FACTORS ASSOCIATED WITH LONGITUDINAL BONE MINERAL DENSITY LOSS IN A TOBACCO-EXPOSED COHORT

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ABSTRACT

The prevalence of low bone mineral density (BMD) is increased in individuals with chronic obstructive pulmonary disease (COPD) independent of traditional osteoporosis risk factors. COPD-specific guidelines for BMD assessment do not exist and general guidelines for osteoporosis screening do not recognize COPD as a risk factor for fracture. Nonetheless, osteoporotic fractures are of major public health importance given the negative impact of fractures on morbidity and mortality in COPD patients. Furthermore, increased bone turnover independently contributes to fracture risk yet cannot be determined by cross-sectional dual x-ray absorptiometry (DXA) assessment alone. We established a longitudinal cohort of male and female smokers with and without smoking-related lung disease (i.e. emphysema or airflow obstruction) to determine factors related to accelerated BMD loss in smokers. We obtained baseline and 2-year measurements of pulmonary function, BMD, radiographic emphysema, and demographic and clinical characteristics. We assayed baseline blood samples for markers of bone metabolism, vitamin D, matrix metalloproteinases (MMP) and their inhibitors, interleukin-6, and tumor necrosis factor alpha. Of those with lung disease, 48.6% had evidence of osteopenia or osteoporosis at baseline compared to 29.4% without lung disease (p=0.01). The incidence of accelerated hip or lumbar spine BMD loss was 33.8% in subjects with lung disease versus 19.6% in those participants without

lung disease (p=0.01). Both type I collagen C telopeptide (CTx), a marker of bone resorption, and radiographic emphysema were independent predictors of accelerated BMD loss (OR 5.5 [2.1,14.4] for moderate/severe emphysema; OR 2.9 [1.3,6.4] for highest tertile CTx) when adjusted for age, gender, steroid use, and current smoking status. Tissue inhibitor of matrix metalloproteinase (TIMP) 4 was independently associated with BMD decline at the hip (p=0.04) and higher in subjects with emphysema progression (p=0.03). TIMP1 and MMP7 levels were also higher in subjects with emphysema progression. These findings suggest that accessible and easily measured biomarkers may be used to guide early and serial DXA BMD assessments in a subset of smokers at risk for accelerated BMD decline. TIMP4 and MMPs may further be involved in the regulation of lung and bone matrix degradation in individuals with emphysema-related BMD decline.

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1.0 INTRODUCTION

Osteoporotic fractures contribute to increased morbidity and mortality in patients with chronic obstructive pulmonary disease (COPD) (1-3). Whereas osteoporosis risk factors including decreased physical activity levels, increased steroid use, and frailty are common in severe obstructive lung disease, numerous studies have demonstrated an increased prevalence of low bone mineral density (BMD) even in those individuals with mild airflow obstruction (4-9). Criteria for BMD assessment in COPD patients remain undefined while general osteoporosis screening guidelines fail to recognize chronic lung disease as a major risk factor for fracture (10-12). As such, clinicians caring for patients with COPD may miss a substantial proportion of patients with significant bone loss who are at high risk for debilitating fractures. Subsets of patients, notably men, are at particular risk of missed diagnosis given low general screening rates in these populations (2).

Dual x-ray absorptiometry (DXA) assessment of BMD in all patients with COPD is not common clinical practice. BMD assessments limited to those patients with the most severe lung disease may exclude patients with significant bone loss whereas universal screening of all COPD patients is probably not cost-effective. Further, a single cross-sectional BMD assessment provides no information regarding the rate of BMD loss, a separate and important risk factor for osteoporotic fracture (13, 14). Thus, a need exists for an easily measureable biomarker associated with accelerated BMD decline that may be used to target screening in COPD patients at highest fracture risk. Early detection of

COPD patients with low BMD, or accelerated BMD loss, with targeted DXA BMD assessments will lead to earlier treatment strategies for fracture prevention.

Large cohort studies designed to study the influence of genetic variation or patterns of inflammation on disease expression have advanced the field of clinical phenotyping in COPD (15, 16). However, the study of BMD loss in these cohorts is challenged by the fact that, without systematic assessments of BMD, osteoporosis remains silent until fracture occurs, thus impacting the accuracy of a patient-reported osteoporosis diagnosis. We have established a cohort of current and former smokers with and without smoking-related lung disease, defined as either radiographic emphysema or airflow obstruction (9). We have followed this cohort for 2 years with serial assessments of clinical and demographic data, pulmonary function, radiographic emphysema, and BMD. We have also measured baseline circulating serum biomarker levels. This cohort provides the opportunity to study the natural history of BMD loss in smokers with and without lung disease and to examine factors associated with BMD loss. We hypothesize that accelerated BMD loss is prevalent in smokers with lung disease and that clinical, radiographic, and molecular biomarkers can be used to identify smokers at risk for rapid BMD decline.

