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CLINICAL STUDY

Prevalence of subclinical contributors to low bone mineral density and/or fragility fracture

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Abstract

Objective: The prevalence of subclinical contributors to low bone mineral density (BMD) and/or fragility fracture is debated. We evaluated the prevalence of subclinical contributors to low BMD and/or fragility fracture in the presence of normal 25-hydroxyvitamin D (25OHVitD) levels.
Design: Prospective observational study.
Methods: Among 1095 consecutive outpatients evaluated for low BMD and/or fragility fractures, 602 (563 females, age 65.4±10.0 years) with apparent primary osteoporosis were enrolled. A general chemistry profile, phosphate, 25OHVitD, cortisol after 1-mg overnight dexamethasone suppression test, antitissue transglutaminase and endomysial antibodies and testosterone (in males) were performed. Serum and urinary calcium and parathyroid hormone levels were also evaluated after 25OHVitD levels normalization. Vertebral deformities were assessed by radiograph.
Results: In total, 70.8% of patients had low 25OHVitD levels. Additional subclinical contributors to low BMD and/or fragility fracture were diagnosed in 45% of patients, with idiopathic hypercalciuria (IH, 34.1%) and primary hyperparathyroidism (PHPT, 4.5%) being the most frequent contributors, apart from hypovitaminosis D. Furthermore, 33.2% of IH and 18.5% of PHPT patients were diagnosed only after 25OHVitD levels normalization. The subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D were associated inversely with age (odds ratio (OR) 1.02, 95% CI 1–1.04, $P=0.04$) and BMI (OR 1.1, 95% CI 1.05–1.17, $P=0.0001$) and directly with fragility fractures (OR 1.89, 95% CI 1.31–2.73, $P=0.001$), regardless of BMD.
Conclusions: Subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D are present in more than 40% of the subjects with apparent primary osteoporosis. Hypovitaminosis D masks a substantial proportion of IH and PHPT patients.

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Introduction

Osteoporosis encompasses two different forms: primary and secondary. Primary osteoporosis is due to postmenopausal and age-related bone loss. Secondary osteoporosis is defined as bone loss that results from specific, well-defined, clinical disorders or medical treatments (1). The prevalence of secondary osteoporosis, in both men and women, is widely debated (1, 2, 3, 4, 5, 6, 7, 8). In some studies 20–30% of postmenopausal women and more than 50% of men with osteoporosis are affected with the secondary form of the disease. Some of the secondary causes of osteoporosis may be identified by medical history and/or physical examination, while others remain hidden unless additional diagnostic testing is performed. Diagnosing the subclinical contributors to secondary osteoporosis is important, since these conditions are reversible with appropriate intervention, leading to a rapid and substantial increase of bone mineral density (BMD) (9, 10, 11, 12). On the other hand, if these conditions are not identified, medical treatment may be suboptimal or ineffective (1, 13).

Nowadays, guidelines on osteoporosis advocate the evaluation of osteoporotic patients to identify the presence of subclinical contributors to secondary osteoporosis before therapy (8, 14, 15). However, the diagnostic protocols differ among the various guidelines. As a consequence, the actual prevalence of the subclinical contributors to secondary osteoporosis remains uncertain, owing to the inclusion of different populations and different approaches, often not taking into account all the possible causes (i.e. subclinical hypercortisolism (SH), celiac disease, etc.) (1, 3, 5, 6, 7, 16). Moreover, the criteria used to define which patient should undergo additional evaluation (i.e. the

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presence of a clinical fracture and/or densitometric osteoporosis) are still a matter of debate (17, 18, 19, 20). Finally, no studies have evaluated the patients after the normalization of serum 25-hydroxyvitamin D (25OHVitD) levels. This is a crucial point, since hypovitaminosis D may lead to underestimate the real prevalence of some diseases, as it may mask the presence of primary hyperparathyroidism (PHPT) and idiopathic hypercalciuria (IH) (21, 22).

Therefore, the aim of the present study was to evaluate, in a large sample of patients, the prevalence of the different subclinical contributors to low BMD and/or fragility fracture before and after correction of hypovitaminosis D.

Subjects and methods

Study design, population and inclusion/exclusion criteria

We prospectively evaluated 1095 patients (937 females and 158 males) consecutively admitted to our Osteoporosis and Metabolic Bone Diseases Outpatients Clinic from September 2009 to December 2011, and referred by their primary care physician for reduced BMD and/or a history of an adult fragility fracture. The inclusion criteria were the presence of osteopenia or osteoporosis, defined by the WHO established cut-offs (23), and/or of a BMD low for age (i.e. Z-score < -1.0) and/or a history of an adult fragility fracture (hip, vertebrae T5–L4, wrist, ribs, and proximal humerus) caused by low trauma, such as falling from a standing height or less) (Fig. 1). The characteristics of the whole sample of the patients evaluated for the enrollment are reported in Table 1.

A written informed consent before entering the study, which was approved by the Local Ethical Committee and in accordance with the Declaration of Helsinki II, was obtained from all patients.

Since the aim of our study was to investigate the true prevalence of the different subclinical contributors to low BMD and/or fragility fracture, we decided to exclude all subjects affected with an already known or clinically evident cause of secondary osteoporosis at the time of the first visit. Therefore, 427 patients (317 females and 110 males) were not considered eligible for the study on the basis of the following exclusion criteria: a history of gastrectomy, bowel disease or resection, eating disorders, alcoholism, malnutrition, premature menopause (before 40 years of age), prolonged premenopausal amenorrhea (≥ 1 year), a period of prolonged immobility (≥ 3 months), PHPT, overt endogenous hypercortisolism, hyperthyroidism, liver diseases, rheumatoid arthritis or other rheumatologic

