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## The association between iliocostal distance and the number of vertebral and non-vertebral fractures in women and men registered in the Canadian Database For Osteoporosis and Osteopenia (CANDOO)

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### Abstract

**Background:** The identification of new methods of evaluating patients with osteoporotic fracture should focus on their usefulness in clinical situations such that they are easily measured and applicable to all patients. Thus, the purpose of this study was to examine the association between iliocostal distance and vertebral and non-vertebral fractures in patients seen in a clinical setting.

**Methods:** Patient data were obtained from the Canadian Database of Osteoporosis and Osteopenia (CANDOO). A total of 549 patients including 508 women and 41 men participated in this cross-sectional study. There were 142 women and 18 men with prevalent vertebral fractures, and 185 women and 21 men with prevalent non-vertebral fractures.

**Results:** In women multivariable regression analysis showed that iliocostal distance was negatively associated with the number of vertebral fractures (-0.18, CI: -0.27, -0.09; adjusted for bone mineral density at the Ward's triangle, epilepsy, cerebrovascular disease, inflammatory bowel disease, etidronate use, and calcium supplement use) and for the number of non-vertebral fractures (-0.09, CI: -0.15, -0.03; adjusted for bone mineral density at the trochanter, cerebrovascular disease, inflammatory bowel disease, and etidronate use). However, in men, multivariable regression analysis did not demonstrate a significant association between iliocostal distance and the number of vertebral and non-vertebral fractures.

**Conclusions:** The examination of iliocostal distance may be a useful clinical tool for assessment of the possibility of vertebral fractures. The identification of high-risk patients is important to effectively use the growing number of available osteoporosis therapies.

## Background

Osteoporosis is one of the most prevalent chronic health conditions. This condition is characterized by low bone mineral density and microarchitectural deterioration of bone tissue leading to increased bone fragility and risk of fracture [1]. It is estimated that approximately 40% of white women and 13% of men 50 years and older will experience at least one clinically recognized hip, spine or distal forearm fragility fracture in their lifetime [2]. These fractures result in physical, psychological and emotional disabilities, and increased pain that can negatively influence quality of life [3,4].

Osteoporosis can be identified early during the course of the disease by diagnostic tests. Bone mineral density measurements provide the single best method for predicting fracture risk [5] but densitometers are occasionally not available and since the pathogenesis of fragility fractures is multifactorial, bone mass is not the only factor that determines risk.

A number of factors have been found to be associated with fragility fractures, they include advanced age, positive family history, height, existing fracture, propensity to falls, and postural instability [5-7]. Unfortunately, our understanding of risk factors is still inadequate and thus, there is a need for further research. The identification of new risk factors should focus on their usefulness in clinical situations such that they are easily measured, applicable to all patients, and contribute prognostic information that is independent of bone mineral density. The size of the gap between the costal margin and pelvic ridge (iliocostal distance) may be validated to be a surrogate measure for the presence of osteoporosis and/or vertebral fragility fractures and thereby may be a risk factor for future fracture. Hence, the purpose of this cross-sectional study was to examine the association between iliocostal distance and vertebral and non-vertebral fractures in women and men who were seen in a clinical setting.

## Methods

### Study design

Patient data were obtained from the Canadian Database of Osteoporosis and Osteopenia (CANDOO). CANDOO consists of approximately 10000 patients and involves 8 sites across Canada (Calgary, Saskatoon, Winnipeg, Hamilton, Toronto, Montreal [2 sites], and Quebec City). This database is a prospective registry designed to compile a comprehensive set of osteoporosis-related clinical information [8]. All patients referred to us and seen during the course of routine specialist care were enrolled in CANDOO. Patients data are aggregated using anonymous patient identifiers into a centrally maintained, fully keyed and encoded relational database. In particular, the CANDOO contains electronically stored information regard-

ing basic patient demographics, fracture history, gynecological history, past use of osteoporosis-related drug treatment, drug side effects, past use of corticosteroids and other medications, dietary calcium intake, smoking habits, type and quantity of physical activities, fall history, past medical history and family history including fractures, a self administered osteoporosis health related quality of life instrument, basic laboratory results, and bone density measurements. One database record, with over 400 data fields per patient, is generated for each patient at each clinical visit.