2.0 METHODS

2.1 Subject Selection

The study population consisted of 240 male and female subjects participating in the COPD Specialized Center for Clinically Oriented Research (SCCOR) at the University

of Pittsburgh. Subjects were current or former smokers ages 40-79 with a minimum of 10 pack-years tobacco history at enrollment. One hundred thirty nine subjects had smoking-related lung disease, defined as either visual emphysema on CT scan or airflow obstruction [forced expiratory volume in 1 second to forced vital capacity ratio (FEV1/FVC) less than 0.70]. The remaining 101 participants had no evidence of visual emphysema or airflow obstruction. Exclusion criteria included chronic daily prednisone use, clinical or radiographic evidence of another significant pulmonary diagnosis (e.g. interstitial lung disease), history of lung cancer or a new, suspicious nodule on CT scan, obesity with a BMI > 34, active treatment with bisphosphonate therapy, calcitonin, denosumab, raloxifene, or parathyroid hormone replacement, or active treatment with hormone replacement therapy. Each subject completed a chest CT scan, pre and post-bronchodilator spirometry and plethysmography, measurement of lung diffusion capacity, demographic and medical history questionnaires, and DXA measurements of hip and lumbar spine BMD at baseline and 2 years. Physical activity levels were quantified using a validated, five-point activity questionnaire that addressed both on-the-job and leisure activity levels (17). Symptom scores were assessed with the Saint George's Respiratory Questionnaire (18). Blood specimens were collected at each study visit. The study protocol was approved by the University of Pittsburgh Institutional Review Board and written informed consent was obtained for each subject.

2.2 Dual X-Ray Absorptiometry

BMD at the hip and lumbar spine were determined using a Hologic 4500A Discovery bone densitometer. BMD is reported as an absolute value (gm/cm^2) and a T score, the

number of standard deviations from a young, gender and ethnic specific reference mean, using the NHANES III database reference population for hip BMD measurements (19) and the Hologic database reference population for spine BMD measurements. T scores were used to classify subjects as having normal BMD (T score $\geq$ -1.0), osteopenia (-2.5 < T score < -1.0), or osteoporosis (T score $\leq$ -2.5).

2.3 CT Scans

Noncontrast CT examinations were performed with a General Electric (GE) LightSpeed VCT (64-detector) scanner. A single chest radiologist, blinded to subject identities and other characteristics, interpreted the CT images using a 6-point semi-quantitative visual scoring system to define emphysema severity (0=none, 1=trace, 2=mild, 3=moderate, 4=severe, 5=very severe) which corresponded to 0%, <10%, 10-25%, 26-50%, 51-75%, and > 75% visual emphysema. Our group has validated this methodology in a previous publication (20). The percentage of low attenuation areas (LAA%) was defined as the fraction of voxels less than -950 HU as a percent of total voxels per identification of lung regions similar to the traditional density mask (21). Radiographic progression was defined as greater than a 0.5% annual increase in LAA.

2.4 Biomarker Measurements

Baseline blood samples were analyzed for the following biomarkers. Type I collagen c-telopeptide (CTx), a marker of bone resorption (14, 22), receptor activator of nuclear factor kappa beta ligand (RANKL) and osteoprotegerin (OPG) were measured by a commercially available electrochemiluminescence-based immunoassay using Roche

Elecsys2010 (Roche Diagnostics). Amino-terminal propeptide of type I procollagen (P1NP), a marker of bone formation (14, 22), was measured with a commercial radioimmunoassay kit from Immunodiagnostic Systems Inc. Matrix metalloproteinase (MMP) 1, MMP7, tissue inhibitor of matrix metalloproteinase (TIMP) 1, TIMP2, and TIMP4 were measured using Performance Assay MMP and TIMP multi-plex kits (R and D Systems) on a Luminex system. Interleukin (IL) 6 , TNF-alpha, and vitamin D were measured by enzyme-linked immunoabsorbant assay (ELISA) in duplicate. All assays and kits were used according to manufacturers' instructions. All samples were above the detection level for each measured biomarker.

2.5 Statistical Analysis

Annual percent change in total hip and lumbar spine BMD was calculated for each subject. A Monte Carlo simulation with 10,000 iterations was performed separately on both male and female subjects with no evidence of emphysema or airflow obstruction to obtain separate, smoothed estimates of the distribution of annual BMD percent change at the hip and lumbar spine among both male and female cohort participants without smoking-related lung disease. A prior population-based study (23) showed that every one standard deviation below the mean annual BMD percent change at the femoral neck (corresponding to approximately the lower 16% of the population under normal distribution assumptions) was associated with 3.9 excess hip fractures per 100 persons. Given the non-normal distribution of BMD change in our longitudinal cohort, we thus chose a conservative 10^{th} percentile cut-off to define accelerated BMD loss at the hip and lumbar spine. Definitions of accelerated BMD loss were then applied to both those

