Journal of Internal Medicine 2002; **252**: 389–397

Search for occult secondary osteoporosis: impact of identified possible risk factors on bone mineral density

H. A. DEUTSCHMANN, M. WEGER, W. WEGER, P. KOTANKO, M. J. DEUTSCHMANN & F. SKRABAL
From the Department of Internal Medicine, Krankenhaus der Barmherzigen Brüder, Teaching Hospital of the Karl-Franzens University Graz, Austria

Abstract. Deutschmann HA, Weger M, Weger W, Kotanko P, Deutschmann MJ, Skrabal F (Krankenhaus der Barmherzigen Brüder, Marschallgasse, Teaching Hospital of the Karl-Franzens University Graz, Austria). Search for occult secondary osteoporosis: impact of identified possible risk factors on bone mineral density. *J Intern Med* 2002; **252:** 389–397.

Objectives. To determine whether the use of more elaborate diagnostic tests can identify possible risk factors for secondary osteoporosis and to evaluate the impact of these possible risk factors on the severity of bone disease in the study population.
Design. Cross-sectional study.
Setting and participants. We have investigated 377 subjects (285 females, 92 males) with osteoporosis (T-score less than −2.5 in dual energy X-ray absorption) or nontraumatic lumbar vertebral fractures; these patients were referred to our hospital, a secondary care centre, for evaluation and treatment of osteoporosis.
Results. Osteoporosis without attributable risk factor was diagnosed in 106 women (37%) and 30 men (33%). In 241 patients (179 women, 62 men) one or more possible risk factors for osteoporosis (in this paper also called subclinical disease) were revealed. The most common were lactose malabsorption, disturbed exocrine pancreatic function and renal tubular disturbances, including renal hypercalciuria, incomplete renal tubular acidosis and mild phosphate diabetes. The number of possible risk factors in the individual patient was significantly related to the severity of osteoporosis as assessed by Z-scores (Spearman correlation $r = -0.43$, $P < 0.001$, $n = 172$ for females; $r = -0.28$, $P < 0.05$, $n = 65$ for males).
Conclusions. All the identified subclinical diseases would have remained undetected if the currently accepted guidelines for the investigation of patients with osteoporosis were applied. The statistically significant correlation between the number of identified possible risk factors and the severity of bone disease in the individual patient strongly suggests the pathogenetic significance of the identified subclinical diseases. It is yet to be shown, whether specific treatment of these subclinical diseases yields additional improvement of bone mass as compared with standard treatment of osteoporosis.

Keywords: bone mineral density, clinical guidelines, lactose malabsorption, renal tubular acidosis, risk factors, secondary osteoporosis.

Introduction

The bone calcium content is the result of whole body calcium balance, which is determined by uptake in the gut and faecal and renal calcium excretion [1]. Over time, even a minor negative calcium balance leads to reduced bone mass [2]. Such a negative calcium balance is not detectable by the commonly applied diagnostic tests. It is therefore important when investigating patients with reduced bone mineral density, that every mechanism possibly contributing to a negative calcium balance is carefully considered. Special emphasis should also be placed on past diseases that may lead to permanently reduced bone mass. Considering the above, we have performed very careful case histories of the patients referred to our hospital for the investigation and treatment of osteoporosis, and supplemented the

case histories with an extended diagnostic work-up. The diagnostic protocol included tests which are not part of the currently recommended work-up of osteoporosis [3–6]. We report the results of the clinical work-up of 377 consecutive patients with osteoporosis.

Materials and methods

Study population

The study cohort comprised 285 women and 92 men with osteoporosis (WHO stages I to III) [7], who were investigated at our hospital between 1997 and 2000. All patients were referred from primary care physicians after the diagnosis of osteoporosis was made on the basis of vertebral fractures or reduced bone density on dual energy X-ray absorption (DXA) measurements. A radiographic vertebral fracture was defined as a decrease of 20% and at least 4 mm in the height of any vertebral body [8]. Baseline characteristics of the study population are given in Table 1. Excluded from the study were 441 osteopenic patients and 126 patients with apparent or suspected diseases known to be associated to osteoporosis (i.e. glucocorticoid therapy, chronic obstructive pulmonary disease, chronic inflammatory bowel disease, gastrectomy, chronic pancreatitis, known malabsorption syndrome, hyperparathyroidism, hyperthyroidism, Cushing's disease, chronic parenchymatous renal disease and chronic hepatic disease).

