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ORIGINAL ARTICLE

High level of serum undercarboxylated osteocalcin in patients with incident fractures during bisphosphonate treatment

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Abstract To evaluate the possible interaction of metabolic effects in the mevalonate pathway between amino-bisphosphonates (amino-BP) and vitamin K, the serum level of undercarboxylated osteocalcin (ucOC) was measured in amino-BP users in relationship to incident fracture occurrence. Osteoporotic patients (mean age, 70.7 ± 9.1 years; $n = 231$) treated with alendronate or risedronate were followed for 3.4 ± 2.1 years, and observations regarding the presence or absence of incident fractures in their vertebrae were made based on vertebral X-ray films every year. During the observation period, new fractures were found in a total of 71 patients (incident vertebral fracture, $n = 61$; the remaining 10 patients had long bone fractures). The baseline data of the patients with incident fractures indicated that incident fractures are more likely to occur in older patients who have a higher number of prevalent vertebral fractures and lower baseline lumbar bone mineral density (LBMD) as compared to patients without incident fractures. There was no significant difference in the changes of LBMD and urinary excretion of NTX after treatment. On the other hand, the serum level of ucOC in patients with incident fractures and with amino-BP treatment was significantly higher (2.75 ± 0.19 ng/ml) than that in patients without incident fractures and with amino-BP treatment (2.28 ± 0.13 ng/ml) ($P = 0.038$). These results indicate that older age, a greater number of prevalent fractures and higher ucOC levels, and lower LBMD are risks for incident fractures despite use of amino-BP. The time-dependent incident fracture rate was higher in accordance with an increase in the number of risk items ($P < 0.001$ in log-rank and Wilcoxon tests). In conclusion, measurement of undercarboxylated osteocalcin may be useful for assessing fracture risk in patients receiving amino-BP treatment.

Keywords Undercarboxylated osteocalcin (ucOC) · Bisphosphonates treatment · Osteoporosis · Incident fractures · Bone mineral density

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Introduction

Recent progress in key pathogenesis of osteoporosis has focused on bone resorption through increased osteoclastic activity. Bisphosphonates specifically inhibit osteoclastic activity through inhibition of the mevalonate pathway [1], achieving a decrease in bone turnover followed by an increase in secondary mineralization of bone. Such actions of bisphosphonates are connected to prevention of bone fractures in osteoporosis [2] because bisphosphonates turn the negative bone balance positive. Although bisphosphonate has been established as a first-line drug for preventing fractures in osteoporosis, complete inhibition of new fractures in osteoporosis has not been achieved [3–5]. This failure may be partly explained by the concept that complex pathogenesis of osteoporosis and the reduction in bone turnover or increase in bone density induced by bisphosphonates may not be sufficient to achieve thorough inhibition of incident fractures. In fact, deficiencies of many

nutrients such as vitamin D, calcium, and vitamin K have accounted for possible risk factors of incident fractures in osteoporosis [6, 7]. Among these nutrients, vitamin K deficiency or insufficiency has been consistently reported as a risk factor for osteoporotic fractures [7–9]. Vitamin K is thought to maintain bone strength through gamma-carboxylation of matrix glutamic acid residues of protein. In vitamin K insufficiency or deficiency, a small amount of undercarboxylated osteocalcin (ucOC) is released from the osteoblasts into circulation. Thus, the serum concentration of ucOC has been recognized as being a sensitive marker of vitamin K deficiency in bone. Serum ucOC decreases significantly after menatetrenone (vitamin K_2) [10, 11] or vitamin K_1 [12] treatment, suggesting that vitamin K homologues may improve bone osteocalcin content and may be linked to reduction of the incident fracture rate. Furthermore, Okano et al. [13] reported that phylloquinone (vitamin K_1) can be converted to menaquinone (vitamin K_2) in various cells, including osteoblasts, through geranylgeranylation in the side chain, and that menaquinone 4 was considered to be an active form of vitamin K because menaquinone 4 was reported to bind to nuclear receptor SXR [14]. It is possible that this metabolic process of vitamin K activation may be inhibited by bisphosphonates as a result of inhibition of geranylgeranylation of protein through reduction of farnesyl diphosphate (FPP) synthase activity [1, 2]. Therefore, there may be a close relationship between the effect of bisphosphonates on cell function and vitamin K activation in the same cell. However, until now, there have been no data regarding the relationship between the state of vitamin K and the effect of bisphosphonates on fracture prevention. In this study, the authors attempted to investigate preliminarily whether the state of vitamin K in bone modulates the effect of bisphosphonates on fracture prevention.

Materials and methods

Subjects

Ambulatory postmenopausal women more than 45 years old with primary osteoporosis and undergoing amino-bisphosphonates (amino-BP) treatment during the period from January 2000 to June 2008 were eligible for participation in the study. Exclusion criteria consisted of endocrine disorders such as hyperthyroidism or hyperparathyroidism, a history of extensive gastrointestinal surgery or chronic renal failure, and current use of medications known to result in secondary osteoporosis. The patients were participants in a Nagano cohort study, and therefore baseline examinations such as bone density measurement and measurements of serum levels of calcium, phosphate, and urinary excretion of N-telopeptide of type I collagen (NTX) had been performed for baseline data with informed consent. Baseline X-ray examinations to confirm the presence or absence of preexisting fractures were also performed at the time that the patients registered for the Nagano cohort study. The period for conducting follow-up observations of each participant was calculated as the time from their inclusion into the study up to their death, minus 1 year after the occurrence of incident fractures or to the end of June 2009, whichever occurred first. Follow-up was conducted on all the subjects in this study for more than 1 year.

Intervention

Study subjects were started on amino-BP treatment, either alendronate 5 mg/day orally or risedronate 2.5 mg/day orally by Japanese dosage regulation. Alternatively, some patients received equivalent weekly doses of alendronate (35 mg/week) or risedronate (17.5 mg/week). Patients were continued on amino-BP treatment for the duration of their participation in the study. Vitamin K_2 administration was prohibited in the participants. All the patients were treated with amino-BP alone during the entire observation period.

Bone mineral density (BMD) measurements

Lumbar spine bone mineral density (LBMD) was measured by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L or DPX-IQ (Lunar Corporation, Madison, WI, USA). The interassay variance of LBMD in the laboratory was 0.5 ± 0.5% [coefficient of variation (CV) ± SD] [15]. To guard against machine drift, a quality assurance test was carried out for every measurement. The baseline value of LBMD was used to diagnose osteoporosis, and measurements of LBMD were repeated every 6 months. The value of the last observation was used as the value of LBMD after the treatment.

Detection of prevalent and incident vertebral fractures

Prevalent and incident vertebral fractures were diagnosed by a semiquantitative visual method using lateral thoracolumbar spine radiographs in accordance with the method described by Genant et al. [16]. To detect incident vertebral fractures, spine radiographs were routinely taken at 1-year intervals, and additional X-rays were taken whenever the subjects complained of symptoms suggestive of new clinical vertebral fractures. Both new clinical and morphometric fractures were counted as incident vertebral fractures. Incident long bone fractures were identified from medical records or confirmed using X-ray films. Although incident clinical (symptomatic) fractures in vertebrae or

other parts of the bone structure were easily recognized when they occurred, morphometric vertebral fractures were sometimes difficult to detect clinically. Because the exact timing when a morphometric incident vertebral fracture occurred could not be determined for some of the patients with such fractures, the time of the spinal radiograph showing the fractures was considered as being the time of the fracture. Fractures induced by major trauma were excluded from the analysis; namely, fractures induced by a fall from standing height were categorized as incident fractures, but fractures induced by a fall from a point higher than body height were excluded.

Diagnosis of osteoporosis

Diagnosis of osteoporosis was made in accordance with the osteoporosis diagnostic criteria (2000 version) proposed by the Japanese Society for Bone and Mineral Research [17]. Osteoporosis is diagnosed as the presence of fragility fractures in any bone lesion in a person with a BMD less than 80% (–1.63 SD) of the BMD of the young adult mean (YAM). Osteoporosis is also diagnosed when the LBMD is less than 70% (–2.45 SD) of the BMD of a YAM, even if the person has no prevalent fragility fractures.

Biochemical indices

Nonfasting serum and urine samples were collected as baseline data at the time of enrollment. Routine biochemical data including serum levels of calcium and phosphate were analyzed immediately using an autoanalyzer. Urinary N-terminal telopeptides of type I collagen (NTX) were measured with an enzyme-linked immunosorbent assay (ELISA) kit (Osteomark Ostex, Princeton, NJ, USA), and the value of NTX was standardized by the concentration of creatinine in the same urine sample. Urine samples were collected during the second voiding of the day. Urinary NTX was measured before and at the end of the observation.

Measurement of undercarboxylated osteocalcin

Serum level of undercarboxylated osteocalcin (ucOC) was measured using a new electrochemiluminescence immunoassay (Sanko Junyaku, Ibaraki, Japan) [8]. Because ucOC measurements were not available when the study began, serum level of ucOC could not be measured at baseline for any of the participants, but during treatment values could be obtained for all participants. Measurement of ucOC in patients without incident fracture was conducted at the end of the observations. On the other hand, for patients with incident fractures, serum samples were taken 1 or more years after the occurrence of incident fractures to determine ucOC.

Ethical considerations

The study protocol was reviewed by the ethical committee of the Research Institute and Practice for Involutional Diseases (RIPID), and comprehensive written informed consent was obtained from all study subjects.