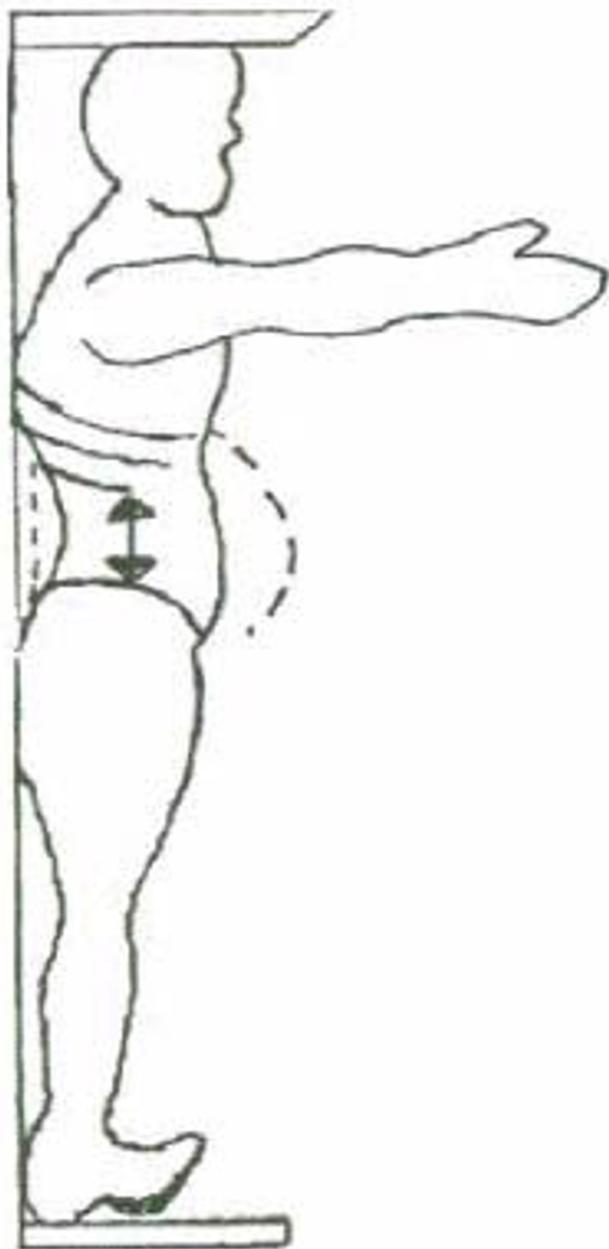
For the current analysis, the database was searched for women and men who had iliocostal distance measurements, and who were seen at the Saskatoon site. The Saskatoon location was chosen because it was the only CANDOO site that recorded iliocostal distance values.

### Iliocostal distance measurements and the number of prevalent fractures

Iliocostal distance was defined as the number of cm between the costal margin and the pelvic ridge of a patient, measured in the midaxillary line (figure 1). The measurement was determined by one investigator (WPO) using fingerbreadths (1 finger = 2 cm). All patients were standing during the measurement. Prevalent vertebral and non-vertebral fractures were determined using the CANDOO questionnaire ("Have you ever had any fractures?"). Vertebral fractures may or may not have been confirmed by x-ray. Non-vertebral fractures included the ankle, arm, clavicle, elbow, foot, heel, hand, hip, knee, leg, nose, pelvis, rib, shoulder, sacrum, and wrist. Multivariable linear regression analyses were conducted to determine the relationship between iliocostal distance and the number of vertebral and non-vertebral fractures.