Diagnostic protocol

Bone density at the lumbar site (L2–L4) and/or the neck of the femur was assessed using DXA on a Sophos XRA machine (Sophos Medical, Saulnier France). The measurements were evaluated against the female and male reference population of Caucasian origin as supplied by the manufacturer. Osteoporosis was diagnosed according to WHO criteria in all patients, namely a reduced bone mineral density in DXA at the lumbar site with a T-score less than or

Table 1 Baseline characteristics of patients and findings

	Female *n* (%)	Male *n* (%)
Number	285 (75.6)	92 (24.4)
Premenopausal	28 (9.8)	
Postmenopausal	257 (90.2)	
Age (years; mean ± SD)	66 ± 13.8	54.3 ± 15.3
(years; median)	68	56
Range (years)	23–88	12–89
BMI (mean ± SD)	24.3 ± 4.5	24.5 ± 4.3
Number of patients with lumbar vertebral fractures	113 (39.7)	43 (46.7)
Smoking status		
Past	33 (11.5)	11 (11.8)
Current	54 (18.9)	24 (26.1)
Never	198 (69.6)	57 (62.1)
Pack-years	9.2 ± 15.8	14.3 ± 20.4
Identified possible risk factors		
Short reproductive period/hypogonadism	57 (20)	2 (2.2)
Lactose malabsorption	53 (18.6)	20 (21.7)
Past history of hyperthyroidism	38 (13.3)	3 (3.3)
Hypercalciuria	22 (7.7)	19 (20.7)
Borderline hyperparathyroidism	14 (4.9)	2 (2.2)
Renal tubular acidosis type I (RTA I)	12 (4.2)	15 (16.3)
Interstitial nephritis other than analgesic	8 (2.8)	1 (1.1)
Analgesic abuse nephropathy	7 (2.5)	3 (3.3)
Exocrine pancreatic insufficiency	6 (2.1)	11 (12)
Other malabsorption syndrome	3 (1.1)	2 (2.2)
Mild phosphate diabetes	2 (0.7)	3 (3.3)
Myeloma/lymphoma	1 (0.4)	5 (5.4)
Osteogenesis imperfecta	1 (0.4)	4 (4.3)
Vitamin D_3 deficiency	1 (0.4)	1 (1.1)

BMI, body mass index [body weight (kg) body height $(m)^{-2}$].

equal to −2.5 SD or radiological evidence of spontaneous vertebral fractures, respectively [4]. One of these conditions had to be present. Bone mineral density was not measured in patients with radiologically proven nontraumatic lumbar vertebral fractures or severe degenerative changes of the lumbar vertebral spine, because these measurements are distorted by these two conditions.

Routine investigation. Personal history was obtained by an experienced clinician, placing emphasis on the consumption of analgesic drugs, any gonadal disturbances such as late menarche and a short period of reproduction, past history of thyroid disease, past history of gastrointestinal disease and a history of urinary tract diseases such as renal stones, cystitis or kidney disease during pregnancy. It was not attempted to assess the dietary intake of dairy products quantitatively. All patients consumed average amounts of milk products such as cheese and yoghurt and there were no strict vegetarians amongst the patients studied. None of the patients reported longer periods of immobilization. Basic laboratory tests included serum creatinine, serum electrophoresis, immunoelectrophoresis, free tri- and tetraiodothyroxine (fT3, fT4), thyroid stimulating hormone (TSH), serum aminotransferases (ASAT and ALAT), alkaline phosphatase, intact parathyroid hormone (iPTH), 24-h urinary excretion of calcium, urinalysis, quantitative urinary sediment and Bence Jones paraproteins. The laboratory methods used and normal ranges are given in the appendix. In this paper, an abnormal result of the diagnostic work-up is either referred to as 'possible risk factor for osteoporosis' or 'subclinical disease'.

Additional diagnostic procedures and definition of abnormal results. (i) Analgesic nephropathy was diagnosed when two of the three major diagnostic criteria were met: (a) history of regular consumption of analgesics of the phenacetin type totalling >1–2 kg during lifetime; (b) small and bumpy kidneys by ultrasound; (c) papillary necrosis [9]. If interstitial nephritis was suspected on other grounds, additional tests were performed based on the clinical picture. (ii) Chronic pyelonephritis was identified by personal history, a quantitative sediment and ultrasound studies of the kidneys. (iii) Definition of distal renal tubular acidosis (RTA I): urinary pH was measured in five random urine samples. If urine pH was consistently above 5.5, renal acidification was further studied by the ammonium chloride loading test [10]. (iv) Phosphate diabetes: repeated measurements (at least twice) of serum phosphate and index of phosphate excretion (IPE) were performed to identify even mild phosphate diabetes in patients with normal serum phosphate levels [11]. Mild phosphate diabetes was diagnosed by at least one measurement of serum phosphate below 2.5 mg dL^{-1} and/or of an IPE > 0.45 [11].

$$\mathrm{IPE} = \frac{[\text{urinaryphospate}] * [\text{serum creatinine}]}{[\text{urinary creatinine}]} \quad \frac{-[\text{serum phosphate} - 2.5]}{2}$$

all concentrations in mg/dL.