with and without smoking-related lung disease and cohort participants below the 10th percentile threshold at either the hip or lumbar spine were classified as having accelerated BMD loss. The entire tobacco-exposed cohort, regardless of lung disease status, was included in all analyses. The characteristics of subjects with and without accelerated BMD loss were compared using Student's t-test for continuous variables and chi-square analysis for categorical variables. Wilcoxon rank-sum test was used for non-normally distributed variables. Univariable and multivariable logistic regression analyses were performed to assess factors related to accelerated BMD loss. Biomarkers were included in the models as both continuous and categorical (tertile) variables. Biomarker tertiles and median levels were determined using the entire tobacco-exposed cohort. Biomarkers that were not normally distributed were log-transformed. Associations between biomarkers were assessed by Spearman correlation. Models were adjusted for age, gender, steroid use, and smoking status. The relationship between plasma biomarkers and absolute change in BMD at the total hip and lumbar spine were assessed with univariable and multivariable regression analyses adjusting for age, gender, smoking status, and steroid use. All statistical analyses were performed using SAS 9.2 and Stata 13.1.

3.0 RESULTS

3.1 Subject Characteristics

The mean age of the cohort was 66.7 years with an equal distribution of men and women. Although cohort participants had a significant degree of tobacco use and 40%

continued to smoke, lung disease in the cohort was mild with only approximately 29% of the cohort having moderate or severe airflow obstruction (**Table 1**).

Table 1: Subject Characteristics (N=240)

Age, mean (SD)	66.7 (5.6)
Gender male, n (%)	126 (52.5)
Pack Years, mean (SD)	48.7 (25.3)
Current Smokers, n (%)	97 (40.4)
BMI, mean (SD)	28.2 (4.4)
FEV1%, mean (SD)	85.7 (21.1)
GOLD, n (%)	
At risk	141 (58.7)
I (mild)	29 (12.1)
II (moderate)	59 (24.6)
III (severe)	11 (4.6)
Emphysema present, n (%)	115 (47.9)
Emphysema or Obstruction, n (%)	139 (57.9)
Bone Mineral Density	
Normal, n (%)	97 (40.4)
Osteopenia, n (%)	125 (52.1)
Osteoporosis, n (%)	18 (7.5)
ICS Use, n (%)	35 (14.6)
Oral Steroid Use, n (%)	15 (6.3)
Bisphosphonate Use, n (%)	0 (0)

BMI=body mass index; ICS=inhaled corticosteroids; oral steroids=temporary use within the past six months.

As a result, both inhaled and oral steroid use was low. However, radiographic emphysema was present in almost half of the cohort participants with 57.9% of the cohort having evidence of either radiographic emphysema or airflow obstruction.

3.2 Prevalence of Low Bone Mineral Density and Accelerated Bone Mineral Density Loss

The prevalence of low BMD was high with 60% of the entire cohort having low BMD on baseline DXA assessment (**Table 1**). Osteopenia and osteoporosis were significantly more common in cohort participants with smoking-related lung disease compared to unaffected smokers (p=0.01, **Figure 1**). Notably, 65% of cohort participants with lung