### Potential confounding variables

Potential confounding variables collected from CANDOO included age; height; weight; menopausal status; age at menopause; lumbar spine, trochanter, femoral neck, and Ward's triangle bone mineral density (measurements were made by dual energy x-ray absorptiometry using Hologic or Lunar densitometers); prevalent vertebral fracture status (yes/no); and prevalent non-vertebral fracture status (yes/no); smoking status (never, previously, previously with interruptions, currently, currently with interruptions); family history of fracture (yes/no); number of alcoholic beverages consumed per week (including beer, wine and liquor); number of falls during the last 12 months; dietary calcium intake per day (measured as mg/d and estimated by a food frequency questionnaire); number of minutes spent exercising per week (such as walking, stair climbing, jogging, swimming, bicycling, dancing, skiing and others); current medication use (etidronate, alendronate fluoride, raloxifene, hormone re-



**Figure 1**  
**Measurement Technique for Iliocostal Distance.** Represents iliocostal distance. Iliocostal distance was defined as the number of cm between the costal margin and the pelvic ridge of a patient, measured in the midaxillary line.

placement or corticosteroids); calcium supplement status (yes/no); vitamin D supplement status (yes/no); and comorbid conditions (lung disease, liver disease, thyroid disease, cancer, visual problems that are not corrected by eyeglasses or contacts, osteoporosis, inflammatory bowel

disease, epilepsy, coronary disease, cerebrovascular disease, rheumatoid arthritis, diabetes, and kidney failure).

**Statistical analysis**

All multivariable regression analyses were conducted separately for women and men. We determined regression coefficient estimates as well as 95% confidence intervals (CI) of the estimates. All factors listed in the potential confounding variable section were assessed separately for their association with the number of fractures. Variables with a p-value < 0.2 were included in the multivariable analysis. Variables with a high degree of multicollinearity were removed. Model selection was determined using a stepwise procedure. If necessary, the iliocostal distance variable was force into the final model. All statistical analyses were performed on a Dell computer using the SAS/STAT (version 8.1; SAS Institute Inc., Cary, NC, USA) software package.

**Results**

A total of 549 patients including 508 women and 41 men participated in this cross-sectional study. Table 1 shows patients' characteristics at study entry for both women and men. A total of 142 women and 18 men had prevalent vertebral fractures, and 185 women and 21 men had prevalent non-vertebral fractures. The number of prevalent vertebral and non-vertebral fractures varied from 0 to 9 and 0 to 7 in women and 0 to 8 and 0 to 6 in men, respectively. Of the patients who sustained a vertebral fracture, 57 (40.1%), 34 (23.9%), 23 (16.2%) and 28 (19.7%) women, and 11 (61.1%), 3 (16.7%), 2 (11.1%), and 2 (11.1%) men had 1, 2, 3, or 4+ vertebral fractures. A total of 112 (60.5%), 48 (25.9%), 16 (8.6%), and 9 (4.9%) women and 10 (47.6%), 7(33.3%), 1 (4.8%), and 3 (14.3%) men had had 1, 2, 3, or 4+ non-vertebral fractures, respectively. The mean (SD) iliocostal distance for women with a vertebral, non-vertebral and no fracture was 3.49 (1.81) cm, 3.84 (1.69) cm and 4.53 (1.52) cm as compared with 4.22 (1.52) cm, 4.67 (1.43) cm, and 4.77 (1.54) cm for men. Iliocostal distance decreased as the number of vertebral fractures increase in both women and men (table 2). Tables 3 and 4 present univariate regression analysis results in women and men modelled for the number of vertebral and non-vertebral fractures.

**Iliocostal Distance**

*Women*

In women, univariate regression analysis revealed a statistically significant negative association between iliocostal distance and the number of vertebral and non-vertebral fractures (Table 3). After adjustments were made for confounding variables, multivariable regression analysis showed that iliocostal distance was negatively associated with the number of vertebral fractures (-0.18, CI: -0.27, -0.09; adjusted for bone mineral density at the Ward's tri-

