

K961562

Summary of Safety and Effectiveness

Osteomark is a competitive enzyme-linked immunosorbent assay (ELISA) which utilizes a horseradish peroxidase labeled monoclonal antibody directed against the cross-linked N-telopeptides (NTx) present in urine specimens. An Osteomark® kit is comprised of the following reagents:

Antigen Coated 96-Well Plate

Calibrators:

1 nM BCE

30 nM BCE

100 nM BCE

300 nM BCE

1000 nM BCE

3000 nM BCE

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Antibody Conjugate Concentrate

Antibody Conjugate Diluent

Level I and Level II Urine Controls

30X Wash Concentrate

Buffered Substrate

Chromogen Reagent

Stopping Reagent

The solid phase utilizes microwells onto which NTx has been adsorbed. NTx in the specimen or Calibrator competes with the solid phase NTx for antibody binding sites. The resulting amount of Antibody Conjugate bound to the solid phase is indirectly proportional to the amount of NTx in the specimen or Calibrator. The quantity of NTx in the specimen is determined from a standard calibration curve using reagents supplied in the kit. Assay values are standardized to an equivalent amount of bone collagen, and are expressed in nanomole bone collagen equivalents per liter (nM BCE). BCE reflects the amount of immunoreactive NTx, as measured by Osteomark, liberated from human bone collagen following digestion with bacterial collagenase, as measured by hydroxyproline by high performance liquid chromatography (HPLC).

Intended Use of Osteomark®

Osteomark® is a urinary assay that provides a quantitative measure of the excretion of cross-linked N-telopeptides of type I collagen (NTx) as an indicator of human bone resorption.

Elevated levels of urinary NTx indicate elevated human bone resorption. Measurement of NTx is intended for use in:

- A. Predicting skeletal response (bone mineral density) to hormonal anti- resorptive therapy in postmenopausal women
- B. Therapeutic monitoring of:
 - 1. anti-resorptive therapies in postmenopausal women
 - 2. anti-resorptive therapies in individuals diagnosed with osteoporosis
 - 3. anti-resorptive therapies in individuals diagnosed with Paget's disease of bone
 - 4. estrogen-suppressing therapies

The measurement range of Osteomark is 20 to 3000 nM Bone Collagen Equivalents (BCE) of NTx.

Expected Values

Urine Collection:

A multi-center, cross-sectional study was conducted to determine the reference range for normal premenopausal women (mean age 36 years, range 25-49). Male reference range was determined from a study conducted at a large reference laboratory (mean age 46 years, range 24-71). The mean, standard deviation, and the mean \pm 2 standard deviation ranges for these two populations are presented in Table 1 below.

Table 1 - Expected Osteomark® Values for Premenopausal Women and Men

	Mean*	Std Dev	Range (mean \pm 2 std dev)*	N
Women	35	15	5-65	258
Men	27	12	3-51	81

*expressed as nanomoles BCE/millimole creatinine

In a separate study, the expected within-subject variability was determined from urine specimens from eight healthy subjects collected every 2-3 days over approximately 2 months. The average of the individual within-subject longitudinal variation was 19.3%. The average between-subject longitudinal variation was 38.3%.

Limitations of the Procedure

Lower Limit of Detection

The lower limit of detection of the Osteomark® assay is 20 nM BCE. This value represents a concentration which is greater than the value which can be distinguished from zero, and was calculated by subtracting 3 standard deviations optical density (A450-A630) from established variability of the 1nM BCE Calibrator. Assay precision below this value is insufficient for accurate results.

Interfering Substances

Common urine components and contaminants, as well as microbiological contaminants, that are known to interfere with many laboratory urine analyses were evaluated for an interfering effect with Osteomark®. The evaluations were performed by adding normal and excessive quantities of each potential inhibitor to normal urine specimens and analyzing for an effect on the final results. Results show that specimens obviously contaminated with whole blood or that have extensive hemolysis may show interference in the assay. These specimens should be avoided, and the specimen recollected.

Limitations of the Procedure

While Osteomark® is used as an indicator of bone resorption, use of this test has not been established to predict development of osteoporosis or future fracture risk. Use of this test has not been established in primary hyperparathyroidism or hyperthyroidism. When using Osteomark® to monitor therapy, results may be confounded in patients afflicted with clinical conditions known to affect bone resorption, e.g., metastases to bone. While an

Osteomark® value provides a measure of the level of bone resorption, a single Osteomark® value can not provide the rate of bone resorption as reported results do not contain a measure of time. Osteomark® results should be interpreted in conjunction with clinical findings and other diagnostic results.

Performance Characteristics

Reproducibility

Assay reproducibility was evaluated for intra-assay and inter-assay variability of normal urine specimens across the range of the calibration curve.

Intra-assay variability, or within assay precision, was assessed using eight urine specimens tested in replicates of 10 by each of four operators. Results demonstrate an average intra-assay variability estimate of 8% CV, with a range of 5-19% CV along the calibration curve.