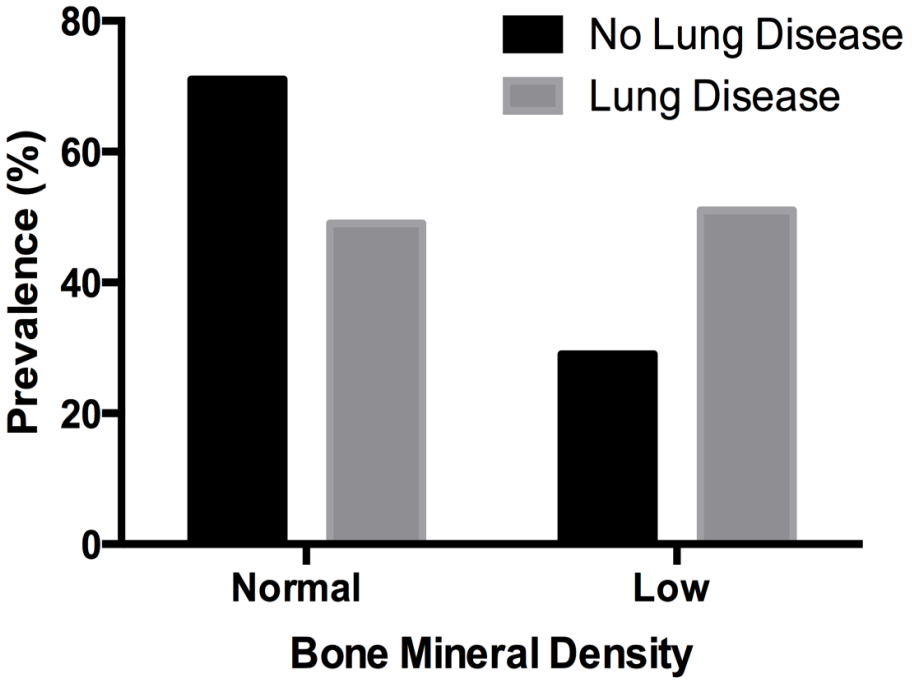
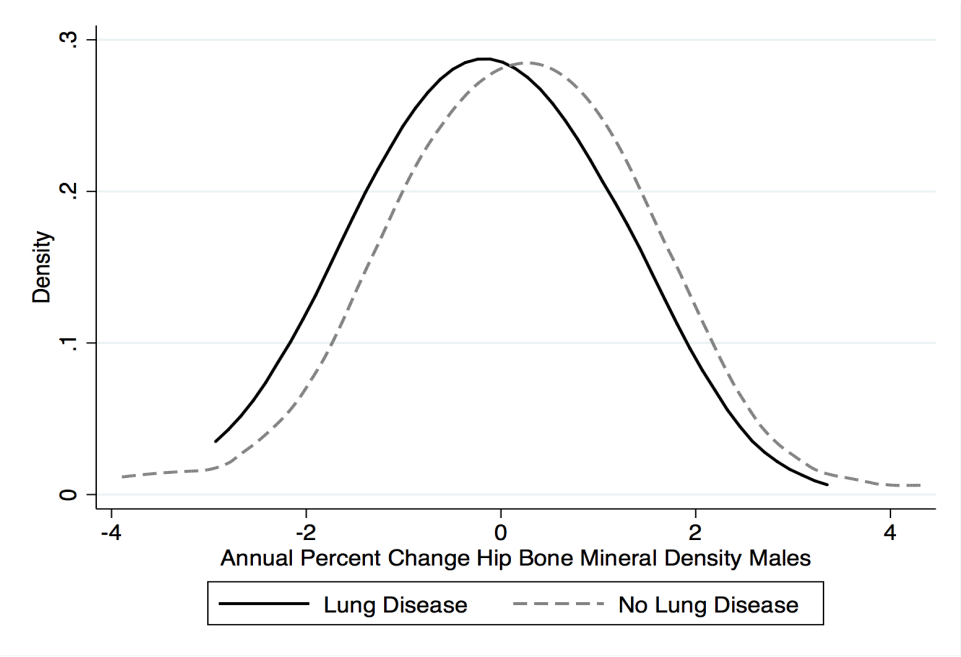


Figure 1: Prevalence of normal bone mineral density, osteopenia, and osteoporosis in those with and without smoking-related lung disease (radiographic emphysema and/or airflow obstruction). P=0.01

disease and osteopenia and 29% of those with lung disease and osteoporosis did not meet the established age criteria for osteoporosis screening (≥ 65 year of age in women, ≥70 years of age in men). Annual median percent loss in BMD at the hip was 0.2% and 0.5% for males and females with lung disease respectively. Males without

lung disease had a 0.2% annual median percent increase in BMD at the hip while females without lung disease still had 0.7% median percent loss in hip BMD (**Figure 2**).



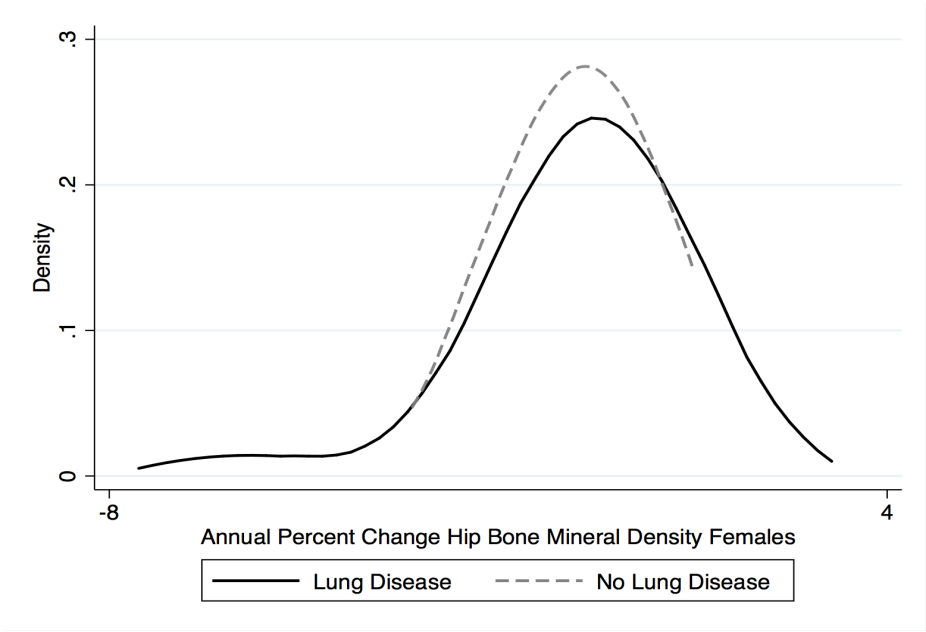


Figure 2: Distribution of annual percent change in hip bone mineral density for males and females with and without lung disease

