) were studied by Bauer et al. (1999). The women were at least 65 years old (mean age of 73 years) and not taking estrogen replacement therapy. Mean bone loss for the total hip was 0.6% per year. Baseline biochemical markers were weakly correlated with rate of change of hip BMD ($r=-0.01$ to $r=0.19$). The age-adjusted percentage loss in hip BMD increased across quartiles of the urine markers CTx, dPyr, NTx, and Pyr (all trends $p<0.03$). A similar, but non-significant, pattern was observed for the serum markers. The distribution of changes in BMD among those with baseline marker levels above the median was compared with the distribution of changes in BMD among patients with levels below the median. There was little difference in the distribution of the changes for the different markers indicating that the probability of an increase or decrease in total BMD is similar regardless of baseline marker level. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of a marker level either above the median or in the 4th quartile for identifying the highest tertile of total hip bone less (>1.1% per year) was determined. For a marker level above the median, the sensitivities (across the 6 biochemical markers studied) ranged from 56% to 72%, specificities ranged from 46% to 56%, PPVs from 35% to 42%, and NPVs from 69% to 79%. For a marker level in the 4th quartile, sensitivities ranged from 28% to

38%, specificities from 74% to 79%, PPVs from 36% to 46%, and NPVs from 68% to 71%. The authors concluded that the predictive value of the biochemical markers included in this study was limited and their clinical utility uncertain.

In the study presented by Garnero, Sornay-Rendu, Duboeuf, and Delmas (1999), baseline levels of OC, BAP, PICP, PINP, NTx, and CTx were correlated with the rate of change of forearm BMD. The follow-up period was 4 years with BMD measurements at baseline, 2, 3, and 4 years. The study included 305 women ages 50 to 88 years (mean of 64 years) none of whom had taken any treatment that might affect bone metabolism for 12 months prior to the study. Significant correlations were observed between OC, PINP, NTx, and CTx and rate of bone loss ($r=-0.16$ to $r=-0.30$; $p<0.01$) with higher values ($r=-0.20$ to $r=-0.53$) when the analysis was limited to women within 5 years of menopause. In the high turnover group (defined as more than 2 standard deviations above the level of healthy, premenopausal women), the odds ratios for fast bone loss (in the upper tertile of the population) were increased by 1.8- to 3.2- fold over those in the normal turnover group. Sensitivity for the identification of fast bone losers from high and low bone turnover rates ranged from 16% to 59%, specificity ranged from 66% to 93%, positive predictive value ranged from 44% to 56%, and negative predictive value ranged from 68% to 76%. The ability to identify individual fast bone losers was limited. It was concluded that increased levels of selected markers are associated with greater radial bone loss and because increased bone loss is associated with an increased risk of fracture, the markers may be useful for improving the prediction of the risk of osteoporosis in postmenopausal women.

Two studies used a retrospective approach measuring the bone turnover markers after the measurement of bone loss were complete. It is unknown whether prospectively the relationship between bone turnover markers and future bone loss can be predicted based on retrospective data. To assess the relationship between biochemical markers of bone turnover and femoral bone loss in the elderly, 36 female and 17 male community-dwelling individuals over age 65 were followed for 3 years (Dresner-Pollak, Parker, Poku, Thompson, Seibel, & Greenspan, 1996). Annual BMD measurements of the total hip, femoral neck, trochanter, intertrochanter, and lumbar spine were done using DXA. Rate of bone loss was determined as the slope of the best fit regression line representing average change in BMD over time. Serum and urine were collected at year three. Dietary calcium and vitamin D were estimated using a food frequency questionnaire. The urine specimens were analyzed for NTx, free Pyr, total Pyr, total dPyr, Hyp, Cr, and Ca. Serum tests for bone formation included OC and BSAP. Serum parathyroid hormone, Ca, albumin, phosphate, and ALP were also assessed. Over the course of the study, the elderly women had a statistically significant bone loss at the total hip ($p<0.01$) and intertrochanter ($p<0.01$) with a significant increase in BMD at the spine ($p<0.05$). The elderly men also showed an increase in spine BMD ($p<0.01$). There were no significant correlations between the initial BMD measurements of the total hip, femoral neck, trochanter, and intertrochanter and the rate of bone loss at each site. In the elderly women, rate of bone loss at the total hip was negatively correlated with NTx ($r=-0.52$, $p<0.01$), free Pyr ($r=-0.39$, $p<0.05$), Pyr ($r=-0.44$, $p<0.01$), dPyr ($r=-0.51$, $p<0.01$) and BSAP ($r=-0.38$, $p<0.05$). Trochanteric and intertrochanteric bone loss were similarly correlated. Calcium and OC were not significantly correlated with bone loss. Several of the biochemical markers were positively correlated with each other. The biochemical markers were not correlated with bone loss in men. It was suggested that the biochemical markers may help identify women at greatest risk for bone loss.

Data from a subset of patients participating in the Hawaii Osteoporosis Study were presented by Ross and Knowlton (1998). Rate of change of bone density was determined from BMD values obtained over a mean of 13.4 years. Biochemical markers (BSAP, OC, dPyr, and Pyr) were measured at the time of the final BMD measurement. dPyr and Pyr were corrected for the creatinine values. The subset of patients studied included those who provided serum and urine samples at the final BMD assessment. The study excluded

patients with new vertebral fractures on radiographs taken at the end of BMD follow-up; patients with nonspine fractures during the previous 12 months; and patients who used corticosteroids, active forms of vitamin D, bisphosphonates, or calcitonin. Of the total eligible sample, the analysis focused on the 99 women with the most rapid declines in BMD and the 100 women with the slowest declines in BMD. Levels of BSAP (28%), OC (21%) Pyr/Cr (16%), and dPyr/Cr (17%) were higher in the fast loss group (all $p<0.0001$). Each of the markers was significantly (all $p<0.001$) associated with rapid bone loss (increase in odds of rapid bone loss corresponding to a one standard deviation increase in the marker ranged from 1.8 to 2.0). When the patients were categorized by marker levels, women with higher levels of BSAP had an odds of rapid loss 4.7 times that of women with the lowest levels (the odds were 3.3 for OC, and 2.4 for both Pyr and dPyr). In clinical terms, women with BSAP values 2 standard deviations below the mean of the entire sample had a 20% probability of rapid bone loss while those with BSAP values 2 standard deviations above the mean had an 80% probability of rapid loss. Similar results were found for the other markers.

Monitoring Changes in Bone Formation or Resorption as a Result of Therapy

It has been widely suggested that the most beneficial use of biochemical markers would be in monitoring the effects of treatment. Changes in biochemical markers would be evident within a few months of the initiation of treatment (rather than 1 to 2 years as required for BMD measurements). To date, there is no consensus that the information is useful in guiding the choice of treatment or appropriate dose for an individual patient. Since the available treatments are anti-resorptive agents, the use of markers of bone resorption would appear to be most appropriate. However, given that the available markers of resorption are urinary markers and that urinary markers have greater variability than serum markers, individual results must be interpreted cautiously.

Postmenopausal women treated with HRT were found to have lower levels of markers of bone turnover than untreated women (Cosman et al., 1996) and to experience decreases in marker levels with treatment relative to women receiving calcium only (Rosen et al., 1997; Gonnelli et al., 1997) vitamin D only (Heikkinen et al., 1997), placebo (Marcus et al., 1999; Ettinger et al, 1998; Heikkinen et al., 1997), or no treatment (Hannon et al., 1998). Significant correlations of biochemical markers with bone loss at 12 and 24 months have been reported (Riis et al., 1995) and odds ratios have indicated that changes in biochemical markers (from baseline levels) were predictive of future BMD (Rosen et al., 1997). However, other studies have observed only low correlations (Cosman et al., 1996; Heikkinen et al., 1997) and have concluded that changes in biochemical markers are of no value in predicting changes in BMD (Marcus et al., 1999).

Postmenopausal women treated with alendronate have shown decreases in markers of bone turnover relative to placebo (Garnero et al., 1994; Greenspan et al., 1998; Kress et al., 1998; Ravn et al., 1999b), intermittent cyclical etidronate (ICE) (Watts et al., 1999), or intranasal salmon calcitonin (Adami et al., 1995). Changes in marker levels at 3 to 6 months have been found to be moderately, but significantly, correlated with changes in BMD at 24 months (Garnero et al., 1994; Greenspan et al., 1998; Kress et al., 1999; Ravn et al., 1999a; Ravn et al., 1999b).

Several studies have shown that marker levels decrease and BMD increases in postmenopausal women treated with HRT and/or ICE regardless of initial levels of BMD (Wimalawansa et al., 1995; Wimalawansa et al., 1998). Women receiving HRT and alendronate experience a greater decrease in biochemical markers levels and greater increases in BMD than women receiving either therapy alone (Lindsay et al., 1999; Bone et al., 2000). Changes in marker levels predicted no more than 22% of the variation in BMD (Bone et al., 2000).

Intranasal salmon calcitonin treatment resulted in increases in BMD (Cecchettin et al., 1995, Overgaard & Christiansen, 1996). Marker levels were decreased during treatment (Cecchettin et al., 1995), especially resorption markers (Overgaard & Christiansen, 1996;

Kraenzlin et al., 1996). Correlations between changes in marker levels and changes in BMD have not been reported.

Overall, there is no strong relationship between baseline markers levels and the degree of change in BMD as a result of treatment with either HRT or a bisphosphonate. The correlations between changes in markers and changes in BMD are inconsistent. No more than 23% of the variability in the 1 to 3 year change in BMD with treatment was accounted for by changes in bone formation marker levels during the first 3 to 6 months of treatment. For resorption markers, no more than 13% of the variability is accounted for. The clinical value of repeated measures of the biochemical markers is not clear (Looker et al., 2000).

Monitoring the Response to Hormone Replacement Therapy

The goal of hormone replacement therapy in the context of osteoporosis is to prevent postmenopausal bone loss. Riis, Overgaard, and Christiansen (1995) investigated whether biochemical markers of bone turnover would be of value in monitoring the skeletal effect of HRT. The study included 119 randomly selected healthy postmenopausal women (ages 45 to 55 years) with a history of natural menopause 6 months to 3 years prior to the start of the study. None were taking medications known to influence calcium metabolism. Seventy-six were taking HRT and 43 were taking placebo (this investigation was part of two large randomized trials). The treatment period was 24 months with bone mass measurements made every 3 months and serum and urine sampling at 3, 6, 12, and 24 months. Forearm BMD was measured with single photon absorptiometry while spine BMD was measured with DXA. Serum OC and ALP and urinary Hyp and Cr were assessed. Over the 2 year period, the HRT group did not lose bone mass while the placebo group lost an average of 2.4% in the forearm and 1.6% in the spine (both $p<0.001$). In multiple regression analyses, bone loss was correlated with changes in the biochemical markers (OC, Hyp, and ALP) with $r=0.77$ at 24 months for the forearm (and slightly lower values for the spine). In general, the correlation coefficients between bone loss and changes in the individual biochemical markers increased from 3 to 6 months and then were relatively stable. The maximum values, achieved at 12 or 24 months, ranged from $r=0.52$ to $r=0.72$ for the forearm and $r=0.51$ to $r=0.68$ for the spine.

Cosman, Nieves, Wilkinson, Schnering, Shen, and Lindsay (1996) followed three groups of women over a 3-year period. The purpose was to determine whether biochemical markers of bone turnover were related to rates of bone loss. The groups included untreated premenopausal women (n=17), untreated postmenopausal women (n=40), and estrogen treated postmenopausal women (n=24). Fifteen (37.5%) of the untreated postmenopausal women and 20 (83%) in the treated group were classified as having osteoporosis according to the WHO definition. Forty percent of the untreated group had a history of osteoporotic fracture compared to 67% in the treated group. BMD of the lumbar spine and femoral neck was measured with dual photon absorptiometry (with results subsequently converted to DXA equivalent data) or DXA approximately every 6 months. Serum assays included OC, PICP, ALP, BSAP, TRAP, ICTP, and CTx. Urine assays included Hyp, Ca, Pyr and D-Pry (both expressed relative to Cr values). Baseline levels of the biochemical markers including ALP, BSAP, OC, ICTP, urine dPyr/Cr and Pyr/Cr were higher in the untreated postmenopausal than the treated postmenopausal or the premenopausal women. TRAP was higher in the untreated postmenopausal than the premenopausal women. PICP and Hyp/Cr were not different in any group. Based on data from all patients, significant correlations were observed between OC, PICP, ALP, BSAP, and Hyp and percent rate of change of BMD (PRC-BMD) of the spine ($r=-0.24$ to $r=-0.49$, all $p<0.05$). PRC-BMD of the femoral neck was significantly correlated with PICP, ICTP, Hyp, Pry, and dPyr ($r=-0.26$ to $r=-0.35$, all $p<0.05$). For the untreated postmenopausal women, PRC-BMD in the spine was significantly correlated with calcium intake, ALP, and Hyp/Cr (p values not reported). PRC-BMD in the hip was significantly correlated with PICP, ICTP, Hyp/Cr, Pyr/Cr, and dPyr/Cr. For PRC-BMD of the spine, the best multiple regression model (predicting 42% of

the variance in BMD) included Hyp/Cr, ICTP, and BSAP. For PRC-BMD of the hip, the best model (predicting 32% of the variance) included body mass index, ICTP, and OC. For the women treated with estrogen (both pre- and postmenopausal), only OC and PICP were at all predictive, together able to predict only 4-5% of the variance in PRC-BMD at the hip or spine. In a subpopulation of all patients who lost bone in the femoral neck (n=46), all bone resorption measures except TRAP were significantly related to PRC-BMD in the femoral neck ($r=-0.30$ to -0.45; $p<0.05$). PICP, ALP, BSAP, but not OC were also correlated with PRC-BMD in the hip in this subgroup. There were no significant correlations between any of the biochemical markers and PRC-BMD in the spine in a subgroup of those who lost bone in the spine. It was concluded that biochemical markers cannot replace serial bone densitometry for accurate determination of change in bone mass at the most clinically relevant sites.

