

Serum osteocalcin and procollagen as markers for the risk of osteoporotic fracture in corticosteroid-treated asthmatic adults

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Background: Dual energy x-ray absorptiometry provides the definitive measure of osteoporotic fracture risk.

Objective: We sought to determine whether metabolic measures of bone formation and/or common features of clinical hypercortisonism provide a useful guide in selecting corticosteroid-treated asthmatic patients for referral for bone densitometry.

Methods: We measured bone density and 8 AM serum osteocalcin, procollagen, and cortisol levels in 52 asthmatic adults aged 60.7 ± 12.6 years (mean $\pm$ SD). Years of steroid exposure for these patients was 11.8 ± 10.7 (prednisone) and 11.78 ± 4.98 (inhaled steroid). Using stepwise logistic regression, we assessed the capacity of the osteocalcin and procollagen levels, with or without the cortisol level, age, clinical features of hypercortisonism, and different lifetime exposures to inhaled and oral steroids for distinguishing between patients with greater or lesser risk of fracture.

Results: Osteoporosis, defined as a bone density *T* score below -2.5 , affected 26% of the group at the spine and 63% at the hip. At the spine, greater risk was associated only with lower cortisol levels ($P = .003$). Diagnostic accuracy was 71%, the false-positive rate was 26%, and the false-negative rate was 31%. At the hip, greater risk was associated with lower cortisol levels ($P = .002$), longer prednisone exposure, ($P = .003$), lower current doses of prednisone ($P = .01$) and inhaled steroid ($P = .02$), and older age ($P = .01$). Diagnostic accuracy was 83%, the false-positive rate was 13%, and the false-negative rate was 21%.

Conclusions: Neither osteocalcin nor procollagen nor any of

the clinical criteria analyzed proved sufficiently accurate to be reliable as indicators of the risk of fracture in these elderly, corticosteroid-treated asthmatic adults. They are therefore not useful for selecting such patients for diagnostic densitometry. (*J Allergy Clin Immunol* 1999;104:769-74.)

Key words: Asthma, glucocorticoids, inhaled steroids, steroid complications, osteoporosis, fracture risk, bone densitometry, osteocalcin, Type I procollagen

Historically, osteoporosis and fracture have constituted the most common major complication of long-term oral or parenteral corticosteroid therapy for asthma.^{1,2} Additionally, high-dose inhaled corticosteroid therapy (eg, >1 mg/day of budesonide or beclomethasone [nominal dose]) may be associated with reduced bone mineral density.^{3,4}

Bone density, measured at the spine and hip by dual energy x-ray absorptiometry (DXA), is currently the preferred method for quantifying the risk of osteoporotic fracture.⁵⁻⁷ Identification of patients at increased risk before the actual occurrence of fracture is important to avoid major morbidity.^{7,8}

Cost and regional differences in accessibility to DXA may restrict its application in patients potentially at risk.⁹ In particular, there is concern about the costs to the health care system of surveying populations in whom the yield of positive diagnoses may be low.⁹ It is therefore of interest to the practicing physician and health care management to know the actual prevalence of osteoporosis among asthmatic patients chronically exposed to oral and inhaled corticosteroid therapy in ordinary clinical practice. It is also of interest to know whether clinical and/or laboratory data may provide a reliable guide in selecting those corticosteroid-treated asthmatic patients most likely to benefit from referral for diagnostic densitometry.

The serum levels of osteocalcin and the carboxypropeptide of Type I collagen (PICP) are sensitive and specific biochemical measures of bone formation,¹⁰ and each may be suppressed by corticosteroid treatment.¹¹⁻¹³ Their diagnostic efficiency in clinical practice as markers for identifying steroid-treated asthmatic patients at increased risk of fracture has not been systematically assessed previously.

We therefore evaluated the capacity of a 1-time measurement of these metabolic parameters, taken alone or in conjunction with other laboratory or clinical data read-

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Abbreviations used

DXA: Dual energy X-ray absorptiometry
 PICP: Carboxypropeptide of Type I procollagen
 ROC: Receiver operating characteristic

TABLE I. Patient characteristics: Osteoporosis risk factors*

	No.
M/F	20/32
Age >60 y	28
Postmenopausal	32
With estrogen replacement	19
No estrogen replacement	13
Corticosteroid exposure	
Prednisone	51
Inhaled	52
Smoker (current or ex-smoker)	2
Low physical activity	6†
Male impotence-infertility	0
Fractures with minor trauma	0
Parental history of fracture	0
Alcoholism	0

*Prepared from Arth Rheum 1996;39:1791-801.