Inter-assay variability, or assay to assay precision, was assessed using three urine specimens tested in duplicate by one operator over 20 separate assay runs. Results demonstrate an average interassay variability estimate of 4% CV, with a range of 3-5% CV along the calibration curve.

Antigen Recovery

Antigen recovery was evaluated by adding known amounts of NTx to each of three normal urine specimens. Recovery represented the observed assay value of the "spiked" specimens, calculated as a percent of the expected urine value (baseline urine value plus added antigen value). Results demonstrate an average antigen recovery of 105%, over an assay value range of 200-2500 nM BCE.

Dilutional Linearity

Dilutional linearity was evaluated by performing serial dilutions of four urine specimens with high nM BCE values into a urine specimen with a low nM BCE value. Results demonstrated correlation coefficients of $r=0.999$ to $r=1.000$ across an assay range of 44-2940 nM BCE.

Clinical Studies

Use of Osteomark® in Therapeutic Monitoring of Hormonal Anti-Resorptive Therapy and in Predicting Skeletal Response (Bone Mineral Density) in Postmenopausal Women

A multi-center, randomized, prospective clinical trial was conducted to determine the ability of the Osteomark® assay to monitor the effect of hormonal anti-resorptive therapy on bone resorption in early postmenopausal women, and to predict response to hormone replacement therapy (HRT). Subjects were randomized to estrogen (0.625 mg) and medroxyprogesterone (2.5 - 5 mg cyclic or continuous) plus a daily 500 mg calcium supplement (HRT group), or a daily 500 mg calcium supplement only (calcium group). A total of 227 women, 109 in the HRT group and 118 in the calcium group, completed the 12 month study (Campodarve et.al., 1995).

The following data support the clinical utility of Osteomark® to monitor hormonal anti-resorptive therapy in early postmenopausal women and to predict changes in bone mineral density (BMD) measured by dual energy x-ray absorptiometry (DEXA) in response to HRT, thereby identifying who will receive the greatest benefit from such therapy, and to identify those at risk for bone loss.

Osteomark® monitors the effect of therapy

Figure 1 provides the Osteomark® values throughout the study. Figure 2 provides the percent change from baseline Osteomark® throughout the study.

- The baseline Osteomark® value in the two groups combined was 59 ± 2.1 (mean \pm sem) nM BCE/mM creatinine.
- In the HRT Group, the values decrease toward the premenopausal mean.