Raisz et al. (1996) compared the effects of estrogen alone to estrogen plus androgen using biochemical markers to monitor the skeletal response. The patients were all postmenopausal (with the last menstrual cycle at least five years prior). The initial treatment consisted of calcium (1000-1500 mg/day) for 3 weeks after which they were randomized to either esterified estrogens plus methyltestosterone (n=13) or conjugated equine estrogens (n=15). At the end of 9 weeks, patients with an intact uterus received medroxyprogesterone acetate for 2 weeks. After an additional week (3 weeks post-treatment) they returned for follow-up evaluation. The patients selected for the study were within 25% of ideal body weight; were nonsmokers; had not taken estrogens within the last 6 months; had no prior history of estrogen-dependent cancer, hypercortisolism, hyperthyroidism, or metabolic bone disease; and had no prior treatment with drugs that might affect bone metabolism (other than calcium supplements and estrogens) or drugs known to alter hepatic enzymes. A dietary questionnaire was used to assess calcium intake. DXA was used to measure the BMD of the lumbar spine. A lipid profile, hormonal testing, and a menopausal symptom assessment were also part of the study. Serum OC, BSAP, and PICP, and urinary Pyr, dPyr, and Hyp were assessed at baseline and at 3 week intervals. At baseline, the two groups were similar (although the mean age of the estrogen only group was 6 years older than the estrogen plus androgen group). Based on pill counts, the compliance with the treatment was greater than 90%. The two treatments had a similar effect on the markers of bone resorption (Hyp, Pyr, and dPyr) with significant decreases in Pyr and dPyr and little change in Hyp during treatment. At three weeks after treatment, there was a trend toward a return to baseline levels for Pyr and dPyr. The response of the formation markers differed between the two treatment groups. In response to estrogen only, OC declined throughout the 9 week trial, BSAP decreased initially and then leveled off, and PICP increased somewhat through week 6 and then decreased to below baseline levels. In response to estrogen plus androgen, OC and BSAP increased throughout the study period while PICP increased at week 3 and then decreased through week 9 (not quite returning to baseline level). Adverse events were comparable in the two treatment groups. It appeared that short term administration of androgen with estrogen might reverse the inhibitory effect of estrogen on bone formation.

In a study comparing the sensitivity and specificity of markers of bone turnover for predicting subsequent BMD, Rosen, Chesnut, and Mallinak (1997) randomly assigned women to receive either HRT plus calcium or calcium supplementation only (500 mg/day) for 1 year. The women were all postmenopausal and ranged in age from 40 to 58 years. All had experienced natural menopause from 6 months to 3 years before the study, were willing to be randomized, and had follicle stimulating hormone levels of greater than 30 IU/mL. The authors excluded patients based on prior medications that might interfere with bone metabolism, presence of a disease known to affect skeletal turnover, ideal body weight greater than 130%, renal insufficiency, recent fracture, positive mammogram within 12 months, or baseline BMD (spine or hip) more than four standard deviations below the mean of normal young subjects. Biochemical measurements were made at baseline and at 1, 3, 6, and 12 months after the start of the study. Included in the analysis were urinary NTx

and free dPyr, and serum OC and BSAP. BMD at the spine and femoral neck was assessed using DXA. A total of 227 women completed the study (out of 236 randomized). After 12 months of treatment, BMD increased significantly in the HRT group at both the spine (+2.5%, $p<0.0001$) and femoral neck (+1.0%, $p<0.05$). Markers of bone remodeling decreased consistently in the HRT group. At 1 month the decreases for NTx, dPyr, and OC were -28%, -10%, and -15%, respectively (all $p<0.0001$). BSAP increased initially but then decreased at 6 and 12 months (-25%, $p<0.0001$). Higher baseline levels of NTx, OC, and BSAP were associated with greater increases in spine BMD. Similarly, greater decreases in NTx, dPyr, and BSAP at 6 months were associated with greater increases in spine BMD. In addition, baseline levels of NTx differed ($p=0.0002$) between those who lost spine BMD and those who gained spine BMD following 12 months of HRT. For the HRT group, the odds ratios for the odds of a gain or loss in BMD associated with an increase of 1 standard deviation above the premenopausal mean of the different biochemical markers were as follows: OC (OR=6.5, 95%CI:2.6-9.99); NTx (OR=5.4, 95%CI:1.95-15.2); BSAP (OR=1.9, 95%CI:1.03-3.45); dPyr (OR=1.2, 95%CI:0.8-1.73). In the calcium only group, BMD decreased significantly at the spine and hip (-1.1%, $p<0.01$). Mean resorption marker levels did not change significantly after 12 months of treatment. Of the bone formation markers, BSAP increased from baseline to 12 months while OC did not change. Higher levels of NTx or OC at baseline were associated with the greatest decrease in spine BMD. For the calcium group, the odds ratios for the odds of a gain or loss in BMD associated with an increase of 1 standard deviation above the premenopausal mean of the different biochemical markers were as follows: OC (OR=1.6, 95%CI:1.0-2.41); NTx (OR=2.1, 95%CI:1.29-3.41); BSAP (OR=1.1, 95%CI:0.77-1.68); dPyr (OR=1.5, 95%CI:0.96-2.37). Overall, baseline urinary NTx and serum OC were the most sensitive predictors of change in BMD of the spine after 1 year of either HRT or calcium supplementation. The best prediction of bone gain or loss was the percent change in NTx and OC from baseline to 6 months.

Heikkinen et al. (1997) compared HRT with and without the addition of vitamin D_3 to determine whether levels of biochemical markers of bone turnover were changed with treatment and to determine their correlation with long-term BMD changes. The full data set included 464 postmenopausal women who had experienced their last menstrual period within 6 to 24 months before the study and who had no contraindications for HRT. Participants were randomized to either HRT, vitamin D, HRT plus vitamin D, or placebo. The data presented in this reference were from 18 women randomly selected from each treatment group (with 3 subsequently excluded when they stopped taking the assigned medications). The participants were questioned about their calcium consumption, physical activity level, and smoking and drinking habits. BMD of the lumbar spine and femoral neck were obtained with DXA. Serum concentrations of OC, BSAP, and ICTP were assessed at baseline and after 6 and 12 months of treatment. Vitamin D, estradiol, FSH, calcium, and phosphate levels were assessed at baseline. There were no differences between groups at baseline. After 6 months, OC levels had increased by 19.2% in the placebo group ($p=0.04$) but not in the other groups. At 12 months, OC levels decreased by 29.2% in the HRT group ($p=0.017$) and by 37.3% in the HRT + D group ($p=0.004$) but were not significantly different from baseline in the other two groups. BSAP levels decreased in subjects taking HRT (34.4%, $p<0.001$ after 12 months), HRT + D (36.2%, $p<0.001$ after 12 months), and vitamin D only (11.7%, $p=0.04$ after 12 months). ICTP concentrations decreased in both hormone groups (21.6%, $p=0.012$ after 12 months in the HRT group and 14.1%, $p=0.011$ after 12 months in the HRT+D group). BMD, assessed at baseline and after 2.5 years of treatment (total number with data at both points was 67), decreased in the vitamin D group (2.1%, $p=0.022$ at the spine and 3.6%, $p=0.019$ at the femoral neck) and in the placebo group (3.3%, $p=0.009$ at the spine and 2.7%, $p=0.010$ at the femoral neck). BMD was not significantly changed over 2.5 years in either of the HRT groups. The one-year changes in the biochemical markers were inversely correlated with the 2.5 year changes in BMD at the spine and femoral neck ($r=-0.24$ to $r=-0.36$, all $p<0.05$ except change

in OC with change in lumbar spine BMD, r=-0.24, p=0.06). HRT appeared to counteract the biochemical changes caused by increased bone turnover associated with menopause.

Gonnelli, Cepollaro, Pondrelli, Martini, Monaco, and Gennari (1997) randomly assigned post-menopausal women who were diagnosed with osteoporosis for the first time, to 2 years of treatment with either transdermal estrogen plus calcium (E+Ca) or calcium (Ca) alone. The goal was to compare the effect of treatment on high, normal, and low bone turnover patients. Of 90 patients randomized, 81 completed the study. Whole body retention (WBR), a measure of the skeletal uptake of bone-seeking radiopharmaceuticals, was evaluated at baseline as a measure of bone turnover. BMD (lumbar spine) was evaluated at baseline and at 12 month intervals as were ALP, OC, Hyp, and Pyr. There were no significant differences between groups at baseline. BMD was significantly increased from baseline in the E+Ca group and significantly decreased in the Ca group (both $p<0.001$ at 24 months) resulting in significant differences ($p<0.001$) between groups both at 12 and 24 months. In the E+Ca group, OC, Hyp/Cr, and Pyr/Cr decreased significantly at both 12 and 24 months of treatment (all $p<0.001$ at 24 months). Changes in ALP were not significant. There were no significant changes from baseline in the Ca group. At 24 months, differences between groups were significant ($p<0.001$) for all of the biochemical markers. The patients were subdivided into high-turnover (HT) or low-turnover (LT) osteoporosis groups according to their baseline WBR values. All of the markers of bone turnover were significantly higher ($p<0.001$) in the HT patients. HT patients treated with E+Ca had a greater increase in BMD than did LT patients with a significant difference between groups at 24 months ($p<0.05$). Both HT and LT patients in the Ca treatment group showed similar decreases in BMD. Among patients treated with E+Ca, ALP, OC, Hyp/Cr, and Pyr/Cr were decreased to a greater degree in the HT group than in the LT group with significant differences between the groups for all markers at 24 months. The authors suggested that bone turnover might be useful to identify those women with osteoporosis who could benefit from treatment with estrogen.

Hannon, Blumsohn, Naylor, and Eastell (1998) measured the response of biochemical markers of bone turnover to HRT over a 24 week period. The subjects were all within 8 years of menopause with low bone density (determined by DXA). None were taking any medications known to affect bone metabolism (including no HRT within the previous 6 months). Of 37 women who met the entry criteria, 21 agreed to participate. Assignment to a study group was by self-selection. Controls were allowed to switch to a treatment group following 24 weeks of no treatment. Overall, complete data was obtained from 11 who completed the treatment (24 weeks of transdermal HRT) and 11 who completed 24 weeks without treatment. Lumbar spine, total body, and left femoral neck BMD were measured at baseline and at 24 weeks. Serum PICP, PINP, OC, BSAP, TRAP, and ICTP were assessed along with urinary dPyr, free Pyr and dPyr, NTx, CTx, Hyp, Ca, and Cr. A least significant change (LSC) value was determined for each variable using estimates of analytical variability and within subject variability. Despite the non-random assignment, there were no baseline differences between groups for age, years post-menopause, BMD or any of the biochemical marker values. In general, markers of bone formation increased during the first 4 weeks of the treatment phase and then decreased to below baseline for the remainder of the 24 weeks. Most of the markers of bone resorption decreased following the introduction of HRT and continued to show a decrease throughout the study period. The exceptions were ICTP, which increased at the start and then decreased below baseline at 16 weeks, TRAP (another serum marker of resorption), which showed little change, and Hyp/Cr, which showed great intraindividual response to treatment. The lowest variability was observed for markers of bone formation (OC, BSAP, PICP, PINP) and for the two serum markers of bone resorption (ICTP and TRAP). Overall, LSC values were lower for serum markers than for urinary markers. For the treatment group, the mean percent change from baseline exceeded the LSC value for OC, PINP, Pyr, and free dPyr (all $p<0.05$). The number of responders (those whose pre- to post-treatment changes exceeded the LSC values) varied depending on the marker chosen to reflect a

response to HRT. If Hyp/Cr was the marker chosen, there were no responders while if OC or PINP were chosen, 9 of 11 were considered responders. If markers were measured twice at both baseline and at the end of treatment, the number of responders increased for most of the markers. The control group experienced a significant loss of BMD at the lumbar spine while the treatment group experienced a significant gain at that site (both $p<0.05$). No other differences were significant. The authors encouraged the use of two measurements at each time point to reduce the variability and thus lower the LSC value and suggested that individual laboratories should establish their own LSC values appropriate for the population(s) of patients being monitored.

Marcus et al (1999) presented data from the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial. This was a randomized trial designed to study the effect of different hormonal regimens (estrogen alone or estrogen plus progestins) versus placebo on coronary heart disease risk factors and bone mineral density in healthy postmenopausal women. For the purposes of this study, results from the women assigned to the different hormonal regimens were pooled to form an active treatment group. At the time of randomization, the women ranged in age from 45 to 64 years. Resorption markers (ICTP, NTx, Pyr, and dPyr) and formation markers (BSAP-1, BSAP-2, PICP, and OC) were assessed at baseline, 12, and 36 months (serum markers were also assessed at 24 months). Spine and hip BMD were assessed at baseline, 12, and 36 months. Fifty-four placebo group members and 239 active treatment group members took greater than 80% of their assigned medication and completed both baseline and 1 year biochemical marker and BMD assessments. For the placebo group, decreases in spine (1.43%, 3.31%) and hip (1.59%, 2.61%) BMD were observed at 1 year and at 3 years, respectively. For the active treatment group there were gains at the spine of 3.23% at 1 year and 5.4% at 3 years and gains at the hip of 1.12% at 1 year and 1.76% at 3 years. In the placebo group, there were no changes from baseline in any of the biochemical marker levels at either 1 year or 3 years. The treatment group experienced decreases in all marker levels with the greatest decline observed at the 12 month measurement point and a return toward baseline by 36 months. Baseline BMD level accounted for 4.4% of the variance in the one-year change in spine BMD among the placebo group. Baseline ICTP level accounted for 5.3% and baseline NTx level accounted for 4.1% of the variance in the change in spine BMD; no other marker accounted for more than 2%. At the hip, baseline BSAP-1 level accounted for 3.6% of the variance in hip BMD change at 1 year; no other marker accounted for greater then 1.3% and baseline BMD did not account for any of the variance. Combining baseline BMD, age, BMI, plus all baseline and all % change marker levels accounted for 44% of the variance in one-year percent spine BMD change (33% for the hip) leaving much of the variance in bone mass change unexplained and suggesting that the use of markers to predict the magnitude of change in bone mass at 1 year was of limited practical value in patients not taking estrogen. In the active treatment group, baseline BMD level accounted for 8.6% of the variance in the one-year change in spine BMD. Baseline NTx level accounted for 2.6% of the variance in spine BMD change; OC accounted for 2.1%. No individual baseline marker level accounted for more than 1% of the variance in hip BMD change. The percent change in BSAP-2 from 0 to 12 months accounted for 9% of the variance in one-year change in spine BMD and 4.1% of the variance in hip BMD. Percent change in PICP accounted for 7.2% and percent change in OC accounted for 5.1% of the variance in spine BMD change. No other marker accounted for more than 4.2% of the variance in spine BMD change. Other than BSAP-2, no marker accounted for more than 3.2% of the variance in one-year change in hip BMD. Baseline values for age, BMI, and spine (or hip) BMD together accounted for 12.6% of the variance in spine BMD change (4.3% of the change in hip BMD) at 1 year. The addition of individual baseline marker levels did not improve these values. The addition of percent change in marker levels accounted for more of the change in both spine and hip BMD with maximum values of 19.6% (spine) and 8.1% (hip) with the addition of change in BSAP-2. With all baseline marker levels and all percent change in marker values added, 28% of the variance in one-year change in spine BMD and 12% of the variance in one-year change in hip BMD was accounted for. The authors concluded that bone turnover

markers were not a surrogate for BMD in identifying women with low bone mass. The markers were not useful in predicting BMD changes for either treated or untreated women.

