In April 2022, Osaka City University and Osaka Prefecture University marge to Osaka Metropolitan University

Association of cystatin C- and creatinine-based eGFR with osteoporotic fracture in Japanese postmenopausal women with osteoporosis: sarcopenia as risk for fracture

Masafumi Kurajoh, Masaaki Inaba, Yuki Nagata, Shinsuke Yamada, Yasuo Imanishi, Masanori Emoto

Citation	Journal of Bone and Mineral Metabolism, 37(2); 282–291
Issue Date	2018-02-20
Type	Journal Article
Textversion	Author
Relation	This is a post-peer-review, pre-copyedit version of an article published in Journal of Bone and Mineral Metabolism. The final authenticated version is available online at: https://doi.org/10.1007/s00774-018-0913-4 . See Springer Nature's AM terms of use: https://www.springernature.com/gp/open-research/policies/accepted-manuscript-terms
DOI	10.1007/s00774-018-0913-4

Self-Archiving by Author(s)
Placed on: Osaka City University

KURAJOH M, INABA M, NAGATA Y, YAMADA S, IMANISHI Y, & EMOTO M. (2019). Association of cystatin C- and creatinine-based eGFR with osteoporotic fracture in Japanese postmenopausal women with osteoporosis: sarcopenia as risk for fracture. *Journal of Bone and Mineral Metabolism*. 37, 282-291. doi:10.1007/s00774-018-0913-4

Association of cystatin C- and creatinine-based eGFR with osteoporotic fracture in Japanese postmenopausal women with primary osteoporosis: sarcopenia a risk for fracture

Rnning title: eGFRcys, eGFRcr, and osteoporotic fracture

Masafumi Kurajoh, MD, PhD.*, Masaaki Inaba, MD, PhD., Yuki Nagata, MD, PhD., Shinsuke Yamada, MD, PhD., Yasuo Imanishi, MD, PhD., and Masanori Emoto MD, PhD.

Department of Metabolism, Endocrinology, and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan

***Corresponding author:**

Masafumi Kurajoh, MD, PhD.

Department of Metabolism, Endocrinology, and Molecular Medicine

Osaka City University Graduate School of Medicine

1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Tel: +81-6-6645-3806, Fax: +81-6-6645-3808

Email: m1155129@med.osaka-cu.ac.jp

Key words: eGFRcys, eGFRcr, eGFRcys/eGFRcr ratio, osteoporotic fracture, sarcopenia

ABSTRACT

Coexistence of chronic kidney disease (CKD) is regarded as a risk for osteoporotic fracture particularly in postmenopausal women, not only because of increased parathyroid hormone level but also uremic sarcopenia. We examined the relationships of cystatin C-based glomerular filtration rate (eGFRcys) and creatinine-based GFR (eGFRcr), as well as their ratio with occurrence of osteoporotic fracture in postmenopausal osteoporotic women. This cross-sectional study included 555 postmenopausal women with primary osteoporosis. eGFRcr and eGFRcys were simultaneously measured, while occurrence of osteoporotic fracture was obtained by a medical chart review. Patients with osteoporotic fractures (n=211) exhibited significantly lower levels of physical activity, eGFRcr, eGFRcys, and eGFRcys/eGFRcr ratio, while a higher percentage were CKD stage 3 or more, estimated by eGFRcr or eGFRcys (CKDcys), than those without (n=344). Lower eGFRcys, but not lower eGFRcr, was independently associated with osteoporotic fracture in the entire cohort and that association was retained in CKDcys patients. Of great interest, higher eGFRcr was associated with osteoporotic fracture independent of eGFRcys in CKDcys patients. Furthermore, lower eGFRcys/eGFRcr ratio was independently associated with osteoporotic fracture in both CKDcys patients and the entire cohort. eGFRcys reduction

might be associated with osteoporotic fracture in postmenopausal osteoporotic women, indicating the involvement of renal osteopathy in its occurrence. Furthermore, the association of higher, but not lower, eGFRcr with osteoporotic fracture in CKDcys cases might be explained by underestimation of renal dysfunction by eGFRcr resulting from decreased muscle mass and quality in those patients.