Males and females with or without lung disease demonstrated slight increases in lumbar spine BMD over the 2-year study interval. Using the 10^{th} percentile cut-off of the Monte Carlo simulated distribution of annual percent BMD change for cohort participants without smoking-related lung disease, accelerated BMD loss was defined as an annual percent BMD loss of greater than 1.9% at the hip or 1.2% at the lumbar spine for women and greater than .74% at the hip or 0.24% at the lumbar spine for men. Of those with smoking-related lung disease, 47 (33.8%) had evidence of accelerated BMD loss at either the hip or lumbar spine over the 2-year study period. Again, the majority (68%) of those with lung disease and accelerated BMD loss did not meet age criteria for osteoporosis screening. In contrast, only 20 (19.6%) of unaffected smokers demonstrated rapid BMD decline at either the hip or lumbar spine (p=0.01).

3.3 Factors Associated with Accelerated Bone Mineral Density Decline

Age, gender distribution, BMI, steroid use, and current smoking status did not differ between those subjects with and without rapid BMD decline; although annual percent change in hip BMD did decrease with increasing age (p=0.013) and decreasing BMI (p=0.001). A greater proportion of subjects with accelerated BMD loss had evidence of either radiographic emphysema or airflow obstruction compared to those with stable BMD over time (70.1% vs. 52.6%, p=0.01, **Table 2**). This increased prevalence of rapid BMD loss appeared to be driven by the presence of emphysema rather than the severity of airflow obstruction. In fact, the odds of accelerated BMD loss were 2.1 times

Table 2: Characteristics of Subjects with and without Accelerated Bone Mineral Density Loss

	No Accelerated Loss (-) n=173	Accelerated Loss (+) n=67	P-Value
Age, mean (SD)	64.4 (0.4)	64.2 (0.8)	0.85
Gender, male, n (%)	87 (50.3)	39 (58.2)	0.27
BMI, mean (SD)	28.6 (0.3)	27.8 (0.5)	0.16
Pack Years, mean (SD)	47.3 (1.9)	50.3 (2.9)	0.41
Current Smoker, n (%)	74 (42.8)	28 (41.8)	0.89
Emphysema or Obstruction, n (%)	91 (52.6)	47 (70.1)	0.01
Emphysema Present, n (%)	74 (42.8)	41 (61.2)	0.01
LAA%, median (IQR)	0.5 (0.8)	0.8 (3.1)	0.08
FEV1 %, mean (SD)	86.5 (1.4)	81.7 (2.7)	0.18
ICS Use, n (%)	16 (9.2)	11 (16.4)	0.11
Oral Steroid Use, n (%)	10 (5.8)	5 (7.5)	0.62
Activity Score, median (IQR)	3 (1)	2.5 (2)	0.46
Symptom Score, median (IQR)	14.2 (19.5)	13.9 (25.7)	0.45
Vitamin D ng/ml, median (IQR)	33.7 (25.9)	32.1 (32.2)	0.52
CTx pg/ml, median (IQR)	248 (192)	322 (205)	0.01
P1NP pg/ml, median (IQR)	28.5 (14.2)	28.2 (18.8)	0.66
OPG pg/ml, median (IQR)	1092.6 (580.3)	1216.5 (631.7)	0.05
RANKL pg/ml, median (IQR)	94.2 (113.1)	98.2 (116.2)	0.98
TIMP1 pg/ml, median (IQR)	65490.3 (19518.4)	64189.7 (19383.8)	0.67
TIMP2 pg/ml, median (IQR)	63679.9 (10436.5)	64360.3 (10304.7)	0.84
TIMP4 pg/ml, median (IQR)	1193.6 (647.7)	1247.5 (753.6)	0.21
MMP1 pg/ml, median (IQR)	1789.1 (1693.0)	2093.6 (1941.8)	0.24
MMP7 pg/ml, median (IQR)	11278.15 (6736.2)	10683.5 (8813.4)	0.41
IL6 pg/ml, median (IQR)	1.22 (1.39)	1.38 (1.15)	0.73
TNF-alpha pg/ml, median (IQR)	1.89 (1.27)	1.94 (1.23)	0.62

BMI=body mass index; FEV1=forced expiratory volume in 1 second; LAA=low attenuation areas; ICS=inhaled corticosteroids; CTx=type I collagen C-telopeptide; P1NP=amino terminal propeptide of type I procollagen; OPG=osteoprotegerin; RANKL=receptor activator of nuclear factor kappa beta ligand; TIMP=tissue inhibitor of matrix metalloproteinases; MMP=matrix metalloproteinase; TNF=tumor necrosis factor

higher in subjects with any evidence of radiographic emphysema (p=0.01) and 5.2 times higher in subjects in whom emphysema was moderate or severe (p<0.001). These findings remained significant after adjustment for age, gender, current smoking status, and steroid use (**Table 3**). Whereas a trend for greater odds of accelerated BMD decline did exist in subjects with airflow obstruction (OR 1.7, p=0.06), this effect was lost after emphysema severity was included in the model.