Although the primary purpose of the study was to evaluate the effects of raloxifene on vertebral fracture risk in a group of 7705 women, Ettinger et al. (1999) also measured serum OC and urinary CTx in 2622 of the women. To be eligible for the overall study, women had to be at least 2 years postmenopausal, have low bone mineral density or radiographically apparent vertebral fractures, have not taken an androgen, calcitonin, or bisphosphonate within the previous 6 months, have not taken oral estrogen within the previous 2 months, and have not received fluoride therapy for more than 3 months during the previous 2 years. The women were randomly assigned to receive either placebo, 60 mg of raloxifene, or 120 mg of raloxifene (plus daily supplements of calcium and vitamin D). The data presented were 36 month follow-up results. Women treated with raloxifene (either dose) had fewer new vertebral fractures than those taking placebo. OC concentrations decreased by a median of 8.6%, 26.3%, and 31.1% in the placebo, 60 mg, and 120 mg groups, respectively; CTx excretion decreased by 8.1%, 34.0%, and 31.5%, respectively. The differences between either of the raloxifene groups and the placebo group were significant ($p<0.001$). Bone mineral density of the femoral neck and spine also increased significantly ($p<0.001$) in the raloxifene group compared to the placebo group. The authors concluded that 3 years of raloxifene treatment would preserve bone density, reduce bone turnover, and reduce the incidence of vertebral fractures in postmenopausal women with osteoporosis.

Eastell et al. (2000) evaluated the short-term and long-term intrasubject variability of urine and serum NTx. The study included 277 healthy, postmenopausal women, 150 who had not taken HRT for at least 6 months prior to enrollment and had never taken bisphosphonates and 127 who took HRT on a regular basis for at least 6 month before enrollment. Short-term variability was assessed from specimens collected once a day for three consecutive days. Long-term variability was assessed from specimens collected at baseline and during 2 subsequent months. Lumbar spine and femoral neck BMD values (with DXA) were also determined at baseline. The two groups were similar at baseline with respect to age (overall mean of 63.6 years), years since menopause (overall mean of 16.3 years), height, weight, BMD, smoking status, exercise status, family history of osteoporosis, and race. NTx values (both urine and serum) were lower among the HRT users ($p<0.001$). The baseline urine NTx values were not significantly different at the 4 different test sites; the serum NTx values differed by site for both the HRT and the non-HRT groups ($p<0.001$ for both groups). The median short-term intrasubject variability (%CV) of urine NTx was 13.1% for the entire group. The median %CV values were higher for nonwhites and women who had never smoked or were former smokers. Risk factors for osteoporosis (including age, family history, HRT use, and BMD) were not associated with variability. The 2 test sites located in more southern regions had higher median %CV values than those in more northern regions. Median long-term variability for urine NTx was 15.6%; the value was higher for HRT users than non-users (17.2% vs. 14.2%; $p=0.05$). Long –term variability was weakly but significantly correlated with dietary vitamin D intake, serum calcium, and dietary calcium intake ($r=-0.17$ to -0.22; $p\leq0.01$). The median short-term variability for serum NTx was 6.3%. The value was higher among those who never smoked or were former smokers ($p=0.03$). Values were again higher at the more southern test sites. The median long-term variability for serum NTx was 7.5%. Long-term variability was correlated with body mass index, femoral neck BMD, and serum creatinine ($r=-0.14$ to -0.19; $p\leq0.02$). There were differences between clinic sites but the differences were not associated with the clinic location. A signal-to-noise ratio was developed with the signal indicating the effect of HRT (the difference in mean levels of NTx between HRT users and non-users) and the noise indicating long-term variability (median %CV). The signal-to-noise ratio for urine NTx was $-45.7/15.6=2.9$; for serum NTx the ratio was $-21.3/7.5=2.8$. The similar ratio values indicate a similar diagnostic ability. The authors computed LSC values to indicate that a decrease of 31% in urine NTx and 14% in serum NTx would be required to achieve a 90% confidence level that a decrease between two

sequential measurements (taken after the start of therapy) is clinically relevant rather than due to variability alone. Those differences were exceeded in the present study suggesting that NTx may be of clinical utility in monitoring response to antiresorptive therapy.

Monitoring the Response to Treatment with Bisphosphonates

Changes in biochemical markers as a result of treatment with alendronate were evaluated by Garnero, Shih, Gineyts, Karpf, and Delmas (1994). The study included 85 women ages 43-74 years, all of whom were at least 5 years past natural menopause and with a lumbar spine BMD of more than 2 standard deviations below the mean for premenopausal women. The women were randomly assigned to receive either a placebo, 5 mg of alendronate, or 10 mg of alendronate for 24 months. All of the women received supplemental calcium (500 mg/day). In addition to BMD measures (DXA), serum OC (total and intact), BSAP, PICP, and ICTP and urinary total Pyr, dPyr, free Pyr, and NXT were assessed. The markers of bone formation (OC, BSAP, PICP) were suppressed as a result of either dose of alendronate. Maximum suppression was reached at between 6 and 12 months of treatment. The response of the bone resorption markers was less consistent. NTx and dPyr levels dropped within one month of treatment and remained suppressed. Pyr levels decreased to a lesser degree while neither free Pyr or ICTP were affected by the treatment. Among those markers showing a response to treatment, a dose-response pattern was observed for OC, BSAP, and NTx. To assess whether early changes in biochemical markers could predict long-term response to treatment, percent changes in the biochemical markers from baseline to 3 months of treatment were correlated with percent change in lumbar spine BMD after 24 months. All of the markers of bone formation were significantly correlated with the change in BMD ($r=-0.63$ to $r=-0.67$, $p<0.0001$) while for bone resorption, only dPyr and NTx were significantly correlated ($r=-0.48$ and $r=-0.53$, respectively, $p<0.0001$).

Adami et al. (1995) examined the safety, efficacy, and tolerability of 2 daily oral doses of alendronate as well as the efficacy of intranasal salmon calcitonin (sCT). The primary outcome measure was percent change from baseline in BMD of the lumbar spine. A total of 286 women ages 48-76 years were enrolled in the trial. All were at least 2 years past natural menopause. Their lumbar spine BMD was more than 2 standard deviations below the mean for young premenopausal women. Prevalent fractures were noted in 5%. Patients with metabolic bone disease or thyroid disease; those who received calcitonin, estrogen, progestogen, steroids, glucocorticoids, or high doses of vitamin A or D for more than 2 weeks within 6 months prior to baseline; and those who had ever been treated with fluoride in doses of more than 1 mg/day or any bisphosphonate were excluded. The subjects were randomized to 2 treatment arms one of which was double blind (with respect to placebo) and one open label (sCT) since there was no suitable placebo for the intranasal therapy. In the double blind arm, patients received either placebo, 10 mg/day of alendronate or 20 mg/day of alendronate. All patients also received 500 mg elemental calcium supplements (to be taken daily). Follow-up visits were scheduled for 3, 6, 9, 12, 18, and 24 months. BMD (spine and hip as determined with DXA) was assessed every 6 months; biochemical analyses (urinary Pyr and dPyr, and serum OC and ALP) were done at each visit. Daily treatment with alendronate increased bone mass at the lumbar spine by 5.2% and 7.3% ($p<0.001$) for the 10 and 20 mg/d doses, respectively. The placebo group experienced a 0.01% decrease and the sCT group a 0.81% decrease. The increases in BMD occurred primarily during the first year of treatment but the gains did continue in the second year. BMD of the proximal femur increased by 1.2% with 10 mg of alendronate and 2.04% with 20 mg. Trochanter BMD increased by 6.81% in the 10 mg group and 7.21% in the 20 mg group. Both alendronate doses produced increases in BMD at all hip sites (relative to treatment with placebo) while sCT failed to increase BMD at any site and resulted in decreased BMD at the femoral neck and Ward's triangle. Urinary dPyr excretion decreased at 12 months with both doses of alendronate while there were no changes from baseline in either the placebo or the sCT groups. Pyr changes followed a similar pattern with the exception of a slight decrease in placebo-treated patients. ALP and OC were

both decreased following alendronate treatment. Neither placebo nor sCT were effective in decreasing ALP. OC was decreased with sCT but the difference was not significant from the decrease observed with the placebo treatment. Changes following both sCT and placebo were less than those following treatment with alendronate. The overall safety profile did not differ across groups (including the placebo group).

Greenspan et al. (1998) evaluated the early changes in biochemical markers associated with the use of alendronate in women 65 years of age and older. All of the women were healthy, ambulatory, and community-dwelling. They excluded patients with a history of any illness affecting bone and mineral metabolism, those taking any medications known to affect bone metabolism, or those treated for osteoporosis within the past year but did not select patients on the basis of their bone density. In a double-blind trial, the patients were assigned to receive either alendronate (5 mg/day initially, increased to 10 mg/day for the final year of the study) or placebo for 2.5 years. Patients in both groups with estimated daily dietary calcium intakes of less than 1000 mg received supplemental calcium and vitamin D. BMD (with DXA) was evaluated at the hip, lumbar spine, total body, and radius at 6 month intervals. NTx, D-Pry, OC, and BSAP were also assessed at 6 month intervals. There were no differences between groups at baseline for any clinical measure (height, age, weight, BMI, dietary calcium and vitamin D, and various serum measures) or any of the biochemical markers. Baseline BMD was similar with the exception of the intertrochanteric site. Of 120 enrolled, 77% of the treatment group and 73% of the placebo group completed the study. An intention-to-treat analysis was used carrying forward the last available measurement from those with incomplete data. Over 2.5 years, significant increases in BMD (1.3% to 10.6%, $p<0.01$) were observed at all sites in the patients treated with alendronate. BMD values for the placebo group remained stable with the exception of significant increases at the spine (1.9% to 2.1%, $p<0.05$). Compared with placebo, by 12 months, treatment with alendronate significantly increased mean BMD at all sites ($p<0.05$). The number of fractures reported by the two groups over the 2.5 years of the study did not differ. Following 6 months of treatment, there were significant decreases in NTx (-53%), dPyr (-10%), OC (-20%), and BSAP (-24%) in the alendronate group (all $p<0.01$). In the placebo group, smaller but significant decreases were observed for NTx (-14%) and BSAP (9%) (both $p<0.01$) at 30 months and for OC (-8%, $p<0.05$) at 24 months. At 6 months, there were differences between the alendronate and placebo groups in the mean levels of biochemical markers ($p<0.01$), a difference that was maintained for 2.5 years. Baseline levels of NTx were associated with changes over 2.5 years in total body, vertebral, and trochanteric BMD in the alendronate group ($r=0.27$ to 0.36, all $p<0.05$). The change in NTx after 6 months of treatment with alendronate was significantly correlated with changes over 2.5 years in total hip, trochanteric, femoral neck, spine, and total body BMD ($r=-0.28$ to -0.41; all $p<0.05$). The 6 months change in OC was associated with changes at 2.5 years in total hip, trochanteric, intertrochanteric, and spine BMD ($r=-0.31$ to -0.43, all $p<0.05$). There were no consistent correlations between changes in the markers at 6 months and changes at 2.5 years in BMD in the placebo group. The alendronate was well-tolerated with gastrointestinal complaints from 47% of the patients taking alendronate and 43% of those taking the placebo. The authors suggested that early decreases in NTx might be useful for predicting changes in BMD, for motivating patients to remain compliant with the treatment, and for indicating poor compliance or a decreased absorption of the treatment.