Introduction

Osteoporosis is associated with increased risk for fragile fracture and most commonly seen in post-menopausal women [1]. Because of the higher incidence of osteoporotic fracture in aged women [2], the clinical significance of osteoporotic fracture as a health problem is increasing not only on the basis of fracture-associated impaired quality of life [3] but also because of the fracture-associated increased rate of mortality [4]. The rates of osteoporosis and chronic kidney disease (CKD) were found to be higher in older women due to age-related increased prevalence [1,5]. Furthermore, coexistence of those conditions is much higher in aged women, as osteoporosis may impair kidney function by increasing phosphate release from bone [6] and patients with CKD stage 3 or more have a greater risk for osteoporosis [7] due to development of secondary hyperparathyroidism [8,9].

Osteoporosis and CKD are both major diseases associated with sarcopenia [10,11]. Although estimated glomerular filtration rate (eGFR) obtained using a creatinine-based formula (eGFRcr) provides a clinically useful index for renal function [12], sarcopenia might falsely elevate the eGFRcr value due to decreased release of creatine from muscle mass [13]. In contrast, estimated GFR, determined by a cystatin C-based formula (eGFRcys), is not affected by low muscle mass or muscle quality [13,14], and

provides a more precise measurement of renal function than eGFRcr [15].

Thus far, limited studies have examined the association of osteoporotic fracture with renal dysfunction using only eGFRcr [16,17]. Based on its underestimation of renal function, particularly in patients with sarcopenia, it is clear that eGFRcys should be measured to determine the association of renal dysfunction with prevalence of osteoporotic fracture. However, to the best of our knowledge, no such investigation has been presented, which led us to examine the relationship of renal function, determined by either eGFRcr or eGFRcys, with the prevalence of osteoporotic fracture in postmenopausal osteoporotic women. Furthermore, a decreased eGFRcys/eGFRcr ratio, which theoretically provides a measure for sarcopenia, might affect the prevalence of osteoporotic fracture in post-menopausal osteoporotic women after separation based on a eGFRcys cutoff value of 60 mL/min/1.73 m^2.

Patients and Methods

Study design

A previous cross-sectional, observational, and epidemiological study was performed at 41 medical institutions conducting osteoporosis diagnosis and treatment throughout Japan between July 2015 and November 2015, and designed to assess renal function

status and treatment patterns in Japanese patients aged 50 years and older with osteoporosis undergoing clinical examinations (in preparation). The present investigation was performed as a sub-analysis of the above-mentioned study. All assessments, including laboratory measurements, of the enrolled participants were performed once at the baseline visit, while all other data were obtained from medical chart reviews.

Participants

Subjects aged 50 years and older who were diagnosed with osteoporosis according to Japanese guidelines [18], and regularly visited their physician during the study period were considered eligible to participate (n=988). The study population included patients currently receiving treatment, previously treated but not currently receiving treatment, and never treated for osteoporosis. The exclusion criteria were presence of Paget's disease, currently or previously diagnosed with a malignant neoplasm, and current or previous participation in a clinical trial within the past 6 months. This study was registered at www.clinicaltrials.jp as #JapicCTI-152989 and informed consent was obtained from all individual participants in the study. In this sub-analysis, to avoid the influence of gender on serum creatinine, male patients (n=41) were excluded. In

addition, patients younger than 55 years old (n=7), with rheumatoid arthritis (n=51), being treated with glucocorticoids (n=10), with secondary osteoporosis (n=56), or missing data for analysis (148) were also excluded, as were those with a body mass index (BMI) less than 18.5 kg/m^2 (n=120), because serum creatinine has been shown to be reduced in subjects with a BMI lower than that value [19]. As a result, 555 postmenopausal women with primary osteoporosis were analyzed in the present study.