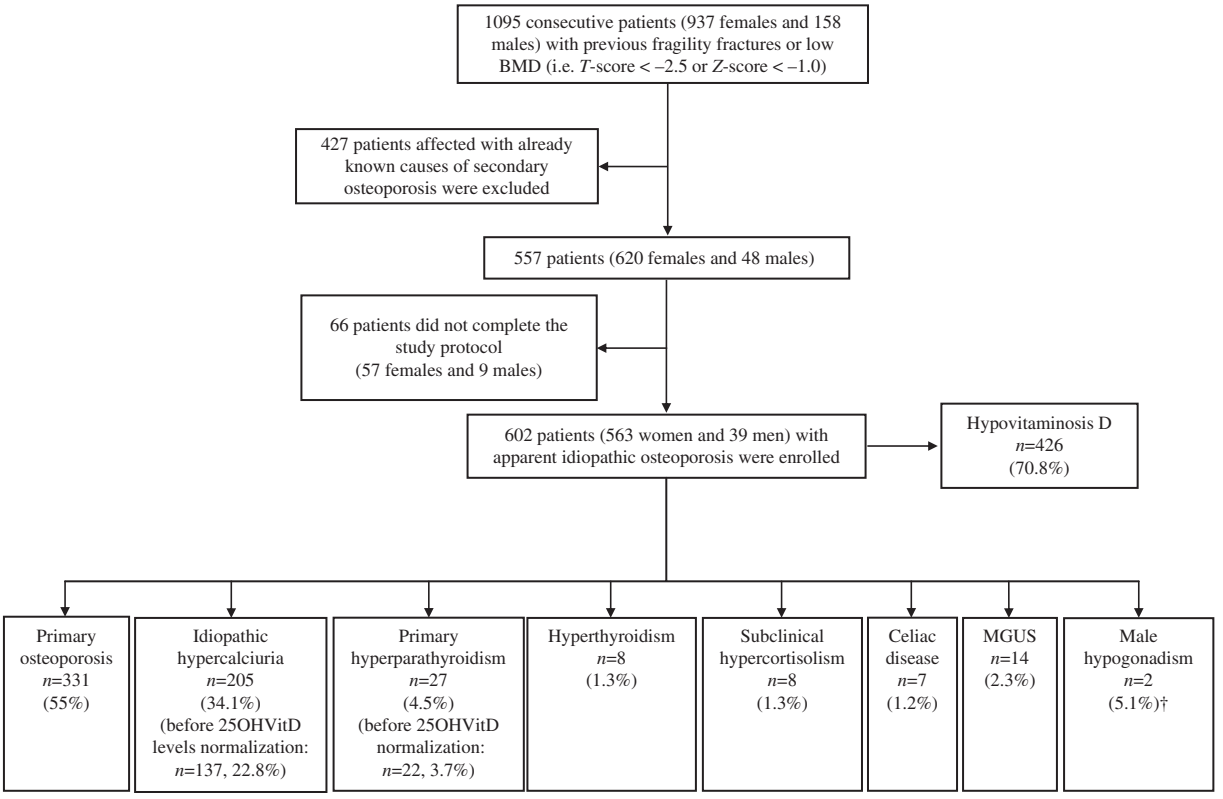

Figure 1 Study of flow diagram. BMD, bone mineral density; MGUS, monoclonal gammopathy of undetermined significance; 25OHVitD, 25-hydroxyvitamin D; †among 22 male patients with apparent primary osteoporosis.

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Table 1 Characteristics of the 1095 patients evaluated for the enrollment.

	Patients evaluated for the enrollment ($n=1095$)
Females	943 (86.1)
Age (years)	62.9±12.3 (19–91)
BMI (kg/m^2)	24.4±3.6 (16.1–38.6)
Patients with already known	
Primary hyperparathyroidism	25 (2.3)
Hyperthyroidism	23 (2.1)
Overt hypercortisolism	3 (0.3)
Hypogonadism[a]	26 (2.3)
Celiac and other bowel diseases	19 (1.7)
Liver diseases	60 (5.5)
Multiple myeloma	4 (0.4)
Paget's disease of bone	3 (0.3)
Patients with history of organ transplantation	46 (4.2)
Patients with other diseases influencing bone metabolism	50 (4.6)
Patients with medication history of glucocorticoid therapy (>1 month)	16 (10.6)
Patients with medication history of other drugs influencing bone metabolism[b]	52 (4.7)
Patients with apparent primary osteoporosis	668 (61)

[a]In males with already known hypogonadism, in females with prolonged premenopausal amenorrhea (≥1 year) or premature menopause (before 40 years of age).
[b]Systemic chemotherapy, current anticonvulsant therapy, TSH-suppressive L-thyroxine therapy, heparin, methotrexate, cyclosporine, isoniazid, lithium, GnRH agonists or antagonists and aromatase inhibitors.

diseases, organ transplantation, renal failure (creatinine clearance estimated by Cockcroft–Gault equation <60 ml/min) (24), Paget's disease, osteomalacia, childhood rickets, advanced Parkinson's disease, sarcoidosis, hematologic malignancy, Gaucher's disease and metastatic cancer (Table 1). We also excluded all subjects ($n=7$) with alcohol consumption ≥12 U/day, and those with medication history of systemic chemotherapy, prolonged glucocorticoid use (>1 month), current anticonvulsant therapy, TSH-suppressive L-thyroxine (T_4) therapy, heparin, methotrexate, cyclosporine, isoniazid, lithium, GnRH agonists or antagonists, aromatase inhibitors and other drugs known to affect bone metabolism (Table 1).

Among the remaining 668 patients (620 females and 48 males) who were eligible for the study, 66 (57 females and nine males) did not complete the study protocol.

A full personal history was collected from the 602 eligible patients who completed the study protocol (563 females and 39 males). The availability of testing results was mandatory for the enrollment.

Moreover, family history (first- and second-degree relatives) of osteoporosis or fragility fractures, history of nephrolithiasis, cigarette smoking (ever smoked vs never smoked) and number of falls within the last 12 months (0, 1 and >1) were obtained from all subjects. All subjects were asked about quantity and type of alcoholic drinks consumed and data were converted as units per day (8 g of pure alcohol) (25).

The patients were also asked about previous clinical fragility fractures at spine, ribs, wrist and hip and proximal humerus. Fractures of shoulder, pelvis, skull, jaw, coccyx, phalanx, ankle, cervical and thoracic vertebrae (C1 to T4), toes and fingers and of posterior arches of the vertebra were not considered as osteoporosis-related fractures and were excluded from the analysis. In all patients, the presence of previous fragility fractures was ascertained by self-report and no additional validation of this information was conducted. Calcium intake, expressed as milligrams per day, was assessed using a validated questionnaire (26). In particular, usual calcium intake coming from some selected calcium-rich foods (milk and dairy products) was estimated by a 7-day food frequency questionnaire. The foods checked include milk, aged cheese, soft cheese, cottage cheese and yoghurt. The portion sizes were quantified by means of household measures (slices and cups). To standardize the slice weight, three cardboard samples of different sizes were used (about 100, 50 and 25 g). The number of standardized servings was assessed, each containing ~300 mg of calcium (a 250 ml cup of milk or yoghurt, a portion of about 100 g of cottage cheese, a 50 g slice of soft cheese and a 25 g slice of aged cheese) (26). In all patients height and weight were measured and BMI was calculated.

Methods

All patients underwent the following testing on a blood venous sample obtained after overnight fasting under a free diet: complete blood cell count and differential, serum calcium, phosphate, serum creatinine, alkaline phosphatase, γ-glutamyl transpeptidase, erythrocyte sedimentation rate, C-reactive protein, serum protein electrophoresis, serum aminotransferases, intact plasma parathyroid hormone (PTH, normal values 10–55 ng/l), 25OHVitD, TSH, total serum IgA, IgA antitissue transglutaminase and IgA endomysial antibodies were measured. On a separate day, the serum cortisol levels after 1-mg overnight dexamethasone suppression test (1-mg DST) were measured in all subjects. In all males serum total testosterone was evaluated. Urinary calcium, creatinine and calcium clearance were measured in 24-h urine collections.