A subset of postmenopausal women with osteoporosis (defined as a lumbar spine BMD at least 2.5 standard deviations below the premenopausal mean) and enrolled in a larger trial of alendronate was studied by Kress, Mizrahi, Armour, Marcus, Emkey, & Santora (1999). The purpose of the study was to determine whether BSAP could be used to monitor changes in bone turnover associated with the use of alendronate. To be eligible, the women had to have BSAP values from baseline, 3 months, and 6 months with at least one additional BSAP value at 12 or 24 months after baseline. The women also had to have lumbar spine BMD values at baseline and 24 months with at least one additional

measurement at 3, 6, or 12 months. Eighty percent of the women in the larger trial were eligible, 148 who had been assigned to the placebo group and 74 who had been assigned to the alendronate (10 mg/day) group. The mean age of these 222 women was 64 years (range 45-78 years) and all were at least 5 years postmenopausal. BSAP values from apparently healthy premenopausal (n=228) and postmenopausal (n=529) women comprised the reference group. Compared to the healthy premenopausal women (mean=8.7μg/L), the BSAP mean was 52% higher (mean=13.2μg/L) in the healthy postmenopausal women and 101% higher (mean=17.5μg/L) in the postmenopausal osteoporotic women (both $p \leq 0.0001$). In the alendronate group, BSAP decreased 35.5% from baseline at 3 months and 45.7% from baseline at 6 months. The decreased levels were maintained after 12 and 24 months of treatment. The values were significantly different from baseline at all times ($p \leq 0.0001$). In the placebo group, BSAP decreased by 11.4% at 3 months, remained constant at 6 months, and then increased to baseline levels by 24 months. The BSAP values were significantly different from baseline at 3, 6, and 12 months ($p \geq 0.0001$) but not at 24 months. After 3 months of treatment, the mean BSAP values from the alendronate group decreased to within 1 standard deviation of the mean BSAP for the healthy premenopausal women. By 6 months, the mean BSAP values did not differ significantly. The change in BMD over 24 months was significantly correlated with percent decrease in BSAP at 3 months ($r=0.43$; $p \leq 0.0001$)) and at 6 months ($r=0.49$; $p \leq 0.0001$) when the alendronate and placebo groups were considered together. The correlation was not significant for either group, alone. To assess the response to therapy in individual patients, those with a ≥25% decrease in BSAP from baseline after 6 months of treatment were termed "biochemical responders" while those with a BSAP that fell below the mean for healthy premenopausal women were termed "normalizers." In the alendronate group, 63 (85.1%) were biochemical responders by 6 months (60 or 95% of whom also were normalizers within 6 months of starting treatment). Of the remaining 11 (14.9%) who did not respond by 6 months, 9 achieved a decrease in BSAP of ≥25% by 24 months of therapy. In the placebo group, 36 (24.3%) were biochemical responders by 6 months. At 24 months, the number had decreased to 17 (11.5%). BSAP was considered to be a sensitive and reliable tool for monitoring reduction in bone turnover after treatment with alendronate.

Biochemical markers were assessed in a subset of the patients in a trial designed to evaluate the effects of risedronate on fracture risk (Harris et al., 1999). The subset consisted of 775 patients (32% of the total). All were ambulatory, age ≤85 years (mean age approximately 68.5 years), at least 5 years post-menopausal, with either ≥2 vertebral fractures or at least 1 fracture and a BMD of 2 standard deviations below the mean for young adults. Patients in the larger trial had been randomly assigned to receive either 2.5 mg or 5 mg of risedronate or placebo. The 2.5 mg treatment arm was discontinued after data from other trials indicated that this dosage was less effective. All patients also received calcium supplements (1000 mg/day); vitamin D (up to 500 IU/day) was provided if baseline levels were low. In the overall study, the incidence of both vertebral (41%) and non-vertebral (39%) fractures was reduced in the 5 mg risedronate group over the 3 years of the study. Bone mineral density increased by 5.4% at the lumbar spine, 1.6% at the femoral neck, and 3.3% at the femoral trochanter. After 3 years, BSAP levels had decreased 33% in the risedronate group and 7% in the placebo group. dPyr levels had decreased by 26% in the risedronate group and 1% in the placebo group.

Ravn et al. (1999b) evaluated the prediction of long-term response to bone mass based on short-term changes in biochemical markers of bone turnover. The women in the study, all ages 45 to 59 years and at least 6 months postmenopausal, were participants in a randomized trial of alendronate. There were 407 women in the 2.5 mg/day group, 387 in the 5 mg/day group, and 408 in the placebo group. BMD (hip and spine) was measured at baseline and annually. Enrollment was restricted so that at least 90% of the participants had a spine BMD above 2 standard deviations below the mean for young, normal women (i.e., most participants had normal BMD at baseline). Biochemical markers (NTx and OC) were assessed at baseline and every 6 months. In the 2.5 mg/day group, NTx decreased

41% at 6 months and 48% at 24 months. In the 5 mg/day group, NTx decreased 56% and 58% at 6 and 24 months, respectively. The placebo group had decreases of 14% and 16% respectively. OC decreased by 21% at 6 months and 33% at 24 months in the 2.5 mg/day group and by 29% at 6 months and 41% at 24 months in the 5 mg/day group. The placebo group had decreases of 10% and 17%, respectively. Baseline values of NTx and OC were only weakly correlated with the change in BMD from baseline to 24 months in the placebo group (r=-0.12 to r=0.07) and in the 5 mg/day group (r=-0.09 to r=0.17). The correlations between percent change from baseline for NTx and OC at 6 months and change from baseline in BMD at 24 months were statistically significant (r=-0.12to r=-0.31 for NTx [all $p<0.01$]; r=-0.11 to r=-0.25 for OC [all $p<0.05$]). To evaluate the relationship for individual patients (the 5 mg/day group), cut points of decreases from baseline of 40% (NTx) and 20% (OC) were identified. These cut points gave the highest possible sensitivities and specificities and reflected that NTx decreased about twice as much as OC. For spine BMD, the sensitivity, specificity, positive predictive value, and negative predictive value of a decrease in NTx at month 6 of 40% or more were 86%, 48%, 92%, and 33%, respectively. For OC, the corresponding values were 79%, 53%, 92%, and 37%, respectively. The accuracy of predicting changes in hip and total body BMD was similar; there was lower accuracy in predicting change in forearm BMD. A decrease in NTx or OC below the cut point at 6 months was therefore a valid indicator of stabilized or increased BMD in response to treatment with alendronate. A lack of decrease below the cut point could not accurately identify women with bone loss during treatment with alendronate.

A subsequent publication focused on the Danish cohort of a multicenter alendronate trial (Ravn et al., 1999a). This study was designed to look at the ability of biochemical markers to predict long-term response in BMD. The cohort consisted of 79 women, 67 of whom completed the second year of the study. The eligibility criteria were similar to those described above: age 40-59 years at baseline, menopause 6 months to 3 years previously, spinal BMD within ± 2 standard deviations of the young normal mean, and no established osteoporosis. Patients were randomized to receive either placebo, or 1, 5, 10, or 20 mg of alendronate daily plus a 500 mg calcium supplement. Lumbar spine, total hip, and total body BMD values were determined at baseline and every 6 months thereafter. dPyr, NTX, total OC, BSAP, N-terminal mid-fragment OC (N-MID OC), urinary ICTP, and serum ICTP were measured at baseline and every 3 months thereafter. A dose-related response was observed for the different biochemical markers and for BMD. In the groups receiving 5 to 20 mg/day of alendronate, baseline marker values tended to be negatively associated with baseline BMD and positively associated with change in BMD over 2 years. There were few significant correlations. Changes from baseline marker levels at 3, 6, and 12 months were more highly correlated with two-year change in BMD. For N-MID OC, the correlation coefficients ranged from r=-0.40 to r=-0.78 (all $p<0.001$). For total OC, the range was r=-0.33 to r=-0.70 (all $p<0.01$). Urinary ICTP (r=-0.40 to r=-0.71; all $p<0.001$), serum ICTP (r=-0.47 to r=-0.77; all $p<0.001$), and NTX (r=-0.27 to r=-0.62; all $p<0.05$ except total body BMD at 5 months with r=-0.20) were also significantly correlated with two-year change in BMD. The ability of the biochemical marker values to predict prevention of bone loss over 2 years was also determined. A change from baseline after 12 months of treatment in N-MID OC, total OC, urinary ICTP, serum ICTP, and NTX were similar in their ability to predict (area under ROC curve of 92%-96%). For N-MID OC, total OC, urinary ICTP, serum ICTP, and NTX, the sensitivity of a change from baseline at 6 months for the prediction of prevention of bone loss over 2 years at the spine ranged from 78% to 98%, specificities from 59% to 100%, positive predictive values from 82% to 100%, and negative predictive values from 67% to 93%.

Watts and Becker (1999) studied 24 women who had been treated with intermittent cyclical etidronate (ICE, 400 mg/day for 14 days, repeated every third month) for one year or more with no change or a decrease in spine BMD. The purpose of the study was to evaluate the effect of alendronate for women who failed to respond to ICE therapy. The women were switched to alendronate (10 mg/day). Under both treatments, the women

were advised to take at least 500 mg of supplemental calcium and a multivitamin with 400 IU of vitamin D daily. BMD of the lumbar spine and proximal femur was assessed before the ICE treatment, at the time of the switch to alendronate, and after at least 4 months of alendronate treatment. Biochemical markers (dPyr and BSAP) were measured at the end of ICE treatment and at the first follow-up visit after switching to alendronate. Due to different lengths of treatment, BMD was expressed as annualized percent change. The mean age of the women was 65 years (range 45-83 years). At baseline, they had moderate to severe osteoporosis (based on mean t scores of –3.6 at both the spine and hip). The mean duration of treatment was 3.3 years (range 0.8-7.7) for ICE and 1.3 years (range 0.4-2.1) for alendronate. The mean annualized percent changes in BMD after ICE ranged from –1.6 (spine) to –0.8 (Ward's region of the proximal femur). After an average of more than 1 year of alendronate, the mean annualized percent changes ranged from 1.2 (intertrochanteric region of the proximal femur) to 4.4 (spine). The changes in BMD after alendronate treatment were significantly greater ($p \leq 0.02$ for all sites) than after ICE. The dPyr/Cr ratio decreased and the BSAP level increased significantly (both $p<0.0001$) following treatment with alendronate. There was no correlation between change in bone markers and change in BMD after alendronate. Four patients (16% of an initial group of 25) discontinued alendronate because of gastrointestinal side effects (one patient discontinued treatment immediately and was not included in the results presented above). The authors acknowledged problems in defining nonresponders and noted that many patients did experience significant increases in BMD with ICE treatment.

Monitoring the Response to Treatment with Bisphosphonates and Hormone Replacement Therapy

In the study presented by Wimalawansa (1995), the purpose was to determine whether there was an added benefit if HRT was combined with alendronate. Early postmenopausal women (within 1 to 5 years of the onset of natural menopause) with BMD within the reference range established for healthy women ages 30 to 40 years, were randomly assigned to receive HRT plus calcium (n=15), ICE plus calcium (n=14), HRT plus ICE plus calcium (n=15), or calcium only (n=14). The calcium dose was 1000mg/day for all groups. A nonrandomized control group of 4 patients received no treatment. BMD (femoral neck and lumbar spine) and biochemical marker (BSAP and Hyp) data were obtained at baseline and after 2 years in 86% of the patients enrolled and after 4 years in 74% of the patients enrolled. The mean age of the women was 53 years (range 45-57 years). In the HRT, ICE, and HRT plus ICE groups, both BSAP and Hyp were significantly decreased ($p<0.001$) from baseline values at both 2 and 4 years. In the calcium only and the no treatment groups, BSAP and Hyp were significantly increased ($p<0.01$) from baseline at both 2 and 4 years. A similar pattern was observed for BMD at both the spine and hip. BMD increased relative to baseline in the HRT, ICE, and HRT plus ICE groups (all $p<0.05$) with significantly greater increases ($p<0.05$) in BMD in the patients receiving the combined treatment. BMD decreased relative to baseline in both the calcium only group and the no treatment group ($p<0.05$).

In a subsequent study, postmenopausal women with established osteoporosis (based on radiographically demonstrated fractures and spine BMD of at least 2 standard deviations below the reference range) were enrolled (Wimalawansa, 1998). They excluded women who had taken medications that affect calcium metabolism within the past 2 years and women who had taken HRT, anabolic steroids, glucocorticoids, calcitonin, fluoride, or bisphosphonates at any time since menopause. Patients were randomly assigned to calcium only (n=18), HRT plus calcium (n=18), ICE plus calcium (n=17), or HRT plus ICE plus calcium (n=19). The calcium dose was 1000mg/day for all groups; 400 IU of vitamin D was also provided. BSAP and Hyp were measured at baseline and after 2 years of treatment in 90% of the patients and after 4 years of treatment in 81% of the patients. BMD of the lumbar spine and femoral neck were also determined. The mean age of the patients was 65 years (range of 58-72 years). Both BSAP and Hyp were reduced relative to baseline at both 2 and 4 years of treatment in women treated with HRT and/or ICE ($p<0.001$). Non-

significant increases were observed in the calcium only group. At the spine, BMD increased significantly at both 2 and 4 years for the HRT and/or ICE groups (all $p<0.001$ relative to the calcium only group) with the greatest increases for the combined therapy group. At the hip, increases in BMD at 4 years in the ICE group were modest ($p<0.05$ relative to the calcium only group and $p<0.05$ relative to the other treatment groups). The HRT plus ICE therapy group experienced the greatest increase in BMD at both the spine and hip. Spine and hip BMD were decreased in the calcium only group ($p<0.05$).

Lindsay et al. (1999) recruited women who had been receiving HRT for at least 1 year and who had BMD values at the lumbar spine or femoral neck that were at least 2 standard deviations below the mean for a reference population of young women. The BMD of the other site had to be at least 1.5 standard deviations below the mean. The purpose was to study the effects of adding alendronate to ongoing HRT. The 428 patients eligible for the study were randomized to receive either 10 mg of alendronate daily or placebo in addition to their HRT regimen. BSAP and NTx were measured at baseline and after 6 and 12 months of treatment as were BMD of the lumbar spine, trochanter, and femoral neck. The mean age of the study participants was 62 years. The combined treatment group experienced greater decreases in both BSAP and NTx (relative to baseline) than did the HRT only group with the difference between groups significant ($p<0.001$) at both 6 and 12 months. Increases in BMD at the spine ($p<0.001$) and the trochanter ($p<0.01$) were significantly greater for the combined therapy group. At the femoral neck, the percent change from baseline did not differ significantly in the two treatment groups.