†In lowest quantile of physical activity rating

ily accessible to the practitioner, to distinguish between asthmatic patients with greater or lesser degrees of risk of osteoporotic fracture.

METHODS

This was a cross-sectional survey of bone density at one point in time. The study was approved by the review board of the University of Western Ontario for health sciences research involving human subjects.

The study group comprised 32 female and 20 male adults attending a specialty clinic for ambulatory care of chronic asthma. The patients were stratified and selected to reflect a broad range of different lifetime exposures to prednisone or inhaled corticosteroid (mostly budesonide). The purpose of this selection process was to yield independent information about the respective effects of exposure to oral versus inhaled corticosteroids.

Patient characteristics

The prevalence of the major risk factors for osteoporosis in the survey group is shown in Table I. Their current and past exposures to corticosteroid up to the time bone density was measured are shown in Table II. Their current corticosteroid doses averaged 1.34 mg/d inhaled steroid (range, 0-3.2 mg/d [nominal dose]) and 1.32 mg/d prednisone (range, 0-20 mg/d). In all but 8 patients, chronic exposure to corticosteroids began after age 30 years when peak bone density is normally attained. No patient had used any bone-depleting drug other than corticosteroid.

There were no black, Hispanic, or Asian patients. The body mass index of the patients averaged 25.8 ± 3.5 (SD) (mode 24; range, 18.6 to 38). None had a body mass index in the subnormal range associated with osteoporosis consequent to undernutrition.¹⁴ Their physical activity rating, determined by using an empiric 10-point ordinal scale, ranged from 9 (strenuous physical exercise daily) to 2 (moderately sedentary), with a mean of 5.58 ± 1.9 (SD) and a mode

TABLE II. Patient characteristics: Corticosteroid exposure

	No.
Daily or alternate-day prednisone	
Regular (≥ 6 mo)	23
Intermittent only	28
Current	14
Never	1
Inhaled steroid	
Budesonide only	5
Beclomethasone only	2
Budesonide and beclomethasone	45
Current	52
Intranasal steroid	24

of 5. The distributions of the body mass index and physical activity ratings were unimodal, bell-shaped, and symmetric.

Outcome measurements

Bone density was measured at the hip and lumbar spine by using the Hologic QDR 1000 densitometer (Hologic Corp, Boston, Mass). Measurement at the hip improves the diagnostic sensitivity of densitometry by avoiding the confounding effects of coexisting degenerative joint disease, which is commonly present at the spine in elderly patients. The measured bone density in grams per square centimeter was adjusted for age and sex to yield a *T* score from which to estimate the risk of fracture as defined in current guidelines for osteoporosis management.^{7,8}

Blood for metabolic studies was collected at $8 \text{ AM} \pm 1.5$ hours on or close to the day densitometry was performed. Serum was separated and stored at -70°C for subsequent batch assay of the osteocalcin, PICP, and serum cortisol levels. Osteocalcin was assayed by RIA (IncStar Corp, Stillwater, Minn); intra-assay and inter-assay coefficients of variation were 4.5% and 5.3%, respectively, and the normal adult range was 0.52 to 2.24 nmol/L. PICP was measured by RIA (IncStar Corp.); intra-assay and inter-assay coefficients of variation were 3.0% and 5%, respectively, and the normal adult range was 38 to 202 $\mu\text{g/L}$. Cortisol was measured by RIA (IncStar Corp), and the normal range was 193 to 690 nmol/L. The purpose of measuring the serum cortisol was to provide an objective surrogate marker for the intersubject differences in corticosteroid pharmacokinetics, which are thought to account for much of the variation among patients in their susceptibility to corticosteroid-induced systemic side effects.^{15,16}

The relationships of these laboratory parameters, along with selected clinical risk factors, to the degree of osteoporosis as determined by DXA were explored by regression analysis.

The clinical risk factors selected for analysis are readily accessible to the practicing physician and are commonly used in practice to estimate the systemic tolerability of chronic corticosteroid therapy by particular patients and/or cited as risk factors in current guidelines for osteoporosis management.^{7,8} These are listed in Table III.