Figure 1: Osteomark® Values Throughout the Study

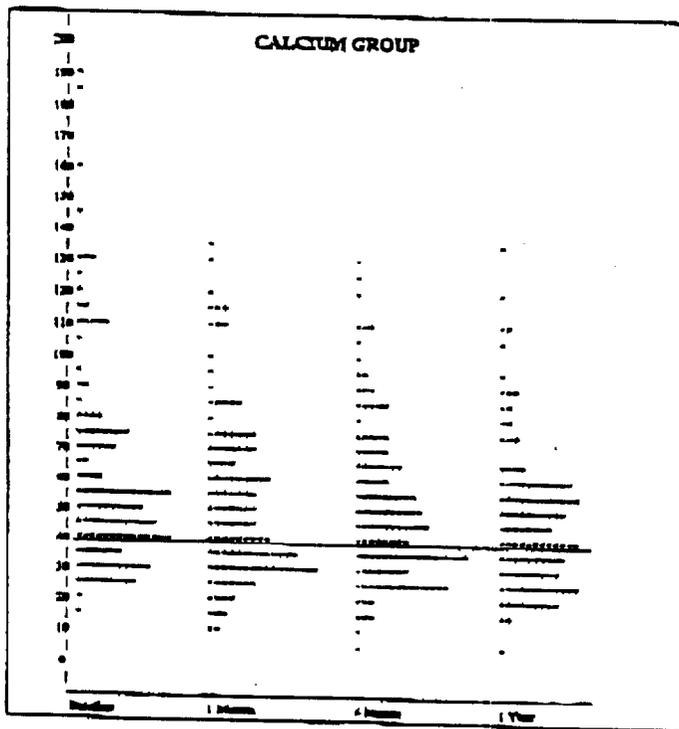
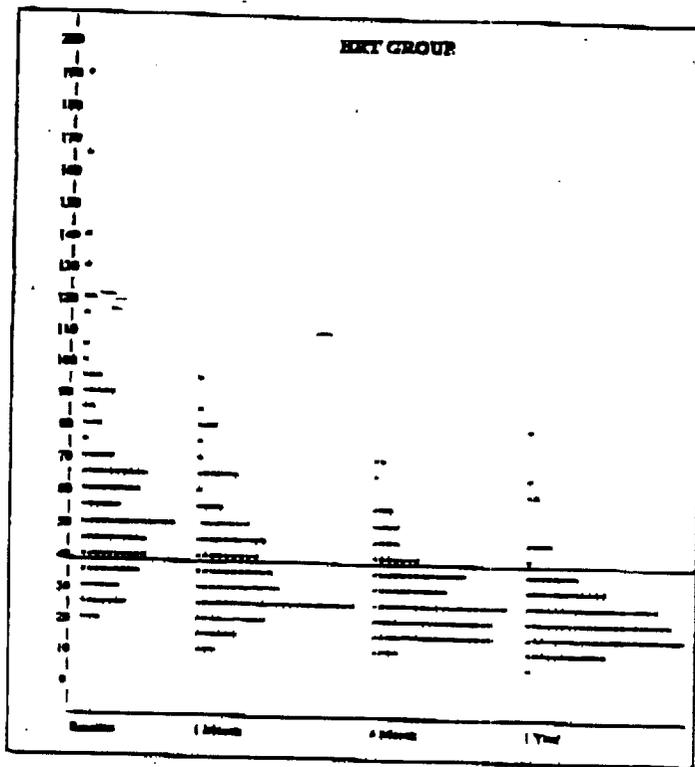
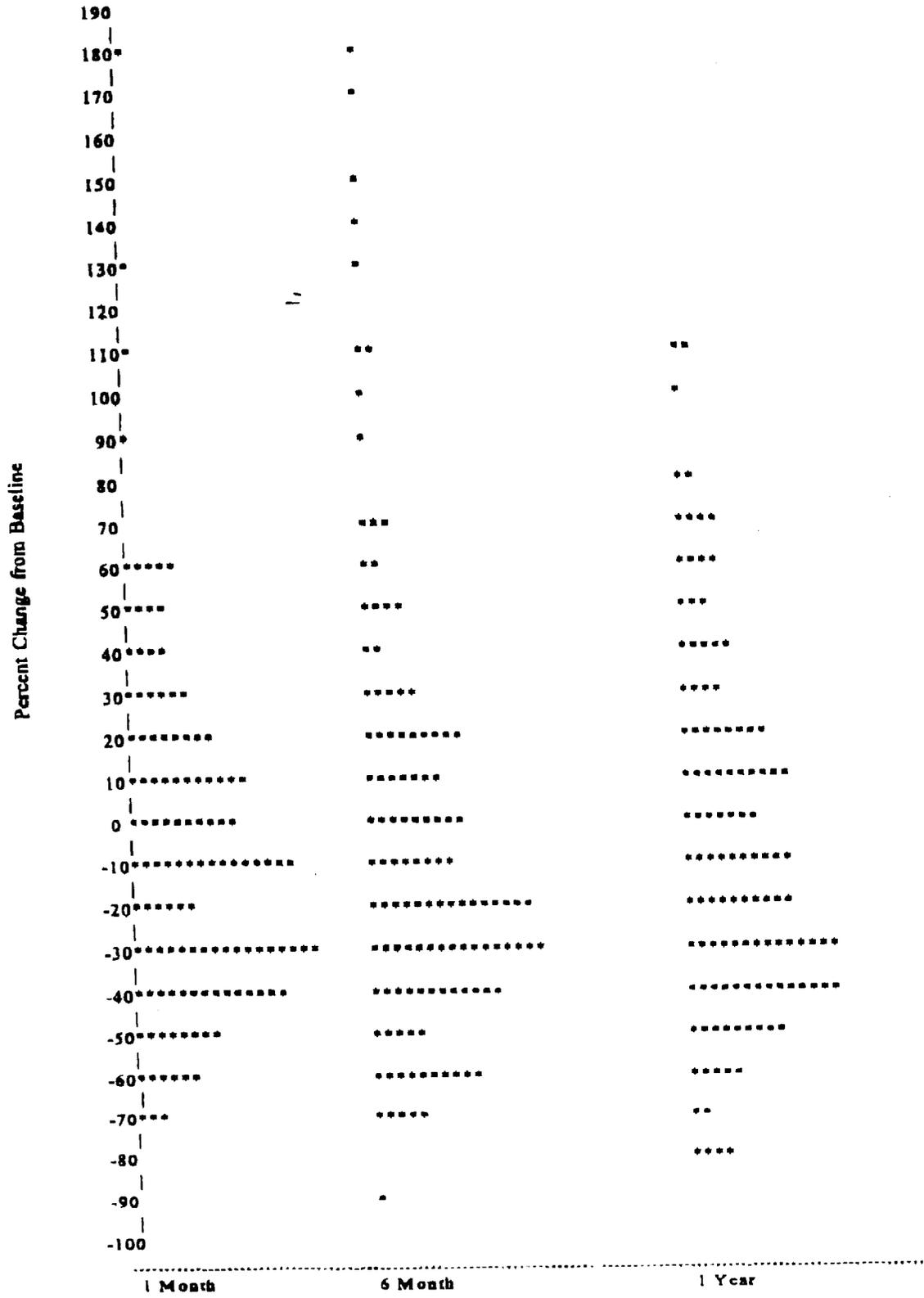


Figure 2: Percent Change from Baseline Osteomark® Throughout the Study

Osteomark® S100b Substitution
Osteix International, Inc.