All patients with 25OHVitD levels below 75 nmol/l received supplementation. An oral bolus of 100 000 or 300 000 IU of cholecalciferol was administered in patients with 25OHVitD levels between 25 and 75 nmol/l and below 25 nmol/l respectively (15). Serum calcium, 25OHVitD and PTH and urinary calcium and creatinine in 24-h urine collections were re-evaluated once 25OHVitD levels above 75 nmol/l were achieved, at least 1 month after cholecalciferol administration. We decided to use 25OHVitD levels

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below 75 nmol/l as the threshold for vitamin D supplementation, since several evidences suggest that, below this 25OHVitD concentration, the calcium absorption and PTH levels are reduced and increased respectively, while above this threshold they reach a steady state (27, 28). In all subjects, after vitamin D supplementation, 25OHVitD levels were between 75 and 200 nmol/l.

Dual energy X-ray absorptiometry (DXA) scans were carried out to measure BMD at the spine and hip. Seventy-nine percent of subjects underwent DXA evaluation at our Hospital (Hologic Discovery, Waltham MA, USA) at lumbar spine (LS; *in vivo* precision 1.0%), total femur (FT; *in vivo* precision 1.7%) and femoral neck (FN; *in vivo* precision 1.8%), while the remaining 21% had measurements on other instruments at other centers (Hologic Discovery and Lunar GE). The different machines were not calibrated against the same phantom. However, to accommodate results from different machines and manufacturers, densitometry results were recorded as *T*- and Z-scores (S.D. from young and age-matched normals respectively) using the manufacturer's normative reference groups (29). Data from LS scans were used only if at least three vertebrae were visualized without interfering artefacts. Fractured vertebrae were excluded from BMD measurement. In 45 patients LS BMD could not be evaluated.

Conventional spinal radiographs in lateral and anteroposterior projection (T4–L4) were obtained at baseline in all subjects with standardized technique. Two trained physicians, who were blinded to BMD and hormonal data, independently reviewed the radiographs, and they discussed questionable cases to agree on a diagnosis. The interrate reliability between the two radiologists was good ($k=0.8$).

Vertebral fractures were diagnosed on visual inspection using the semiquantitative (SQ) visual assessment previously described by Genant *et al.* (30). According to this technique, fractures assessed on lateral thoracolumbar spine radiographs were defined as reductions of more than 20% in anterior, middle or posterior vertebral height. From lateral spine radiographs, 13 vertebrae from T4 to L4 were assessed visually as intact (SQ grade 0) or as having approximately mild (20–25% compression), moderate (25–40% compression) or severe (>40% compression) deformity (SQ grades 1, 2 and 3 respectively). Subsequently, for each subject, the spinal deformity index (SDI) was calculated by summing the SQ grade for each vertebra (SDI=SQT4+…+SQT12+SQL1+…+SQL4) (31). For example, an SDI of 3 can mean one grade 3 fracture or three grade 1 fractures.

Abnormal laboratory tests

Participants with altered TSH levels were tested for free T_4 (fT_4), antithyroglobulin, antithyroperoxidase and anti-TSH receptor (TRAb) antibodies. Overt hyperthyroidism was defined by TSH levels <0.3 μU/ml (mU/l) with elevated fT_4 and subclinical hyperthyroidism by TSH levels <0.3 μU/ml (<0.3 mU/l) with normal fT_4 levels. Total calcium was corrected for serum albumin according to the formula: total calcium (mg/dl)+(4.4−albumin (mg/dl))×0.8 (reference interval 8.4–10.2 mg/dl, 2.1–2.55 mmol/l) (32). Patients with increased serum calcium levels together with increased or inappropriately normal PTH levels were tested for ionized serum calcium levels. PHPT was diagnosed by hypercalcemia and elevated or inappropriately normal PTH levels, after the evaluation of calcium clearance:creatinine clearance ratio, in order to rule out familial hypocalciuric hypercalcemia (21).

In patients with normal IgA antitissue transglutaminase and IgA endomysial antibodies and a selective IgA deficiency, IgG antitissue transglutaminase antibodies and IgG endomysial antibodies were evaluated. Patients with positive serologic tests underwent duodenal biopsy. The diagnosis of celiac disease was established in the presence of the typical histological findings (33).

Participants with 1-mg DST >50 nmol/l underwent further diagnostic investigations (i.e. serum cortisol levels measured at 0900 h after 2 days of low-dose (0.5 mg every 6 h) dexamethasone suppression, two measurements of 24-h urinary free cortisol and measurement of ACTH at 0800 h). SH was diagnosed in the presence of cortisol level >50.0 nmol/l after a 2-day low-dose DST and/or elevated 24-h urinary free cortisol in the absence of signs or symptoms of cortisol excess, including moon facies, striae rubrae, skin atrophy or buffalo hump (34).

Males with at least two determinations of serum total testosterone levels below the lower limit of the normal range for age (<10.4 and <6.9 nmol/l, before and after the age of 70 respectively) were classified as hypogonadic and underwent additional evaluations (35).

The diagnosis of monoclonal gammopathy of undetermined significance (MGUS) required a serum monoclonal protein, no evidence of other B-cell proliferative disorders and no end-organ damage due to the plasma cell proliferative process (i.e. bone lesions, hypercalcemia, renal insufficiency and anemia) (36, 37).

Hypercalciuria was established in the presence of urinary calcium excretion >0.1 mmol/kg body weight (22), both in men and women, in at least two 24-h urinary samples, in conditions of normal dietary sodium (1.5 g/day between 19 and 30 years of age, 1.3 g/day between 31 and 50 years of age and 1.2 g/day older than 51 years of age), protein (46 g/day for females and 56 g/day for males older than 19 years of age) and calcium intake (1.0 g/day for males and females between 19 and 50 years of age, 1.2 g/day for females older than 51 years of age) (38). After the exclusion of known causes of hypercalciuria (i.e. PHPT, sarcoidosis, Cushing's syndrome, cancer, excess vitamin D intake,

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hyperthyroidism, glucocorticoid use, Paget's disease or renal tubular acidosis), the diagnosis of IH was established.

Patients with polyarticular pain and elevated erythrocyte sedimentation rate and/or C-reactive protein underwent rheumatologic evaluation, in order to exclude an autoimmune rheumatologic disease. In selected patients serum tryptase levels and bone marrow examination were needed in order to exclude mastocytosis and multiple myeloma.

Statistical analysis

Statistical analysis was performed by SPSS version 18.0 statistical package (SPSS, Inc.). The normality of distribution was checked by Kolmogorov–Smirnov test. The results are expressed as mean $\pm$ S.D. if not differently specified. The comparison of continuous variables between patients with and without subclinical contributors to low BMD and/or fragility fractures was performed using Student's *t*-test or Mann–Whitney *U* test, as appropriate. The univariate general linear modeling has been used to compare SDI values between patients to adjust for age variables between patients with and without subclinical contributors to low BMD and/or fragility fractures, if needed. Categorical variables were compared by χ^2 test.