Table 3: Predictors of Accelerated Bone Mineral Density Loss Multivariable Analysis

	Odds Ratio	P-Value
Emphysema Present	2.2 (1.2, 4.0)	0.009
Age	1.0 (0.9, 1.0)	0.8
Gender (female)	0.6 (0.4, 1.2)	0.1
Current Smoking	1.1 (0.6, 2.1)	0.7
Steroid Use	1.5 (0.3, 8.0)	0.6

CTx levels were higher among those cohort participants with accelerated BMD decline (322 [205 IQR] vs. 248 [192 IQR], p=0.01, **Table 2**) whereas vitamin D levels and other circulating biomarker levels did not differ between groups.

As shown previously (24), CTx levels were highly correlated with P1NP levels (r=0.61, p<0.0001). Participants with CTx levels above the median value had a 1.9 greater chance of having accelerated BMD loss (OR 1.9, p=0.03) and those with the highest tertile of CTx levels were 3 times more likely to have rapid BMD decline (OR 3.05, p=0.01). The addition of age, gender, current smoking status, and steroid use to the model did not impact the significance of these findings. A multivariable model including emphysema severity and CTx tertile showed that both the severity of radiographic

emphysema and circulating CTx levels are independent predictors of accelerated BMD loss (**Figure 3**) when adjusted for age, gender, steroid use, and smoking status. The

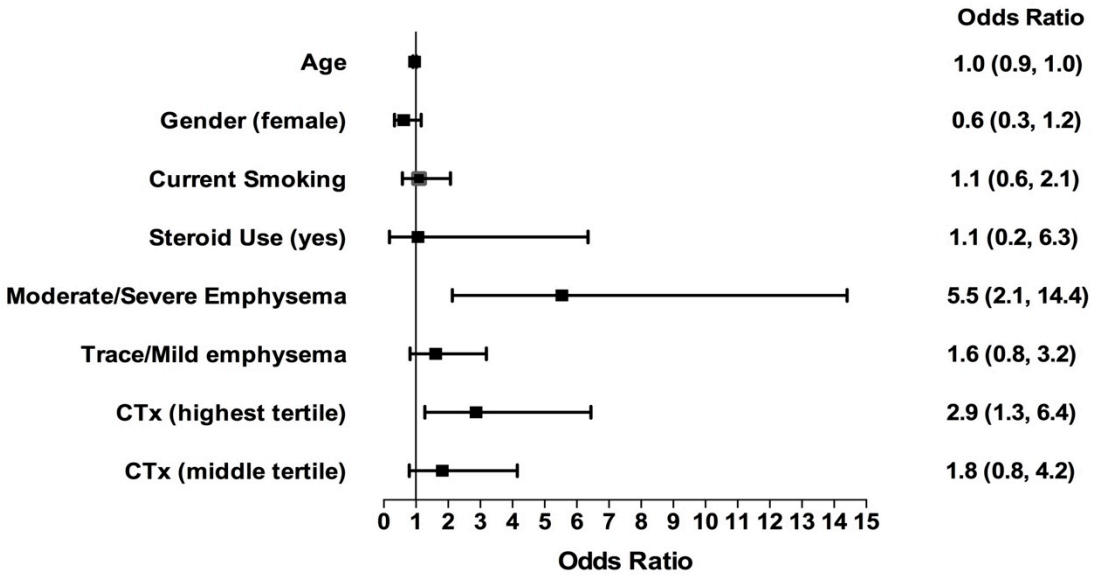


Figure 3: Forest Plot showing predictors of accelerated bone mineral density loss in multivariable logistic regression analysis.

presence of any radiographic emphysema or CTx values above the median level likewise predicted rapid BMD loss after accounting for other potential confounders (**Table 4**).

Table 4: Predictors of Accelerated Bone Mineral Density Loss Multivariable Analysis

	Odds Ratio	P-Value
Emphysema Present	2.2 (1.2, 4.1)	0.01
CTx (above median)	2.1 (1.1, 3.8)	0.02
Age	1.0 (0.9, 1.0)	0.3
Gender (female)	0.6 (0.3, 1.1)	0.08
Current Smoking	1.2 (0.6, 2.2)	0.6
Steroid Use	1.2 (0.2, 6.8)	0.8

3.4 Serum Biomarkers Associated with Bone Mineral Density Change

TIMP4 levels were associated with a decrease in annual percent change in hip BMD in univariable regression analysis ($p<0.001$). This relationship remained significant after adjustment for age, gender, BMI and current smoking status. In fact, multivariable modeling showed that TIMP4 levels, age, gender, and BMI were all independently associated with BMD loss at the hip ($R^2=17.8$, $p<0.001$, **Table 5**).