In the study presented by Bone et al. (2000), women ages 42 to 82, with prior hysterectomy and lumbar spine BMD below 0.862g/cm^2 for at least three vertebrae in the L1-L4 region, and who had not taken any form of systemic HRT within the past 6 months, were randomized to receive either placebo alendronate and placebo estrogen (n=50), 10mg/day alendronate plus placebo estrogen (n=92), estrogen plus placebo alendronate (n=143), or alendronate plus estrogen (n=140). Each patient also received 500mg/day of calcium. The study was designed to study whether the combined use of alendronate and estrogens might lead to increases in bone mass beyond that observed with the individual components. BMD measures of the lumbar spine, hip, and total body were assessed at 6, 12, 18, and 24 months. Biochemical markers (BSAP and NTx) were assessed at 3, 6, 12, 18, and 24 months. At baseline, the groups were similar. The mean age was 61.5 years and the mean t-score for the lumbar spine BMD was –2.5. During the course of the study, 29 patients discontinued treatment because of an adverse experience, 40 patients withdrew consent, 17 patients were lost to follow-up, and 9 patients were dismissed for protocol violations. In all, 75.3% of the patients completed the study (with no differences between groups); data were analyzed by intention to treat. Increases in BMD (relative to baseline) in the combination group at both the lumbar spine and the femoral neck were significantly greater than those in either the alendronate or estrogen groups (all $p\leq0.02$). The increases were greatest in the first year of the study but continued to 2 years with no evidence of a plateau being reached. NTx was significantly reduced in all of the treatment groups (all $p\leq0.005$ with respect to baseline), reaching a low point by 6 or 12 months that continued through 24 months. The placebo group decreased initially but returned to baseline levels by 12 months. BSAP levels followed a similar pattern (all $p\leq0.002$ with respect to baseline) with the low point reached by 18 months. Neither baseline BMD measures nor changes in BMD at 2 years were consistently correlated with baseline levels of BSAP or NTx. In all active treatment groups, changes in BSAP and NTx were weakly correlated ($r=-0.19$ to $r=-0.47$) with changes in lumbar spine and total hip BMD. No more than 22% of the variation in BMD was predicted by changes in the biochemical markers levels. The marker levels were not useful in identifying women whose BMD decreased despite active treatment.

Monitoring the Response to Treatment with Intranasal Salmon Calcitonin

Cecchettin, Bellometti, Cremonesi, Solimeno, and Torri (1995) used BMD and biochemical markers of bone turnover to assess the metabolic and bone effects of 12 months of treatment

with either ipriflavone (IP) or sCT. IP is a synthetic flavonoid derivative that is being investigated for the treatment of osteoporosis. The 40 osteoporotic (BMD at the radius of greater than 2 standard deviations below the mean value for healthy age matched individuals) women were between 55 and 80 years of age. Assignment to a treatment condition was random. Evaluations took place at baseline, 3, 6, 9, and 12 months. Serum calcium, phosphate, ALP, and OC, and urinary calcium, phosphate, and the Hyp/Cr ratio were assessed. BMD of the radius was determined using DXA at 0, 6, and 12 months. There were no significant differences between the groups at baseline. After 6 and 12 months, both treatments increased BMD with greater improvements seen in the IP group ($p<0.001$). The mean increase after 12 months of IP treatment was 4.3% compared to a 1.9% increase in the sCT group. Both groups experienced significant ($p<0.001$) decreases (from baseline) in ALP, OC, urinary calcium, and the Hyp/Cr ratio. There were significant differences between groups ($p<0.05$) for urinary calcium and the Hyp/Cr ratio with greater changes in the IP group. No patient discontinued treatment because of adverse effects.

The response of biochemical markers of bone resorption to 3 different doses of sCT (50 IU, 100 IU, or 200 IU) or placebo, administered for 2 years, was evaluated by Overgaard and Christiansen (1996). The subjects of the study were healthy, postmenopausal women ages 68 to 72 who were randomly assigned to a treatment group. All of the subjects received daily calcium supplements (500mg). Of 208 enrolled in the study, 164 completed the 2 year follow-up. In addition to BMD of the spine (assessed with DXA), serum ALP and OC, and urinary Pyr, dPyr, CTx, and Hyp (with all urinary parameters expressed as a ratio with Cr) were determined. Vertebral fractures were determined using radiographs of the spine. At baseline, there were no differences between groups for BMD of the spine or for any of the biochemical markers. At 2 years, a significant dose-response was observed for BMD ($p=0.001$). The biochemical markers showed maximum decreases of 10% to 23% between 6 and 9 months with a leveling-off of the therapeutic effect after that. For ALP, OC, Hyp/CRr, Pyr, and dPyr there were no differences between any of the treatment groups (including the placebo group). For CTx/Cr, there was a significant difference ($p<0.01$) between groups with the greatest decrease in the 100 IU group (-42.7% compared to -15.7% in the placebo group). When women who suffered fractures during the follow-up period ($n=14$) were compared to those who did not ($n=150$), the CTx/Cr measure was unchanged in those who experienced fractures but decreased by a mean of 30% in those who had no fractures.

The effects of three months of treatment with sCT on bone turnover as assessed by biochemical markers was evaluated by Kraenzlin et al. (1996). The study included 10 women who were early postmenopausal (1 to 5 years after menopause) with a high rate of bone resorption (based on the Hyp/Cr ratio), a BMD of more than 2 standard deviations below the mean for young normal females, no evidence of vertebral fractures, and having taken no medication that would act on bone metabolism for at least 2 years prior to the study. The women were given intranasal sCT for 12 weeks (100 IU twice per day) along with 500 mg of calcium per day. After 12 weeks, the sCT was discontinued but the calcium maintained. Blood and urine samples were taken at baseline and at 4, 8, 12, 14, 16, 20, and 24 weeks after the start of treatment. Serum ALP, OC, and PICP, and urinary Hyp, Pyr, dPyr, ICTP, and NTx were assessed. BMD of the lumbar spine and femoral neck was measured using DXA at baseline and after 24 weeks. For the bone formation markers, OC decreased ($p<0.05$) but returned to baseline after treatment. Changes in PICP and ALP were not significant. All of the bone resorption markers, with the exception of ICTP, were significantly reduced ($p<0.01$) during sCT treatment. All of the parameters returned to pretreatment levels after cessation of sCT treatment. BMD did not change significantly during the 6 month observation period.

Monitoring the Response to Treatment with Calcium and Vitamin D

In the study presented by Prestwood, Pannullo, Kenny, Pilbeam, and Raisz (1996), 12 women (all over 70 years of age) were given 1500mg/day of elemental calcium plus 1000

IU/day of vitamin D_3 for a 6 week period. The purpose of the study was to assess the effect of a short course of calcium and vitamin D on biochemical markers of bone turnover. The markers were monitored at baseline (2 measures at 1 week intervals), at 5 and 6 weeks of treatment, and at 5 and 6 weeks after treatment. Serum OC, BSAP, and type I procollagen peptide were assessed as markers of bone formation while urinary Hyp, Pyr, dPyr, and NTx were assessed as markers of bone resorption. BMD values of the proximal femur, lumbar spine, and total body were measured at baseline using DXA. Calcium intake was estimated using a 4-day food record. All 12 of the women completed the study with no reported adverse effects. The combination of calcium and vitamin D did not alter the levels of the bone formation markers. The markers of bone resorption showed a curvilinear response to the treatment, decreasing during the treatment phase but returning to baseline following treatment. The only significant changes from baseline during treatment were for NTx ($p<0.01$) and dPyr ($p<0.05$). Parathyroid hormone (PTH) levels were also decreased during treatment and it was speculated that reduced bone resorption with calcium and vitamin D therapy was a result of the suppressed PTH levels.

Risks and Limitations

Testing for biochemical markers of bone turnover is limited by large intra- and inter-individual variability in the levels of the biochemical markers. The variability has been attributed to a variety of factors including technical difficulties in performing the measurements; differences in normal values between different laboratories; seasonal, circadian, and day-to-day differences; aging; disease status; and the use of various medications (Douglas, Miller, Reid, Hutchison, Porter, & Robins 1996; Seibel et al., 1997; Beck Jensen, Kollerup, Sørensen, Pors Neilsen, & Sørensen, 1997a; Beck Jensen, Kollerup, Sørensen, & Sørensen, 1997b; Hough, 1998). As a result, although group data (e.g., means between treatment groups, pre- to post-treatment means, or correlations with BMD) may suggest differences and associations, the large variability makes the markers unsuitable for prediction of outcomes in individual patients (Beck Jensen et al., 1997a). Despite this shortcoming, producers of both test equipment and supplies and therapeutic agents have actively marketed their products to individuals.

Although the best therapeutic use of biochemical markers is for follow-up of treatment programs there are several limitations associated with the use of biochemical markers for this purpose. Under best use conditions, the treatment effectiveness is reported to be 90% to 95%. The cost of multiple tests must be considered. It is unknown whether the results of a test of biochemical markers can be generalized to all bones. The level of any marker in the serum or urine reflects a cumulative effect over the entire body. In contrast, DXA provides a measure of bone mineral density at a specific site. There is also a concern over whether any test, in general, should be used to monitor compliance. A recent NIH Consensus Development Conference concluded that patients should not stop or change therapies with demonstrated efficacy solely because of modest loss of bone density or adverse trends in markers of bone turnover since short-term results are not predictive or long-term results (NIH, 2000). Finally, even it there is a way to identify a failed treatment program, at present there are limited data to indicate that other therapies would be more effective.

There are also concerns about the overall quality of the research on this topic. Specifically, many of the studies involve small sample sizes and wide age ranges. The studies are largely cross-sectional in nature (which does not allow for changes in risk factors over time) or longitudinal studies (which show better correlations between biochemical markers and initial BMD rather than changes in BMD) (Hayes, 1998). With data from retrospective studies involving fracture patients it is impossible to know whether the changes in bone turnover observed following a fracture occurred before the fracture (and thus were a contributing factor) or after the fracture. Prospective studies must include a large sample size and an adequate follow-up time to allow for inclusion of a sufficient number of fracture cases (Delmas, 1998).

A recent report from the National Osteoporosis Foundation (Looker et al., 2000) outlined the direction for future studies. It was suggested that work continue to identify better markers or combinations of markers as well as better indices reflecting the relationship between resorption and formation. It was also suggested that there be more studies involving direct comparisons of different markers. Studies with fracture endpoints should be prospective in nature and should relate treatment induced changes in the marker levels to the fracture endpoints. More complete studies of the range and variability of markers in response to treatment are also called for with calculation of sensitivity, specificity, negative predictive value, and positive predictive value included. To date, the majority of studies have involved white populations. There is a need for studies of people from other racial backgrounds. There is also a need for studies of premenopausal women, men, individuals who require long-term corticosteroid use, and growth hormone deficient patients treated with growth hormone. It is presently unknown whether marker values at the menopause are predictive of future fracture risk. Finally, it was suggested that efforts continue to improve the analysis and interpretation of the biochemical markers (including assay standardization, minimizing variability, uniform reporting of results, and optimizing control of preanalytical variables) (Christenson, 1997; Looker et al., 2000).

Alternative Forms of Assessment

Alternative methods for the assessment of osteoporosis include the following (ICSI, 1996; Christenson, 1997; Formica, Nieves, Cosman, Garrett, & Lindsay, 1998).

Histomorphometry is a technique that provides critical information on the activation frequency of bone remodeling units and the rate of bone turnover. However, it is generally limited to the iliac crest.

Ultrasound shows promise as a technique for assessing the quantity and quality of bone. However, as a screening assay, it lacks precision (typically reported to be 3% to 5%) and therefore cannot be used to follow-up on therapy effectiveness.

Bone mineral density assessment (with DXA) allows a physician to identify a decrease in bone mass and to assess fracture risk but, due to the precision of the measurements (typically reported to be 1% to 2%), one to three years are needed between measurements to reliably assess the efficacy of any intervention.

Quantitative computed tomography (QCT) provides a measure of volumetric density (g/cm^3) rather than mass per unit area (as in DXA) This enables assessment of trabecular bone. However, QCT is not used for serial monitoring of patients. Peripheral QCT is a recent development.

Additional information on these techniques is included in the ICSI report on *Densitometry as a Diagnostic Tool for the Identification and Treatment of Osteoporosis in Women* (ICSI, 2000).

Costs

The biochemical markers with commercially available assays (see Table, pages 5-6) can typically be assessed for a fee of $75-$200. The retail cost of DXA screening is approximately $150 for 1 site and $225 for 2 sites.

Summary

With regard to the use of biochemical markers of bone turnover in osteoporosis, the ICSI Technology Assessment Committee finds the following:

1. The assessment of serum and urine biochemical markers of bone turnover is a safe, minimally invasive procedure.
2. While there is some population based evidence that increased values for biochemical markers are associated with increased fracture risk and that uncoupling of the bone formation/resorption mechanism is greater in fracture cases, it is not possible to predict an individual's fracture risk from biochemical marker measurements. A combination of bone mineral density and biochemical marker measurements may be of greater value but the data are inconclusive. The clinical value of serial measurements of marker levels is unclear. To make predictions about individual patients, more information is needed about the positive and negative predictive values of the test and how those values change as the pre-test probability of fracture changes (as it would with age). (Conclusion Grade II based on Class B and Class C evidence; see Appendix A)
3. Although population trends have been observed, biochemical markers do not have adequate sensitivity and specificity to predict osteoporosis in individual, untreated patients. The diagnosis of osteoporosis is based on a reduced BMD and/or the presence of fragility fractures. (Conclusion Grade II based on Class C and Class D evidence; see Appendix B)
4. Several biochemical markers are responsive to various therapeutic options. However, there is no conclusive evidence that biochemical markers may be used to assist in selecting the type of therapy or to predict the amplitude of the BMD response for an individual patient. (Conclusion Grade II based on Class A and Class C evidence; see Appendix C)
5. Although biochemical markers have the potential to be used to motivate individuals to maintain a therapy program, there are no studies of the use of biochemical markers for this purpose.

References

Evidence is classed and graded as described below.

I. CLASSES OF RESEARCH REPORTS

Primary Reports of New Data Collection:

Class A:	Randomized, controlled trial
Class B:	Cohort study
Class C:	Non-randomized trial with concurrent or historical controls Case-control study Study of sensitivity and specificity of a diagnostic test Population-based descriptive study
Class D:	Cross-sectional study Case series Case report

Reports that Synthesize or Reflect upon Collections of Primary Reports:

Class M:	Meta-analysis Decision analysis Cost-benefit analysis Cost-effectiveness study
Class R:	Review article Consensus statement Consensus report
Class X:	Medical opinion

II. CONCLUSION GRADES

Key conclusions (as determined by the work group) are supported by a conclusion grading worksheet that summarizes the important studies pertaining to the conclusion. Individual studies are classed according to the system defined in Section I, above, and are assigned a designator of +, -, or ø to reflect the study quality. Conclusion grades are determined by the work group based on the following definitions:

Grade I: The evidence consists of results from studies of strong design for answering the question addressed. The results are both clinically important and consistent with minor exceptions at most. The results are free of any significant doubts about generalizability, bias, and flaws in research design. Studies with negative results have sufficiently large samples to have adequate statistical power.