CALCIUM GROUP

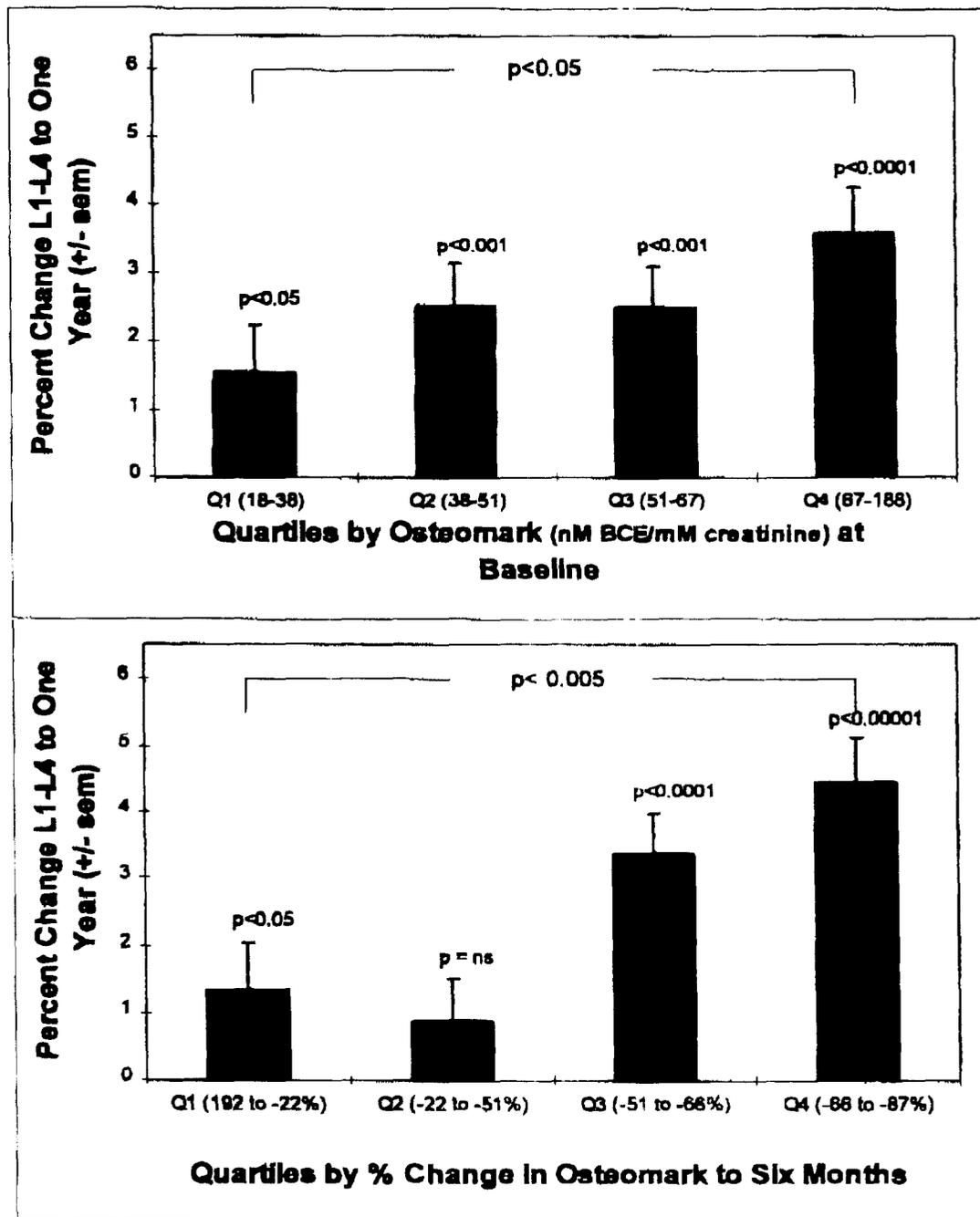


Baseline Osteomark® values and the percent change from baseline to 6 months are predictive of the change in BMD and/or response to HRT.

Figure 4 provides the baseline Osteomark® values and the percent change from baseline to six months stratified into quartiles, with the corresponding percent change in spine BMD after 1 year of HRT.

- Subjects in the highest quartile of Osteomark® values at baseline (67 - 188 nM BCE/mM creatinine), or with the greatest decrease from baseline to 6 months of therapy (-66 to -87%), showed the greatest gain in spine BMD in response to HRT.

Figure 4: HRT Group - Values of Baseline and Percent Change in Osteomark® to Six Months Stratified by Quartile and Corresponding Percent Change of L1-L4 BMD at 1 Year



Contingency table analysis showed that a $\geq 30\%$ decrease at 6 months was significantly associated ($p < 0.005$) with a positive BMD response to HRT (maintenance or gain in BMD) at 1 year. The binomial 95% confidence intervals for the sensitivity and specificity of using a 30% change in Osteomark® for predicting a response to HRT are: Sensitivity = 80% (95% C.I. 70%, 88%)

Specificity = 59% (95% C.I. 36%, 79%)

The results of using Baye's Rule to define the predictive value positive (PVP) and predictive value negative (PVN) of a 30% change in Osteomark for predicting response are tabulated below for a range of prevalence values. A prevalence of 80% was seen in this study. With higher prevalence rates, low specificity and PVN percent values are indicative of a low number of subjects in the negative response category.