The logistic regression analysis assessed the association between the presence of subclinical contributors to low BMD and/or fragility fractures including and excluding hypovitaminosis D (dependent variable) and the variables that were found to be significantly different between patients with and without subclinical contributors to low BMD and/or fragility fracture (independent variables). Then, for the continuous independent variables, the receiver operating characteristic (ROC) curve analysis was performed to assess their best cut-offs for individuating patients with subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D. Finally, we assessed the sensitivity and specificity and overall accuracy for detecting patients with subclinical contributors to low BMD and/or fragility fracture of a diagnostic two-step protocol, in which a first-line testing is performed in all patients and a second-line testing is performed only in patients showing the characteristics that were found by the logistic regression analysis to be associated with the presence of a subclinical contributor of low BMD and/or fragility fracture (using for the continuous variables the cut-offs individuated by the ROC curve analysis). Values of $P<0.05$ were considered significant.

Results

The final sample of patients enrolled in the study and screened for the presence of subclinical contributors to low BMD and/or fragility fracture was composed of 602 patients (563 females and 39 males; Fig. 1). The characteristics of these patients are shown in Table 2. At baseline, 70.8 and 45.3% of patients had 25OHVitD levels below 75 and 50 nmol/l respectively. Among these, 10.3% of patients had 25OHVitD below 25 nmol/l.

The prevalence of subclinical contributors to low BMD and/or fragility fracture is reported in Table 3. A status of hypovitaminosis D (25OHVitD levels <75 nmol/l) was found to be the most prevalent contributor of low BMD and/or fragility fracture (Table 3). After vitamin D supplementation, a diagnosis of additional subclinical contributors to low BMD and/or fragility fracture apart from hypovitaminosis D was made in 271 subjects (45%). However, given its high prevalence even in the healthy population (39, 40, 41), hypovitaminosis D might be considered a concomitant cause of secondary osteoporosis. In addition, we do not have information regarding the vitamin D status of our patients before the study entry. Therefore, in Table 3 we also report the prevalence of each subclinical contributor to low BMD and/or fragility fracture besides hypovitaminosis D.

Table 2 Characteristics of the 602 enrolled patients. Data are mean $\pm$ S.D. with range or percentage in parenthesis.

	Patients enrolled ($n=602$)
Females	563 (93.5)
Age (years)	65.4 $\pm$ 10.1 (33 to 89)
BMI (kg/m^2)	24.6 $\pm$ 3.6 (18 to 38.6)
No. of patients with history of current or previous smoking	189 (31.4)
No. of patients experiencing falls (0/1/2)	515/64/23 (85.5/10.6/3.8)
Dietary calcium intake (mg/day)	533 $\pm$ 254 (50 to 2000)
No. of patients with history of nephrolithiasis	101 (16.8)
No. of patients with family history of osteoporosis	297 (49.3)
No. of patients with family history of fragility fractures	126 (20.9)
No. of patients with hypovitaminosis D (0/1/2)	176/364/62 (29.2/60.5/10.3)
LS BMD (*Z*-score)	-1.03 ± 1.14 (-4.2 to 3.1)
FT BMD (*Z*-score)	-0.66 ± 0.9 (-3.5 to 2.1)
FN BMD (*Z*-score)	-0.74 ± 0.86 (-3.5 to 2.0)
No. of patients with *T*-score ≤ -2.5 at any site	412 (68.4)
No. of patients with history of clinical fractures	136 (22.6)
SDI	2.2 $\pm$ 3.4 (0 to 27)
SDI ≥ 1	335 (55.6)
No. of patients with clinical and/or morphometric fractures	361 (60)

Fall: 0, no fall within previous 12 months; 1, one fall within previous 12 months; 2, greater than or equal to two falls within previous 12 months; hypovitaminosis D: 0, 25-hydroxyvitamin D at baseline ≥ 75 nmol/l; 1, 25-hydroxyvitamin D at baseline ≥ 25 and <75 nmol/l; 2, 25-hydroxyvitamin D at baseline <25 nmol/l; BMD, bone mineral density; SDI, spinal deformity index; SDI ≥ 1, patients with at least one morphometric vertebral fracture.

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Table 3 Hypovitaminosis D and other additional contributors to low BMD and/or fragility fracture in patients affected with apparently idiopathic primary osteoporosis after vitamin D supplementation. Data are number of patients with percentage in parentheses.

Main contributors of low BMD and/or fragility fracture (no. of patients)	All enrolled patients ($n=602$)	Patients with hypovitaminosis D ($n=426$)
Hypovitaminosis D	426 (70.8)	–
Idiopathic hypercalciuria	205 (34.1)	47 (34.5)
Primary hyperparathyroidism	27 (4.5)	23 (5.4)
Subclinical and overt hyperthyroidism	8 (1.3)	5 (1.2)
Subclinical hypercortisolism	8 (1.3)	6 (1.4)
Celiac disease	7 (1.2)	7 (1.6)
Hypogonadism	2 (5.1)[a]	2 (9.1)[a]
MGUS	14 (2.3)	–
Low calcium intake (10)	10 (1.7)	–

Hypovitaminosis D, 25-hydroxyvitamin D <75 nmol/l; MGUS, monoclonal gammopathy of undetermined significance.
[a]Among 22 male patients.

Looking at the effect of vitamin D supplementation, we found that before vitamin D supplementation IH was present in 137 patients (22.8% of the whole sample), while after vitamin D supplementation, IH was diagnosed in an additional 68 patients. Thus, IH was found in 205 patients, about one out of three patients with apparent idiopathic primary osteoporosis, representing 75.6% of subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D. Most of these patients well tolerated the therapy with hydrochlorothiazide (25 mg daily), which is suggested in order to reduce urinary calcium excretion.

Similarly, before vitamin D supplementation, PHPT was found in 22 subjects (3.7% of the whole sample, all females). After vitamin D supplementation, PHPT was diagnosed in an additional five subjects (one male and four females). Therefore, the prevalence of PHPT among the whole sample of 602 patients was 4.5% (27 patients). Fifteen were surgically treated and in all subjects PTH and serum and urine calcium normalized.

Hypogonadism was diagnosed in 2 out of 39 male patients (5.1% of male patients). All these patients initially denied, at medical history, alteration of sexual function. Both patients, after the exclusion of prostatic diseases, started testosterone substitutive therapy.

Hyperthyroidism was diagnosed in 1.3% of patients (subclinical in seven patients and overt in one patient). All patients underwent neck ultrasound, radioiodine uptake and TRAb antibodies were measured. In three patients (one overt and two subclinical) a Graves–Basedow disease was diagnosed. One patient underwent radioactive iodine treatment and the remaining two started medical treatment with methimazole. In the other five patients we found a partial autonomous multinodular goiter. Three patients underwent radioiodine treatment and two underwent surgery.