Grade II: The evidence consists of results from studies of strong design for answering the question addressed, but there is uncertainty attached to the conclusion because of inconsistencies among the results from different studies or because of minor doubts about generalizability, bias, research design flaws, or adequacy of sample size. Alternatively, the evidence consists solely of results from weaker designs for the question addressed, but the results have been confirmed in separate studies and are consistent with minor exceptions at most.

Grade III: The evidence consists of results from studies of strong design for answering the question addressed, but there is substantial uncertainty attached to the conclusion because of serious doubts about generalizability, bias, research design flaws, or adequacy of sample size. Alternatively, the evidence consists solely of results from a limited number of studies of weak design for answering the question addressed.

Grade IV: The support for the conclusion consists solely of the statements of informed medical commentators based on their clinical experience, unsubstantiated by the results of any research studies.

The symbols **+**, **–**, **ø**, and **N/A** found on the conclusion grading worksheets are used to designate the quality of the primary research reports:
+ indicates that the report has clearly addressed issues of inclusion/exclusion, bias, generalizability, and data collection and analysis;
– indicates that these issues have not been adequately addressed;
ø indicates that the report is neither exceptionally strong or exceptionally weak;
N/A indicates that the report is not a primary reference and therefore the quality has not been assessed.

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Beck Jensen J-E, Kollerup G, Sørensen HA, Sørensen OH. Intraindividual variability in bone markers in the urine. *Scand J Clin Lab Invest* 1997b;57(Suppl 227):29-34. (Class C)

Bone HG, Greenspan SL, McKeever C, et al. for the Alendronate/Estrogen Trial Research Group. Alendronate and estrogen effects in postmenopausal women with low bone mineral density. *J Clin Endocrinol Metab* 2000;85:720-726. (Class A)

Bouman A, Scheffer PG, Ooms ME, Lips P, Netelenbos C. Two bone alkaline phosphatase assays compared with osteocalcin as a marker of bone formation in healthy elderly women. *Clin Chem* 1995;41:196-199. (Class D)

Caulfield MP. Alternate markers for biochemical assessment of bone turnover. *J Clin Lig Assay* 1998;21:149-158. (Class R)

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Dessauer A. Analytical requirements for biochemical bone marker assays. *Scand J Clin Lab Invest Suppl* 1997;227:84-89. (Class R)

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Dresner-Pollak R, Parker RA, Poku M, Thompson J, Seibel MJ, Greenspan SL. Biochemical markers of bone turnover reflect femoral bone loss in elderly women. *Calcif Tissue Int* 1996;59:328-333. (Class D)

Eastell R, Blumsohn A. The value of biochemical markers of bone turnover in osteoporosis. *J Rheumatol* 1997;24:1215-1217. (Class R)

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Garnero P, Hausherr E, Chapuy M-C, et al. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS prospective study. *J Bone Miner Res* 1996;11:1531-1538. (Class C)

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Woitge HW, Scheidt-Nave C, Kissling C, et al. Seasonal variation of biochemical indexes of bone turnover: results of a population-based study. *J Clin Endocrinol Metab* 1998;83:68-75. (Class D)

Appendices

See next pages

Appendix A: Conclusion Grading Worksheet

Work Group's Conclusion: While there is some evidence that increased values for biochemical markers are associated with increased fracture risk and that uncoupling of the bone formation/resorption mechanism is greater in fracture cases, it is not possible to predict an individual's fracture risk from any one marker measure.

Conclusion Grade: II

Author/Year	Design Type	Class	Quality +,−,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Åkesson et al. (1995)	Cohort	B	ø	-328 women of Scandinavian ethnic background aged 40, 50, 60, 70, and 80 years selected randomly from city population files -Forearm bone mineral content with SPA at 1cm and 6cm proximal to tip of styloid process (BMC1, BMC6) -Serum assays for OC, PICP, and ICTP[a] -Followed for 5 years for fracture	-127 fractures had occurred at time of baseline testing -During 5 year follow-up there were 52 fractures in 43 women (including 12 hip, 15 vertebral, 8 radius, and 6 pelvic) -Correlations: OC PICP ICTP BMC6 BMC1 Age 0.36 0.13 0.44 -0.71 -0.54 BMC1 -0.31 -0.16 -0.25 0.73 BMC6 -0.35 -0.08* -0.31 ICTP 0.32 0.10* PICP 0.23 (All p<0.05 except *) -No difference in baseline marker levels among those who sustained a fracture in follow-up vs. those who did not	-High bone turnover was not linked to the occurrence of common fractures in this sample. NOTES: there were no selection criteria for menopausal status or use of hormone replacement therapy; minor fractures of the hand or foot were not included
Garnero et al. (1996)	Case-control (nested)	C	ø	-7,598 healthy volunteers ages 75 yrs and older -Baseline measures of gait speed; femoral neck bone mineral density (BMD) with DEXA; and OC, BSAP, NTx, CTx, dPyr, Ca, Cr, albumin, and AlP[a] -Followed for mean of 22 months -126 hip fractures (cases) matched with 3 with no fracture (controls) -Excluded: primary hyperpara-thyroidism, chronic hemodialysis, or taking substances that influence calcium metabolism	-Studied 109 with fracture (cases) and 292 controls -Fracture cases had lower BMD and gait speed and higher CTx and dPyr than did controls (p<0.05) -Odds Ratios for fracture risk: CTx: 1.3 (95%CI: 1.0-1.6) dPyr: 1.4 (95%CI: 1.1-1.7) BMD: 1.7 (95%CI: 1.3-2.2) -Combination of low hip BMD (>2.5 SD below young adult mean) and high bone resorption (above upper limit of premenopausal range) resulted or ORs of 4.8 (95%CI: 2.4-9.5) for CTx & BMD and 4.1 (95%CI: 2.0-8.2) for dPyr & BMD	-Increased levels of some markers of bone resorption, but not formation, are associated with increased risk of hip fracture (independently of BMD level); combining BMD and resorption rate improved the prediction of hip fracture risk. NOTES: subjects were participants in EPIDOS cohort study

Appendix A: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,−,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Ross et al. (2000)	Cohort	B	ø	-512 community-dwelling post-menopausal women from the Hawaii Osteoporosis Study; mean age at baseline of approx. 74 ± 5 yrs -Baseline calcaneus BMD, CTx, and BSAP -Recorded new fractures (spine or non-spine) for a mean of 2.7 years	-55 patients (10.7%) with new fractures; 33 (6.5%) vertebral, 25 (4.9%) non-spine -Baseline BSAP & CTx higher in those who experienced a fracture; baseline BMD lower ($p \leq 0.007$) -BSAP and CTx values correlated ($r=0.54$, $p=0.0001$) -BSAP, CTx, and calcaneus BMD significant predictors of new fractures (OR=1.61 [BMD], OR=1.53 [BSAP], OR=1.54 [CTx]; all $p<0.05$) -In multivariate analysis, BMD ($p=0.002$) and BSAP ($p=0.017$) significant predictors of fracture -In hierarchical regression incremental contribution of BSAP (beyond BMD) was significant ($p=0.0009$)	-High bone turnover (as assessed by BSAP and CTx) was significantly associated with increased risk of osteoporotic fractures. The association of high bone turnover with fracture risk was largely unaffected after adjustment for BMD, estrogen use, and anthropometric and performance-based measures.

[a]OC=osteocalcin, PICP=carboxyterminal propeptide of Type I procollagen, ICTP=carboxyterminal crosslinked telopeptide of Type I collagen, BSAP=bone-specific alkaline phosphatase, NTx=aminoterminal crosslinked telopeptide of Type I collagen, CTx=Type I C-telopeptide breakdown products, dPyr=urinary free deoxypyridinoline, Ca=calcium, Cr=creatinine

Appendix B: Conclusion Grading Worksheet

Work Group's Conclusion: Although population trends have been observed, biochemical markers do not have adequate sensitivity and specificity to predict osteoporosis in individual, untreated patients.

Conclusion Grade: II

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Eastell et al. (1993)	Non-Random	C	ø	-67 postmenopausal women (ages 50-79 yrs) with lumbar spine BMD within age-specific normal range (controls) -63 postmenopausal women (ages 53-74 yrs) with vertebral fractures and lumbar spine BMD at or below fracture threshold; no prior treatment with fluoride or bisphosphonates, no estrogen in past 6 months, and no calcium in past 3 months -Measured serum OC and urinary dPyr, Pyr, and Hyp; also free crosslinks of dPyr, Pyr, and glycosylated Pyr[a]; all but serum OC were normalized by Cr	-Mean levels of markers of bone turnover were higher in osteoporosis patients than controls ($p<0.01$ for OC, urinary Hyp, and crosslinks Pyr; $p<0.001$ for urinary Pyr and dPyr and crosslinks dPyr) -Variances were greater in osteoporosis patients than controls for dPyr ($p<0.05$), Pyr ($p<0.001$), and Hyp ($p<0.001$) -Greater increase (above control values) in resorption markers (dPyr and Pyr) than in formation marker (OC) -Uncoupling indices for dPyr, Pyr, and Hyp were 0.45 ($p=0.02$), 0.83 ($p=0.002$), and 0.18 (NS), respectively; no correlation between uncoupling index and lumbar spine BMD or age	-Pyridinium crosslinks of collagen enable better discrimination between normal and osteoporotic women than does hydroxyproline. In osteoporosis, there appears to be a heterogeneity of bone resorption. An uncoupling index indicated that in osteoporosis, bone resorption was increased to a greater extent than bone formation as compared with normal postmenopausal women.
Rosso et al. (1995)	Case Series	D	ø	-45 healthy women ≤3 yrs since last menstrual period (mean of 20 months); receiving no bone active drugs -Measured urinary Cr, Ca, and Hyp and serum ALP and OC; BMD at ultradistal radius; repeated in 19 patients at a mean of 30 months since menopause and in all 45 patients at a mean of 40 months since menopause -Expressed scores as standard units in respect to data from a fertile, age-matched control population (n=23)	-Mean initial values of each biomarker were significantly higher in the post-menopausal women (all $p\leq0.004$) -Levels of Ca/Cr and Hyp/Cr decreased following initial measurement while ALP and OC increased; at final measurement OC levels were significantly higher than the other biomarkers measured -Mean yearly bone loss was 2.83% -Correlation between initial and final BMD values was r=0.91; correlation between individual rates of bone loss and initial BMD was r=-0.11; correlation between initial serum AlP and % yearly bone loss was r=-0.30	-Within a relatively short period of time since natural menopause, markers of bone turnover behave differently. The increase in osteogenesis appears to be delayed compared to that of bone resorption. The actual bone mass in the immediate post-menopausal period should be considered the best predictor of future bone mass.

Appendix B: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Miura et al. (1995)	Non-Random	C	–	-51 pre-menopausal women (ages 28-59) and 30 post-menopausal women (ages 42-59; mean of 2.5 years after menopause); 7 in the post-menopausal group were peri-menopausal at the start of the study; all healthy volunteers -Lumbar spine BMD monitored once a year for 3 years (total of 4 measures) -Measured Pyr, dPyr, OC, PICP, and ALP at time of initial BMD measurement	-BMD decreased by 1.3% in the pre-menopausal women (NS) and by 5.6% in the post-menopausal women ($p<0.05$) -Only PICP was correlated with bone loss ($r=0.48$, $p<0.05$) -Pyr ($r=-0.60$, $p<0.01$), dPyr ($r=-0.66$, $p<0.001$), PICP ($r=-9.64$, $p<0.001$), and ALP ($r=-0.43$, $p<0.05$) were correlated with initial BMD -Higher correlations with initial BMD were observed for combinations of several markers – best model included PICP, ALP, and dPyr ($r=0.84$, $p<0.0001$); for bone loss, the only significant model included PICP, dPyr, OC, and initial BMD ($r=0.76$, $p<0.01$)	-Biochemical markers of bone turnover are good predictors not only of bone loss but also of BMD itself. There were no differences between bone resorption markers and bone formation markers in predicting bone loss or BMD. NOTES: suggested that higher correlation with BMD (rather than bone loss) might be due to smaller errors in measuring BMD once and to smaller changes in BMD (expect improved correlation with bone loss if monitoring period were extended); only the data from the post-menopausal women was used in deriving the correlations/models
Keen et al. (1996)	Cohort	B	–	-141 Caucasian women ages 45-62 years (all within 5 yrs of menopause); normal volunteers; none taking HRT at start of study (or other medication that affects bone metabolism) -BMD of lumbar spine and left hip with DPA at baseline, 12, 24, and 48 months -Measured OC, ALP, estradiol, serum sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), testosterone, Ca, OHP, Pyr, dPyr	-123 did not start HRT during the 4 year follow-up; analysis included only those with 2 or more BMD measurements prior to start of HRT; the numbers of women with 4, 3, and 2 scans at the lumbar spine were 85, 22, and 7; comparable values for the femoral neck were 75, 21, and 11 -No significant correlation was found between the baseline BMD (either site) and demographic variables (age, menopause duration, height, weight); spine and hip BMD measures were correlated ($r=0.41$, $p<0.001$) -No significant correlation between baseline BMD and individual biochemical markers -Mean annual change in BMD for total group (with initial BMD included in slope calculation) was –1.41%/yr for spine and –0.86%/yr for femoral neck; rates of change in density were normally distributed at both sites, showed a linear pattern over 4 years, and no evidence of bimodality; no significant correlation between rates of loss at 2 sites ($r=0.13$) (results were similar if initial BMD was excluded) -No significant correlation between change in BMD and any of the biochemical markers (individually or in combination)	-Annual percentage rates of change in BMD at spine and hip were normally distributed and it was not possible to demonstrate a subgroup of "fast losers." Baseline bone density did not correlate with demographic variables (perhaps because of narrow age range) and baseline biochemical marker values (alone or in combination) did not correlate with bone density or subsequent bone loss over 4 years at spine or hip. NOTES: in calculating the rate of change of BMD it was assumed that the expected change in BMD is linear with time for each subject; a linear regression equation was fitted for individual subjects; estimated that study had 80% power to detect a correlation of $r=0.50$ between bone loss and biochemical marker values (assuming 50% error in rate of loss and 20% error in marker values); markers used are "first generation" and not all are bone-specific; delay in performing assays may have effected measurement of some markers; DPA has been replaced by DXA