Prevalence	PVP	PVN
60%	66.1%	66.3%
70%	82.0%	55.8%
80%	88.6%	42.4%
90%	94.6%	24.7%
99%	99.5%	2.9%

Figure 5 provides the linear regression analysis ($y = -0.03x + 1.3$, $r = -0.34$, $p = 0.0003$) of the percent change from baseline to 6 months Osteomark® and percent change from baseline to 1 year BMD in the HRT Group ($R^2 = 0.12$)

Figure 5. HRT Group - Linear Regression of Percent Change from Baseline to 6 Months Osteomark and Percent Change from Baseline to 1 Year L1-L4 BMD

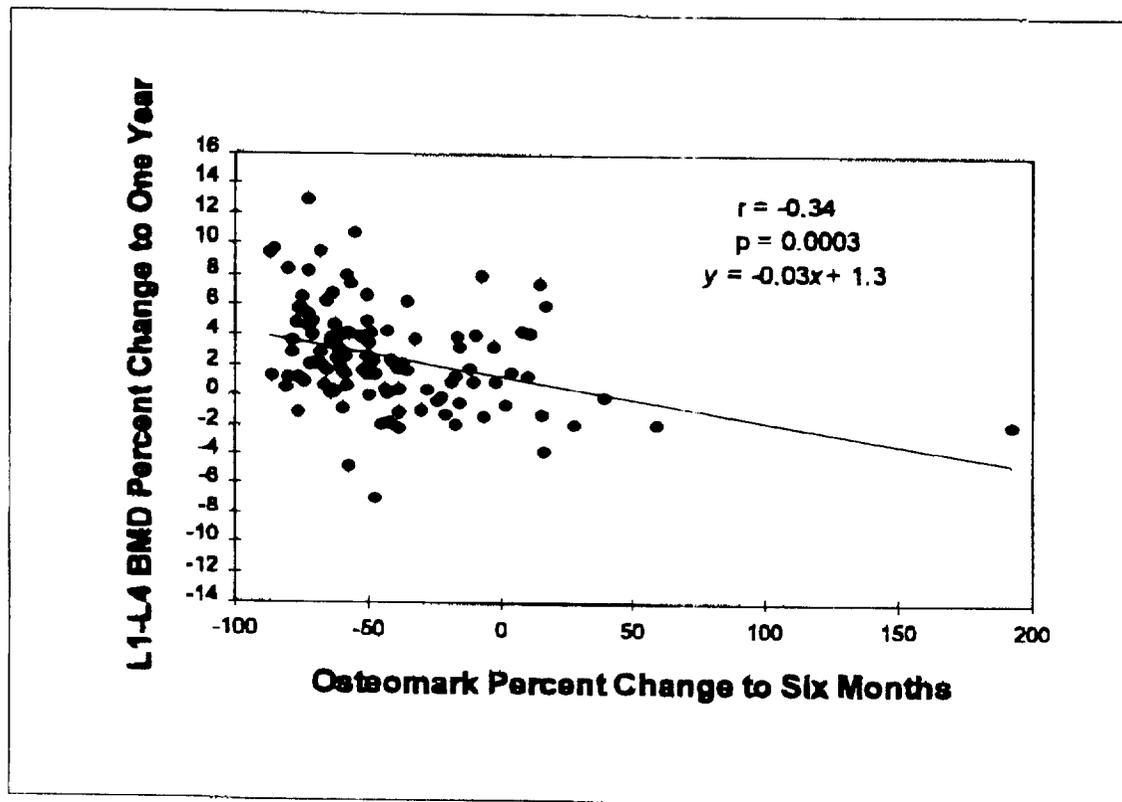


Figure 6 provides the average on-study Osteomark® value for the calcium group stratified into quartiles, with the corresponding percent change in spine BMD after 1 year.

- Subjects maintaining the highest Osteomark® values (>64 nM BCE/mM creatinine) had the greatest decrease in spine BMD.

Figure 6: Calcium Group - Average On-Study Osteomark® Values Stratified by Quartile and Corresponding Percent Change L1-L4 BMD at 1 Year