**Table 1: Baseline characteristics of women and men\***

	Women n = 508	Men n = 41
Age-years (SD)	61.5 (11.3)	57.8 (12.4)
Height-cm (SD)	160.8 (9.6)	174.8 (18.3)
Weight-kg (SD)	67.8 (14.4)	86.2 (22.0)
Iliocostal distance-cm (SD)	4.2 (1.6)	4.6 (1.5)
Menopausal status-#post/#pre	424/78	NA
Age at menopause-years (SD)	47.3 (6.8)	NA
Lumbar spine BMD-g/cm <sup>2</sup> (SD)	0.867 (0.165)	0.918 (0.165)
Femoral neck BMD-g/cm <sup>2</sup> (SD)	0.664 (0.112)	0.713 (0.124)
Trochanter BMD-g/cm <sup>2</sup> (SD)	0.608 (0.107)	0.676 (0.123)
Ward's triangle BMD-g/cm <sup>2</sup> (SD)	0.483 (0.133)	0.471 (0.155)
Currently smoking-#	64	6
Family history of fracture-#	122	12
Alcohol-beverages/week (SD)	1.4 (3.0)	5.5 (8.7)
Number of falls – min to max	0–12	0–4
Calcium intake-mg/d (SD)	539.0 (435.9)	553.5 (375.4)
Exercise-minutes/week (SD)	166.7 (165.9)	187.9 (293.0)
Etidronate use-# (%)	50 (9.8)	3 (7.3)
Alendronate use-# (%)	41 (8.1)	7 (17.1)
Fluoride use-# (%)	2 (0.4)	0 (0.0)
Raloxifene use-# (%)	9 (1.8)	0 (0.0)
Hormone replacement use-# (%)	146 (28.7)	0 (0.0)
Corticosteroids use-# (%)	77 (15.2)	8 (19.5)
Calcium supplement use-# (%)	366 (72.0)	16 (39.0)
Vitamin D supplement use-# (%)	173 (34.1)	10 (24.4)
Lung disease <sup>a</sup> -# (%)	59 (11.6)	3 (7.3)
Liver disease <sup>b</sup> -# (%)	13 (2.6)	2 (4.9)
Thyroid disease <sup>c</sup> -# (%)	69 (13.6)	0 (0.0)
Cancer <sup>d</sup> -# (%)	14 (2.8)	0 (0.0)
Visual impairment-# (%)	51 (10.0)	2 (4.9)
Osteoporosis-# (%)	187 (36.8)	13 (31.7)
Inflammatory bowel disease-# (%)	15 (3.0)	5 (12.2)
Epilepsy-# (%)	7 (1.4)	2 (4.9)
Coronary disease-# (%)	12 (2.4)	1 (2.4)
Cerebrovascular disease-# (%)	19 (3.7)	1 (2.4)
Rheumatoid arthritis-# (%)	5 (1.0)	0 (0.0)
Diabetes-# (%)	18 (3.5)	0 (0.0)
Kidney failure-# (%)	24 (4.7)	0 (0.0)

\* BMD= bone mineral density; SD= standard deviation; # = number of patients; %=percent of patients; NA= not applicable. <sup>a</sup> Lung disease includes asthma, chronic bronchitis and other lung diseases. <sup>b</sup> Liver disease includes cirrhosis, hepatitis and cholangitis. <sup>c</sup> Thyroid disease includes hyper, hypo, nodule, and other. <sup>d</sup> Cancer includes breast, ovaries, cervix, uterus and colon.

**Table 2: Iliocostal distance and the number of vertebral and non-vertebral fractures in women and men**

	Women	Men
Vertebral Fractures		
0*	4.53 (1.52)	4.77 (1.54)
1	3.90 (1.73)	4.54 (1.57)
2	3.79 (1.75)	4.00 (2.00)
3	2.91 (1.41)	4.00 (0.00)
4+	3.75 (2.06)	3.00 (1.41)

\* Indicates patients without vertebral or non-vertebral fractures.