Table 5: Factors Associated with Annual Percent Change in Hip Bone Mineral Density

	Coefficient	P-Value
TIMP4 (log)	-0.49	0.04
Age	-.03	0.06
Gender	0.50	0.001
Body Mass Index	0.05	0.03

TIMP=Tissue inhibitor of matrix metalloproteinase

Baseline TIMP4 levels, as well as TIMP1 and MMP7 levels, were likewise higher in subjects with radiographic progression of emphysema (n=27, 11.3%, **Table 6**)

Table 6: Matrix Metalloproteinases and Their Inhibitor Levels in Subjects with and without Emphysema Progression

	(+) Progression	(-) Progression	P-Value
TIMP1 pg/ml	73626.2 (16740.9)	66470.4 (14556.0)	0.04
TIMP2 pg/ml	64403.1 (8337.1)	65048.9 (10634.7)	0.8
TIMP4 pg/ml	1570.3 (703.2)	1305.9 (549.0)	0.03
MMP1 pg/ml	3554.9 (3334.6)	2677.7 (3055.8)	0.09
MMP7 pg/ml	15090.1 (6885.8)	12387.8 (6559.0)	0.02

All values expressed as median (interquartile range)
MMP=matrix metalloproteinase
TIMP=tissue inhibitor of matrix metalloproteinase

compared to those with stable lung disease over the 2-year study interval. Annual percent change in spine BMD was small in the overall cohort and was not associated with circulating biomarker levels or other osteoporosis risk factors (data not shown).

4.0 DISCUSSION

4.1 Summary of Findings and Review of the Literature

We have shown that the prevalence of osteopenia and osteoporosis is increased in smokers with smoking-related lung disease (i.e. emphysema or airflow obstruction) when compared to smokers with similar demographic and clinical characteristics. The incidence of accelerated BMD loss is high in this group with the positive correlation between CTx, a marker of bone resorption, and P1NP, a marker of bone formation, suggesting that BMD decline in smokers is due to a high turnover state. The majority of subjects with osteopenia or osteoporosis were below the recommended screening age for osteoporosis (10, 11) and traditional risk factors, including age, gender, and steroid use, did not differentiate those subjects with accelerated BMD loss from those without. Two easily accessible biomarkers, radiographic emphysema and serum CTx levels, independently predicted accelerated decline in BMD over a short follow-up interval. Further, a circulating inhibitor of matrix metalloproteinases, TIMP4, was independently associated with BMD loss at the hip after adjustment for other factors commonly attributed to BMD decline.

Osteoporosis and osteoporotic fractures are a significant public health problem in individuals with smoking-related lung disease. Individuals with COPD who sustain hip

fractures have 60-70% higher rates of mortality compared to individuals with hip fractures who do not have evidence of lung disease (1). Further, in those patients with COPD, hip fracture increases 1-year mortality up to 3 to 5 times that of COPD patients without fracture (1). Osteoporotic vertebral fractures can lead to reductions in forced vital capacity and FEV1, further impairing lung function in individuals who already have low pulmonary reserve (3). Yet, osteoporosis screening rates remain low in persons with COPD, particularly in subgroups, such as men, where general osteoporosis screening guidelines have not reached a uniform consensus. For example, a study of the Veterans Affairs electronic database revealed a high fracture rate (3.99 and 1.31 per 1000 person years for hip and wrist fractures respectively) with only rare BMD assessments (4.4%) and osteoporosis treatment (2.8%) in male veterans with COPD (2). Findings from our study further highlight the complexities of osteoporosis screening in smoking-related lung disease with only 35.1% of those with low BMD and 32.0% with accelerated BMD loss meeting screening criteria for BMD assessment.

The finding that both radiographic emphysema and serum CTx levels independently predict rapid BMD decline coupled with literature linking emphysema with cross-sectional assessments of BMD (9, 25) offers a potential means of identifying patients with smoking-related lung disease who should undergo early and serial DXA BMD assessment. Studies have shown that the rate of bone loss may be as important as absolute bone mineral density when determining fracture risk (13, 14). Cross-sectional assessments of BMD, even if performed at an earlier age than guidelines currently advise, provide no information regarding rate of BMD decline. As evidence in support of

lung cancer CT screening (26) and experimental options for endoscopic lung volume reduction increase (27, 28), the frequency of routine chest CT scans in the management of patients with COPD will continue to rise. Meanwhile, commercial assays for CTx are readily accessible and available in most clinical laboratories. A strategy incorporating these two biomarkers to direct targeted osteoporosis screening will likely be more cost-effective than universal screening in all patients with COPD. The age at which screening should commence remains unclear, although our findings would suggest that initial BMD assessment before the age of 65 is warranted. The impact of age on prevalence of low BMD and BMD decline should be systematically studied in a COPD cohort with a more diverse distribution of age and disease severity to establish age criteria for osteoporosis screening in COPD patients.