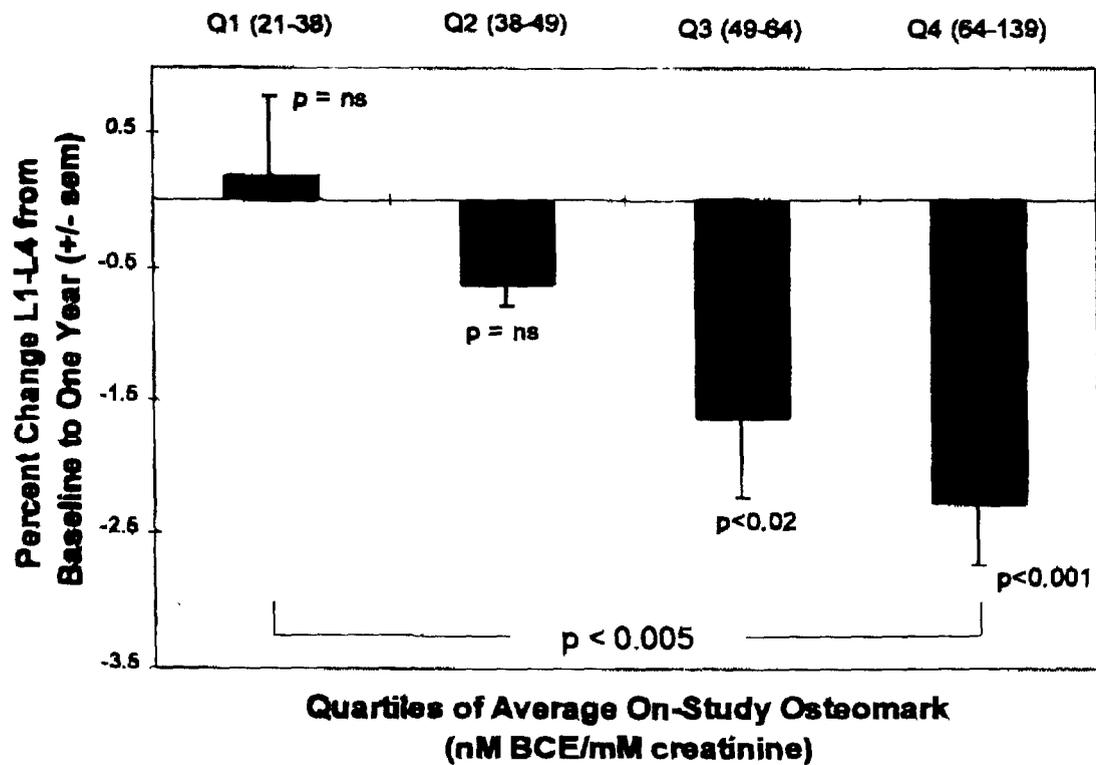


Table 1 compares the two treatment groups using Relative Risk analysis.

- A high baseline Osteomark® value (≥ 67 nM BCE/mM creatinine) indicated a 17.3 times higher risk of loss of spine BMD if not treated with HRT.

Table 1: Relative Risk of Loss of BMD Comparing Calcium and HRT Groups

Baseline NTx (nM BCE/mM creatinine)	Relative Risk	95% CI
18-38	1.4	0.8 - 2.5
38-51	2.5	1.0 - 6.1
51-67	3.8	1.6 - 9.1
67-188	17.3	2.5 - 118.6

Use of Osteomark® to Monitor Estrogen Suppressing Therapy

A multi-center, non-randomized, prospective, longitudinal clinical trial was conducted to determine the ability of the Osteomark® assay to monitor the effect of estrogen suppressing therapy on bone resorption in premenopausal women (Marshall et.al., 1996). Subjects were given GnRH-agonist therapy 3-6 months for treatment of varied gynecologic disorders.

The following data support the clinical utility of Osteomark® to monitor the effect of estrogen suppressing therapy.

Figure 7 represents the mean (\pm sem) Osteomark® values obtained throughout the study along with corresponding mean (\pm sem) estradiol values for each timepoint.

- The mean Osteomark® value at baseline was 44 ± 3 nM BCE/mM creatinine.
- The mean Osteomark® value while estrogen suppressed was 61 nM BCE/mM creatinine, a 68% increase from baseline.
- The mean serum estradiol level was 21 pg/mL during this time period.
- The Osteomark and estradiol values while estrogen suppressed were concordant with postmenopausal ranges of each analyte.

Figure 7: Osteomark® Values and Serum Estradiol Levels (mean ± sem) During and After Estrogen Suppression Therapy