Appendix B: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Bauer et al. (1999)	Cohort	B	ø	-295 women age 67 or older (mean=73 years); not receiving estrogen replacement therapy -Excluded black women, women unable to walk without assistance of another person, history of bilateral hip replacement -Baseline OC, BSAP, NTx, CTx, dPyr, Pyr -Hip BMD at baseline and mean of 3.8 years later	-Baseline markers weakly correlated with bone loss (r=-0.01 to r=0.19); those with baseline levels of NTx, CTx, Pyr, dPyr, and OC greater than median had higher levels of bone loss ($p<0.05$) -Probability of increase or decrease in total hip BMD was similar regardless of baseline marker levels -Sensitivity for identifying those in highest tertile of bone loss with marker levels in the 4th quartile ranged from 28% to 38%, specificity from 74% to 79%, PPV from 36% to 46%, and NPV from 68% to 71%	-Higher levels of selected bone turnover markers are associated with faster bone loss at hip in elderly women not receiving estrogen replacement therapy but the markers have limited value in predicting rapid loss in individual patients NOTES: subjects were a subset of the Study of Osteoporotic Fractures; limitations of study include single measure of turnover markers and only 2 measures of BMD, limited generalizability (beyond elderly, ambulatory, Caucasian women)
Garnero et al. (1999)	Cohort	B	ø	-305 women post-menopausal women ages 50-88 years (mean 64 years); no treatment that might affect bone metabolism -Baseline OC, BSAP, PICP, PINP, NTx, & CTx (serum & urinary) -Mid- and distal radius BMD at baseline, 2, 3, & 4 years -Premenopausal levels obtained from 135 healthy premenopausal women 35-55 yrs old -High turnover group defined as 2 SD above premenopausal control mean	-OC, NTx, CTx (serum & urinary) negatively associated with baseline BMD (r=-0.14 to r=-0.23) -Mean % change in BMD was –0.22%/yr at mid-radius and –0.59%/yr at distal radius -Higher marker levels associated with faster BMD loss (r=-0.16 to r=-0.30; $p<0.01$) (except BSAP with mid- & distal radius & PICP with mid-radius BMD); correlations were higher in early post-menopausal women with faster bone loss (r=-0.20 to r=-0.53) -Rate of bone loss in high turnover group was 2- to 6-fold higher (depending on marker) than in those with low turnover ($p\leq0.01$ except BSAP, PICP) -ORs for fast bone loss (upper tertile) were 1.8- to 3.2-fold higher in fast turnover group vs. normal turnover group; sensitivity ranged from 16%-59%; specificity from 66%-93%; PPV from 44%-56%; NPV from 68%-76%	-Increased levels of some newer sensitive and specific biochemical markers are associated with a faster rate of bone loss from the forearm over 4 years. The value of bone markers to predict the rate of bone loss for an individual was quite limited. NOTES: strengths of study are prospective design, large n, long duration of follow-up, multiple BMD measurements, state-of-the-art bone markers; limitations include measurement of bone mass at radius only, excluded about 50% of cohort for various bone metabolic diseases

[a]OC=osteocalcin, dPyr=urinary free deoxypyridinoline, Pyr=urinary pyridinoline, Hyp=hydroxyproline, ALP=total alkaline phosphatase, Ca=calcium, Cr=creatinine, PICP=carboxyterminal propeptide of Type I procollagen, BSAP=bone-specific alkaline phosphatase, NTx=aminoterminal crosslinked telopeptide of Type I collagen, CTx=Type I C-telopeptide breakdown product, PINP=aminoterminal propeptide of Type I procollagen

Appendix C: Conclusion Grading Worksheet

Work Group's Conclusion: There is no conclusive evidence that biochemical markers may be used to assist in selecting the type of therapy or to predict the amplitude of the BMD response for an individual patient.

Conclusion Grade: II

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Cosman et al. (1996)	Non-Random	C	ø	-3 groups of women: untreated pre-menopausal (PRE, n=17), untreated post-menopausal (UPOST, n=40), estrogen-treated post-menopausal (EPOST, n=24); all Caucasian; in UPOST group37.5% met WHO definition of osteoporosis (vs. 83% of EPOST group); EPOST group had received different forms of estrogen for from 1-19 years -BMD tested every 6 months for 3 years; at initial BMD testing OC, PICP, ALP, BSAP, TRAP, ICTP, CTx, Hyp, Ca, Pyr, and dPyr[a] were also measured	-Baseline biochemical turnover variables were higher in UPOST group than EPOST or PRE groups except for PICP and Hyp -Percent rate of change in BMD (PRC-BMD) of spine for UPOST group significantly different from baseline and other groups; PRC-BMD of femoral neck significantly different from baseline (all $p<0.05$); PRC-BMD of spine and hip not related ($r=0.02$) -For the whole sample, PRC-BMD of the spine significantly (all $p<0.05$) related to OC ($r=-0.40$), PICP ($r=-0.24$), ALP ($r=-0.49$), BSAP ($r=-0.47$), and Hyp ($r=-0.31$); PRC-BMD of the hip related to PICP ($r=-0.30$), ICTP ($r=-0.31$), Hyp ($r=-0.26$), Pyr ($r=-0.35$), and dPyr ($r=-0.23$) -For the UPOST group alone, the significant relationships remained except PRC-BMD of the spine was no longer related to PICP, BSAP, or OC -Based on data from 30 in UPOST group with all data available, the best model (with Hyp, ICTP, BSAP, and Ca intake) predicted 42% of variance in PRC-BMD of the spine; Body Mass Index, ICTP, and OC were included in the best model for PRC-BMD of the hip (predicting 32% of variance) -Among BMD losers from the femoral neck (n=46) all resorption variables except TRAP were significantly related to PRC-BMD in femoral neck ($r=-0.30$ to $r=-0.45$, $p<0.05$); PICP, ALP, and BSAP were also related ($r=-0.32$ to $r=-0.44$); none of the markers correlated with PRC-BMD of the spine in a subpopulation of spine losers	-Data show generally weak relationships between individual biochemical turnover variables and subsequent rates of bone loss in a heterogeneous group of women. When relatively high bone turnover was identified by biochemical criteria, only 60% of high bone loss individuals were identified indicating that the markers do not have adequate sensitivity for use as diagnostic screening tests. A combination of 4 markers and demographic variables could predict less than half of the variance in bone loss in both the spine and hip of post-menopausal women. The combined errors in bone density and biochemical measurement make it impossible to detect the small changes in bone density observed in a longitudinal study. NOTES: EPOST group contained a higher proportion of osteoporotic and high-risk patients and calcium intake was higher in estrogen group (but these factors would not likely impact a longitudinal study); there was no relationship between spine and hip bone mass change thus the use of a single measure to reflect an average of change throughout the skeleton would be difficult; bone loss and bone turnover may not occur linearly so that a single measure might be misleading about turnover rates in future years

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Rosen et al. (1997)	RCT	A	–	-236 women ages 40-58 yrs -Included: natural menopause from 6 mos to 3 yrs before study, willingness to be randomized, FSH >30 IU/mL -Excluded: medications that might interfere with bone metabolism, disease known to affect skeletal turnover, ideal body weight >130%, renal insufficiency, recent fracture or immobilization, positive mammogram in past 12 mos, baseline spine or hip BMD >4 SD below mean of young normals -Randomized to either calcium supplement (500 mg/day) or HRT (0.625 mg estrogen and continuous or cyclic medroxyprogesterone) plus calcium; instructed to maintain total calcium of 800-1200 mg/day -Biochemical markers (NTx, dPyr, OC, BSAP) at baseline, 1, 3, 6, & 12 mos -BMD (spine and femoral neck) at baseline, 3, 6, & 12 mos	-HRT GROUP: -Increased BMD at spine (+2.5%, p<0.0001) and femoral neck (+1.0%, p<0.05); more than 2/3 had positive skeletal response to HRT; at 1 month mean decreases from baseline were 28% for NTx, 10% for dPyr, and 15% for OC (all p<0.0001); BSAP increased initially but then decreased at 6 & 12 mos -Those with higher initial NTx, OC, or BSAP levels had greater increases (p<0.05) in spine BMD; those with the greatest decreases in NTx, dPyr, or BSAP from baseline to 6 mos had the greatest increases in BMD (P<0.05); with ROC curves the percent change in NTx provided the best prediction of gain or loss of spine BMD after 1 yr of HRT; baseline NTx values were higher (P=0.0002) among those who gained BMD compared to those who lost BMD at the spine; ORs for gain or loss of BMD with 1 SD increase in baseline marker value were 6.4 (95%CI 2.6-9.99) for OC, 5.4 (95%CI 1.95-15.2) for NTx, 1.9 (95%CI 1.03-3.45) for BSAP, and 1.2 (95%CI 0.8-1.73) for dPyr -CALCIUM GROUP: -BMD decreased significantly at spine and hip (-1.1%, p<0.01); NTx, dPyr, & OC values did not change significantly over 12 mos; BSAP increased; those with highest NTx and OC values over course of study had greater decreases in spine BMD than those with low values (p<0.05); ORs for loss of spine BMD with a 1 SD increase in baseline marker value were 2.1 (95%CI 1.29-3.41) for NTx and 1.6 (95%CI 1.0-2.41) for OC	-Biochemical markers of bone turnover can be used to determine skeletal responsiveness to HRT; select baseline biochemical markers in postmenopausal women provide clinically useful information about future change in bone mass after therapeutic intervention. NTx and OC provided the greatest sensitivity and specificity. NOTES: used percent change in L1-L4 BMD at 1 yr to classify subjects as maintaining/gaining (zero or positive change) or losing (negative change) BMD; 9 women discontinued study (4 relocation, 3 by MD request, 2 stopped taking drug); compliance was not monitored; uncertain whether changes in BMD at 1 yr are sufficient to determine final skeletal responsiveness to HRT; significant intrasubject variability; women were healthy and not overtly osteoporotic *Work Group's Comments:* *-Used lumbar BMD to classify direction of change – it was not specified whether this was a lateral or A/P assessment; not stated whether analysis was by intention-to-treat; no baseline comparison of groups reported*

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,−,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Heikkinen et al. (1997)	RCT	A	ø	-From a study of 464 women randomly selected 18 from each of 4 groups for this study -Included: willingness to undergo 5 yr clinical trial, last period within 6-24 months, no contraindication for HRT -Had been randomized to one of 4 groups: *HRT* (estradiol valerate [2 mg days 1-21] and cyproterone acetate [1mg days 12-21]); *Vitamin D* (cholecalciferol [300 IU/day] and calcium lactate [500 mg/day] - no intake June-August); *HRT + D*, and *placebo* (calcium lactate (500 mg/day) -Assessed calcium intake, physical activity, smoking, and alcohol consumption at baseline -Tested BMD of lumbar spine and femoral neck (used L2-L4 data for analyses) -Tested serum OC, BSAP, and ICTP at baseline, 6, & 12 mos -Assessed vit D metabolites, estradiol, FSH, Ca, & phosphate at baseline	-69 completed the 1 yr study (2 in HRT and 1 in Vit D group stopped taking medication); lumbar and femoral neck BMD was available from 63 after 2.5 yrs of treatment -No differences between groups at baseline (age, BMI, duration of menopause, prior HRT use) -OC: decrease at 12 mos in HRT groups ($p<0.01$), increase in placebo group ($p<0.05$) at 6 mos (with return to near baseline at 12 mos) -BSAP: decrease at 12 mos in HRT groups ($p<0.001$) and D group ($p<0.05$) -ICTP: decrease at 12 mos in HRT groups ($p<0.05$) -BMD: lumbar and femoral BMD decreased at 2.5 yrs in the D and placebo groups (all $p<0.05$); there were no significant changes in either HRT group -Relative changes in bone biochemical markers (1 yr) correlated with changes in BMD (2.5 yrs) ($r=-0.24$ to $r=-0.36$, $p=0.06$ to $p=0.005$)	-HRT appears to counteract the biochemical changes caused by increased bone turnover associated with menopause. Serum markers of bone formation and resorption are persistent and their changes reflect those in BMD. They may be used to motor the effect of HRT in healthy, early post-menopausal women. They may also be useful in finding women with a high rate of bone turnover after menopause. Low dose vitamin D seems to have only marginal effects on bone metabolism in early post-menopausal healthy women. NOTES: likely that clinically rational use of bone markers requires a combination of markers rather than reliance on a single marker; major shortcomings of biochemical markers are their great intra- and inter-individual variations

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,−,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Gonnelli et al. (1997)	RCT	A	ø	-90 consecutive post-menopausal women (46-66 yrs) diagnosed as osteoporotic (lumbar spine BMD >2.5 SD below reference range) for 1st time; ≥ 2 yrs past natural menopause; within 20% of ideal weight -Excluded: disorders affecting mineral metabolism, history of alcohol abuse, habitual smoking, medications known to interfere with calcium metabolism -Randomized to 2 yr treatment with ERT (transdermal, 0.05 mg/day in 4 wk cycles with medroxyprogesterone acetate) + calcium (Ca) (500 mg/day) (n=45) or Ca alone (n=45) -Measured whole body retention (WBR) (^{99m}Tc-methylendiphosphonate) at baseline; BMD of lumbar spine and ALP, OC, Hyp, & Pyr were measured at baseline and at 12 and 24 mos; Hyp & Pyr were expressed relative to Cr	-81 completed study (40 in ERT+ group and 41 in calcium only group); groups were similar at baseline -Over 2 yrs, BMD of lumbar spine increased by 3.8% in ERT+Ca group and decreased by 2.3% in Ca group ($p<0.001$ between groups) -In ERT+Ca group OC, Hyp/Cr, and Pyr/Cr levels at 24 months were significantly ($p<0.001$) lower than baseline; in Ca group there was little change in any of the markers; at 24 months all marker levels were lower in ERT+Ca group ($p<0.001$) -Determined high (HT) vs. low (LT) turnover osteoporosis using WBR; in each treatment group HT patients had higher levels of all markers of bone turnover ($p<0.001$) (BMD was not different); in ERT+Ca group there was a greater increase in BMD in the HT group (6.6% for HT and 2.7% for LT, $p<0.05$ at 2 yrs); in the Ca group, both HT and LT patients decreased BMD with no difference between groups at 2 yrs; WBR and change in BMD values after 1 and 2 years were significantly correlated ($r=0.39$, $p<0.05$ at 2 yrs) -No significant correlations found between the biochemical markers and changes in BMD -For the ERT+Ca group markers of bone formation and resorption showed greater decrease in the HT patients (all $p<0.05$ at 2 yrs) -WBR was correlated with Hyp/Cr ($r=0.59$), Pyr/Cr ($r=0.67$), OC ($r=0.66$), and ALP ($r=0.57$)	-ERT+Ca resulted in a significant reduction of bone resorption (as documented by a decrease in Hyp/Cr and Pyr/Cr) and bone formation (decreased OC levels); bone markers and bone mass changed little after the 1st year of treatment. -The response to estrogen in women with established osteoporosis depends on bone turnover – HT patients have greater increase in BMD. -WBR was confirmed as a valid estimate of skeletal turnover showing good correlation with the biochemical markers. Based on OC and Pyr/Cr measures, it was possible to divide patients into HT or LT almost as well as WBR.