**Table 3: Univariate parameter coefficient estimates and P-values for the association between risk factors and the number of vertebral and non-vertebral fractures in women**

	No. Vertebral fractures		No. Non-Vertebral Fractures	
	Coefficient	P-value	Coefficient	P-value
Age	0.034	<0.001	0.018	<0.001
Height	-0.012	0.083	0.004	0.454
Weight	-0.001	0.768	0.002	0.534
Iliocostal distance	-0.271	<0.001	-0.115	<0.001
Menopausal status	0.516	0.003	0.305	0.015
Age at menopause	0.001	0.917	-0.006	0.405
Lumbar spine BMD	-0.813	0.048	-0.722	0.026
Femoral neck BMD	-3.069	<0.001	-1.901	<0.001
Trochanter BMD	-3.381	<0.001	-2.415	<0.001
Ward's triangle BMD	-2.928	<0.001	-1.951	<0.001
Prevalent vertebral fractures	NA		0.310	0.002
Prevalent non-vertebral fractures	0.391	0.003	NA	
Currently smoking	0.018	0.759	0.049	0.258
Family history of fracture	-0.158	0.305	0.121	0.272
Alcohol-beverages/week	-0.013	0.529	-0.012	0.435
No. of falls	-0.004	0.935	0.079	0.017
Calcium intake	-0.000	0.499	0.000	0.536
Exercise	-0.000	0.248	-0.000	0.895
Etidronate use	0.315	0.003	0.188	0.014
Alendronate use	0.250	0.009	0.062	0.366
Fluoride use	0.392	0.541	0.843	0.067
Raloxifene use	-0.053	0.807	0.167	0.280
Hormone replacement use	-0.069	0.551	-0.001	0.992
Corticosteroids use	0.113	0.308	-0.046	0.564
Calcium supplement use	0.302	0.006	0.107	0.171
Vitamin D supplement use	0.147	0.115	0.094	0.163
Lung disease <sup>a</sup>	0.223	0.264	0.334	0.020
Liver disease <sup>b</sup>	-0.068	0.866	-0.143	0.621
Thyroid disease <sup>c</sup>	-0.185	0.318	0.060	0.656
Cancer <sup>d</sup>	0.327	0.400	0.190	0.495
Visual impairment	0.136	0.526	0.174	0.258
Osteoporosis	-0.109	0.409	0.002	0.982
Inflammatory bowel disease	-0.634	0.091	0.618	0.022
Epilepsy	1.047	0.054	-0.175	0.656
Coronary disease	0.582	0.164	0.401	0.174
Cerebrovascular disease	1.042	0.002	1.016	<0.001
Rheumatoid arthritis	0.083	0.898	0.403	0.383
Diabetes	0.157	0.648	0.241	0.328
Kidney failure	0.115	0.700	0.200	0.352

\* BMD= bone mineral density, NA= not available. <sup>a</sup> Lung disease includes asthma, chronic bronchitis and other lung diseases. <sup>b</sup> Liver disease includes cirrhosis, hepatitis and cholangitis. <sup>c</sup> Thyroid disease includes hyper, hypo, nodule, and other. <sup>d</sup> Cancer includes breast, ovaries, cervix, uterus and colon.

angle, epilepsy, cerebrovascular disease, inflammatory bowel disease, etidronate use, and calcium supplement use) and for the number of non-vertebral fractures (-0.09, CI: -0.15, -0.03; adjusted for bone mineral density at the trochanter, cerebrovascular disease, inflammatory bowel disease, and etidronate use).

#### Men

In men, univariate regression analysis did not demonstrate a significant association between iliocostal distance and the number of vertebral or non-vertebral fractures (Table 4). The iliocostal distance variable remained non-significant following multivariable adjustments modelled for the number of vertebral fractures (-0.12, 95% CI: -0.41, 0.16; adjusted for age and the number of fall during

**Table 4: Univariate parameter coefficient estimates and P-values for the association between risk factors and the number of vertebral and non-vertebral fractures in men\***