Assessment of risk factors of osteoporotic fracture

Medical charts were reviewed to determine baseline demographics and risk factors for osteoporotic fracture, with smoking habit, alcohol use (≥3 units/day), parental history of femoral neck fracture, hours of physical exercise per week, use of glucocorticoids, and co-existence of rheumatoid arthritis, secondary osteoporosis, diabetes, and hypertension noted. Dyslipidemia was defined as low-density lipoprotein cholesterol ≥140 mg/dl, high-density lipoprotein cholesterol ≤40 mg/dl, triglycerides ≥150 mg/dl, or treatment for dyslipidemia [20]. Urine albumin-to-creatinine ratio (UACR) was determined based on measurement of a spot urine sample, with albuminuria defined as UACR ≥30 mg/g [13]. Fractures attributed to osteoporosis comprised those of the hip, vertebra, ribs, pelvic, proximal humerus, tibia, fibula, and

distal radius, as previously described [21].

Assessment of eGFR based on serum creatinine and serum cystatin C

The concentration of creatinine in serum was measured using an enzymatic method and that of cystatin C using a colloidal gold immunoassay (Alfresa Pharma) [22]. eGFR based on serum creatinine (eGFRcr) and serum cystatin C (eGFRcys) were calculated using equations for women reported by the Japanese Society of Nephrology, as follows [22,23].

$$\text{eGFRcr (mL/min/1.73 m}^2\text{)} = 194 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287} \times 0.739$$

$$\text{eGFRcys (mL/min/1.73 m}^2\text{)} = \{104 \times \text{serum Cystatin C}^{-1.019} \times 0.996^{\text{age}} \times 0.929\} - 8$$

For the present study, CKD was defined as eGFRcr <60 mL/min/1.73 m^2 (CKDcr) or eGFRcys <60 mL/min/1.73 m^2 (CKDcys) [24].

Statistical analysis

Physical exercise per week including values of zero were natural logarithm-transformed [ln (x+1)] to achieve a normal distribution. A non-repeated t-test (continuous variables with normal distribution) and a chi-square test (categorical variables) were used to compare variables between groups. Pearson's correlation test

was used to determine correlations between continuous variables. Multivariate logistic regression analyses were used to calculate odds ratio (OR) and 95% confidence interval (CI) values. Analysis of covariance was used to compare regression lines. All statistical analyses were performed using the Statistical Package for the Social Sciences software (PASW Statistics version 22.0). All reported p values are 2-tailed and were considered statistically significant at a level <0.05.

Results

Clinical characteristics of subjects with and without history of osteoporotic fracture

The characteristics of the enrolled osteoporotic female patients are shown in Table 1. Patients with osteoporotic fractures were significantly older and had lower physical activity level as compared to those without, and also tended to have a higher BMI value. Also, the mean levels of eGFRcr and eGFRcys were significantly lower in patients with osteoporotic fractures. Based on the finding that the percentages of CKDcr and CKDcys were significantly higher in patients with osteoporotic fracture, we considered that impaired renal function might be associated with osteoporotic fracture in female osteoporotic patients. Notably, eGFRcys/eGFRcr ratio was significantly lower in those with osteoporotic fractures than in those without. In contrast, the presence of diabetes,

hypertension, dyslipidemia, alcohol drinking habit, smoking habit, parental history of femoral neck fracture, and albuminuria were not significantly different between the groups.

Multivariate logistic regression analysis of factors associated with osteoporotic fractures in entire cohort

To examine whether eGFRcys, eGFRcr, and/or eGFRcys/eGFRcr ratio are independently associated with osteoporotic fracture in female osteoporotic patients, multivariate logistic regression analyses were performed (Table 2). In basic model 1, which included age, BMI, physical activity, presence of diabetes, hypertension, dyslipidemia, alcohol drinking habit, current smoking habit, albuminuria, and parental history of femoral neck fracture as covariates, age and physical activity were significantly associated with osteoporotic fracture, while higher BMI tended to show an association. When eGFRcr was added as a covariate to the basic model (model 2), eGFRcr was not significantly associated with osteoporotic fracture. On the other hand, when eGFRcys was replaced with eGFRcr (model 3), eGFRcys in addition to physical activity emerged as significant factors associated with osteoporotic fracture. Furthermore, when both eGFRcr and eGFRcys were simultaneously included as

covariates (model 4), eGFRcys, but not eGFRcr, was significantly associated with osteoporotic fracture. Of interest, when eGFRcys/eGFRcr ratio was added as a covariate to the basic model (model 5), that as well as age were significantly associated with osteoporotic fracture, while physical activity had a tendency to show an association.