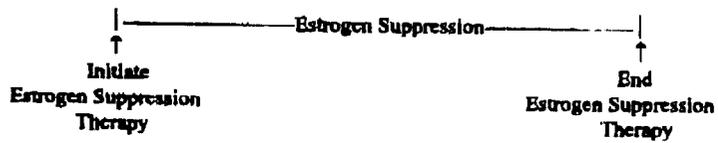
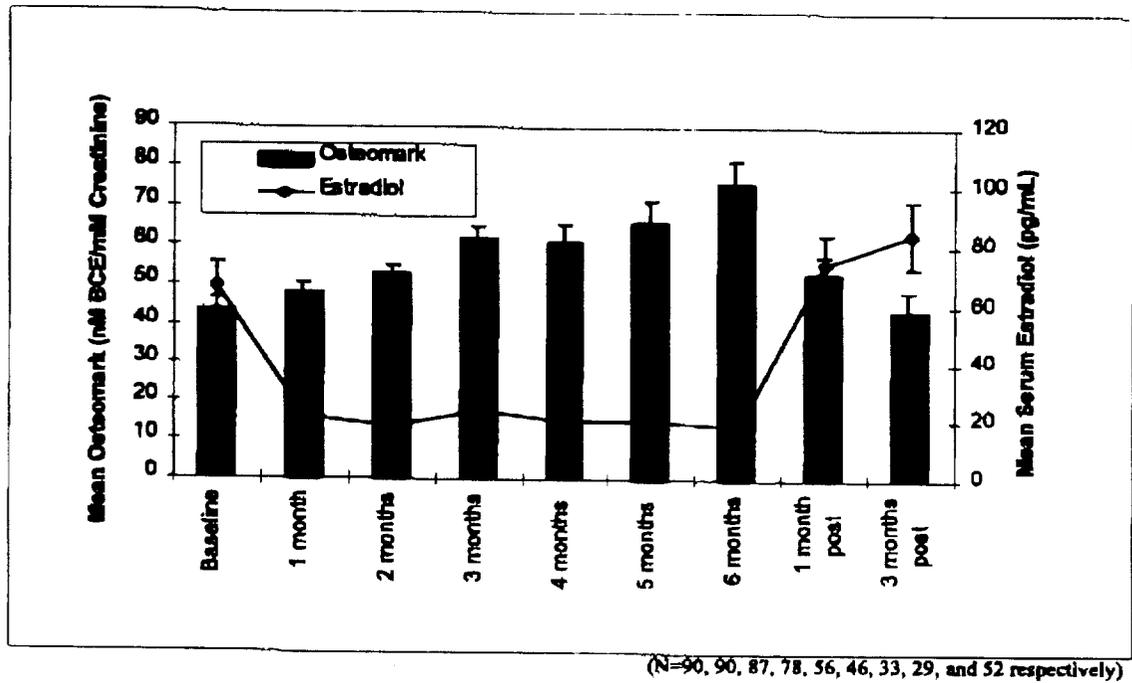


Figure 8 provides percent change in Osteomark® from baseline throughout the study for each subject.

- Sixty three percent (55/88) of the subjects had a mean percent change from baseline of $\geq 30\%$ ($p=0.025$).
- Subjects exhibiting $< 30\%$ change (33/88, or 37%) had a mean baseline Osteomark® value that was higher (60 nM BCE/mM creatinine) than those with a $\geq 30\%$ change (35 nM BCE/mM creatinine), accounting for the lesser percent change in Osteomark® in these individuals.
- The average on-therapy Osteomark value for the $< 30\%$ change group was lower (51 nM BCE/mM creatinine) than the $\geq 30\%$ group (66 nM BCE/mM creatinine). The 30% change group also tended to lose less bone at the spine than those who had a $\geq 30\%$ change.

Further analysis showed:

- The mean 68% increase from baseline Osteomark® correlated to a mean percent decrease at six months of -3.7% in lumbar spine (L1-L4) BMD ($r = -0.46$, $p < 0.01$). Three months after cessation of estrogen therapy, the mean Osteomark® value returned to baseline (44 nM BCE/mM creatinine) as serum estradiol levels returned to normal premenopausal levels.
- Lumbar spine BMD remained below baseline (-2.4%) at the 3 month post estrogen suppression therapy timepoint.

Use of Osteomark® in Therapeutic Monitoring of Anti-Resorptive Therapy in Paget's Disease of Bone

Paget's disease of bone is characterized by abnormally elevated rates of bone resorption coupled with elevated bone formation. A study was conducted to determine the ability of Osteomark® to monitor the effect of bisphosphonate therapy on bone resorption in Paget's disease patients. Subjects (65% male, 81% caucasian) were diagnosed with Paget's disease based upon radiographic evidence and a serum total alkaline phosphatase level at least twice the upper limit of normal, and were treated with one of three bisphosphonates; alendronate 40 mg/day, orally (n=28), etidronate 400 mg/day, orally (n=24), and pamidronate 60 mg intravenous x3 doses (n=20). Serum and urine specimens were collected at baseline and 1, 3, and 6 months after initiation of therapy. The following data support the clinical utility of Osteomark® to monitor the effect of bisphosphonate therapy on the rate of bone resorption in patients diagnosed with Paget's disease of bone.

Table 2 provides Osteomark® values at each timepoint for the three treatment groups combined.

Table 2: Osteomark® Values (nM BCE/mM creatinine) at Baseline and On Therapy (All Therapies Combined)

Visit*	Mean	sem
Baseline	1195	155
Month 1	599	87
Month 3	443	77
Month 6	307	63

*N= 72, 59, 71, and 69 respectively

Table 3 provides the mean percent change from baseline Osteomark® for each therapy.

- At each timepoint and with all therapies, a clinically significant change in Osteomark® ($\geq 30\%$) was achieved.