Statistical analysis

In the descriptive analysis of the baseline characteristics, numerical data are expressed as mean ± SD. Comparisons of baseline characteristics between subjects with and without incident fractures were performed using two levels of one-way analysis of variance (ANOVA). Comparisons between the values before and after treatment were based on a paired t test. To assess confounding effects of the risks, stepwise multiple regression analysis was used. After confirmation of independent risks for incident fractures in amino-BP users, secondary analyses were carried out: the sum of the existing risk factors in individual subjects was calculated, and the patients were categorized by the calculated number of risks. Subsequently, time-dependent incident fracture rates were analyzed using a Kaplan–Meier plot. Here, the number of patients with high ucOC in each category of risk was tested by Pearson's Chi-square test. The level of significance was set at less than 0.05 (Table 1).

Results

Demography of the subjects

From among the patients visiting the outpatient care unit of Research Institute and Practice for Involutional Diseases, a total of 269 patients with osteoporosis were recruited for this study. Of these patients, 38 were excluded from the study because of lack of baseline data or missing follow-up data. The remaining 231 patients were followed for 1 or more years and were adapted to the following analyses. The mean ± SD age of participants was 70.0 ± 9.1 years old, and 140 subjects (60.6%) had prevalent fractures. The average observation period was 3.4 ± 2.1 years, with the longest observation period being 9 years. After bisphosphonate treatment, urinary excretion of NTX decreased significantly, from 55.2 to 30.0 nM/mM Cr (45.7% of the baseline; $P < 0.0001$ in paired t test), and LBMD increased significantly, from 0.774 to 0.844 g/cm^2 (+9.0% increase from baseline; $P < 0.0001$ in paired t test).

Table 1 Comparison of baseline data and data at end of observation of subjects

Item	Baseline	End of observation
Age (years)	70.7 ± 9.1	74.1 ± 8.9*
Body weight (kg)	49.1 ± 7.4	47.5 ± 7.6
Body height (cm)	149.2 ± 6.3	148.2 ± 5.8
Serum Ca (mg/dl)	9.19 ± 0.41	9.24 ± 0.43
Serum Pi (mg/dl)	3.49 ± 0.46	3.50 ± 0.50
NTX (nM/mM Cr)	55.2 ± 30.7	30.0 ± 19.3*
Initial bone mineral density (BMD) (g/cm^2)	0.774 ± 0.129	0.844 ± 0.144*

Values are expressed as mean ± SD. *$P < 0.0001$ versus baseline in paired t test

Table 2 Fracture outcomes during the observation

Site of fractures	Baseline	Incident fractures
None	208	160
Vertebrae	117	61
Colles	9	4
Hip	5	2
Other sites	9	4

Multiple prevalent fractures in multiple bone sites were observed in 14 cases; incident fractures were counted as the first incident fracture

Prevalent and incident fractures in the participants

A total of 154 sites of prevalent fractures were counted in 140 patients, indicating that 14 cases had multiple prevalent fractures. Incident fractures were observed in 71 cases during the observation period, and the most prominent fracture site was the vertebral body, with both morphometric and clinical symptomatic fractures (Table 2).

Baseline data of patients with and without incident fractures

To screen for risks for incident fractures in amino-BP users, comparisons were made on baseline data between patients with incident fractures and those without incident fractures during the observation period. Table 3 shows the comparison of baseline data between patients with and without incident fractures. Patients with incident fractures during amino-BP treatment were characterized by older age and a lower initial lumbar BMD as compared to the patients without incident fractures. The number of prevalent vertebral fractures in the patients with incident fractures was higher than that of the patients without incident fractures, suggesting that incident fractures may occur in more severe cases of osteoporosis even during bisphosphonate treatment.

Bone outcomes after treatment in patients with and without incident fractures

Follow-ups were conducted on all the patients treated with amino-BP in the form of LBMD, urinary NTX, serum levels of calcium and phosphate, and measurement of ucOC at the end of the observation period. Comparisons were made in the values obtained between patients with and without incident fractures to determine what kinds of changes occurred in bone parameters after the treatment in association with incident fractures (Table 4).

Among the various outcomes related to bone metabolism, only serum level of ucOC was significantly higher in the patients with incident fractures than in those without, suggesting that vitamin K deficiency in bone may exist in the patients with incident fractures.

Stepwise regression analysis for the risk of future fractures in amino-BP users

From the primary analyses, the baseline age, LBMD, number of prevalent vertebral fractures, and ucOC after treatment were considered to be risks for fracture susceptibility in bisphosphonate users. To exclude confounding factors, multiple stepwise regression analysis among these risks was performed. The four risks just mentioned were recognized as independent risks for incident fractures in bisphosphonates users (Table 5).

Logistic regression analysis and receiver operating characteristic (ROC) analysis for each risk to evaluate the risk assessment of each patient (Fig. 1)

To evaluate the time-dependent fracture rate, secondary analyses using a Kaplan–Meier plot analysis were performed. After deciding the cutoff value for each risk using ROC analysis, the sum of the risks (0–4) was calculated for each participant. The cutoff values for each risk were as follows: 75 years or older for age, 0.763 g/cm^2 or less for LBMD, two or more for number of prevalent vertebral fractures, and 2.6 ng/ml or more for ucOC. The patients were divided into five categories in accordance with the presence of risks. Group 0 consisted of patients without any risk ($n = 31$), group 1 consisted of patients with one risk ($n = 67$), group 2 consisted of patients with two risks ($n = 75$), group 3 consisted of patients with three risks ($n = 47$), and group 4 consisted of patients with four risks ($n = 11$). Groups 0, 1, 2, 3, and 4 consisted of 2, 11, 20, 24,

Table 3 Baseline data in the patients with or without incident fractures during amino-bisphosphonates (amino-BP) treatment

Items	Incident fracture (−)	Incident fracture (+)	*P*
Number of cases	160	71	–
Age (years)	69.0 ± 0.7	74.3 ± 1.1	<0.0001
BMI (kg/m^2)	21.8 ± 0.2	22.3 ± 0.4	0.272
Serum Ca (mg/dl)	9.23 ± 0.03	9.14 ± 0.05	0.060
Serum P (mg/dl)	3.53 ± 0.04	3.41 ± 0.05	0.083
NTX (nM/mM Cr)	54.1 ± 2.5	57.2 ± 3.7	0.448
Initial BMD (g/cm^2)	0.788 ± 0.010	0.743 ± 0.015	0.0157
Number of prevalent vertebral fractures	1.09 ± 0.16	2.58 ± 0.25	<0.0001

The baseline data were compared between the patients with or without incident fractures, retrospectively. A total of 71 patients had new fractures during amino-BP treatment. The patients with incident fractures were of higher age, with a greater number of prevalent vertebral fractures and lower lumbar bone mineral density (LBMD) at baseline. Data are expressed as mean ± SE. Statistical analysis was made by analysis of variance (ANOVA)

Table 4 Bone-related outcomes at the end of observation in the patients with or without incident fractures

Outcomes	Incident fracture (−)	Incident fracture (+)	*P*
LBMD (g/cm^2)	0.854 ± 0.011	0.823 ± 0.017	0.131
Ca (mg/dl)	9.26 ± 0.03	9.16 ± 0.05	0.091
P (mg/dl)	3.54 ± 0.04	3.43 ± 0.06	0.140
NTX (nM/mM Cr)	28.6 ± 1.5	33.2 ± 2.3	0.104
ucOC (ng/ml)	2.28 ± 0.13	2.75 ± 0.19	0.038

Data listed in Table 4 were obtained at the end of the observations. In a case having incident fracture, serum and urinary samples were taken 1 or more years after the occurrence of incident fracture or 1 or more years after the recognition of morphometric fracture (2.6 ± 0.6 years after the recognition of incident fracture). Data are expressed as mean ± SE. ucOC, undercarboxylated osteocalcin

Table 5 Stepwise regression analyses of the risks for fracture susceptibility in amino-BP users

Risk	χ^2	*P*	R^2
Number of prevalent vertebral fracture	21.999	0.0000	0.0772
Age (years)	9.1164	0.0025	0.1092
ucOC (ng/ml)	4.4385	0.0351	0.1247
Baseline LBMD (g/cm^2)	3.8923	0.0485	0.1384

Stepwise regression analysis was performed among the four risks, which may relate to having incident fractures during amino-BP treatment. All four risks were significantly associated with incident fractures

and 9 patients with incident fractures, respectively. The Kaplan–Meier plot indicated a significantly higher susceptibility of incident fractures in accordance with an increasing number of risks (see Fig. 1). Furthermore, the number of patients with high ucOC (>2.6 ng/ml) in each category is shown in Table 6. The percentages for the patients with high ucOC were significantly increased in

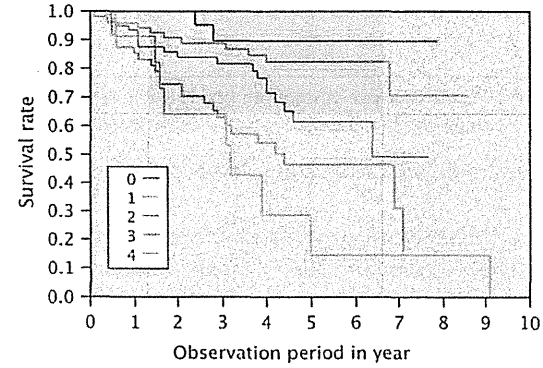

Fig. 1 Kaplan–Meier plot of the categorized patients under amino-bisphosphonate (amino-BP) treatment in accordance with the number of fracture risks (0–4). The lower survival rate from incident fractures was observed in accordance with the number of fracture risk in patients with amino-BP-treated osteoporosis ($P < 0.001$ in log-rank and Wilcoxon test)

accordance with increase in number of risks ($P < 0.0001$), providing further proof that high ucOC was an independent risk factor for incident fractures in osteoporosis during bisphosphonates treatment.