Association of eGFRcys, eGFRcr, and eGFRcys/eGFRcr ratio with osteoporotic fracture in patients with and without CKDcys

In patients with CKDcys (eGFRcys <60 mL/min/1.73 m^2), eGFRcys and eGFRcys/eGFRcr ratio, but not eGFRcr, were significantly lower in the group with osteoporotic fracture (Table 3). The percentage of patients with osteoporotic fracture was significantly higher in the group with an eGFRcys/eGFRcr ratio <1.0 as compared to a ratio ≥1.0 (Table 4). The relationship between eGFRcr and eGFRcys in CKDcys patients both with and without osteoporotic fracture is shown in Figure 1. Although the slope of the regression line between eGFRcr and eGFRcys in those with osteoporotic fracture did not differ significantly from that in those without such fracture (p=0.979), the regression line was shifted significantly to the right for patients with osteoporotic fracture (p=0.001). On the other hand, in patients without CKDcys (eGFRcys ≥60 mL/min/1.73 m^2), eGFRcys, eGFRcr, and eGFRcys/eGFRcr ratio did not differ

significantly between those with and without osteoporotic fracture (Table 3), and the percentage of osteoporotic fracture was also not significantly different between the groups (Table 4). Furthermore, the relationship between eGFRcr and eGFRcys was not significantly different between non-CKDcys patients with and without osteoporotic fracture (data not shown).

Multivariate logistic regression analysis of factors associated with osteoporotic fracture in patients with CKDcys

Multivariate logistic regression analyses of patients with CKDcys were performed again to further examine whether eGFRcys, eGFRcr, and/or eGFRcys/eGFRcr ratio were independently associated with osteoporotic fracture (Table 5). When eGFRcr (model 2) or eGFRcys (model 3) was added as a covariate to the basic model, neither eGFRcr nor eGFRcys showed a significant association with osteoporotic fracture. However, we found it interesting that when both eGFRcr and eGFRcys were simultaneously added as covariates to the basic model (model 4), higher eGFRcr and lower eGFRcys were significantly and independently associated with osteoporotic fracture. Also, when eGFRcys/eGFRcr ratio was included as a covariate with the basic model (model 5), a lower ratio was found to be significantly associated with

osteoporotic fracture. With all models, physical activity, diabetes, hypertension, dyslipidemia, smoking habit, alcohol drinking habit, parental history of femoral neck fracture, and albuminuria were not significantly associated with osteoporotic fracture, while higher BMI exhibited a significant or borderline association. In patients without CKDcys, the factors eGFRcys, eGFRcr, and eGFRcys/eGFRcr ratio were not associated with osteoporotic fracture (data not shown).

Discussion

The primary findings in the present study are as follows. First, lower eGFRcys, but not lower eGFRcr, was found to be independently associated with osteoporotic fracture in postmenopausal women with primary osteoporosis. Second, the association of lower eGFRcys with osteoporotic fracture was retained even when the analyzed patients were restricted to those with CKDcys. Of great interest is the finding that higher eGFRcr, independent of eGFRcys, was associated with osteoporotic fracture in CKDcys patients, suggesting the presence of a mechanism other than renal dysfunction in the association of eGFRcr with osteoporotic fracture in CKDcys patients. Finally, in support of this finding is that a lower eGFRcys/eGFRcr ratio was shown to be independently associated with osteoporotic fracture in CKDcys patients as well as the entire cohort.