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,−,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Ettinger et al. (1999)	RCT	A	+	-Included: osteoporosis (low BMD or vertebral fractures on x-ray) at least 2 yrs postmenopausal, no severe or long-term disabling conditions -Excluded: other bone disease; substantial postmenopausal symptoms; cancer; taken androgens, calcitonin, bisphosphonate, estrogen, fluoride, glucocorticoids, antiseizure drugs, or cholecalciferol (within specified time frames); thromboembolic or endocrine disorders; liver or kidney disorders;>4 alcoholic drinks/day; pathologic fractures -2 study groups: a) BMD t-score below -2.5, b) low BMD and/or fractures -Randomized in groups to either placebo or 1 of 2 doses of raloxifene (60 or 120 mg); calcium and cholecalciferol supplements -X-ray, BMD, OC, CTx	-2622 had OC and CTx assessed (of 7705 randomized) -Women receiving raloxifene had fewer new vertebral fractures regardless of presence of existing fractures at start of study (10.1% of placebo group, 6.6% of 60 mg/day group, 5.4% of 120 mg/day group) ($p<0.05$ for all comparisons to placebo); overall incidence of fracture was same in both treatment groups but within group with prior fractures incidence was lower for those receiving 120 mg ($p=0.02$) -BMD increased in both raloxifene groups at 36 months (relative to placebo); for 60 mg group increase was 2.1% at femoral neck and 2.6% at spine; for 120 mg group increases were 2.4% at femoral neck and 2.7% at spine -OC decreased by 8.6% in placebo group, 26.3% in 60 mg group, and 31.1% in 120 mg group -CTx decreased by 8.1% in placebo group, 34% in 60 mg group, and 31.5% in 120 mg group ($p<0.001$ for each dose vs. placebo)	-Three years of raloxifene treatment preserves bone density, reduces bone turnover, and reduces the incidence of vertebral fractures in post-menopausal women with osteoporosis. NOTES: 3..6% of the placebo group, 1.1% of the 60 mg group, and 0.9% of the 120 mg group withdrew from the study for having multiple fractures or for excessive BMD loss

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Garnero et al. (1994)	RCT	A	–	-85 women ages 43-74, ≥5 yrs past natural menopause; lumbar spine BMD >2 SD below normal mean for premenopausal women -Randomized to placebo, or 5 mg or 10 mg alendronate/day; all received 500 mg calcium/day -BMD assessed at baseline and after 3, 6, 12, 15, 18, & 24 mos of treatment -Biochemical markers assessed at baseline (2 visits) and after 1, 3, 6, 12, & 15 mos of treatment; included total and intact OC, BSAP, PICP, total Pyr, dPyr, NTx, free Pyr, ICTP	-Compared to 46 healthy (including BMD in normal range) premenopausal women (ages 31-49), markers of bone turnover (except PICP and ICTP) were elevated ($p<0.001$); ALP, NTx, and free-Pyr were the most responsive markers -Long-term variability over 15 mos was low (12.5% to 17.4% for serum assays and 24% to 29% for urine assays); sensitivity (ratio of mean % increase from premenopausal levels to long term within patient variability) highest for ALP and NTx -OC (total & intact), BSAP, & PICP significantly (50%) suppressed with alendronate; peak suppression at 6-12 mos; dose related for OCs and BSAP -NTx (65%), dPyr (50%) and Pyr (30%) were suppressed with treatment; free Pyr and ICTP were not -Correlations of % change in biochemical marker (at 3 mos) and % change in BMD (at 24 mos) ranged from $r=-0.48$ to $r=-0.67$ ($p<0.001$) except for Pyr, free Pyr, and ICTP	-Early changes in the responsive biochemical markers were significantly correlated with long-term changes in bone mass suggesting that the markers could be used to predict response to therapy. -Treatment with alendronate produced a decrease in bone turnover to premenopausal levels after several months which predicted a significant increase in bone mass after 24 months. The markers with the highest clinical utility were NTx, dPyr, BSAP, and OC. NOTES: study included a single blind, 4 week, placebo run-in period followed by 24 month double blind placebo-controlled treatment period *Work Group's Comments:* *-Compliance with treatment and adverse effects of treatment were not reported*

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Greenspan et al. (1998)	RCT	A	ø	-120 unselected, healthy, ambulatory, community-dwelling women ≥65 yrs -Excluded: history of any illness affecting bone and mineral metabolism, current medication known to affect bone metabolism, treatment for osteoporosis with bisphosphonates, HRT, or calcitonin within 1 yr of screen -Randomized to placebo or alendronate (5 mg/day then 10 mg/day for final year of study) -Calcium + vit D supplements given if needed to bring daily intake to 1000 mg -BMD of hip (4 sites), lumbar spine (AP & lateral), total body, and radius (4 sites) at baseline and every 6 mos to 30 mos -Biochemical markers NTx, dPyr, OC, & BSAP every 6 mos	-Groups were similar at baseline (except intertrochanteric BMD higher in placebo group, $p<0.05$) -Following treatment, alendronate group had increased BMD at total hip (4.0%), femoral neck (3.1%), trochanter (5.5%), intertrochanter (3.8%), AP spine (7.8%), lateral spine (10.6%), total body (2.2%), and 1/3 distal radius (1.3%) (all $p<0.01$); placebo group had stable BMD of total body, radius, and hip (all sites) with increases of 2.1% at AP spine ($p<0.01$) and 1.9% ($p<0.05$) at lateral spine; treatment with alendronate significantly increased BMD at all sites ($p<0.01$) relative to placebo -No differences in numbers of hip or wrist fractures -Alendronate group had significant decreases in NTx, dPyr, OC, & BSAP (all $p<0.01$) at 6 mos; placebo group had significant decreases in NTx & BSAP at 30 mos (both $p<0.01$) & OC ($p<0.05$) at 24 mos -Few significant correlations between baseline biochemical markers and changes in BMD but in alendronate group NTx was correlated with long term changes in total body, vertebral, & trochanteric BMD ($r=0.27$ to $r=0.36$, $p<0.05$) -Decreases in alendronate group NTx at 6 mos were associated with changes in total hip, trochanter, femoral neck, AP spine, and total body BMD at 2.5 yrs ($r=-0.28$ to $r=-0.41$, $p<0.05$); decreases in OC at 6 mos were associated with increases in total hip, trochanter, intertrochanter, AP spine, and lateral spine BMD ($r=-0.31$ to $r=-0.43$, $p<0.05$); no consistent changes in placebo group -Patients with largest decreases in NTx had greatest gains in BMD at total hip, trochanter, and AP spine -Height decreased in both groups; weight was stable -Gastrointestinal complaints from 47% of alendronate group and 43% of placebo group	-Early dynamic decreases in urinary cross-linked collagen (NTx) can be used to monitor and predict long-term response to bisphosphonate therapy in elderly women. NOTES: study was double-blind; dose of alendronate was increased based on data that became available during study; 77% of alendronate group and 73% of placebo group completed study; analysis was intention-to-treat (with last available measurement); >50% had osteoporosis (WHO definition) *Work Group's Comments:* *-It was not stated why 73%-77% of patients completed the study; there were no differences between the groups in numbers of fractures but numbers of fractures were low overall (1 hip, 3 wrist)*

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,–,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Kress et al. (1999)	RCT	A	ø	-Reference population: apparently healthy premenopausal (n=228, ages 22-57 yrs) and postmenopausal (n=529, ages 45-89 yrs) women; *excluded*: bone fracture in past 6 mos, disorders known to affect bone & mineral metabolism, abnormal renal or liver function, prior treatment with bisphosphonate or fluoride; treatment in past 6 mos with calcitonin, androgens, systemic corticosteroids, oral contraceptives, estrogen or progestin, or other medication that influences bone metabolism -Postmenopausal osteoporotic women: randomized to placebo (n=148) or 10 mg/day alendronate (n=74); both groups given supplemental calcium; BSAP and BMD assessed at baseline and selected points in treatment phase; 24 month follow-up	-Alendronate and placebo groups were comparable at baseline in age, years past menopause; all had BMD at least 2.5 SD below mean for premenopausal women; BSAP levels significantly higher (relative to premenopausal women) in postmenopausal healthy group ($p<0.0001$) and postmenopausal osteoporotic group ($p<0.0001$); osteoporotic women had higher BSAP levels than healthy postmenopausal women ($p<0.001$) -BMD increased over baseline in alendronate group and was significantly different from placebo ($p\leq0.0001$) at all times (to 24 months) -BSAP decreased in alendronate group (significantly different from baseline at all points to 24 months; $p\leq0.0001$); within 1 SD of healthy premenopausal women by 3 months and not different from healthy premenopausal women by 6 months -BSAP of placebo group significantly decreased from baseline at 3, 6, and 12 months ($p\leq0.0001$) but not at 24 months -63 of 74 in alendronate group (85.1%) had BSAP decrease from baseline of ≥25% at 6 months (responders); 9 of the 11 who did not respond at 6 months did achieve ≥25% decrease at 24 months -36 of 148 in placebo group (24.3%) had decrease of ≥25% at 6 months; by 24 months only 17 of 148 (11.5%) were responders	-BSAP provides a sensitive and accurate means to monitor the reduction in bone turnover in response to alendronate therapy in individual postmenopausal osteoporotic women. NOTES: the postmenopausal osteoporotic patients were from the U.S. portion of an alendronate Phase III study; only those with BSAP and BMD assessed at the designated times were included in this study; placebo group responders were found to have less baseline calcium intake, higher baseline BSAP concentrations, and smaller decreases in BMD at 3 and 6 months (but not 12 or 24 months)

Appendix C: Conclusion Grading Worksheet (cont)

Author/Year	Design Type	Class	Quality +,−,ø	Population Studied/Sample Size	Primary Outcome Measure(s)/Results (e.g., p-value, confidence interval, relative risk, odds ratio, likelihood ratio, number needed to treat)	Authors' Conclusions/ *Work Group's Comments (italicized)*
Ravn et al. (1999)	RCT	A	ø	-Included: women 45-59 years of age, at least 6 months past menopause at baseline, good general health, no evidence of confounding systemic disease -Randomized to placebo, 2.5 mg/day alendronate, 5 mg/day alendronate -BMD (spine, hip, forearm, total body) assessed annually; BSAP and OC assessed every 6 months	-3 groups comparable at baseline in age, years since menopause, height, weight, BMD, NTx, & OC -Greater decreases in NTx and OC in 5 mg/day group relative to 2.5 mg/day group or placebo group -Placebo group: baseline NTx and OC values weakly correlated with percent change from baseline (to 24 mos) in BMD (r=-0.12 to r=0.17) -Alendronate groups: percent change in NTx and OC at 6 months was correlated with percent change in BMD (all sites) at 24 months (r=-0.28 to -0.31 for NTx, r=-0.16 to -0.25 for OC; all $p<0.001$) -By tertiles (based on percent change from baseline) those with greatest decrease in NTx or OC at 6 mos had 4- to 5-fold increase in spine or hip BMD at 24 mos relative to least decrease in NTx or OC -With 5 mg/day alendronate dose a decrease in NTx at 6 months of 40% or more has sensitivity=86%, specificity=48%, PPV=92%, and NPV=33% for prediction of an increase in spine BMD at 24 months; a decrease in OC at 6 months of 20% or more has a sensitivity=79%, specificity=53%, PPV=92%, and NPV=37% for prediction of increase spine BMD	-Short term (6 months) changes in NTx and OC in women treated with alendronate indicates a long-term response in BMD. Changes in NTx and OC below the cutpoints during alendronate treatment was a valid indicator of long-term prevention of bone loss; a change in NTx or OC above the cut points was a poor indicator of bone loss during treatment NOTES: the participants were part of the Early Postmenopausal Intervention Cohort study; only those with baseline, 6-, and 24-month values of NTx and spine BMD were included; at least 80% of tablets were taken; wanted normal bone mass at baseline so at least 90% of the participants had BMD above 2 SD below the mean

[a]OC=osteocalcin, PICP=carboxyterminal propeptide of Type I procollagen, ICTP=carboxyterminal crosslinked telopeptide of Type I collagen, BSAP=bone-specific alkaline phosphatase, NTx=aminoterminal crosslinked telopeptide of Type I collagen; CTx=Type I C-telopeptide breakdown products, Hyp=hydroxyproline, dPyr=urinary free deoxypyridinoline, Pyr=urinary pyridinoline, Ca=calcium, Cr=creatinine, ALP=total alkaline phosphatase