Discussion

In this study, traditional risk factors, such as older age, low BMD, and the presence of preexisting fractures [18], for incident fractures in osteoporosis were also recognized as risks for incident fractures in bisphosphonate users. However, no significant contribution of incident fractures to changes in LBMD and bone resorption markers was seen, although such changes considerably favored fracture prevention; that is, LBMD increased by about 11% and 8% for patients with and without incident fractures, respectively, and urinary excretion of NTX decreased by about 60% and

Table 6 Rate of patients with high ucOC in five risk categories

Risk category	ucOC < 2.6 ng/ml	ucOC ≥ 2.6 ng/ml	Percent (%)
0	31	0	0
1	48	19	28.4
2	46	29	38.7
3	19	28	59.6
4	0	11	100

The trend for the rate of patients with high ucOC was apparently increased in accordance with increase in number of risks. $\chi^2 = 49.1$ and $P < 0.0001$ by Peason's Chi-square test

50% for patients with and without incident fractures, respectively. These changes in LBMD and urinary NTX were not significantly different between patients with and without incident fractures. These changes in parameters were in good agreement with previous reports obtained from Japanese postmenopausal osteoporosis [19]. Therefore, the biological effects of bisphosphonates on bone density and resorption were identical regardless of the occurrence of new fractures during treatment. Previous reports have indicated that increase in BMD and decrease in bone markers were not strong predictors for future fractures in bisphosphonates users [20–24].

On the other hand, the serum level of ucOC of fractured patients was significantly higher than that of patients without any new fractures. Secondary analysis using stepwise regression analysis revealed that the level of ucOC in amino-BP users was a significant independent risk for incident fractures (Table 5). Because ucOC was measured after an incident fracture occurred, it was thought that the difference in ucOC levels between patients with and without incident fracture may have reflected the occurrence of a fracture but not a vitamin K deficiency in bone. However, this possibility is unlikely because urinary excretion of NTX did not differ between these two groups, indicating that there was no significant difference in bone resorption. It has been recently reported that the biological action of amino-BP and activation of vitamin K have a point of contact, that is, the geranylgeranylation of molecules. Amino-BP inhibits this process [1, 2] and, in contrast, vitamin K activation requires geranylgeranylation of the side chain [13]. Therefore, it is possible that bisphosphonates may interfere with vitamin K activation in bone cells, especially in patients with preexisting vitamin K deficiency. This hypothesis prompted the authors to measure ucOC in patients treated with amino-BP. Results indicate that patients with incident fractures during amino-BP treatment may be lacking vitamin K in bone cell levels consequent to a higher level of ucOC. However, Hirano et al. [24] reported that amino-BP treatment caused a decrease in ucOC level but resulted in no change in carboxylated osteocalcin (cOC), suggesting that amino-BP did not decrease the carboxylation of osteocalcin. So, further clarification is required whether amino-BP treatment affects carboxylation on osteocalcin in both patients with or without vitamin K deficiency in a prospective study design. This is the first report that suggests the usefulness of measuring ucOC in amino-BP users in terms of fracture prediction. Because ucOC decreased after vitamin K_2 treatment [11], concurrent use of vitamin K_2 with amino-BP may be effective in preventing the occurrence of new fractures in patients with higher ucOC levels during treatment with only amino-BP.

The limitations of this study were as follows. First, the present results must be evaluated as a prospective study design because the present study was a retrospective study. Second, a larger number of participants is necessary to increase statistical power. Third, direct evidence that shows that bisphosphonates inhibit vitamin K activation in bone cells is required in vitro, and we have to measure serum carboxylated osteocalcin level together with ucOC to assess gamma-carboxylation during amino-BP treatment in vivo. Last, because other risk factors for fracture such as the physical activity of the participants was not evaluated in the present study, those factors related to increased susceptibility to fractures should be incorporated into the model in future.

In conclusion, it is useful to evaluate the serum level of ucOC in patients with amino-BP to predict future fracture occurrence.

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Design of a pragmatic approach to evaluate the effectiveness of concurrent treatment for the prevention of osteoporotic fractures

Rationale, aims and organization of a Japanese Osteoporosis Intervention Trial (JOINT) initiated by the Research Group of Adequate Treatment of Osteoporosis (A-TOP)

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Abstract The aim of osteoporosis treatment is to prevent future fractures. Although concurrent treatment has been used very frequently for osteoporosis in clinical practice, there are no data on accurate and verified effectiveness of concurrent treatment for fracture prevention in patients with osteoporosis. To clarify the clinical usefulness of concurrent treatment, the Japan Osteoporosis Society has authorized the establishment of the A-TOP (Adequate Treatment of Osteoporosis) research group. The objective of this research is to establish a design for a clinical trial to prove whether concurrent treatment using both alfacalcidol (1-alpha-hydroxycholecalciferol) and alendronate is more effective as compared to treatment using alendronate alone

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in terms of fracture prevention. The present study was named JOINT (Japanese Osteoporosis Intervention Trial) and is based on a method using national, prospective, randomized, open-labeled, blinded endpoints focusing on postmenopausal osteoporosis with a high risk for fracture. The patients were mainly selected by practitioners and allocated randomly by a central registration system into two groups, of which one received 5 mg/day of alendronate alone, and the other received 1 μg/day of 1-alpha-hydroxycholecalciferol (alfacalcidol) in addition to the alendronate. The endpoints focused primarily on fracture prevention, and the patients' quality of life (QOL) and change in body height, as well as adherence and the adverse events of the treatments were evaluated secondarily. To obtain sufficient statistical power in the events during a 2-year observation period, the patients who are expected to have higher risk were selected to participate in this study, and it was decided that the final plan would involve 890 patients per group (two-sided alpha = 0.05, power = 0.8). Data collection began in November 2003. Correspondence regarding the registration of the investigator and the progress of the study was conducted through a web system from the Public Health Research Foundation to practitioners.

Keywords Alendronate · Alfacalcidol · Concurrent treatment · Fracture prevention · Osteoporosis

Introduction

Osteoporosis, which is characterized by compromised bone strength and increased susceptibility to fractures, which lead to deterioration in the QOL and increased mortality, is a national burden on an aging society [1, 2]. However, recent studies indicate that treatment with a parathyroid hormone, bisphosphonates or a selective estrogen receptor modulator (SERM) [3–9] may decrease the risk of fractures in patients with osteoporosis.

Although bisphosphonate treatment currently represents the most powerful form of treatment available for fracture prevention in osteoporotic patients, it has not succeeded in completely preventing osteoporotic fractures [3–5, 7–9]. Therefore, concurrent treatment of osteoporosis has been frequently used by Japanese practitioners without any concrete evidence regarding fracture reduction. Since the concept of evidence-based medicine (EBM) has been introduced to clinical practice since the 1990s [10], the Japan Osteoporosis Society and the Japanese Society of Bone Mineral Research have edited the clinical guideline for treatment of osteoporosis (Chief editor: Hajime Orimo [11]). However, the writers recognized that there was a lack of evidence in the effectiveness of concurrent treatment of osteoporosis. Furthermore, it was expected that the patients who visit clinics have varying degrees of risk of fracture, which may differ from the degree of those who participated in development trials for bisphosphonates. This possibility would make it easier to obtain pragmatic evidence in general clinical practice.

Starting in 2000, the Japan Osteoporosis Society had planned to investigate the effectiveness of treatment of osteoporosis in order to provide evidence to general practitioners. Before constructing evidence, some feasibility studies were required to confirm the consensus in the diagnosis of incident fractures among the researchers and to elucidate the risk of future fractures in the patient population. In addition to these efforts in the field of osteoporosis, the Japanese government also established an

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ethical guideline for clinical trials [12], and the International Committee of Medical Journal Editors launched a clinical trial registry [13]. Such kinds of progress in the circumstances of clinical trials have enabled for investigator-initiated clinical trials in general practice.

The Adequate Treatment of Osteoporosis (A-TOP) study group was established in 2000 [11] in affiliation with the Japan Osteoporosis Society and organized a team for clinical trial management. The team consisted of clinical investigators (planning and analysis), foundation (funding) managers, officers from non-profit organization (data management) and several companies (data collection). This was the first joint team to create the post-making evidence for osteoporosis. In November 2003, A-TOP initiated a randomized clinical trial referred to as the Japanese Osteoporosis Intervention Trial (JOINT). The purpose of JOINT was to confirm the clinical significance of concurrent use of osteoporotic drugs. The first protocol, named JOINT-01, was initiated in 2002, but was suspended the following year due to a change in drug labeling. A second protocol, named JOINT-02, was established to clarify the effect of adding 1-alpha-hydroxycholecalciferol (alfacalcidol) to alendronate (ALN), using the incident fracture rate as the primary endpoint. In this paper, the rationale, organization and study design of JOINT-02 are introduced.