The discrepancy between eGFRcys and eGFRcr might be explained by a falsely elevated value for eGFRcr as compared to eGFRcys in patients with sarcopenia, which are characterized by loss of skeletal muscle mass and muscle strength, and/or low physical performance [25]. Sarcopenia is closely associated with osteoporotic fracture and a prospective study found that osteoporosis patients have increased risk for its development [10]. Since the release of creatine from muscle mass is the major determinant of creatinine serum level, due to its conversion to creatinine in circulation, serum creatinine should be lower and eGFRcr higher, as muscle volume in affected patients becomes lower in a manner independent of renal function [13]. Supportive of this notion are findings of our previous study showing that serum creatinine was lower in hemodialysis patients with lower muscle quality [26]. On the other hand, cystatin C is a cysteine protease inhibitor constantly produced by all nucleated cells, thus is unaffected by muscle mass [13,14], while eGFRcys value has a lower level of bias and greater accuracy for GFR measurement based on the urinary clearance of inulin [27]. In the present study, lower eGFRcys was independently associated with osteoporotic fracture in all patients or CKDcys, but not non-CKDcys patients (Table 2 and 5), supporting the notion that impaired renal function is a risk factor.

eGFRcys/eGFRcr ratio has been suggested to provide a clinically relevant measure

of muscle mass, based on the assumption that eGFRcys is determined only by renal function, while eGFRcr is determined by not only by renal function but also muscle mass [14], thus indicating that a lower eGFRcys/eGFRcr ratio is a clinically useful parameter for reduced muscle mass. As expected, eGFRcys/eGFRcr ratio exhibited a positive correlation with physical activity level ($r=0.144$, $p=0.001$). In addition, that ratio was significantly lower in patients with osteoporotic fracture as compared to those without (Table 1). Addition of eGFRcys/eGFRcr ratio into multivariate analysis made the association between physical activity level and osteoporotic fracture insignificant (Table 2). These results suggest that adoption of eGFRcr as a marker for renal function in osteoporotic patients may result in underestimation of renal function, particularly in osteoporotic subjects with sarcopenia.

Sarcopenia is frequently observed in dialysis patients [28] as well as pre-dialysis CKD patients [11]. CKD-associated sarcopenia, known as uremic sarcopenia, is induced by complex pathways including immunological, myocellular, hormonal, and renin-angiotensin system changes [29]. Consistent with that report, the incidence of osteoporotic fracture was significantly higher in our CKDcys patients with a eGFRcys/eGFRcr ratio <1.0 (Table 4), but not in patients without CKDcys. In addition, in patients with CKDcys, the regression line between eGFRcr and eGFRcys was

shifted significantly to the right (Fig. 1), and the eGFRcys/eGFRcr ratio was significantly lower in those with as compared to without osteoporotic fracture (Table 3). Therefore, it is possible that muscle mass and/or quality are decreased to a greater degree in osteoporotic subjects with CKD than in those without. Also, the association between higher BMI or eGFRcr and greater prevalence of osteoporotic fracture (Table 5) suggested that increased eGFRcr reflects decreased muscle mass and quality in spite of obesity in CKD patients, and that eGFRcr is not a reliable index for renal function, especially in osteoporotic patients with CKD.

Limitations

This study has several limitations. First, it was conducted in an outpatient clinical setting and osteoporotic subjects who were not diagnosed may not have been included. Second, because of the study design, we did not measure serum PTH or vitamin D concentration, which are known to be important factors related to osteoporotic fracture. Third, data for bone mineral density (BMD) were obtained in the 3-month period prior to enrollment at various clinics using different measurement locations, techniques, and devices, thus we could not consider the influence of BMD on osteoporotic fracture. Finally, we did not directly assess muscle volume or muscle quality using MRI, DEXA,

or handgrip test findings, though we assessed physical activity level. Nevertheless, the present results clearly indicate that eGFRcr is not a reliable marker of renal function, especially in osteoporotic patients, since osteoporosis preferentially occurs in aged individuals with frequent coexistence of sarcopenia. Furthermore, the higher prevalence of CKD along with the main diseases responsible for development of sarcopenia might make eGFRcr an unreliable marker for renal function in aged osteoporotic patients.