(v) Hypercalciuria: a urinary Ca^{2+} excretion of more than 4 mg Ca^{2+} (kg body weight)$^{-1}$ day^{-1} was the criterion for hypercalciuria after the patients had been at least 1 week off any calcium supplements [1]. (vi) Hyperparathyroidism: borderline hyperparathyroidism was diagnosed with respect to the iPTH/calcium function curve, if both iPTH (normal range 10–65 pg mL^{-1}) and serum calcium (normal range 2–2.6 mmol L^{-1}) were in the upper quintile of the normal range [12]. (vii) Vitamin D deficiency: calcidiol level <23.0 mmol L^{-1} was the criterion for vitamin D deficiency status. (viii) A short period of reproduction was defined either by a late menarche and/or early menopause resulting in a reproductive period shorter than 30 years or by the occurrence of premature menopause or hysterectomy with or without ovarectomy before the age of 45 years [13]. Serum levels of oestradiol, luteinizing hormone (LH; in women) and serum testosterone and prolactin (in men) were measured when a history of premature menopause in women and disturbed libido or impotence in men was revealed. (a) Lactose malabsorption: testing for lactose malabsorption was performed by measurement of blood glucose and H_2 in breath after the ingestion of 50 g lactose. A rise of blood glucose <20 mg dL^{-1} and/or a rise of H_2 exhalation of >20 ppm was considered to be diagnostic of lactose malabsorption [14]. Both tests were used, as lactose malabsorption may be present even if exhaled breath hydrogen does not increase, because approximately 5% of the Austrian population are lacking lactose producing bacteria in

the colon [15]. (ix) Exocrine pancreatic function was tested by determining elastase in stool. A value <200 μg g^{-1} of formed stool was taken as a marker of impaired exocrine pancreatic function, even if no history of steatorrhoea was present [16]. (x) Other malabsorption syndromes were diagnosed by pathological values for blood iron, prothrombin time and serum albumin, by a pathological xylose test and, if serum vitamin B_{12} levels were abnormally low (<187 pg mL^{-1}), by vitamin B_{12} absorption test (Schilling test). Serum vitamin B_{12} levels were measured only if either mean corpuscular volume (MCV) was above 97.0 fl, or when clinical signs of peripheral neuropathy (pathological light touch, pin prick, cold/warm reception, vibration sense) were present. Antiendomysium antibodies were determined if celiac disease was suspected (i.e. reduced serum iron or prolonged prothrombin time). Bile salt malabsorption was diagnosed by a history of diarrhoea, unexplained by other conditions and a successful therapeutic trial with cholestyramine. (xi) Monoclonal gammopathy of uncertain significance (MGUS) was diagnosed using established criteria [17]. (xii) In cases of particularly severe osteoporosis (i.e. spontaneous vertebral fractures) in patients aged <40 years, with otherwise unexplained severe bone disease, skin fibroblast cultures were established. Altered collagen synthesis is one of the diagnostic features of osteogenesis imperfecta [18]. In none of these patients was osteogenesis imperfecta clinically evident (i.e. no blue sclerae, no marfanoid habitus). The test was abnormal if type I collagen synthesis was reduced in relation to synthesis of type II collagen.

Statistical analysis

Descriptive statistics were used to calculate frequencies and percentage of identified subclinical diseases. Continuous data are given as mean ± SD. To assess the impact of possible risk factors on bone density, the Spearman correlation coefficient for Z-score versus number of identified possible risk factors was computed. A one-way ANOVA was used to test for intergroup differences of age and smoking history (pack-years). Bonferroni's correction was applied to multiple comparisons. A two-tailed *P*-value of <0.05 was considered to be significant. Statistical analysis was performed with the SPSS statistical package (SPSS version 9.0, 1998, Chicago, IL, USA).

Results

Clinical characteristics of the study population and identified possible risk factors are given in Table 1. A total of 377 patients (285 women, 92 men) were studied during a period of 3 years. Despite the extensive diagnostic procedures applied by us, no risk factor or subclinical disease possibly causing osteoporosis was detected in 106 females (37% of female patients) and 30 males (33% of the male patients). A total of 148 patients (39%) had one suspected risk factor for osteoporosis, 71 patients (19%) had two possible risk factors and 26 (7%) had three disorders possibly related to osteoporosis (Table 2). Distribution of age according to the number of identified possible risk factors is given in Table 3. A significant inverse correlation between individual Z-scores and the number of possible risk

Table 2 Distribution of identified possible risk factors for osteoporosis

Number of possible risk factors	Female ($n = 285$)	Male ($n = 92$)
No risk factor	106 (37.2)	30 (32.6)
Premenopausal	9 (8.5)	
Postmenopausal	97 (91.5)	
One risk factor	110 (38.6)	38 (41.3)
Two risk factors	54 (18.9)	17 (18.5)
Three risk factors	15 (5.3)	7 (7.6)

Numbers are given as *n* (%).