SH was found in 1.3% of patients. Thirty-five out of 602 patients failed to suppress cortisol levels after 1-mg DST and underwent additional tests. Eventually, SH was confirmed in eight patients (seven patients had an ACTH-independent SH due to an adrenal mass and one patient had an ACTH-dependent SH of pituitary origin). To date, four SH patients with a unilateral adrenal mass underwent surgery and in all a steroidal replacement therapy was needed.

Among the 602 enrolled patients, 13 underwent small bowel biopsy because of the subsequent findings: positive values of IgA antitissue transglutaminase and/or IgA endomysial antibodies (ten patients), gastrointestinal symptoms without serological alterations (one patient), and severe 25OHVitD deficit hardly correctable (two patients). Among these 13 patients, a histological diagnosis of celiac disease was made in seven (1.2%).

The presence of MGUS was found to be the only possible cause of low BMD and/or fragility fracture in 14 patients (2.3% of the 602 enrolled patients). Five patients with MGUS, with severe osteoporosis, without other abnormalities in the laboratory's tests, underwent bone marrow examination. In all patients the presence of multiple myeloma was excluded.

Ten patients (six males and four females) were found to have secondary hyperparathyroidism, without evidence of hypovitaminosis D, gut malabsorption, hypercalciuria or reduced kidney function. All patients were older than 70 years of age and had a calcium intake lower than 150 mg/day.

Eighteen patients (2.9%), presenting polyarticular pain and elevated erythrocyte sedimentation rate and/or C-reactive protein levels, underwent rheumatologic evaluation. No patients were diagnosed as having a rheumatologic disease. In 23 patients with severe osteoporosis (i.e. SDI ≥ 10), among the 331 without subclinical contributors to low BMD and/or fragility fracture, serum tryptase levels were determined. Two patients with a slight increase of tryptase levels underwent bone marrow examination and in both the diagnosis of mastocytosis was excluded.

By separately analyzing premenopausal females ($n=13$), we found that 11 were affected with subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D (nine with IH and two with SH), while two were not. Among these patients nine and three subjects had 25OHVitD levels below 75 and 25 nmol/l respectively. Seven out of the 11 premenopausal patients with subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D had an asymptomatic and/or clinical fragility fracture, while the two premenopausal patients without subclinical contributors to low BMD and/or fragility fracture were not fractured. Table 4 summarizes the clinical characteristics of patients affected with hypovitaminosis D and with each subclinical contributor of secondary osteoporosis to low BMD and/or fragility fracture besides hypovitaminosis D.

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Table 4 Clinical characteristics of patients with the main subclinical contributors to low BMD and/or fragility fracture. Data are mean±S.D. with range or percentage in parenthesis.

Subclinical contributors to low BMD and/or fragility fracture	Females	Age	*Z*-score <-1.0[a]	*T*-score <-2.5[a]	Prevalence of fragility fracture	Prevalence of hypovitaminosis D
Hypovitaminosis D alone ($n=236$)	217 (91.9)	67.6±9.7 (36–89)	149 (63.1)	162 (68.6)	148 (62.7)	–
Idiopathic hypercalciuria ($n=205$)	195 (95.1)	62.6±9.7 (33–86)	146 (71.2)	143 (69.8)	128 (62.4)	147 (71.7)
Primary hyperparathyroidism ($n=27$)	26 (96.3)	69±10.5 (48–89)	20 (74.1)	22 (81.5)	20 (74.1)	23 (85.2)
Hyperthyroidism ($n=8$)	8 (100)	74.3±7 (63–83)	4 (50)	7 (87.5)	8 (100)	5 (62.5)
Subclinical hypercortisolism ($n=8$)	7 (87.5)	60.3±15 (38–80)	7 (87.5)	7 (87.5)	7 (87.5)	6 (75)
Celiac disease ($n=7$)	6 (85.7)	67.9±9.1 (56–78)	5 (71.4)	6 (85.7)	5 (71.4)	7 (100)

Hypovitaminosis D, 25OHVitD levels <75 nmol/l.
[a]At any site.

The comparison of the clinical characteristics between the whole group of patients with and without subclinical contributors of low BMD and/or fragility fracture is reported in Table 5. Comparing patients with subclinical contributors of low BMD and/or fragility fracture other than hypovitaminosis D with patients without them, we found that the patients in the former group were younger, had a lower BMI and BMD at spine and femur and a higher prevalence of clinical and/or morphometric fragility fractures. The two groups were comparable for gender, history of current or previous smoke, frequency of falls, dietary calcium intake, history of nephrolithiasis, family history of osteoporosis and fragility fractures, SDI, and prevalence of hypovitaminosis D and osteoporosis. Comparing patients with subclinical contributors of low BMD and/or fragility fracture including hypovitaminosis D with patients without them, we did not find differences in gender, age, BMI, history of current or previous smoking, frequency of falls, dietary calcium intake, history of nephrolithiasis, family history of osteoporosis and fragility fractures and BMD at spine and femur, while the former group showed higher prevalence of osteoporosis, clinical and morphometric vertebral fractures and SDI.

The logistic regression analysis showed that the presence of subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D was associated inversely with age and BMI and directly with the presence of a fragility fracture (clinical and/or morphometric), but not with BMD (Table 6A). When including hypovitaminosis D among the subclinical contributors to low BMD and/or fragility fracture only, the presence of a fragility fracture (clinical and/or morphometric) was associated with the presence of subclinical contributors to low BMD and fragility fractures (Table 6B). The SDI was not associated with any specific contributor of low BMD and/or fragility fracture. Then, the ROC curve analysis, performed to assess the best threshold values of BMI and age for predicting the presence of subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D, showed that the cut-offs with the best compromise between sensitivity and specificity were 24 g/cm^2 for BMI and at 65 years for age. Therefore, on the basis of the logistic regression and ROC curve analyses, the presence of a fragility fracture, a BMI <24 g/cm^2 and an age <65 years were found to be the variables associated with the presence of subclinical contributors of low BMD and/or fragility fracture besides hypovitaminosis D.

Following the Italian guidelines (42), in patients with reduced BMD and/or fragility fracture the assessment of PTH, 25OHVitD, TSH, 1-mg DST, and IgA antitissue transglutaminase and endomysial levels is not included among the routine first-line screening tests and it is considered a second-line test to be reserved for patients at high risk of having a secondary cause of osteoporosis. In our sample, if we had performed complete blood cell count and differential count, serum calcium, phosphate, serum creatinine, alkaline phosphatase, erythrocyte sedimentation rate, serum protein electrophoresis and 25OHVitD as first-line screening tests in all patients and the second-line tests (i.e. PTH, TSH, 1-mg DST, and IgA antitissue transglutaminase and endomysial levels) in patients with abnormal screening tests and/or the concomitant presence of BMI <24 g/cm^2, age <65 years and a fragility fracture, after the normalization of 25OHD levels, we would have correctly individuated 549 out of the 602 patients (accuracy 91.2%). Indeed, if this protocol had been applied, 236 and 313 subjects would have been correctly diagnosed as affected (true positives) or not affected (true negatives) with a subclinical contributor of low BMD and/or fragility fracture besides hypovitaminosis D respectively while 21 affected patients would have been missed (false negatives, sensitivity 91.8%) and 32 patients not affected would have been erroneously considered at risk (false positives, specificity 90.7%). In total, 61.9 and 85.7% of false-negative patients showed a Z-score <-1.0 and the presence of at least one fragility fracture respectively.