Statistical analyses

To achieve a clinically meaningful evaluation of the 14 risk factors, we categorized the 52 patients as being at lower or higher risk of fracture, as determined from their bone density measurements by DXA. This was done separately for the spine and hip outcomes by dividing the group at the median spinal and hip bone density *T* scores (-1.6 and -2.8 , respectively) as shown in Fig 1. Thus there were 26 patients in the lower risk and 26 in the higher risk group for each of the spine and hip locations. The classifications were significantly associated with one another, with 40 of the 52 patients being classified in the same risk group (20 high and 20 low) for both loca-

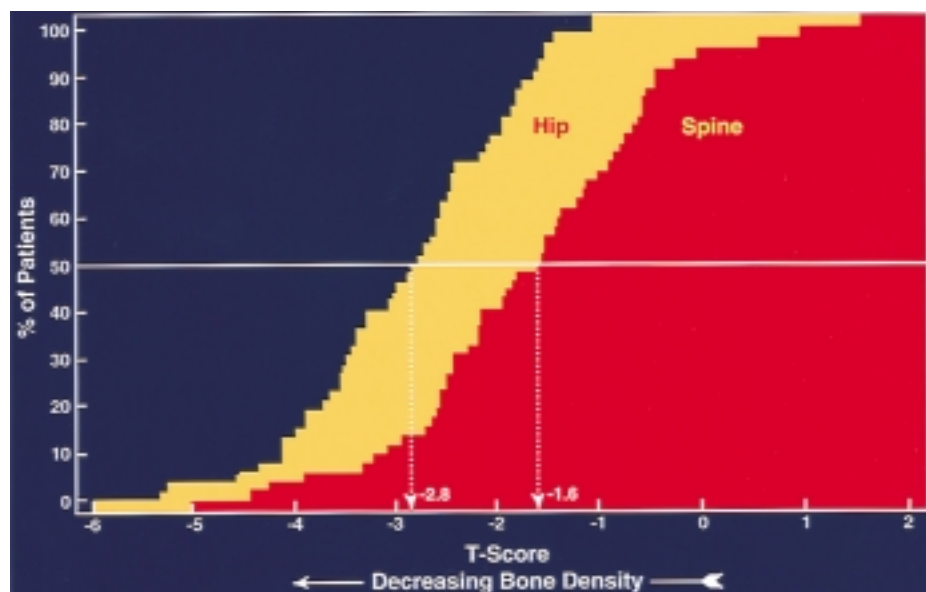


FIG 1. Cumulative frequency distribution of *T* scores determined by DXA at the hip and lumbar spine (*n* = 52). Each *T* score quantifies the number of SDs by which the measured bone density (in grams per square centimeter) differs from the mean bone density of young healthy adults of the same sex as the patient. *T* scores between -1 and -2.5 signify osteopenia. Scores to the left of -2.5 signify osteoporosis and a clinically important increase in fracture risk.^{7,18} The dashed vertical lines indicate the median scores for the group: -2.8 at the hip and -1.6 at the spine.

tions. Using the risk category as the outcome for each patient, stepwise logistic regression (SAS/LOGISTIC) was used to build diagnostic models from the 14 risk factors considered, including PICP and osteocalcin. A significance level of .10 was used for both the inclusion and deletion of variables at each step in this exploratory analysis.

For the logistic regression analysis, the 4 categorical values in Table III (eg, moon face) were coded 1 or 0 to represent their presence or absence, respectively. These variables were then included with the measured variables as candidates for stepwise entry. The statistical significance of such a factor in logistic regression is basically the same as that of a significant χ^2 test for the relevant 2×2 contingency table. However, the other variables are controlled in the logistic regression model rather than ignored, as in a 2×2 table.

Each logistic regression model provides a formula that combines a patient's risk factors to produce a predicted probability that the patient belongs to the higher risk group. The threshold or cut-off point for deciding what predicted probabilities represent higher risk can vary from 0 to 1. Each such cut-off point defines a screening test for which the usual diagnostic characteristics (eg, accuracy and specificity) may be calculated.¹⁷ The plot of true positives (%) versus false positives (%) as a function of the changing cut-off point from 0 to 1 is the receiver operating characteristic (ROC) curve for each screening test defined by the logistic regression model. We report here the diagnostic characteristics of the screening tests that correspond to the particular point on the ROC curve with the highest predictive accuracy; that is, the point at which the highest percentage of patients are correctly classified as being in the higher or lower risk category, as determined by DXA.