Rationale and aims

In 2002, the Japan Society of Osteoporosis sent a letter to randomly selected practitioners and enclosed a questionnaire regarding whether concurrent treatment using bisphosphonate and another drug was being utilized to treat osteoporosis. Surprisingly, 87.8% (79/90 practitioner) of the doctors who responded did have experience using concurrent treatment [14]. The most frequent drugs used in concurrent treatment with amino-bisphosphonate were alfacalcidol (93.7%), followed by calcitonin (50.6%), as there were expectations for these drugs to exhibit more potent inhibition of fracture occurrence or more significant increase in BMD, even though there was no apparent evidence. In addition to the lack of evidence related to fracture prevention, the safety profile of concurrent treatment had not been evaluated. Thus, evaluations of the effectiveness and safety of concurrent treatment were urgently required. Etidronate [15] and ALN [8, 9] were used as the drugs to confirm anti-fracture effectiveness in comparison to alfacalcidol in Japanese osteoporotic patients. However, these clinical trials were carried out at specific institutions and were initiated by experts in accordance with tight regulations. As a result, there may have been differences in the selected treatment and in the backgrounds of the patients between treatments conducted at these institutions and those conducted in general practice. In addition, the adherence of the treatment is expected to be lower in general practice than in institutions with experts who are committed to developmental trials. Thus, pragmatic study is urgently needed to evaluate whether bisphosphonates are effective to the same extent at the level of general practitioners as compared to the prior study (Phase III study).

Feasibility studies

The Japan Osteoporosis Society started discussions to execute a national clinical trial for obtaining evidence regarding the effectiveness of concurrent treatment in 2000. An executive committee of A-TOP was organized in 2002 and planned on forming a consensus regarding judgment standards for pre-existing fractures and incident vertebral fractures [16]. Morphometric criteria for incident fractures combined with a semi-quantitative assessment were thought to provide useful information on the study of clinical osteoporosis, especially for international comparisons. Next, to assume the number of participants in the clinical trial, the incident fracture rate and the risk of incident fracture were analyzed in the patient population, and the number of participants with sufficient statistical power [17] was calculated. Bone resorption marker was an independent risk factor for incident vertebral fractures in Japanese women. When the newly discovered risk factor was incorporated into the inclusion criteria in addition to conventional selection criteria such as age, prevalent fractures and bone mineral density, a reduction of about 40% in the estimated sample size was achieved. Thus, measurement of bone resorption markers is useful in reducing the sample size and the observation period in fracture-prevention studies carried out for developing drugs used to treat osteoporosis.

Materials and methods

Study design

Objective

JOINT was the first national, prospective, randomized, multicenter, open-labeled, blinded endpoints, controlled trial for osteoporosis made up mainly of practitioners of investigators in Japan. The objective of JOINT-02 was to clarify additive efficacy in terms of fracture prevention and safety, QOL and adherence in simultaneous use of alfacalcidol and ALN.

Subjects, intervention and endpoints

Confirmations regarding the patients were made by practitioners based on the inclusion and exclusion criteria (Table 1) after obtaining written informed consent. The

Table 1 Inclusion and exclusion criteria

Inclusion criteria
Postmenopausal osteoporosis[a]
Over 70 years old
Ambulatory patients who do not require any help
Able to answer QOL questionnaire
Corresponds to more than one of A-TOP's risk factors for fracture[b]
Exclusion criteria
Metabolic bone diseases other than osteoporosis[c]
Contraindication to the drugs (ALN or alfacalcidol)
Dysfunction in communication of intentions
Severe degenerative deformation of vertebra
Abnormal heart function
Abnormal hepatic function
Abnormal kidney function
Treatment of osteoporosis by bisphosphonate within 6 months prior to the present study

These thresholds were decided by risk analysis by the A-TOP research group

[a] Over 1 year after menopause

[b] Pre-existing vertebral fracture number ≥1; BMD ≤ Young Adult Mean -3 SD; Urinary DPD ≥7.6 nmol/mmol Cr; or NTX ≥ 54.3 nmol BCE/mmol Cr

[c] Hyperparathyroidism and hyperthyroidism were excluded

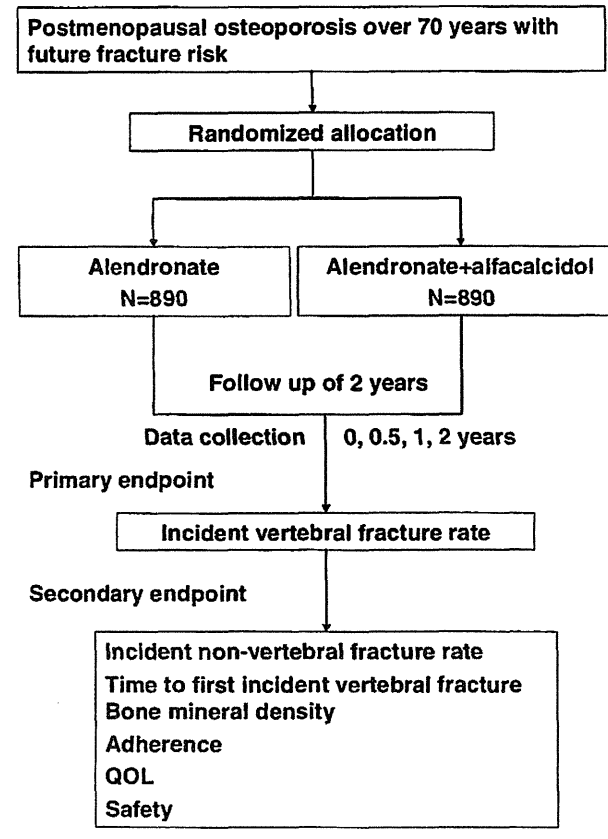

Fig. 1 Study design and outcomes

participants were selected by the practitioners and registered by Japan Clinical Research Support Unit (JCRSU), and then randomly allocated with a modified minimization method using age, number of pre-existing vertebral fracture number, bone mineral density (BMD) and value of bone metabolic marker into the group to be administered only ALN or the group that was to be administered both ALN and alfacalcidol. Registration and allocation of the participants were carried out on the Internet. After initiation of the assigned treatment, clinical data were collected at intervals of half a year for 2 years by I'cros Co., Ltd., through their visiting data collection service. Data were input to the database using a web system developed by ING Corporation. The primary endpoint was to compare the incident vertebral fracture rate between the intervention arms. The secondary endpoints were to compare the differences in the time to first incident vertebral fracture, non-vertebral fracture rate, bone mineral density, adherence, QOL and safety (Fig. 1). In addition, sub-group analyses categorized by baseline characteristics such as age, body mass index (BMI), serum 25-hydroxyl vitamin D levels, the number of pre-existing vertebral fractures and fracture grade were candidate factors. If participants wanted to change the designated treatment because of side effects or occurrence of fractures, they were permitted to do so, and follow-up observations were continued. Please see Table 1 and Fig. 1.

Sample size

Assumptions regarding the fracture rate in the ALN group were made based on a paper by Kushida et al. (Phase III trial for alendronate), in which it is reported that there was a 12.2% fracture rate during observations conducted over 2 years [8]. Since there are not much data on concurrent use of ALN and alfacalcidol [18], the authors' expectations were such that the effects of ALN would be added to those of alfacalcidol and that the hazard ratio of the alfacalcidol combined arm to ALN alone would be 0.64 [19]. The sample size was then estimated to be 890 cases per arm (two-sided alpha = 0.05, power = 0.8), taking account of a dropout rate of 10% referring to the value of the prior clinical trial of fracture intervention [4].

Fracture evaluation

X-ray films of conventional lateral radiographs of lumbar and thoracic vertebrae were taken and collected by I'cros Co., Ltd. After masking the patient's information, two

independent readers (orthopedist, TN and radiologist, MF) simultaneously reviewed films from T4 to L4 in chronological sequence and graded vertebral fracture based on a semi-quantitative method [20]. Before the start of the study, these two observers held meetings to make adjustments between their own criteria for grading vertebral fractures. When the diagnosis of pre-existing fractures made by the reviewers differed from those made by the practitioners, the reviewers' diagnosis was adopted preferentially. If inconsistencies arose between the readers in diagnosing pre-existing and incident vertebral fractures, the two readers negotiated between themselves to reach a consensus. Incident bone fractures other than those of the vertebrae were comprehended from the chart, and the occurrence of fractures was confirmed based on X-ray films or a record of the operation.

Clinical data

BMD at the lumbar vertebrae, hip (proximal femur), distal radius (dual energy X-ray absorptiometry) or left-sided second metacalpal bone (microdensitometry) was measured at baseline and at 6-month intervals for 2 years at each institute. The data of BMD obtained from the different machines and from different bone sites were calculated as the percentage change in each time point from the baseline value. The statistical difference in change of BMD between the group that received combined treatment and the group that was administered only ALN was compared based on each set of data for BMD for the different bone sites. Body height was measured at baseline, 12 and 24 months. Bone turnover markers (urinary type I collagen cross-linked N-telopeptide or urinary excretion of deoxypyridinoline) were measured at baseline and again 6 months after initiating treatment. QOL was assessed by using self-administered questionnaires (JOQOL and EQ-5D) at baseline and at 6, 12 and 24 months after initiating treatment [21]. Serum samples were sent to the central laboratory (SRL Co., Japan), and 25-hydroxyl vitamin D concentrations were measured. Other routine biochemical examinations were carried out at baseline and at 2 years after initiating treatment in order to estimate biochemical adverse events. All adverse events were reported to JCRSU, coded by MedDRA, and categorized as either "known" or "unknown." If an unknown adverse effect occurred, it was reported to the investigator and ethical committee.