Table 3 Age distribution of the patients presented in Figs 1 and 2 (patients without lumbar vertebral fractures in whom Z-scores of the lumbar spine could be measured)

	Number of identified risk factors			
	No	One	Two	Three
Female				
n	63	68	30	11
Mean age	67a	61.3a	58.9a[a]	63a
Median age	68a	64.5a	58.5a	60a
Range	23–85a	23–86a	30–80a	46–83a
Male				
n	16	29	12	8
Mean age	60.3a	50.2a	53.7a	54.5a
Median age	61.5a	54.3a	53.5a	53.6a
Range	31–75a	28–71a	31–76a	46–69a

[a]There was no statistically significant difference in mean age between the groups with the exception of female patients with and without two possible risk factors for osteoporosis (67 vs. 58.9 years, $P < 0.05$).

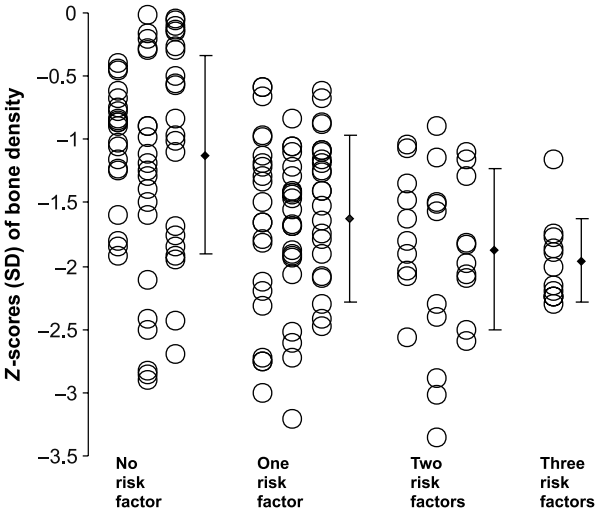

Fig. 1 Individual values of Z-scores, mean ± SD of 172 female patients without and of patients with one, two and three possible risk factors for osteoporosis. There was a significant inverse correlation between the number of identified possible risk factors and the severity of bone disease as assessed by Z-scores (Spearman $r = -0.43$, $P < 0.001$). Z-scores were not measured in patients with lumbar vertebral fractures. These patients had to be excluded from quantitative data analysis. Therefore, the number of patients presented in this graph is lower than the number of patients presented in Tables 1 and 2.

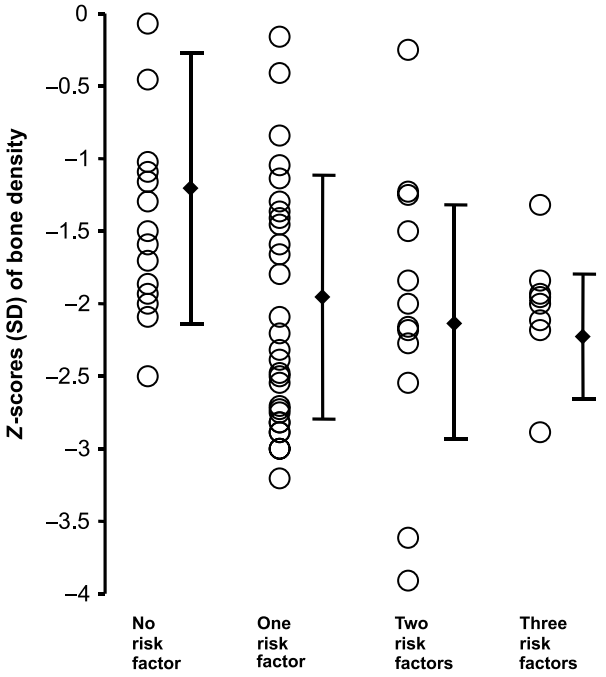

Fig. 2 Individual values of Z-scores, mean ± SD of 65 male patients without and of patients with one, two and three possible risk factors for osteoporosis. There was a significant inverse correlation between the number of identified possible risk factors and the severity of bone disease as assessed by Z-scores (Spearman $r = -0.28$, $P < 0.05$). Z-scores were not measured in patients with lumbar vertebral fractures. These patients had to be excluded from quantitative data analysis. Therefore, the number of patients presented in this graph is lower than the number of patients presented in Tables 1 and 2.

factors was found (Figs 1 and 2). Correlation coefficients (Z-scores versus number of possible risk factors) were $r = -0.43$, $P < 0.001$ for women ($n = 172$) and $r = -0.28$, $P < 0.05$ for men ($n = 65$). Lowest bone mineral density was found in patients with the highest number of possible risk factors for osteoporosis (Figs 1 and 2). There was no statistically significant difference in mean age between the groups with the exception of female patients with and without two possible risk factors for osteoporosis (67 vs. 58.9 years, $P < 0.05$; Table 3). No significant difference concerning smoking history (pack-years) was observed between the groups.