RESULTS

The frequency distributions of the *T* scores are illustrated in Fig 1 for the hip and spine, respectively. The

TABLE III. Risk factors included in multiple regression analysis

Risk factor	Mean $\pm$ SD
Age (y)	60.7 $\pm$ 12.6
Current prednisone dosage (mg/d)	1.32 $\pm$ 3.6
Current inhaled steroid dosage (mg/d)	1.34 $\pm$ 0.82
Years of prednisone*	11.92 $\pm$ 10.93
Inhaled steroid cumulative dose (g)*	4.17 $\pm$ 2.51
Serum osteocalcin level (nmol/L)	0.267 $\pm$ 0.219
Serum procollagen level (μ g/L)	203.36 $\pm$ 154.74
Serum cortisol level (nmol/L)	422.5 $\pm$ 183.83
Moonface	0.13 $\pm$ 0.35
Steroid purpura	0.19 $\pm$ 0.4
Hypertension	0.23 $\pm$ 0.42
Weight gain	0.13 $\pm$ 0.34
Years of estrogen supplement	2.52 $\pm$ 4.96
Physical activity score (1-10)	5.38 $\pm$ 1.86

*Primary selection criteria for patients studied (*n* = 52).

median value, which was the threshold between lower and higher risk categories in this analysis, was -2.8 at the hip and -1.6 at the spine. Negative *T* scores greater than -2.5 signify osteopenia and a clinically important increase in fracture risk.^{7,8} Overall, the *T* scores averaged -2.93 ± 0.98 (SD) at the hip and -1.68 ± 1.21 (SD) at the spine.

In preliminary multiple regression analyses the 14 risk factors listed in Table III could explain a greater proportion of the observed variability in *T* scores at the hip than at the spine (53% vs 26%, respectively; adjusted R^2).

TABLE IV. Risk factors associated with level of fracture risk (results of stepwise logistic regression analysis)

Risk factor	Hip		Spine	
	Standardized coefficient*	P value†	Standardized coefficient*	P value†
Serum cortisol level at 8 AM	-1.62	.002	-0.88	.003
Years of prednisone	1.04	.003	—	>.10
Current dose of prednisone	-0.84	.01	—	>.10
Current dose of inhaled steroid	-0.83	.02	-0.32	.09
Age	0.79	.01	—	>.10

*The standardized coefficients quantify the relative importance or "weight" of the factors. An increase in a factor with a positive coefficient or a decrease in a factor with a negative coefficient increases the odds of being in the higher risk group.

†P values determined by χ^2 analysis.

A preliminary simple correlation analysis demonstrated a significant correlation between the osteocalcin levels and the *T* score at the hip ($r = 0.37$, $P = .007$). However, this disappeared when other factors, such as the serum cortisol level and years of prednisone exposure, were controlled in the stepwise logistic regression. The logistic regression analysis demonstrated that the distributions of 5 of the 14 risk factors of interest listed in Table III differed significantly between the higher versus lower risk patients. These 5 factors are ranked in Table IV according to the amount of variability in bone density that each factor could explain (see coefficients).

At the hip, greater risk was associated with lower serum cortisol levels ($P = .002$), longer exposure to prednisone ($P = .003$), lower current daily doses of prednisone ($P = .01$) or inhaled steroid ($P = .02$), and older age ($P = .01$). At the spine, greater risk correlated only with lower cortisol levels ($P = .003$) and lower current daily doses of inhaled steroid ($P = .09$; Table IV). The association of the morning serum cortisol level with the degree of fracture risk was stronger than that of any of the other statistically significant risk factors identified (compare the standardized coefficients in Table IV). Neither osteocalcin nor PICP correlated significantly with the degree of risk at either site of DXA examination.

When the oral and inhaled steroid daily doses were combined to form an aggregate measure of current corticosteroid exposure, the latter retained the same negative relationship to the risk of fracture exhibited in Table IV by the coefficients for each of the oral and inhaled steroid use components singly (data not shown). Additionally, when sex and its interaction with age were added to the list of variables in the stepwise regression analysis, this did not alter the results because neither variable entered the model (data not shown). Thus the heterogeneity of the group with respect to age and sex did not affect outcome.

The diagnostic accuracy (true positives + true negatives/n%) of the significant variables identified by step-

wise logistic regression is shown in Table V. In combination, age, the current daily doses of prednisone and inhaled steroid, cumulative years of prednisone exposure, and the morning serum cortisol level could achieve a maximum diagnostic accuracy of 82.7% for correctly identifying patients with respect to their levels of risk at the hip. The corresponding false-positive rate was 13%, and the false-negative rate was 20.7%.

The maximum diagnostic accuracy achieved at the spine by using the serum cortisol level plus the current dose of inhaled steroid (ie, the only significant correlates of increased risk at the spine; Table IV) was 71.2%, with an accompanying false-positive rate of 26.1% and a false-negative rate of 31% (Table V).