If the second-line tests had been performed in patients with abnormal first-line tests and/or with two out of the presence of BMI <24 g/cm^2, age <65 years and a fragility fracture, the sensitivity for detecting a

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Table 5 Characteristics of patients with and without subclinical contributors to low BMD and/or fragility fracture, among the whole sample of 602 enrolled patients. Data are mean ± S.D. with range or percentage in parenthesis.

Variables	Patients with subclinical contributors of low BMD and/or fragility fracture $n=493$ (81.9)	Patients without subclinical contributors of low BMD and/or fragility fracture $n=109$ (18.1)	*P*	Patients with subclinical contributors of low BMD and/or fragility fracture other than hypovitaminosis D $n=271$ (45)	Patients without subclinical contributors of low BMD and/or fragility fracture other than hypovitaminosis D $n=331$ (55)	*P*
Females	459 (93.1)	104 (95.4)	0.375	254 (93.7)	309 (93.4)	0.853
Age (years)	65.6±10.2 (33 to 89)	64.6±9.4 (38 to 84)	0.378	64.1±10.3 (33 to 89)	66.4±9.7 (36 to 89)	0.004
BMI (kg/m^2)	24.6±3.7 (18.5 to 38.6)	24.8±3.2 (17.3 to 35.4)	0.488	23.8±3.2 (18.5 to 37.8)	25.3±3.7 (18 to 38.6)	0.0001
No. of patients with history of current or previous smoking	147 (29.8)	42 (38.5)	0.076	82 (30.3)	107 (32.3)	0.586
No. of patients experiencing falls (0/1/2)	416/55/22 (84.4/11.2/4.5)	99/9/1 (90.8/8.3/0.9)	0.13	240/21/10 (88.6/7.7/3.7)	275/43/13 (83.1/13.0/3.9)	0.111
Dietary calcium intake (mg/day)	527.9±242 (50 to 1900)	554±301 (50 to 2000)	0.332	535±231 (50 to 1600)	530±272 (50 to 2000)	0.844
No. of patients with history of nephrolithiasis	83 (16.8)	18 (16.5)	0.935	51 (18.8)	50 (15.1)	0.225
No. of patients with family history of osteoporosis	237 (48.1)	60 (55)	0.188	137 (50.6)	160 (48.3)	0.589
No. of patients with family history of fragility fractures	106 (21.5)	30 (27.5)	0.174	60 (22.1)	76 (23)	0.811
No. of patients with hypovitaminosis D	426 (86.4)	0 (0.0)	0.0001	200 (73.8)	226 (68.3)	0.138
Lumbar spine BMD (*Z*-score)	−1.07±1.12 (−4.2 to 2.9)	−0.86±1.21 (−3.3 to 3.1)	0.083	−1.22±1.14 (−4.2 to 2.1)	−0.88±1.11 (−3.4 to 3.1)	0.0001
Total femur BMD (*Z*-score)	−0.67±0.93 (−3.5 to 2.1)	−0.61±0.77 (−2.9 to 1.5)	0.538	−0.76±0.90 (−3.5 to 2.1)	−0.58±0.90 (−3.2 to 1.8)	0.013
Femoral neck BMD (*Z*-score)	−0.75±0.9 (−3.5 to 2)	−0.71±0.7 (−2.7 to 1.3)	0.669	−0.81±0.86 (−3.5 to 2)	−0.68±0.87 (−3.5 to 1.7)	0.05
No. of patients with *T*-score ≤ -2.5 at any site	348 (70.6)	64 (58.7)	0.016	196 (72.3)	216 (65.3)	0.063
No. of patients with history of clinical fractures	111 (22.5)	15 (13.8)	0.042	67 (24.7)	59 (17.8)	0.038
SDI[a]	2.4±3.4 (0 to 23)	1.4±3.4 (0 to 27)	0.01	2.2±0.2 (0 to 14)	2.3±3.8[a] (0 to 27)	0.32
No. of patients with clinical and/or morphometric fractures	318 (64.5)	43 (39.4)	0.0001	181 (66.8)	180 (54.4)	0.002

Fall: 0, no fall within previous 12 months; 1, one fall within previous 12 months; 2, greater than or equal to two falls within previous 12 months; hypovitaminosis D, 25-hydroxyvitamin D at baseline <50 nmol/l; BMD, bone mineral density; SDI, spinal deformity index; SDI ≥1, patients with at least one morphometric vertebral fracture.
[a]Corrected for age by general linear modeling.

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Table 6 Odds ratio (OR) for detecting the presence of subclinical contributors to low BMD and/or fragility fracture other than hypovitaminosis D (A) and including hypovitaminosis D (B) for potential risk factors using the multivariable logistic regression model.

	OR	95% CI	*P*
A			
Age (1 year decrease)	1.02	1–1.04	0.04
BMI (1 kg/m^2 decrease)	1.1	1.05–1.17	0.0001
Presence of fragility fractures (presence vs absence)[a]	1.89	1.31–2.73	0.01
Z-score LS (1 S.D. decrease)	1.1	0.92–1.3	0.296
B			
Age (1 year decrease)	1.01	0.98–1.03	0.62
BMI (1 kg/m^2 decrease)	1.02	0.95–1.08	0.638
Presence of fragility fractures (presence vs absence)[a]	2.51	1.59–3.96	0.0001
Z-score LS (1 S.D. decrease)	4.01	0.92–1.41	0.88

[a]Clinical or morphometric fracture; *Z*-score LS, BMD at spine expressed as *Z*-score.

subclinical contributor to low BMD and/or fragility fracture besides hypovitaminosis D would have increased to 96.1%, while the specificity would have decreased to 60.3% (accuracy 75.6%).

Discussion

To our knowledge, this is the largest study aimed to assess the true prevalence of subclinical contributors to low BMD and/or fragility fracture. At variance with previous similar studies (3, 4, 5, 6, 7, 18, 19, 20), the present one evaluates the prevalence of subclinical contributors to low BMD and/or fragility fracture even after correction of hypovitaminosis D.

Our study indicates that the most prevalent subclinical contributor to low BMD and/or fragility fracture is hypovitaminosis D, being present in 70.8% of our sample. Without considering hypovitaminosis D, a subclinical contributor to low BMD and/or fragility fracture is present in 45% of patients with apparent primary osteoporosis, without any difference between genders. The presence of subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D was associated inversely with age and BMI and directly with the presence of clinical and/or morphometric fractures, regardless of BMD. IH was diagnosed in about one out of three patients with apparent primary osteoporosis, and represented about 80% of the subclinical contributors to low BMD and/or fragility fracture in addition to hypovitaminosis D.