The most common disorder possibly associated to osteoporosis in this selected group of patients was lactose malabsorption, present in 18.6% of female patients and 21.7% of male patients (Table 1). Furthermore, we found a high prevalence of incomplete RTA I, namely in 12 of 285 females (4.2%) and in 15 of 92 males (16.3%). The diagnosis of incomplete RTA I was based on the finding that all these patients had a pathological NH_4Cl loading test without the presence of overt metabolic acidosis at baseline. In 10 patients an underlying disease for incomplete RTA I was found: chronic analgesic abuse ($n = 2$), type I diabetes ($n = 1$), history of autoimmune thyroiditis ($n = 3$), MGUS ($n = 2$) and hypercalciuria ($n = 2$). We also observed a high prevalence of hypercalciuria without RTA I [21 of 285 female patients (7.4%) and 18 of 92 male patients (19.6%)]. Beside lactose malabsorption (18.6% of females and 21.7% of males) and impaired exocrine pancreatic function (2.1% of females and 12% of males), evidence of other causes of impaired intestinal calcium absorption was found in three female patients (1.1%) and two male patients (2.2%), namely celiac disease ($n = 3$) and diarrhoea responding to cholestyramine ($n = 2$). Furthermore, one female (0.4%) and four male (4.3%) patients had a defective type I collagen synthesis in fibroblast culture.

Discussion

The importance of a thorough diagnostic work-up in patients with reduced bone mineral density is evident from the long list of disorders which may cause osteoporosis [19], and from the assumption that a causal approach to therapy may yield better improvements of bone mass than conventional standard therapy. Currently, the numerous guidelines for the work-up of osteoporosis differ with respect to the diagnostic protocol [3–6]. This leads to an uncertainty on appropriate 'routine tests'. As per definition, the diagnosis of 'primary osteoporosis' is made by exclusion but the question, which diseases or mild disorders should be excluded before 'primary osteoporosis' is diagnosed, is a matter of debate [20]. Supported by the findings of the present study, we propose that the diagnostic protocol for the detection of secondary causes of osteoporosis should be extended.

The role of lactose malabsorption in the pathogenesis of osteoporosis is controversial [21, 22], and this disorder is listed as a cause of osteoporosis in some [23], but not in all textbooks of internal medicine [19] or endocrinology [24]. Results, not only of the present but also of a previous investigation [25], reveal a substantial prevalence of lactose malabsorption in patients with otherwise no risk factors for osteoporosis. This suggests a causal relationship between the two diseases. The prevalence of lactose malabsorption in Caucasians in western Europe is between 10% and 15% [26]. A population-based case–control study would help to detect the impact of lactose malabsorption on the development of osteoporosis. As many as 30% of patients with lactose malabsorption do not experience symptoms related to lactose ingestion [27]. Therefore, the H_2-breathing test cannot be substituted by taking a personal history of milk intolerance. Furthermore, the fact that patients may be lacking H_2-producing bacteria in the large bowel, necessitates additional analysis of the rise of blood glucose after lactose ingestion [15].

In the patients in whom reduced excretory pancreatic function was diagnosed ($n = 17$), no symptoms of steatorrhoea were observed. This finding confirms, that a large proportion of the excretory pancreas must be destroyed before signs and symptoms of excretory pancreatic insufficiency occur [28]. As measurement of elastase in stool is presently not included in the routine work-up of patients with osteoporosis, a reduced excretory pancreatic function will remain unnoticed in a considerable proportion of these patients (14% of the patients in this study). Although some sort of malabsorption was found frequently in our patients, the prevalence of vitamin D deficiency was very low. This was probably the result of the fact that most patients had already received vitamin D supplements by primary care physicians before referral.

Furthermore, 22 of 285 female patients (7.7%), and 19 of 92 male patients (20.7%) exhibited idiopathic hypercalciuria without urolithiasis. Most laboratories define the normal range of calcium excretion between 2.5 and 10 mmol day^{-1} without normalization to body weight [29]. Using this definition many cases of hypercalciuria may remain undetected. It is noteworthy, that in 23 patients (6%) evidence of both a disturbed intestinal calcium absorption (lactose malabsorption and/or chronic excretory pancreatic insufficiency) and a disturbed renal electrolyte handling (hypercalciuria, incomplete RTA I, mild phosphate diabetes) was found. It is conceivable that slightly impaired intestinal calcium absorption in combination with a small extrarenal loss of calcium may lead to particularly severe and early osteoporosis.

We found five patients (two female, three male) with mild phosphate diabetes who had serum phosphate values in the low normal range but the IPE was clearly abnormal. By a sole measurement of serum phosphate this condition would have been missed.

In addition, in 27 patients (7.2% of all patients) incomplete RTA was diagnosed. In 10 of them (37%) a possible cause for the renal acidification defect was identified. We have described the relationship between incomplete RTA I and osteoporosis recently [30]. This finding was confirmed in a subsequent case–control study, demonstrating a normal renal acidification in all 20 control subjects and an impaired renal distal acidification in 10 of 46 (22%) patients with osteopenia or osteoporosis [31]. Defective renal acidification may lead to bone loss through direct physico-chemical effects of even mild acidosis on bone [32]. Furthermore, even incomplete RTA I leads to an osteoblast-mediated activation of osteoclasts [32]. A negative renal Ca^{2+} balance is also likely to occur in RTA I, because Ca^{2+} reabsorption in the cortical collecting tubule is reduced [33].