When osteocalcin and PICP were used alone in the logistic regression model, the best classification achievable was 55.8% at the hip and 51.9% at the spine. When osteocalcin and PICP were added to the best predictor variables listed in Table IV, they did not materially improve the percentage of the group correctly classified by risk category (data not shown).

DISCUSSION

A high proportion of these nonhospitalized asthmatic adults had *T* scores indicating a clinically important increase in the risk of fracture as defined by current criteria for the evaluation of risk in osteoporosis (Fig 1).^{7,18} The generalizability of this finding to other ambulatory care asthma clinics is unknown because of the absence of comparable data from similar facilities, nationally or internationally. The finding is consistent with earlier studies that, on the basis of radiographic evidence of fracture or densitometry of the radius, identified osteoporosis as a relatively common major complication of the long-term treatment of asthma with oral and parenteral corticosteroids.^{1,2} It has been estimated that at least 50% of persons exposed are likely to be affected.¹ As evidenced by Fig 1, such prevalence estimates are strongly influenced by the skeletal site examined.

The substantial unexplained variation in the *T* scores observed at the hip in these patients ($R^2 = 0.54$ after controlling for the effects of the risk factors analyzed) indicates a strong influence from unknown factors, possibly genetic, acting alone or possibly in conjunction with the corticosteroids as major determinants of bone density.

Previous studies in asthmatic adults failed to find a statistical association between the daily dose or duration of oral corticosteroid use and the risk of fracture.² In the current study greater risk at the hip correlated positively with the duration of prednisone exposure but negatively with the current daily doses of oral and inhaled steroid (Table IV). These paradoxical associations could reflect the persistence of osteoporosis established before the introduction of inhaled corticosteroid therapy and subsequent prednisone weaning, coupled with a bias toward conservative steroid dosing in those patients perceived clinically as being potentially at greatest risk. These results were not influenced by the heterogeneity of the

TABLE V. Diagnostic characteristics of logistic regression models*

Predictive factors in model†	Densitometry site	Highest attainable diagnostic accuracy (%)	Sensitivity (%)	Specificity (%)	False positives (%)	False negatives (%)
Serum cortisol level, years of prednisone, current prednisone dose, current inhaled steroid dose, age	Hip	82.7	76.9	88.3	13.0	20.7
Serum cortisol level, current inhaled steroid dose	Spine	71.2	65.4	76.9	26.1	31.0

*These results represent the points on the respective ROC curves corresponding to the highest attainable diagnostic accuracy; that is, the point that represents the cut-off point used for prediction of risk-group membership that yields the maximum number of correctly classified patients.

†Derived from Table IV.

group with respect to age and sex (see the "Results" section). The data do not provide a suitable basis for estimating the relative toxicity to bone of one inhaled steroid formulation versus another.

The absence of a discernible association between osteocalcin or PICP and differences in fracture risk accords with earlier reports that metabolic measures of bone turnover do not accurately reflect the degree of bone depletion complicating chronic corticosteroid use¹⁹ or the postmenopausal state.²⁰ This does not exclude the possibility that change in these markers after the introduction of regular corticosteroid therapy might have prognostic value in respect to the subsequent occurrence of osteoporosis and fracture. However, because such pre-treatment and posttreatment data are not generally available in ordinary clinical practice, their utility as a guide for selecting patients for DXA is problematical.

The strongest risk factor identified in this study proved to be the morning serum cortisol level (Table IV). This accords with observations reported in a survey of a younger population of asthmatic adults treated with high-dose inhaled corticosteroid.³ However, the undesirably high incidence of false-negative categorizations in our patient group (Table V) limits the usefulness of the morning cortisol level as a surrogate or adjunct marker for identifying asthmatic patients at increased risk from corticosteroid-induced bone loss. These false negatives may reflect, in part, the fact that bone densities are known to change at doses of prednisone too low to affect the morning cortisol level.²¹

For the practical purposes of clinical decision making in an ambulatory care setting, it would appear that diagnostic DXA ought to be sought routinely in asthmatic patients similar to those who participated in this survey. This is indicated because a majority may exhibit a clinically important (and potentially treatable) increase in fracture risk (Fig 1) and because these data suggest differences in the degree of risk cannot be accurately estimated from any combination of the common clinical features of hypercortisolemia and/or records of current or accumulated corticosteroid exposure, with or without accompanying measurements of metabolic

indices of bone formation, such as osteocalcin or PICP (Table V).

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