Ethics and registration

Ethical issues regarding protocol were reviewed by the ethical committee for JOINT under the Declaration of Helsinki (Dr. Rikushi Morita, Chairman). If it turned out that a patient was at a disadvantage under observation, the ethical committee was given permission to stop the protocol. This study was registered at UMIN-CTR (University Hospital Medical Information Network—Clinical Trial Registry) with the number C000000001.

Statistical analysis

Analysis of the intent to treat principle was applied to the statistical analysis. Efficacy analysis uses a full analysis set (FAS), and all of the enrolled patients are applied to the analysis except for patients without efficacy data, patients who do not correspond to inclusion criteria and patients who do not receive treatment. The PPS (protocol per set) group is defined as consisting of patients without any serious protocol violation.

Recruitment of the practitioner

The explanatory meeting of the protocol and registration was held in all of Japan. The executive members of the A-TOP research group were responsible for the presentation of the protocol and for the recruitment of study institutions and practitioners. The A-TOP committee created the WEB site on the Internet so that the registration of the study could be executed directly. I'cros Co., Ltd., was also involved as a collaborator in calling practitioners.

Results and discussion

Several principles had to be considered for acceptance of concurrent treatment: firstly, the concurrent treatment should be clinically and statistically significantly more effective than the basic treatment; secondly, the concurrent treatment should be of the same level of safety as single treatment; thirdly, the concurrent treatment should be cost-effective. Although these principles should be evaluated before adopting concurrent treatment, the authors have applied concurrent treatment to osteoporosis widely, without any background evidence. Among these principles, the authors have decided to evaluate the first two issues, effectiveness and safety, in the present study. Since this type of evaluation is absolutely required by a clinician, a researcher-initiative study was considered as being the most suitable type of evaluation. This was the reason why the authors decided to use a researcher-initiative clinical trial in determining the effectiveness of concurrent treatment for osteoporosis. The JOINT-02 protocol was the first randomized, controlled trial conducted nationwide for osteoporosis in Japan initiated by researchers, and its scale was also the largest ever. It was therefore necessary for various organizations to collaborate together, and there

have been no previous reports on how to manage the PROBE trial in Japan. This is why we wanted to report the design of JOINT-02. In this paper, we have presented the organization of the A-TOP research group and execution of the JOINT 02 protocol. This is because we believe that this report should help a researcher who is willing to build a new nationwide investigation that is constructed by an organization of clinical research work.

Since the primary aim of JOINT-02 was to determine whether concurrent treatment using ALN and alfacalcidol is superior to treatment using ALN alone in terms of fracture prevention, true ("hard") endpoints such as vertebral fractures or long bone fractures were selected as the primary endpoint. The diagnosis of whether vertebral fractures were present or not on the X-ray films was made by two independent reviewers who did not have any information about the patient; when the judgment of fractures was split between the two reviewers, the reviewers negotiated with each other. Identifying vertebral fractures is more difficult than identifying long bone fractures due to some cases of new vertebral fractures not showing clinical symptoms and the shape of the vertebral body making it difficult at times to recognize whether there is a fracture. Therefore, to avoid misdiagnosis, diagnosis of pre-existing and incident vertebral fractures was made by two different observers. In cases where there was a discrepancy in the diagnosis of vertebral fractures between the reviewers and the practitioner, which occurred with regard to pre-existing fractures, the reviewers' judgment was given priority over that of the practitioner.

Surrogate ("soft") endpoints such as change in BMD or bone turnover markers were considered to be inadequate as primary endpoints in making conclusive statements regarding the efficacy of concurrent treatment. In this type of study, the soft endpoint (BMD or biomarkers) will connect dropout bias less effectively than the case-selected hard endpoint, because such markers are not able to be made blind to the clinicians. Furthermore, previous studies indicate that changes in BMD or bone markers do not predict future fractures [22–24].

In recent literature, it has been reported that poor adherence of bisphosphonates leads to a decline in the beneficial effects of this drug on bone [25–28]. It is expected that in contrast to prior developmental trials, this current study may have a higher dropout rate, since in a researcher-initiative study, the registered practitioner is not forced to maintain adherence very strictly. As a result, adherence in the present study may resemble the actual circumstances of adherence to bisphosphonate treatment by a general practitioner. It will be interesting to determine whether adherence in this study modifies fracture prevention by alendronate.

Although a careful plan for this study has been set up, the results will be applied to osteoporosis patients with the same background as the present study population, but not adapted to the entire osteoporosis population. Despite this limitation, we believe that the results will give us very important information regarding the concurrent treatment of osteoporosis.

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The Fracture and Immobilization Score (FRISC) for risk assessment of osteoporotic fracture and immobilization in postmenopausal women—A joint analysis of the Nagano, Miyama, and Taiji Cohorts

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ABSTRACT

Introduction: We aimed to (i) explore risk factors for major osteoporotic fracture or immobilization; (ii) develop a prediction model that can be used to assess the risk of fracture and immobilization; and (iii) assess external validity of the final model.
Methods: A total of 1787 postmenopausal Japanese women were followed in a hospital-based cohort study. Endpoints included the annual incidence of major osteoporotic fracture and immobilization. For each endpoint, multivariate Poisson regression models were fitted separately and risk factors were screened through backward variable selection. The predictive accuracy of the final model (FRISC) was evaluated in two independent community-based cohorts.
Results: Over a median follow-up of 5.3 years, a total of 383 major osteoporotic fractures (279 clinical vertebral, 44 hip, 60 distal forearm) and 83 immobilizations occurred in the developmental dataset. Backward variable selection confirmed that the following are risk factors for major osteoporotic fracture: age, weight, prior fracture, back pain, and lumbar bone mineral density (BMD). Age, prior fracture and dementia were significant risk factors for immobilization. Hosmer–Lemeshow tests did not indicate any significant deviation between the observed fracture frequency and prediction from the FRISC in the independent validation dataset. The C statistic for the FRISC was 0.727 (95% confidence interval: 0.660 to 0.794) and was higher than that for BMD alone significantly ($p=0.03$).
Conclusions: We developed a novel prediction model for fracture and immobilization, FRISC, and the clinical risk factors in the FRISC allows better identification of populations at high risk of fracture than BMD alone. A web application is available at http://www.biostatistics.jp/prediction/frisc.

Introduction

Fracture due to osteoporosis results in increased mortality, morbidity and medical expense in the US and Japan [1–3]. As an adjunct to the development of effective treatments, early identification of populations at high risk of fracture is regarded as an effective strategy for decreasing both the burden of illness and the associated cost in countries with aging populations [4]. The US and Japanese guidelines both recommend that bone mineral density (BMD) should be used to determine when to intervene in patients with osteoporosis [5,6]. However, epidemiological studies have demonstrated that many major osteoporotic fractures occur among individuals with a BMD T score value above the intervention threshold value, although the incidence of fracture certainly increases with decreasing BMD [7,8]. As a solution to this problem, a WHO scientific group proposed the use of 10-year probabilities of osteoporotic or hip fracture, calculated using multiple risk factors. The result was the WHO fracture risk assessment tool (FRAX) [9]. Several other prediction models/risk assessment tools were also developed based on data from cohort studies and clinical trials [10–13].

Abbreviations: BMD, bone mineral density; FRAX, fracture risk assessment tool; FRISC, Fracture and Immobilization Score; CHD, coronary heart disease; CVD, cerebrovascular disease; ROC, receiver operating characteristic; CI, confidence interval.

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Although case-finding strategies optimized by risk assessment tool for fracture appear to be promising, there are several problems which must be addressed before clinical application. First, whether combination of BMD and clinical risk factors allows better discrimination of fracture than BMD alone is still controversial; the discriminatory power of the FRAX was excellent in eleven population-based cohorts [14] but was similar to BMD alone in a cohort study [15]. Second, since the Japanese version of FRAX was validated only in the community dwelling cohorts in Japan [9], there has been no available data whether the FRAX probability performs well in hospital-based population. As Justice et al. pointed out, the effectiveness of prediction models in clinical practice depends on the extent to which they can be generalized to the population in question [16]. In general practice, the FRAX was often adapted to assess fracture risk in a patient who has a more complicated risk for fracture, such as atherosclerosis, diabetes or other potential risks to deteriorate bone strength. In this regard, it is necessary to evaluate prediction models for fracture not only in community but also hospital-based populations. Third, outcomes other than fracture, such as immobilization, appear to be also important for optimizing osteoporotic treatment from the health economic perspective. In fact, we have found that osteoporotic fracture was an independent risk for immobilization and this morbid state would require a large amount of resources of care [17].

The present study was therefore performed with three main aims. The first was to explore risk factors for the incidence of major osteoporotic fracture or immobilization. This was done using data from the Nagano Cohort, a hospital-based cohort study of postmenopausal Japanese women. The second aim of this study was to develop a prediction model named as the Fracture and Immobilization Score (FRISC), to assess the risk of major osteoporotic fracture and immobilization, based on the risk factors confirmed in the first part of the study. Finally, we assess the external validity of the FRISC and investigate whether the predictive accuracy was improved from BMD alone using pooled data from the Miyama and Taiji Cohorts, which followed 400 Japanese women from communities over a 10-year period.