We are aware of a number of potential limitations of the present study. First, we consider it possible but unlikely that referral bias may have caused the high prevalence of subclinical diseases found, as the referring physician's only criterion for admission was a low bone density and/or the occurrence of spontaneous lumbar compression fractures. Moreover, 441 patients were excluded from the study, because only osteopenia and not osteoporosis (as defined by WHO criteria) was present [4]. Therefore, not only patients with particularly severe osteoporosis were referred. Secondly, at our unit we were unable to recruit a control group with normal bone density who would agree to perform all the necessary tests. Consequently, it is impossible to compare the frequencies of subclinical diseases or possible risk factors associated with osteoporosis with those found in the healthy population. It would be most interesting to conduct a case–control study to investigate the prevalence of the identified possible risk factors amongst subjects with normal bone density. This would especially be important because we were unable to find prevalence studies for these possible risk factors in the Austrian or Middle European population. However, normal values for each performed test originate from 95% confidence limits in a representative number of healthy subjects. Our findings demonstrate a significant quantitative correlation between the number of detected coexistent subclinical diseases and low bone mass in the individual patient ($P < 0.001$ for females, $P < 0.05$ for males). This correlation is suggestive, but does not necessarily prove a causal relationship.

Keeping these limitations in mind, the results of the present investigation pose the following important questions: is it necessary to extend the currently applied diagnostic work-up [3–6] in patients with osteoporosis? This would only be justified, if the management of the patient is improved by the additional diagnosis. To name a few examples: it has yet to be shown whether the administration of pancreatic enzymes in patients with reduced elastase in stool, but without clinically evident steatorrhoea will improve bone mass and whether the addition of alkali salts to conventional treatment in patients with osteoporosis and incomplete RTA I will further increase bone density. The administration of phosphate supplements may be beneficial in patients with mild phosphate diabetes even in the presence of normal serum phosphate and hydrochlorothiacide therapy may be beneficial in patients with hypercalciuria. The avoidance of lactose containing food may or may not improve the absorption of supplemental calcium in patients with lactose malabsorption [34, 35].

If there were agreement with one of the above suggestions, it should be considered to include the following tests in the diagnostic work-up of asymptomatic patients with osteoporosis: (i) lactose tolerance test with H_2-breathing test; (ii) measurement of elastase in stool; (iii) 24-h urinary calcium interpretation on the basis of body weight; (iv) repeated serum phosphate measurements and calculation of indices of phosphate excretion and (v) repeated urinary pH measurements.

In summary, even after the exclusion of all well established causes of osteoporosis, we found a high number of subclinical diseases and biochemically identified risk factors possibly contributing to the reduction of bone mineral density. As the severity of the bone disease was significantly related to the number of possible risk factors in the individual patient a causal relationship appears likely. Prospective controlled studies are necessary to derive estimates of the true prevalences of possible risk factors in patients with and without osteoporosis. The effectiveness of therapies aimed to treat these specific possible risk factors and subclinical diseases also remains to be shown.

Acknowledgements

This work was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung, FWF Austria, SFB 007 'Biomembranes'.

We would like to thank Ing. Preininger and his staff of the central laboratory of the hospital for their help with the biochemical analyses and University Prof. Dr J. Spranger, Prof. Dr Zabel and Dr Melchior from the Kinderklinik und Kinderpolyklinik Johannes Gutenberg-Universität Mainz, Germany, for performing fibroblast culture and measurement of type I collagen production.

References

1 Broadus AE. Mineral metabolism. In: Baxter JD, Broadus AE, Frohman LA, eds. *Endocrinology and Metabolism*. New York: McGraw-Hill, 1981; 1002–10.