Table 3: Percent Change from Baseline Osteomark® by Therapy

Visit	Alendronate	Etidronate	Pamidronate
Month 1*	-48%	-39%	-71%
Month 3**	-77%	-58%	-67%
Month 6***	-87%	-72%	-71%

*N = 22, 17, and 20 respectively

**N = 28, 23, and 20 respectively

***N = 27, 22, and 20 respectively

Table 4 provides the Osteomark® values for responders and nonresponders at 6 months using total alkaline phosphatase normalization as the criteria for response.

- The level of total alkaline phosphatase is often associated with the severity of Paget's disease of bone. Normalization of total alkaline phosphatase levels has been used to determine response to therapy. Using linear regression of the log-transformed baseline Osteomark® and total alkaline phosphatase levels, a significant positive relationship was found in all three treatment groups ($r=0.88$, 0.74 , and 0.87 in the alendronate, etidronate and pamidronate groups respectively; $p=0.0001$ for all groups). Thus patients with high levels of total alkaline phosphatase also had high levels of Osteomark® at baseline. Again at six months, the correlations of Osteomark and total alkaline phosphatase levels were high, $r = 0.88$, 0.87 , and 0.72 and $p=0.0001$, 0.0001 and 0.0003 in the alendronate, etidronate and pamidronate groups respectively.

Table 4. Six Month Osteomark® Values (mean \pm SD) for Responders and Nonresponders Based on Normalization of Serum Total Alkaline Phosphatase

	Alendronate	Etidronate	Pamidronate
Responder*	31 \pm 36	32 \pm 27	66 \pm 37
Nonresponder**	795 \pm 867	373 \pm 355	487 \pm 759

*N = 18, 2, and 9 respectively

**N = 9, 20, and 11 respectively

Table 5 provides the percentage of responders (all therapies combined), as defined by the normalization of the marker value (Osteomark® 5-65 nM BCE/mM creatinine, total alkaline phosphatase 39-117 IU/L).

- A decrease of serum total alkaline phosphatase into its normal range has been used as an indicator of therapeutic response. An analogous examination of Osteomark® values over time indicates a similar utility as a measure of response.
- Osteomark provides an earlier assessment than total alkaline phosphatase of therapeutic response, defined by normalization of values, to bisphosphonate therapy.

Table 5. Percent Responders as Defined by Normalization of Marker (number of patients with normalized marker value/total number of patients)

Visit*	Osteomark Responders	Total Alkaline Phosphatase Responders
Month 1	19%	2%
Month 3	34%	28%
Month 6	42%	42%

*N = 59, 71, and 69 respectively

Use of Osteomark® to Therapeutic Monitoring of Anti-Resorptive Therapy in Osteoporosis

A multi-center, randomized, prospective study was conducted to establish the safety and efficacy of a new amino-bisphosphonate (alendronate) in the treatment of osteoporosis (Lieberman et.al, 1995). Study subjects were postmenopausal women, age 45 to 80 years diagnosed with osteoporosis (lumbar spine bone mineral density ≥ 2.5 SD below the mean for mature premenopausal women), randomized to receive either alendronate 10 mg plus calcium supplements (500 mg daily) (alendronate group), or placebo and calcium supplements only (calcium group). Fasting second morning void urine specimens were collected at baseline and periodically throughout the three year study. The following data support the clinical utility of Osteomark® to monitor the rate of bone resorption in osteoporotic women treated with anti-resorptive therapy (alendronate 10 mg).

Table 6 provides the Osteomark® values for the two groups.

- Three months after initiating treatment 80% (71/89) of the subjects in the bisphosphonate group had an Osteomark® value ≤ 35 nM BCE/mM creatinine. The mean (\pm SD) percent change from baseline at 3 months was -62% (± 20), 87% (76/87) had a $\geq 40\%$ decrease.
- After 1 year of therapy, the mean percent change from baseline Osteomark® remained similar to the values at 3 months (-65% ± 18); with 90% (77/86) ≤ 35 nM BCE/mM creatinine and 92% (77/84) of the subjects having a $\geq 40\%$ decrease.
- In the calcium only group, the Osteomark® values remained relatively constant, with a mean -13% ± 37 change (mean \pm SD) from baseline at 1 year.

Table 6. Osteomark® Values (nM BCE/mM creatinine) in Osteoporotic Patients Treated with Alendronate 10 mg plus Calcium Supplement or Calcium Supplement Only (mean ± SD)

Treatment Group	Baseline	Month 1	Month 3	Month 6	Month 12	Month 24	Month 36
Alendronate 10mg and Calcium*	70 ± 33	31 ± 21	25 ± 16	22 ± 13	22 ± 13	20 ± 9	18 ± 9
Calcium only**	69 ± 33	57 ± 28	56 ± 26	54 ± 26	54 ± 25	52 ± 21	56 ± 20

*N = 91, 88, 89, 88, 86, 80, and 78 respectively

**N = 188, 182, 183, 180, 174, 157, and 149 respectively

Figure 9 provides the percent change from baseline Osteomark® through 1 year of the study

Figure 9. Percent Change From Baseline Osteomark® Through 1 Year

ALENDRONATE GROUP