The independent association between radiographic emphysema and low BMD that has been demonstrated in numerous studies (9, 25) suggests a common mechanism linking lung and bone matrix destruction. Our findings would suggest that MMPs and MMP inhibitors are important contributors to these synchronous processes. TIMP4, an inhibitor of MMPs, was the only circulating biomarker associated with change in BMD at the hip over the 2-year study interval. TIMP4, as well as TIMP1 and MMP7, was also higher in those subjects demonstrating radiographic emphysema progression over 2 years. MMPs play an important role in tissue remodeling in both emphysema and osteoporosis (29-32). TIMP4 levels may increase in response to lung or bone matrix destruction by MMPs and other pro-inflammatory cytokines. We did not find a significant association between serum MMP levels and BMD change. However, the follow-up

interval was short, a small number of MMPs were assayed, and only baseline MMP levels were assessed. Further investigation into the role MMPs and their inhibitors play in COPD-related BMD loss is warranted given their potential as both diagnostic and therapeutic biomarkers.

4.2 Strengths and Limitations

The strength of our cohort lies in the availability of longitudinal, gold standard assessments of BMD coupled to well-characterized clinical data in smokers with few competing osteoporosis risk factors. Most epidemiologic cohorts to date either provide systematic BMD or fracture assessments with little information regarding both lung function and radiographic emphysema (4, 5) or detailed pulmonary function and CT data without gold-standard assessments of BMD (33). No published studies to date have assessed longitudinal BMD loss in individuals with smoking-related lung disease. Despite the mild severity of underlying lung disease, a significant proportion of our cohort experienced substantial BMD loss in a relatively short time period. We would expect to see even greater BMD decline over several years, given that BMD loss likely does not follow a linear trajectory, particularly in individuals with COPD where systemic inflammation may wax and wane with lung disease activity (34-37).

The absence of longitudinal cohorts examining BMD loss specifically in smokers creates a challenge when defining accelerated BMD loss in our cohort. Population based studies of healthy individuals have used absolute thresholds (>0.4% BMD loss per year in post-menopausal females, no change in men) (38) or standard deviations from the

mean (one standard deviation below the mean associated with an excess of 3.9 hip fractures per 100 persons) (23) to define accelerated BMD loss or to determine clinically meaningful BMD change respectively. Thresholds defined by these studies may not be appropriate for our cohort, all of whom have had a significant smoking history. However, the availability of complete pulmonary function and radiographic data in our cohort participants provided us the opportunity to define a smoking control group that has a common risk factor (smoking) but has not developed lung disease. Because BMD data from our cohort was not normally distributed, we extrapolated the findings of Berry et al. (23) and chose the 10^{th} percentile, well below the 16^{th} percentile that would correspond to one standard deviation below the mean found significant in their study, below which accelerated BMD loss was defined. Thresholds to define accelerated BMD loss established by this methodology were greater than those used in a prior study to define accelerated BMD loss in a healthy population (38).

Finally, the paucity of longitudinal COPD cohorts with systematic measurements of BMD limits our ability to validate our results at present. However, validation of our findings in a separate, more clinically relevant cohort of COPD patients with more severe lung disease will be necessary, and is currently planned, to confirm our findings and identify any additional factors associated with bone loss in those with more extensive disease.

4.3 Public Health Significance

COPD is the third leading cause of death in the United States (39). The increased prevalence of osteopenia and osteoporosis in individuals with COPD has a significant impact on both morbidity and mortality (1-3), yet screening guidelines fail to recognize COPD as a major risk factor to warrant early BMD assessment and monitoring (10, 11). Universal osteoporosis screening of all COPD patients at an earlier age than currently recommended by general osteoporosis screening guidelines is not cost-effective. As such, research addressing which subset of COPD patients should undergo early, and perhaps serial, BMD assessments is of great public health importance.

5.0 CONCLUSION

We have confirmed that low BMD is prevalent in smokers with emphysema or airflow obstruction and are the first to show that significant proportions of individuals with smoking-related lung disease have accelerated BMD decline. Low osteoporosis screening rates combined with detrimental outcomes following fracture in patients with COPD has given rise to a significant public health problem for many current and former smokers. The incorporation of biomarkers associated with accelerated BMD decline into osteoporosis screening algorithms may facilitate selection of individuals for early and serial BMD monitoring and ultimately alter clinical practice for our chronic lung disease patients.

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