	No. Vertebral fractures		No. Non-Vertebral Fractures	
	Coefficient	P-value	Coefficient	P-value
Age	0.035	0.072	-0.005	0.788
Height	-0.034	0.010	-0.017	0.164
Weight	0.007	0.523	0.015	0.155
Iliocostal distance	-0.259	0.095	-0.037	0.796
Lumbar spine BMD	-2.360	0.174	-0.399	0.779
Femoral neck BMD	-1.378	0.511	-0.106	0.953
Trochanter BMD	-1.728	0.410	-0.822	0.646
Ward's triangle BMD	-2.138	0.196	0.275	0.846
Prevalent vertebral fractures	NA		0.396	0.361
Prevalent non-vertebral fractures	-0.090	0.851	NA	
Currently smoking	0.036	0.876	0.161	0.443
Family history of fracture	-0.538	0.330	-0.647	0.184
Alcohol-beverages/week	-0.002	0.933	0.043	0.079
No. of falls	0.691	0.002	0.561	0.006
Calcium intake-mg/d	0.001	0.028	0.000	0.600
Exercise-min/week	-0.000	0.683	-0.001	0.484
Etidronate use	0.259	0.574	-0.180	0.665
Alendronate use	0.589	0.038	0.036	0.891
Fluoride use	NA		NA	
Raloxifene use	NA		NA	
Hormone replacement use	NA		NA	
Corticosteroids use	-0.479	0.286	0.000	1.000
Calcium supplement use	-0.014	0.972	-0.370	0.279
Vitamin D supplement use	0.010	0.977	0.000	1.000
Lung disease <sup>a</sup>	-0.921	0.315	0.000	1.000
Liver disease <sup>b</sup>	1.205	0.276	1.051	0.292
Thyroid disease <sup>c</sup>	NA		NA	
Cancer <sup>d</sup>	NA		NA	
Visual impairment	-0.257	0.810	1.635	0.082
Osteoporosis	1.003	0.046	0.113	0.809
Inflammatory bowel disease	-0.517	0.480	0.000	1.000
Epilepsy	-0.897	0.419	-1.051	0.292
Coronary disease	-0.875	0.574	0.000	1.000
Cerebrovascular disease	3.225	0.033	3.075	0.024
Rheumatoid arthritis	NA		NA	
Diabetes	NA		NA	
Kidney failure	NA		NA	

\* BMD= bone mineral density, NA= not available. <sup>a</sup> Lung disease includes asthma, chronic bronchitis and other lung diseases. <sup>b</sup> Liver disease includes cirrhosis, hepatitis and cholangitis. <sup>c</sup> Thyroid disease includes hyper, hypo, nodule, and other. <sup>d</sup> Cancer includes breast, ovaries, cervix, uterus and colon.

the past year) and the number of non-vertebral fractures (0.11, 95% CI: -0.18, 0.41; adjusted for number of falls during year, visual problems that are not corrected by eye-glasses or contacts, and height).

## Discussion

Osteoporosis is under-diagnosed, under-treated and a large number of individuals are unaware that they have this disease. With the emergence of effective treatments it is essential to detect those patients with a vertebral frac-

ture and those with at higher risk of fracture. At present, there is no universally accepted policy for identifying patients with osteoporosis. Clinical risk assessment is an important step in identifying individuals at high risk for osteoporosis and fractures. To our knowledge, the relationships between iliocostal distance and the number of prevalent vertebral and non-vertebral fractures have not been previously reported.

Our findings indicated that iliocostal distance is negatively associated with the number of vertebral and non-vertebral fractures in women, such that the shorter the distance the greater number of fractures. Iliocostal distance can be used to identify individuals with vertebral fractures and may be an excellent risk factor for future fractures and should be included in a patient risk profile. This measurement is easy to obtain and assess in a clinical setting, can be measured for all patients, and has a high predictive value for prevalent fracture independent of other known risk factors such as bone mineral density. From our clinical experience, healthy adults have an iliocostal distance of approximately 6 cm.