Among the other subclinical contributors besides hypovitaminosis D, PHPT, hyperthyroidism, SH, celiac disease, MGUS and male hypogonadism were found in 4.5, 1.3, 1.3, 1.2, 2.3 and 5.1% of patients respectively. In addition, the correction of hypovitaminosis D consented to individuate 68 patients with IH (out of 205, 33.2%) and five with PHPT (out of 27, 18.5%), who had not been diagnosed before vitamin D supplementation. This is important due to the high prevalence of hypovitaminosis D in Italy, even in the healthy population (39, 40, 41).

Finally, the presence of subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D is more frequent in patients younger than 65 years of age, with a BMI below 24 kg/m^2 and a fragility fracture.

Our study shows a higher prevalence of subclinical contributors to low BMD and/or fragility fracture other than hypovitaminosis D than previously reported (3, 7), possibly because of the extensive laboratory investigations used in the present study. Moreover, the lower cut-off used in the previous reports to define the vitamin D deficiency may also have played a role in underestimating the true prevalence of the subclinical contributors to low BMD and/or fragility fracture (3, 4, 20).

At variance with previous studies (1, 43), we did not find a higher prevalence of subclinical contributors of low BMD and/or fragility fracture in male than in female subjects. This may be due to the fact that we excluded patients with already known causes of secondary osteoporosis. In keeping with this, in the only previous study with a similar design, the authors did not find a difference in the prevalence of secondary osteoporosis between male and female patients (7).

A novel finding is that the prevalence of clinical fragility fractures and/or morphometric vertebral fractures is higher in patients with other subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D than in patients without them. Indeed, this point was not assessed in the study of Tannenbaum *et al.* (3), while in two other studies only fractured subjects were included (6, 7). The presence of a fragility fracture was found to be associated with the presence of subclinical contributors to low BMD and/or fragility fracture regardless of BMD, confirming that, in these patients, BMD explains only in part the increased fracture risk (1, 2, 44).

The prevalence of IH and PHPT in the present report was higher than previously reported (3, 4, 5, 6, 7, 20, 43), with the exception of the study of Romagnoli *et al.* (5). This may be explained by the fact that the previous studies used different criteria to define hypercalciuria and did not evaluate serum and urinary calcium levels after hypovitaminosis D correction (3, 4, 5, 6, 20). Indeed, the prevalence of IH we found before 25OHVitD levels normalization was definitely lower (22.8%) than that found after the achievement of normal 25OHVitD levels (33.2%). Similarly, the normalization of 25OHVitD levels led to an increase of the PHPT prevalence.

As compared with the present one, previous studies found a similar (20), lower (4, 5, 33) or higher prevalence (6, 7) of hyperthyroidism and celiac disease among patients with low BMD. The 'real life' population enrolled in the present study (i.e. patients with low BMD and/or clinical and/or morphometric fragility fractures)

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may explain these discordances. Indeed, the studies finding the highest prevalence of these disorders, in fact, included only osteoporotic patients (33) or those with clinical fractures (6, 7).

Similarly, the lower prevalence of male hypogonadism than previously reported (7, 20) could be explained considering that in the previous studies only patients with clinical fractures (7) or osteoporosis (20) were evaluated. However, the small sample of male patients (possibly explained by the reduced awareness of osteoporosis among males) could have biased this result.

The different patients' selection may also explain the lower prevalence of SH as compared with previous studies (16, 45). On the other hand, the very low SH prevalence reported by other authors (3, 5) is related to the use of urinary free cortisol as a screening test for SH, instead of the more sensitive 1-mg DST (34).

In keeping with previous data (46), MGUS was found in 2.3% of patients with apparent primary osteoporosis. This is important since it seems to be associated with an increased fracture risk (46, 47, 48). Finally, we made no diagnosis of rheumatologic disease. This may result from the fact that, in patients with a rheumatologic disease, pain is the predominant symptom that leads to prompt rheumatologic or orthopedic evaluation.

Overall, as compared with the previous reports, in addition to the large sample studied, the advantage of the present study is related to its 'real life' design that consecutively included all patients with apparently idiopathic bone density decrease and evaluated calcium metabolism after normalization of vitamin D levels. This consented to assess the prevalence of subclinical contributors to low BMD and/or fragility fracture besides hypovitaminosis D in a population not at particular high risk, but, on the other hand, to reliably estimate the prevalence of some disorders (i.e. PHPT and IH), which may be often underscored in the presence of hypovitaminosis D. Unfortunately, we do not have information regarding the duration of the diseases possibly leading to low BMD and/or fragility fracture and the vitamin D status of our patients before the study entry. In addition, since in Italy the hypovitaminosis D is highly prevalent even in the healthy population (39, 40, 41), we do not know in how many patients a long-standing condition of hypovitaminosis D can be reliably considered the main cause of low BMD and/or fragility fracture. To overcome this limitation, the prevalence of the subclinical contributors to low BMD and/or fragility fracture was reported even without considering hypovitaminosis D. Another limit of the present study may be that its protocol is hardly transferable in the clinical practice for because of the high cost. However, it must be noted that the biochemical workup we performed, with the exception of PTH, 25OHVitD, TSH, 1-mg DST, and IgA antitissue transglutaminase and endomysial, is considered the routine first-line testing in patients with reduced BMD and/or fragility fracture (48). On the basis of the present data, in addition to the first-line evaluations, the 25OHVitD levels assessment seems mandatory in all patients with low BMD and/or a fragility fracture, at least in Italy. On the other hand, the results of the present study show that patients showing the concomitant presence of three risk factors (age <65 years, BMI <24 kg/m^2 and a fragility fracture) are at higher risk of being affected with a subclinical contributor to low BMD and/or fragility fracture, regardless for BMD. As a consequence, the second-line evaluations (i.e. PTH, TSH, 1-mg DST and IgA antitissue transglutaminase and endomysial) might be reserved to these latter subjects, even if the first-line tests are negative. Indeed, the application of this protocol would have consented to correctly identifying more than 91% of patients affected with a subclinical contributor to low BMD and/or fragility fracture besides hypovitaminosis D (accuracy 91.2%), but 21 patients would have been missed (sensitivity 91.8%) and in 32 patients the second-line tests would have been useless (specificity 90.7%). However, it is important to underline that the great majority of the 21 false-negative patients had LS Z-score <-1.0 and/or a fragility fracture. In addition, if the second-line tests had been performed in patients with abnormal first-line tests and/or two rather than three risk factors among the presence of BMI <24 g/cm^2, age <65 years and a fragility fracture (i.e. a larger population), only ten patients would have been missed and the sensitivity for detecting a subclinical contributor to low BMD and/or fragility fracture would have increased to 96.1%, but at the expense of a decrease in specificity (60.3%). In our opinion, on the basis of the present data, a protocol including the first-line screening tests in all patients with low BMD and/or fragility fracture and the second-line tests in patients with abnormal first-line tests and/or the concomitant presence of three risk factors (age <65 years, BMI <24 kg/m^2 and a fragility fracture) may be suggested, due to its good specificity (SP) and sensitivity (SN) (Fig. 2). Indeed, even if the SN can be further increased if the second-line tests are performed in patients with abnormal first-line tests and/or two rather than three risk factors, this protocol would have lower SP and, therefore, higher costs. It must be observed, however, that the high costs of the correct diagnosis of the secondary causes of osteoporosis may be justified by the possibility of avoiding the costs of the clinical consequences of these disorders, since the treatment of these conditions leads to a reduction in the fracture risk, while their misdiagnosis can lead to inappropriate or inadequate treatment (1, 2). For these reasons, in the absence of cost-effectiveness data, it is difficult to individuate from the present study the adequate sensitivity and specificity of the first- and second-line tests. However, considering that the great majority of the 21 false-negative patients had LS Z-score <-1.0 and/or a fragility fracture, it is possible to hypothesize