Methods

Development and validation datasets

We used two independent datasets in the current analysis; a developmental dataset from the Nagano Cohort and a validation dataset from the Miyama and Taiji Cohorts. Profiles of these three cohorts have been detailed previously [17–28]. The Nagano Cohort recruited and followed up postmenopausal women who were receiving medical care as outpatients or visitors at a medical institute in Nagano Prefecture, Japan since April 1993 [16–20]. A total of 1787 participants were included in the developmental dataset; exclusion criteria were (i) metabolic bone disease and (ii) secondary osteoporosis (e.g. hyperparathyroidism, hyperthyroidism other than patients on T4 replacement and with euthyroid for more than one year, chronic renal failure or osteomalacia). We excluded those who met the exclusion criteria regardless of BMD.

However, steroid users were enrolled to the present study because the history of steroid use was required in the FRAX. The protocol was approved by the ethics committee at the Research Institute and Practice for Involutional Diseases and we obtained written informed consent from all participants. The Miyama Cohort was set up in 1988 as subsets of nationwide community-based cohort studies sponsored by the Ministry of Education or Ministry of Health and Welfare [21–23]. A total of 1453 inhabitants aged 40–79 years in Miyama Village were listed from the resident registration in December 1988. Then, 200 men and 200 women were recruited and followed up between 1990 and 2000. The Taiji Cohort is a community-based cohort study in Taiji Town, Wakayama Prefecture, Japan [25–27]. From a list of 2261 inhabitants aged 40–79 years obtained from the resident registration in June 1992, 50 men and 50 women in each decade age group between 40 and 79 years (a total of 400 participants) were recruited randomly and followed up between 1993 and 2003. All the sampled participants were contracted and agreed to participate. The validation dataset included all the women in the Miyama and Taiji Cohorts.

Data collection in the Nagano Cohort

At baseline, anthropometric indices, including body weight and body height, were obtained for all patients. Subjects were also interviewed to obtain data about age at menopause, smoking habit, alcohol consumption, past and present occupation, presence of pain, medical history (including rheumatoid arthritis, diabetes mellitus, hypertension, dyslipidemia, cancer, dementia, coronary heart disease [CHD] and cerebrovascular disease [CVD]). Pain was defined as any symptom of pain in the back, hip muscles, ribs, legs, knees, neck, shoulders, wrists, or other joints. Back pain was defined as any symptom of pain in the back trunk area, regardless of the degree or consistency of the pain [18]. Rheumatoid arthritis was diagnosed according to the diagnostic criteria proposed by the American Rheumatism Association [28]. The Japanese version of the Mini-mental State Examination (MMSE) was performed in the subjects who were suspicious dementia and a subject with the total score of MMSE less than 20 points was considered to have obvious cognitive dysfunction [29]. A history of CHD was defined as any previous acute coronary symptoms or events requiring coronary intervention, and was confirmed by coronary angiography, echocardiogram, or MD-CT imaging study. A history of CVD was diagnosed based on evidence of apparent brain attack or an existing brain lesion as observed by magnetic resonance imaging or computer-assisted X-ray tomography. Self-reports of a history of malignancy were confirmed by referring to the patient's medical records [19]. The BMD of the lumbar spine was measured at baseline using dual-energy X-ray absorptiometry (Lunar DPX-L or DPX-IQ; Lunar Corporation, Madison, WI) and a quality assurance test was carried out for every measurement to detect machine drift. The inter-assay variance of the lumbar BMD measurements in our laboratory was $0.5 \pm 0.5\%$ (coefficient of variation ± standard deviation) [20]. T score was calculated by using Japanese standard values [30].

Data collection in the Miyama and Taiji Cohorts

A self-administered questionnaire was used for baseline data collection in the Miyama Cohort, while both self-administered and interviewer-administered questionnaires were used in the Taiji Cohort [22–27]. The items in these questionnaires included birth date, body weight, body height, current smoking status, current alcohol intake, presence of back pain, use of steroids, and medical history such as rheumatoid arthritis. Parental history of fracture was asked only in the Taiji Cohort. The BMD of L2-4 and BMD at femoral neck, Ward's triangle and the trochanteric region were measured by dual-energy X-ray absorptiometry (Lunar DPX; Lunar Corporation, Madison, WI in the Miyama Cohort, Hologic QDR-1000; Hologic Inc., Crosby Drive Bedford, MA in the Taiji Cohort) and treated as T scores. The incidence of clinical fracture was evaluated in both the cohorts. However, radiographs for morphometrical vertebral fracture were available only in the Miyama Cohort. Consequently, parental history or morphometrical vertebral fracture was missing data in either cohort systematically and thus we assumed that participants with these missing data did not have parental history or prior fracture. We calculated the 10-year probability of major osteoporotic fracture by entering the following data into online version of the FRAX; age, sex, weight, height, previous fracture, parental history of hip fracture, current smoking status, glucocorticoid use, rheumatoid arthritis, alcohol intake and femoral neck BMD.

Endpoints

Endpoints included the annual incidence of major osteoporotic fracture and immobilization. Major osteoporotic fracture was defined

as first occurrence of any clinical fracture (hip fracture, surgical neck fracture of the humerus, distal forearm fracture, or clinical vertebral fracture). We also evaluated a radiographical vertebral fracture by the semi-quantitative visual method [31], but major osteoporotic fracture does not include morphometrical fracture by definition. A validation analysis of our semi-quantitative method for analyzing incident vertebral fracture has been reported elsewhere [32]. Prior vertebral fractures were defined as those fractures for which the ratio of the height of the central or anterior vertebral body to that of the posterior vertebral body was less than 0.8, or when any of these three vertebral body heights was less than 80% of the height of the adjacent vertebral body [6]. Immobility was defined in accordance with the subject's locomotive ability. Subjects bed-bound at home (lying in bed almost all day) for more than 6 months or institutionalized in nursing homes (lying in bed or using a wheelchair for locomotion), were defined as immobile [17]. Some immobile subjects could be sitting on a bed and could be going to a portable toilet which located besides the bed. Participants who died from any cause, moved to the home of a relative because they were not able to perform the activities of daily living independently, were lost to follow-up, or were followed up until June 1, 2009 were treated as censored. For each endpoint, the accumulation of person-years at risk started from registration of each patient.

Statistical considerations

For each endpoint, we fitted multivariate Poisson regression models separately and rate ratios for risk factors estimated by the Poisson regression models were reported with 95% confidence intervals (CI) and p values. The following variables were initially identified from the literature as the traditional risk factors for osteoporotic fracture: covariates included in the FRAX other than femoral neck BMD (age, height, weight, prior fracture, parental history of fracture, current smoking status, use of steroids, rheumatoid arthritis, alcohol intake), lumbar BMD, presence of back pain, presence of any pain, and drug treatment for osteoporosis [9,18,22]. These covariates were screened via backward variable selection with a significance level of $p=0.2$. We constructed a prediction model for immobilization using the same procedure, except that three covariates (dementia, history of CVD, and history of malignancy) were used in addition to those used in the osteoporotic fracture analysis. Finally, the FRISC was developed using the following formula:

$$\mathrm{Prob}(t) = \int_0^t \lambda \exp(\mathbf{X}\beta) \exp\left[-\int_0^u \{\lambda \exp(\mathbf{X}\beta) + m(v)\} dv\right] du$$

Here, t is a time point for prediction (i.e. the formula calculates 10-year probability if $t=10$), β is a vector of log-rate ratios for covariates $\mathbf{X}$, λ denotes baseline incidence rate, and $m(v)$ is mortality at time v obtained from sex- and age-specific mortality in Vital Statistics of Japan in 2008 [33].

We assessed the predictive accuracy of the FRISC in terms of calibration and discrimination [34] using occurrence of major osteoporotic fracture within a 10-year period, which was treated as a binary event, in the validation dataset. Calibration, namely how closely the prediction reflects actual events, was assessed using ratio of observed and predicted events and the Hosmer–Lemehshow test. Discrimination, the ability to distinguish between those who experience the event and those who do not, was evaluated using receiver operating characteristic (ROC) curves and Harrell's C statistic. Improvement in the C statistics of the two models from BMD alone was assessed by using contrast tests.

All reported p values for statistical tests are two-tailed, and $p<0.05$ was taken to indicate statistical significance. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Results

Characteristics of participants and follow-up

Baseline characteristics of participants in the Nagano, Miyama and Taiji Cohorts are summarized in Table 1. The Nagano Cohort included older participants and mean lumbar BMD in this cohort was lower than the other cohorts. Prior fracture, back pain and parental history were observed more frequently in the Miyama and Taiji Cohorts. In the Nagano Cohort, 37.4% of participants were being treated with bone resorption inhibitors (bisphosphonates or a selective estrogen receptor modulator), and 16.7% were receiving 1-alpha-OH vitamin D_3 or vitamin K_2 at baseline. Table 2 describes the incidence of fractures and immobilization. In the Nagano Cohort, over a median follow-up time of 5.3 years (range, 0.03–16.5 years), a total of 383 major osteoporotic fractures occurred (279 clinical vertebral fractures, 44 hip fractures, 60 distal forearm fracture, Table 2). In the Miyama and Taiji Cohorts, 337 of 400 participants completed the planned follow-up of the 10-year period (84%) and a total of 60 major osteoporotic fractures occurred (44 clinical vertebral fractures, 8 hip fractures, 8 distal forearm fracture, Table 2). Incidence rates of fractures in the two cohorts were much lower than the Nagano Cohort possibly due to the difference in average age and lumbar BMD at baseline (Tables 1 and 2). Immobilization occurred in 83 participants in the Nagano Cohort.