Vertebral fractures are a well-recognized consequence of postmenopausal bone loss and are the most common osteoporotic fractures [9]. It is estimated that less than one third of all vertebral fractures are clinically diagnosed [10]. A common explanation is that fractures are frequently asymptomatic and patients who suffer them are not prompted to seek medical attention. Furthermore, physicians may not be identifying prevalent fractures among their patients. The early identification of a vertebral fracture is essential. It has been shown that women who develop a vertebral fracture are at increased risk for an additional vertebral fracture [11] and that 20% of women will experience a subsequent fracture within the one year following the first vertebral fracture [12]. Moreover, there is growing evidence that all vertebral fractures are associated with adverse health consequences. Nevitt et al. [13] have found that back pain, functional limitation and disability days are associated with fractures. Among this large cohort of women 65 years of age and older, patients who sustained fractures were 2 to 3 fold more likely to experience more back pain and disability when performing back-dependent activities of daily living as compared with the unaffected comparison group. Likewise, fracture patients were at higher risk of experiencing limited activity days and days confined to bed. Kado et al. [14] observed that women who developed new fractures over a duration of 8 years had a 23% increased risk of mortality. This study also found a dose response effect such that mortality increased with the number of fractures. Accordingly, it is important that physicians recognize patients at risk for vertebral fracture or patients that have sustained fractures.

It is not surprising that iliocostal distance measurements in women were also associated with non-vertebral fractures. Vertebral fractures are early indicators of other osteoporotic fractures [15–17]. For instance, it has been shown that women who have a prevalent vertebral fracture have an increased relative risk (RR) of a subsequent fracture at the wrist (RR = 1.4), hip (RR = 2.3), and all non-spinal sites (RR = 1.8) as compared with unaffected women [11].

Our results showed that iliocostal distance values were associated with fractures in women but not in men. The apparent differences between women and men are difficult to explain; however, others have found gender differences in risk factors for increased bone loss in an elderly population [18]. Nonetheless, due to the low number of men (n = 41) recruited in this study (and the low statistical power) caution should be taken in the interpretation of the results. Further research will need to be conducted in men to confirm or dispute our findings.

Several features of the study are unique, and thus reinforce our conclusions. For example, all participants were "real world" patients who were seen for osteoporosis in a tertiary care setting and thus represent a homogeneous group. Other strengths included the large sample size of women, the careful delineation of potential confounding variables studied, and the wealth of data available about the study cohort. Nonetheless, our study is not without limitations. The study was cross-sectional in design and partially depended on information obtained by recall. Although adjustments were made for several variables, it remains possible that other, unknown determinants of fracture confound the observed associations. Due to the lack of data, no distinction was made between lumbar and thoracic fractures. Moreover, only one investigator assessed iliocostal distance using fingerbreadth as the measurement device, as such future validation of this useful clinical tool in terms of inter-rater reliability is recommended. Not all spinal fractures were confirmed by x-ray. X-rays were performed only in patients with back pain. Therefore, subclinical vertebral fractures may have developed. As a consequence, the actual association between iliocostal distance and the number of vertebral fractures may have been underestimated. The relationship between iliocostal distance and vertebral fractures should be tested in those patients with subclinical fractures.

## Conclusions

At present, only a small number of patients at high risk for fracture are currently recognized. Indeed, vertebral fractures often do not produce symptoms, so that many individuals with fractures will not seek medical attention for the problem. However, all vertebral fractures, whether symptomatic or radiographically identified, are associated with increased mortality and morbidity. The challenge for primary care physicians is to prevent, diagnose, and treat osteoporosis as early as possible. Thus, identification is the first step in osteoporosis management. The examination of iliocostal distance may be an excellent clinical opportunity to identify osteoporotic individuals for referral for diagnosis, preventive counseling and management. The identification of high-risk patients is important to effectively use the growing number of available osteoporosis therapies. Longitudinal studies will need to be

conducted to determine the association between changes in iliocostal distance measurements and fractures.

### Competing interests

None declared.

### Authors' Contributions

WPO participated in the design of the study, critical review of the manuscript; GI participated in the design of the study, drafted the manuscript and statistical analysis; RJS participated in the design of the study, critical review of the manuscript; DAH critical review of the manuscript; AP: participated in the design of the study, critical review of the manuscript; JPB: critical review of the manuscript; RGJ: critical review of the manuscript TMM: critical review of the manuscript; CHG: participated in the design of the study, critical review of the manuscript and statistical analysis; GFS: critical review of the manuscript; AP: critical review of the manuscript; JDA: participated in the design of the study, participated in the design of the study and critical review of the manuscript.

All authors gave final approval of the manuscript.

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