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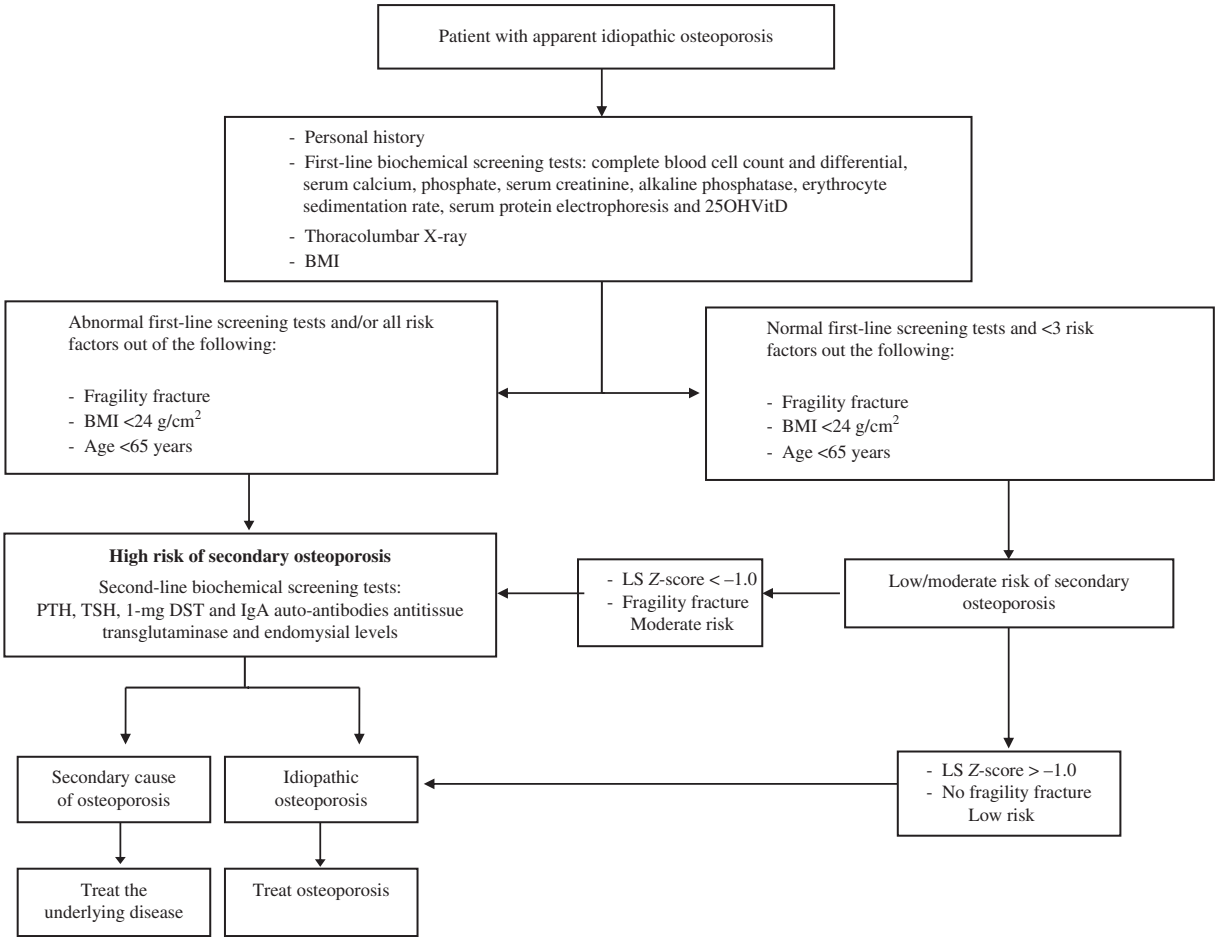

Figure 2 Algorithm for testing individuals with low BMD/fragility fractures for secondary causes derived from the results of the present study. PTH, parathyroid hormone; 1-mg DST, 1-mg overnight dexamethasone suppression test; LS, lumbar spine; fragility fracture, low-trauma clinical or asymptomatic fracture.

that a careful follow-up of patients without abnormal first-line tests and/or three additional risk factors at the first visit, but with a LS Z-score < -1.0 and/or a fragility fracture, may lead to discover an initially concealed subclinical form of secondary osteoporosis (Fig. 2). Large longitudinal studies regarding the cost-efficacy of the diagnostic protocols in patients with osteoporosis are needed in order to determine the best testing strategy for diagnosing the subclinical contributors to low BMD and/or fragility fracture.

In conclusion, our study demonstrates that in patients with apparent primary osteoporosis: i) hypovitaminosis D is a subclinical contributor to low BMD and/or fragility fracture in more than 70% of cases; ii) without considering hypovitaminosis D, a subclinical contributor to low BMD and/or fragility fracture is present in 45% of subjects, with IH being the most frequent cause; and iii) 30% of IH and 20% of PHPT patients are missed if the diagnostic workup is performed in the presence of hypovitaminosis D.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

Study design: C Eller-Vainicher, I Chiodini and A Scillitani. Study conduct: C Eller-Vainicher, I Chiodini, V V Zhukouskaya and E Cairoli. Data collection: C Eller-Vainicher, I Chiodini, V V Zhukouskaya, E Cairoli, V Morelli and S Palmieri. Data analysis: C Eller-Vainicher, I Chiodini, E Cairoli, V V Zhukouskaya, A Scillitani and P Beck-Peccoz. Data interpretation: C Eller-Vainicher, I Chiodini, A Scillitani and P Beck-Peccoz. Drafting manuscript: C Eller-Vainicher, I Chiodini, V Morelli, A Scillitani and S Palmieri. Revising manuscript content: P Beck-Peccoz, A Scillitani and I Chiodini. Approving final version of manuscript: C Eller-Vainicher, E Cairoli, V V Zhukouskaya, V Morelli, S Palmieri, A Scillitani, P Beck-Peccoz and I Chiodini. C Eller-Vainicher and I Chiodini take responsibility for the integrity of the data analysis.

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