Risk factors for fracture and immobilization

We fitted multivariate Poisson regression models to the validation dataset of 1787 participants. Backward variable selection identified the following six risk factors for major osteoporotic fracture: age, weight, lumbar BMD, prior fracture and presence of back pain (Table 3). That is, parental history of fracture, smoking status, alcohol consumption, rheumatoid arthritis, and use of steroids, which are all

Table 1
Characteristics of participants in the three cohorts.

		The Nagano Cohort (N=1787)			The Miyama and Taiji Cohorts (N=400)		
		Mean	SD	5–95 percentile	Mean	SD	5–95 percentile
Age (years)		63.4	11.1	45–81	59.5	11.3	41–77
Height (cm)		150.9	6.6	140–161	150.2	6.2	140–159
Weight (kg)		51.1	8.5	38–65	51.2	9.3	37–66.5
Lumbar BMD (T score)		−1.55	1.22	−3.5–0.5	−1.36	1.19	−3.85–1.57
Femoral neck BMD (T score)		*			−1.61	1.84	−3.29–0.53
				Frequency	%	Frequency	%
Prior fracture				403	22.6	49†	25.0
Presence of pain	Back			572	32.0	251	63.0
	Other sites			449	25.1	†‡	
Parental history				22	1.2	20‡	10.0
Current smoker				38	2.1	16	4.0
Current alcohol drinker				137	7.7	46	11.5
Medication	Bone resorption inhibitors			369	37.4	†‡	
	Active vitamin D_3 or vitamin K_2			299	16.7	†‡	
	Steroids			27	1.5	0‡	0.0
Rheumatoid arthritis				224	12.5	0	0.0
Dementia				97	5.4	†‡	

SD: standard deviation; BMD: bone mineral density.
*Not measured in the Nagano Cohort. †Not measured in the Miyama Cohort (N=200).
‡Not measured in the Taiji Cohort (N=200).

Table 2
Frequencies and incidence rates of fracture and immobilization in participants in the three cohorts.

	The Nagano Cohort (N = 1787)				The Miyama and Taiji Cohorts (N = 400)			
	Frequency	IR	95% CI		Frequency	IR	95% CI	
Major osteoporotic fracture	383	34.1	30.9	37.7	60	16.1	12.5	20.7
Clinical vertebral facture	279	24.9	22.1	28.0	44	11.8	08.8	15.9
Hip fracture	44	3.9	2.9	5.3	8	2.2	1.1	4.3
Immobilization	83	7.4	6.0	9.2	–			

IR, incidence rate per 1,000 person-years; CI, confidence interval.

included in the FRAX, were excluded based on having p values less than 0.2. Importantly, incidence rate of major osteoporotic fracture increased as weight elevated and this direction is opposite to the FRAX and this trend remains significant even when all the other risk factors listed initially in the variable selection procedure are adjusted for (rate ratio for 10 kg increase in weight: 1.22, 95% CI: 1.07 to 1.40, $p<0.01$). Multivariate analysis for immobilization, using the same variable selection procedure, showed that age, prior fracture and dementia were associated with the incidence of immobilization (Table 3).

Input and output of the FRISC

All the risk factors that were retained through the variable selection procedure were incorporated into the final prediction model named as the FRISC. Interface of web application of the FRISC is displayed in Fig. 1. The input comprises the sex risk factors and, menopausal status and secondary osteoporosis which were used only for assessment of the applicability. The output comprises the 1, 3, 5 and 10-year probabilities of major osteoporotic fracture and those of immobilization and is calculated by using the algorithm described in Supplementary Data.

External validation of the FRISC

Fig. 2 displays histograms of the calculated 10-year probabilities of major osteoporotic fracture for the 400 participants in the validation dataset (upper: the FRISC, lower: the FRAX). An apparent difference was observed in the left tail of the two histograms; in the upper figure participants with fracture probability less than 0.05 were very few, while the FRAX gave the fracture probability less than 0.05 to a substantial portion of the participants. As a result, the fracture probabilities from the FRISC were much higher on average. Table 4 compares the predictive accuracy of the two prediction models and prediction from BMD alone. Over the 10-year follow-up, major osteoporotic fracture developed in 60 of 400 participants in the validation dataset. The predicted event frequency calculated from the FRISC was slightly higher than the observation (observed/predicted ratio: 0.74), while the FRAX tended to underestimate (observed/predicted ratio: 1.59). The Hosmer–Lemeshow test did not indicate any significant deviation between the observed event frequency and prediction from the FRISC. The C statistics for the FRISC was 0.727, indicating that the discriminatory power of the FRISC is moderate, while that for prediction from BMD alone was 0.651. That is, the discriminatory power of the FRISC, which combines BMD and additional clinical risk factors, was better than BMD alone significantly even in independent community-based cohort studies ($p=0.03$, Table 4). Fig. 3 shows ROC curves for major osteoporotic fracture probability from the FRISC (solid curve), the FRAX (dashed curve) and BMD alone (dotted curve). Both the ROC curves of the prediction models increased almost identically at first, but the curve for the FRISC was slightly above the curve for the FRAX where sensitivity is higher than 0.7 and where lower probability is used as a cutoff point (i.e. 16% or lower in the FRISC, 14% or lower in the FRAX), indicating that the FRISC is advantageous over the FRAX for screening of low-risk osteoporotic patients.

Table 3
Multivariate Poisson regression analysis of risk factors for major osteoporotic fracture and immobilization in the development dataset of 1,787 participants.

	Major osteoporotic fracture				Immobilization			
	Rate ratio	95% CI		p	Rate ratio	95% CI		p
Age, + 10 years	1.62	1.43	1.83	<0.01	2.80	2.09	3.73	<0.01
Weight, + 10 kg	1.25	1.10	1.42	<0.01	–	–	–	–
Lumbar BMD, + 1 T score point	0.85	0.76	0.94	<0.01	–	–	–	–
Prior fracture, yes/no	2.00	1.57	2.54	<0.01	2.04	1.21	3.44	0.01
Back pain, yes/no	1.58	1.27	1.96	<0.01	–	–	–	–
Dementia, yes/no	–	–	–	–	2.09	1.32	3.29	<0.01

BMD: bone mineral density; CI: confidence interval.

Discussion

In the current study, we explored clinical risk factors for major osteoporotic fracture and immobilization and developed a novel prediction model, the FRISC. Importantly, the assessment of external validity showed that the FRISC allows accurate prediction of major osteoporotic fracture even in the community-based setting and after a long-term follow-up of ten years, although it was developed in a hospital-based cohort study (i.e. for outpatients and visitors to a clinic). Therefore, the FRISC is useful both not only for patients who have a more complicated risk for fracture, such as atherosclerosis, diabetes or other potential risks to deteriorate bone strength, but also general postmenopausal women. Further the discriminatory power of the FRISC was shown to be better than BMD alone. We have previously noted that there is a close relationship between bone fractures and subsequent immobilization in postmenopausal Japanese women, and that these two conditions are morbid states that require a large amount of health resources [17]. Therefore, an accurate measure to predict these two conditions is particularly valuable in the context of an aging society. A web application of the FRISC is available at http://www.biostatistics.jp/prediction/frisc (Fig. 1).

The major finding of the current study is that inclusion of the four clinical risk factors, namely age, weight, prior fracture and back pain, in addition to BMD significantly improved the accuracy of the prediction model for major osteoporotic fracture. In contrast, parental history of fracture, smoking status, alcohol consumption, rheumatoid arthritis and use of steroids, which are all included in the FRAX, were not associated with incidence of fracture in the present analysis. The reason for this observation does not appear to be a lack of power given the number of observed events in the Nagano Cohort. Diet and other lifestyle factors, which were Westernized among smokers in this cohort, may have contributed to this unexpected result. One implication of these findings is that the association between lifestyle factors and fracture risk is possibly biased due to confounding factors, and it is necessary for prediction models to reflect the multidimensional nature of lifestyles. Although there were smokers and drinkers in the present population, the extent of their smoking and drinking was very mild, and smaller percentages of patients had these habits than in comparable Caucasian populations. In the practical point of view, a more parsimonious model is desirable and the FRISC would therefore provide a simple but sufficiently accurate measure for prediction of major osteoporotic fracture.