2 Nordin BE. Calcium and osteoporosis. *Nutrition* 1997; **13**: 664–86.
3 Kanis JA, Delmas P, Burckhardt P, Cooper C, Torgerson D. Guidelines for diagnosis and management of osteoporosis. *Osteoporosis Int* 1997; **7**: 390–406.
4 Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of WHO study group. WHO Technical Report Series 843. Geneva: WHO, 1994.
5 National Osteoporosis Foundation. Physicians's guide to prevention and treatment of osteoporosis. *Excerpta Medica* 1998.
6 Royal College of Physicians. *Osteoporosis: Clinical Guidelines for Prevention and Treatment.* London: Royal College of Physicians, 1999.
7 Kanis JA, Melton LJ III, Christiansen C, Johnston CC, Khaltaev N. The diagnosis of osteoporosis. *J Bone Miner Res* 1994; **9**: 1137–41.
8 Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. *J Bone Miner Res* 1993; **8**: 1137–48.
9 Buckalew VM Jr. In: Greenberg A, Jennette JC, Cheung AK, (eds), Analgesic abuse nephropathy. *Primer on Kidney Diseases*, 1st ed. London: Academic Press 1994: 192–6.
10 Wrong O, Davies HEF. The excretion of acid in renal disease. *Q J Med* 1959; **28**: 259–313.
11 Bijvoet OLM. Kidney function in calcium and phosphate metabolism. In: Avioli LV, Krane SM, eds. *Metabolic Bone Disease*, Vol. 1. New York: Academic Press,1977.
12 Glendenning P, Gutteridge DH, Retallack RW, Stuckey BG, Kermode DG, Kent GN. High prevalence of normal total calcium and intact PTH in 60 patients with proven primary hyperparathyroidism: a challenge to current diagnostic criteria. *Aust N Z J Medical* 1998; **28**: 173–8.
13 Osei-Hyiaman D, Satoshi T, Ueji M, Hideto T, Kano K. Timing of menopause, reproductive years, and bone mineral density: a cross-sectional study of postmenopausal Japanese women. *Am J Epidemiol* 1998; **148**: 1055–61.
14 Newcomer AD, McGill DB, Thomas PJ, Hofmann AF. Prospective comparison of indirect methods for detecting lactase deficiency. *N Engl J Med* 1975; **293**: 1232–6.
15 Vogelsang H, Ferenci P, Frotz S, Meryn S, Gangl A. Acidic colonic microclimate-possible reason for false negative hydrogen breath tests. *Gut* 1988; **29**: 21–6.
16 Löser CH, Möllgaard A, Fölsch UR. Faecal elastase 1: a novel, highly sensitive, and specific tubeless pancreatic function test. *Gut* 1996; **39**: 580–6.
17 Durie BGM. Staging and kinetics of multiple myeloma. *Sem Oncol* 1986; **13**: 300.
18 Minor RR, Sippola-Thiele M, McKeon J, Berger J, Prockop DJ. Defects in the processing of procollagen to collagen are demonstrable in cultured fibroblasts from patients with the Ehlers-Danlos and osteogenesis imperfecta syndromes. *J Biol Chem* 1986; **25**: 10006–14.
19 Krane SM, Holick MF. Metabolic bone disease. In: Fauci A et al., eds. *Harrison's Principles of Internal Medicine*, 14th edn. New York: McGraw-Hill, 1998; 2247–53.
20 Kanis JA, Torgerson D, Cooper C. Comparison of the European and USA practice guidelines for osteoporosis. *TEM* 2000; **11**: 28–32.
21 Honkanen R, Pulkkinen P, Jarvinen R *et al.* Does lactose malabsorption predispose to low bone density? A population-based study of perimenopausal Finnish women. *Bone* 1996; **19**: 23–8.
22 Slemenda CW, Christian JC, Hui S, Fitzgerald J, Johnston CC. No evidence for an effect of lactase deficiency on bone mass in pre- or postmenopausal woman. *J Bone Min Res* 1991; **6**: 1367–71.
23 Moore T, Eastman C. Diseases of bone. In: Simon J, Tailor T, eds. *Diagnostic Endocrinology*, 2nd edn. St Louis: Mosby, 1996; 373–409.
24 Aurbach GD, Marx STJ, Spiegel AM. Metabolic bone disease. In: Williams I, Hardin R, Wilson JD, Foster DW, eds. *Williams Textbook of Endocrinology*, 7th edn. Philadelphia, PA: W.B. Saunders Company, 1985; 1215–55.
25 Finkenstedt G, Skrabal F, Gasser RW, Braunsteiner H. Lactose absorption, milk consumption, and fasting blood glucose concentrations in women with idiopathic osteoporosis. Br Med J (Clin Res Ed) 1986; 292: 161–2.
26 Flatz G, Howell JN, Doench J, Flatz SD. Distribution of physiological adult lactase phenotypes, lactose absorber and malabsorber, in Germany. *Hum Genet* 1982; **62**: 152–7.
27 Suarez FL, Adshead J, Furne JK, Levitt MD. Lactose maldigestion is not an impediment to the intake of 1500 mg calcium daily as dairy products. *Am J Clin Nutr* 1998; **68**: 1118–22.
28 DiMagno EP. Human exocrine pancreatic enzyme secretion. In: Go VLW, Gardner JD, Brooks FP, Lebenthal E, DiMagno EP, Scheele, GA, eds. *The Exocrine Pancreas, Biology, Pathophysiology, and Diseases*. New York: Raven Press, 1986: 193–211.
29 Hallmann L. In: Hallmann L, edr. *Klinische Chemie und Mikrobiologie*, 11th edn. Stuttgart, New York: Thieme-Verlag 1980: 562–3.
30 Weger M, Deutschmann H, Weger W, Kotanko P, Skrabal F. Defective renal acidification in 'primary' osteoporosis. *Osteoporosis Int* 1999; **10**: 325–9.
31 Weger W, Kotanko P, Weger M, Deutschmann H, Skrabal F. Prevalence and characterization of renal tubular acidosis in patients with osteopenia and osteoporosis and in non-porotic controls. *Nephrol Dial Transplant* 2000; **15**: 975–80.
32 Green J, Kleeman CR. Role of bone in regulation of systemic acid–base balance. *Kidney Int* 1991; **39**: 9–26.
33 Alpern RJ, Sakhaee K. The clinical spectrum of chronic metabolic acidosis: homeostatic mechanisms produce significant morbidity. *Am J Kidney Dis* 1997; **29**: 291–302.
34 Cochet B, Jung A, Griessen M, Bartholdi P, Schaller P, Donath A. Effects of lactose on intestinal calcium absorption in normal and lactase-deficient subjects. *Gastroenterology* 1983; **84**: 935–40.
35 Halpern GM, Van de Water J, Delabroise AM, Keen CL, Gershwin ME. Comparative uptake of calcium from milk and a calcium-rich mineral water in lactose intolerant adults: implications for treatment of osteoporosis. *Am J Prev Med* 1991; **7**: 379–83.

Received 23 October 2001; revision received 17 May 2002; accepted 9 August 2002.

Correspondence: F. Skrabal MD, Department of Internal Medicine, Krankenhaus der Barmherzigen Brüder, Graz, Teaching Hospital of the Karl-Franzens University Graz, A-8020 Graz, Marschallgasse 12, Austria (fax: +43 316 7067 598; e-mail: falko.skrabal @kfunigraz.ac.at).

Appendix

Laboratory methods and normal ranges/values

Serum creatinine[a]	0.66–1.09 mg dL^{-1} (women); 0.84–1.25 mg dL^{-1} (men)
Serum calcium[a]	2.0–2.6 mmol L^{-1} (women/men)
Serum 25-hydroxy-vitamin D_2	23.0–113 nmol L^{-1} (women/men)
	^{125}I radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA)
Serum phosphate[a]	2.5–4.2 mg L^{-1} (women/men)
	(Phosphomolybdate method, Daly)
Urinary phosphate[a]	(Phosphomolybdate method, Daly)
Intact parathyroid hormone[a]	10–65 pg mL^{-1} (women/men)
24-h urinary calcium[a]	4 mg calcium (kg body weight)$^{-1}$ day^{-1}
pH, HCO_3, pCO_2	AVL 995Hb Blood Gas Analyser (AVL List, Graz, Austria)
H_2-lactose breathing test	After the ingestion of 50 g lactose; a rise of blood glucose <20 mg dL^{-1} and/or a rise of H_2-exhalation of >20 ppm was considered significant (Stimotron, Exhaled Hydrogen Monitor, Wendelstein, Germany)
Elastase in stool	<200 μg g^{-1} (women/men)
	(Schebo-Tech, Wettenberg, Germany)
Serum albumin[a]	3.5–5.0 g dL^{-1} (women/men)
Serum electrophoresis	(Biolab, Vienna, Austria)
Immunofixation electrophoresis (IFE)	(Beckman Coulter, Fullerton, CA, USA)
fT3[b]	1.45–3.48 pg mL^{-1} (women/men)
fT4[b]	0.71–1.85 ng mL^{-1} (women/men)
TSH[b]	0.49–4.67 μU mL^{-1} (women/men)
ALAT[a]	5–19 U L^{-1} (women); 5–23 U L^{-1} (men)
ASAT[a]	5–15 U L^{-1} (women); 5–17 U L^{-1} (men)
Alkaline phosphatase (AP)[a]	50–170 U L^{-1} (women/men)
Bence Jones paraproteins[a]	
Serum oestradiol[b]	<20–41 pg mL^{-1} (postmenopausal women)
	39–189 pg mL^{-1} (premenopausal-follicular phase)
	94–508 pg mL^{-1} (premenopausal-peak)
	48–309 pg mL^{-1} (premenopausal-luteal phase)
Luteinizing hormone (LH)[b]	15–62 mIU mL^{-1} (postmenopausal women)
	1–18 mIU mL^{-1} (premenopausal-follicular phase)
	24–105 mIU mL^{-1} (premenopausal-peak)
	0.4–20 mIU mL^{-1} (premenopausal-luteal phase)
Testosterone	3.0–12 ng mL^{-1} (men) (Immunotech, Marseille, France)
Prolactin[b]	2.1–17.8 μg mL^{-1}
Blood iron[a]	49–151 μg dL^{-1} (women); 53–167 μg dL^{-1} (men)
Prothrombin time	70–130% (women/men) (Thromborel S; Dade Behring, Marburg, Germany)
Xylose-test[a]	>2 mmol L^{-1} D-xylose
Vitamin B_{12}	187–1059 pg mL^{-1} (women/men) (Amersham International, Clearbrook, IL, USA)

[a]Roche, Vienna, Austria, Basel, Switzerland; [b]Abbott Diagnostics Division, IL, Abbott Park USA.