The present results indicated that incidence of fracture increases with heavier body weight, although low BMI has been considered as a significant risk factor of fracture as proposed in the FRAX. This trend remained even after the adjustment for the other risk factors

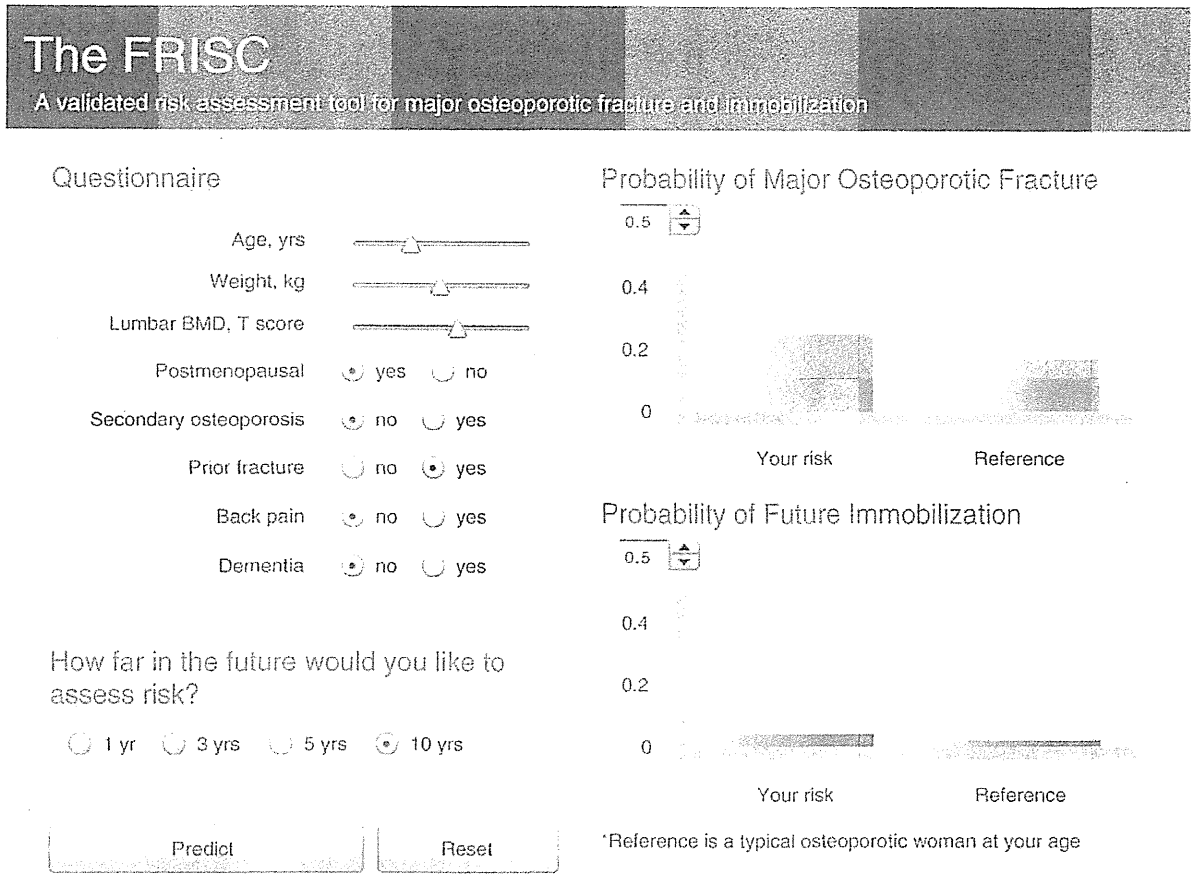

Fig. 1. Input and output of the web application of the FRISC.

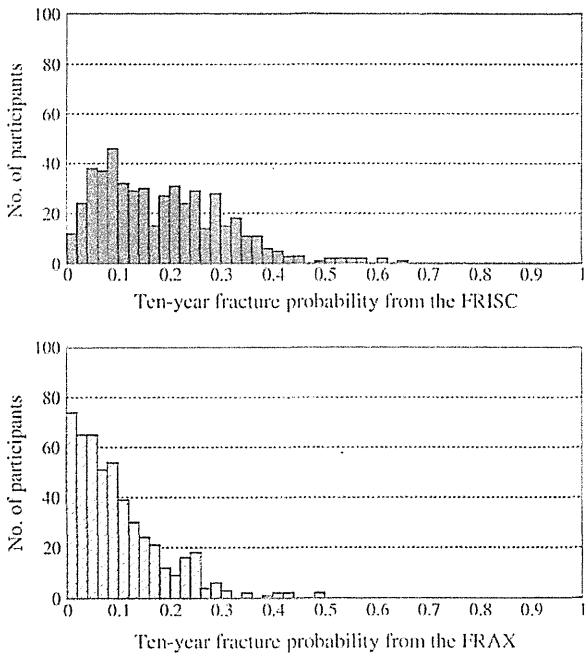

Fig. 2. Histogram of 10-year probabilities of major osteoporotic fracture from the FRISC (upper) and the FRAX (lower) in the Miyama and Taiji Cohorts.

(p<0.01) and therefore seemed to be attributable to confounders. This may be one of the causes of discrepancy in 10-year probability between the FRAX and the actual fracture rate in the three cohorts. Recent report indicated that morbid obesity had a higher susceptibility of fractures comparing to the postmenopausal women with normal weight although the BMD of the obesity was higher than the controls [35]. As it is well known that obesity will connect to have diabetes mellitus or at least to have glucose intolerance and diabetes may deteriorate bone quality due to an increase in non-enzymatic glycation induced cross-links of collagen, which increased collagen

Table 4
Predictive accuracy of major osteoporotic fracture probability from the FRISC compared the FRAX evaluated in the validation dataset from general population.

	Calibration			Discrimination			
	Predicted no. of cases	Observed/ predicted ratio	p^*	C statistics†	95% CI		$p^‡$
BMD alone	–	–	–	0.651	0.575	0.728	–
The FRAX	37.8	1.59	<0.01	0.699	0.629	0.768	0.23
The FRISC	81.2	0.74	0.17	0.727	0.660	0.794	0.03

CI: confidence interval.
* Hosmer–Lemeshow test, p value less than 0.05 indicates a significant deviation between the observed and predicted event frequencies. Number of strata and degree of freedom are 10 and 8, respectively.
† The proportion of all patient pairs in which prediction and observed occurrence of event are concordant.
‡ Contrast test comparing C statistics of the FRAX and FRISC from that of BMD alone, p value less than 0.05 indicates a significant improvement from BMD alone.

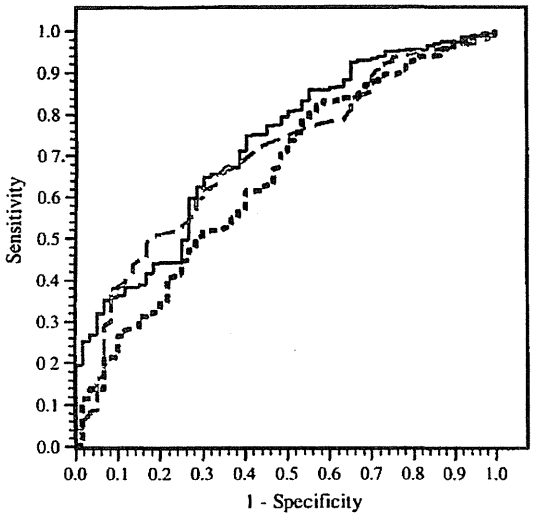

Fig. 3. Receiver operating characteristic curve for major osteoporotic fracture probability from the FRISC (solid curve), the FRAX (dashed curve) and BMD alone (dotted curve) in the Miyama and Taiji Cohorts.

brittleness [36,37]. Lifestyle factors such as diet and exercise may also be other explanations for this observation.

Although the intrinsic properties of the bone are important components of fracture risk, assessment of these factors alone does not adequately reflect the full range of factors associated with the occurrence of fracture [38]. Loss of bone mass and impaired bone quality are commonly held to be the two major causes of increased bone fragility in osteoporosis [2]; however, existing prediction models do not directly take bone quality into consideration. Despite recent progress in understanding the composition and structure of the bone, there are currently no standard assessments of bone quality. Novel bone quality-related markers such as homocysteine [39,40] and pentosidine [41] appear to improve predictive accuracy, but further research is required to determine whether they will be useful in the context of predicting osteoporotic fracture.

The incidence of clinical vertebral fracture in the Japanese population is substantially high (Table 2). As a result, the 10-year fracture probabilities generated using the FRISC is much higher than the FRAX (Fig. 2). The major underlying cause of the discrepancy in the 10-year probabilities is likely to be the difference in population. The FRISC was developed in a cohort study conducted at one medical institute and included subjects who were receiving treatment for osteoporosis, whereas the FRAX was developed using data from a community-based population. Although the effectiveness of bisphosphonate and selective estrogen receptor modulators in reducing fracture risk has been demonstrated, in the current analysis, drug treatment for osteoporosis was not a significant factor at the site of major osteoporotic fracture after adjustment for other risk factors, suggesting that its influence on risk is smaller than that of the risk factors. People who visit a hospital or clinic possibly have a higher prevalence of co-morbid conditions than people in the general population, yielding an increased incidence of fracture because of deterioration in both bone quality and quantity.

Given the large difference in incidence rates of fracture between the Nagano Cohort and the Miyama and Taiji Cohorts (Table 2), it may not seem to be sensible to choose the Miyama and Taiji Cohorts as validation cohorts since a good fit is unexpected. However, as shown in Table 1, the Nagano cohort included older participants and the mean lumbar BMD in this cohort was lower than the other cohorts. Therefore the difference in participants' characteristics may be attributable to the higher incidence rate in the Nagano cohort relative to the other cohorts. Further, the Miyama and Taiji Cohorts followed participants over a 10-year period and are more suitable for the validation analysis. A limitation of our validation analysis was that parental history or morphometrical vertebral fracture was missing data in either of the validation cohorts systematically. We assumed that participants with these missing data did not have parental history or prior fracture, yielding a somewhat lower 10-year probability of major osteoporotic fracture. Given that we did not find any evidence of deviation between the observed fracture frequency and prediction from the FRISC even in independent community-based cohort studies, the FRISC appears to allow accurate prediction of major osteoporotic fracture both in community-based and hospital-based settings.

Supplementary materials related to this article can be found online at doi:10.1016/j.bone.2010.08.019.

Conclusion

We developed a novel prediction model for fracture and immobilization, FRISC, and the clinical risk factors in the FRISC allows better identification of populations at high risk of fracture than BMD alone.

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