Conclusions

In the present study, we found that reduced eGFRcys may be associated with osteoporotic fracture in postmenopausal women, indicating the involvement of renal osteopathy in its occurrence. Furthermore, the association of increased, but not decreased, eGFRcr with osteoporotic fracture in post-menopausal osteoporotic women with CKDcys might be explained by underestimation of renal dysfunction by use of eGFRcr, because of decreased muscle mass and/or quality in those patients.

Acknowledgements

This study was supported by Merck Sharp & Dohme. The authors' institution received research grants from Merck Sharp & Dohme, Asahi Kasei Pharma, Astellas Pharma, Becton Dickinson, Chugai Pharma, Daiichi Sankyo, DS Pharma Biomedical, Eisai, Eli Lilly, Fuji Pharma, Kissei Pharma, Kyowa Hakko Kirin, Mitsubishi Tanabe Pharma, Nippon Boehringer Ingelheim, Ono Pharma, Sumitomo Dainippon Pharma, Sanofi, Taisho Toyama Pharma, Teijin Pharma, and Torii pharma.

Conflict of interest

MI, SY, YI, and ME received lecture fees from Merck Sharp & Dohme. MK and MI received writing and proofreading fees from Merck Sharp & Dohme. YN has no conflicts of interest.

References

1. Yoshimura N, Muraki S, Oka H, Mabuchi A, En-Yo Y, Yoshida M, Saika A, Yoshida H, Suzuki T, Yamamoto S, Ishibashi H, Kawaguchi H, Nakamura K, Akune T (2009) Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women: the research on osteoarthritis/osteoporosis against disability study. Journal of bone and mineral metabolism 27:620-8 doi:10.1007/s00774-009-0080-8
2. Orimo H, Yaegashi Y, Onoda T, Fukushima Y, Hosoi T, Sakata K (2009) Hip fracture incidence in Japan: estimates of new patients in 2007 and 20-year trends. Arch Osteoporos 4:71-77 doi:10.1007/s11657-009-0031-y
3. Al-Sari UA, Tobias J, Clark E (2016) Health-related quality of life in older people with osteoporotic vertebral fractures: a systematic review and meta-analysis. Osteoporos Int 27:2891-900 doi:10.1007/s00198-016-3648-x
4. Center JR, Nguyen TV, Schneider D, Sambrook PN, Eisman JA (1999) Mortality after all major types of osteoporotic fracture in men and women: an observational study. Lancet 353:878-82 doi:10.1016/S0140-6736(98)09075-8
5. Mills KT, Xu Y, Zhang W, Bundy JD, Chen CS, Kelly TN, Chen J, He J (2015) A systematic analysis of worldwide population-based data on the global burden of chronic kidney disease in 2010. Kidney Int 88:950-7 doi:10.1038/ki.2015.230
6. Schulz E, Arfai K, Liu X, Sayre J, Gilsanz V (2004) Aortic calcification and the risk of osteoporosis and fractures. J Clin Endocrinol Metab 89:4246-53 doi:10.1210/jc.2003-030964
7. Naylor KL, McArthur E, Leslie WD, Fraser LA, Jamal SA, Cadarette SM, Pouget JG, Lok CE, Hodsman AB, Adachi JD, Garg AX (2014) The three-year incidence of fracture in chronic kidney disease. Kidney Int 86:810-8 doi:10.1038/ki.2013.547
8. Yamada S, Inaba M, Kurajoh M, Shidara K, Imanishi Y, Ishimura E, Nishizawa Y (2008) Utility of serum tartrate-resistant acid phosphatase (TRACP5b) as a bone resorption marker in patients with chronic kidney disease: independence from renal dysfunction. Clin Endocrinol (Oxf) 69:189-96 doi:10.1111/j.1365-2265.2008.03187.x
9. Kurajoh M, Inaba M, Yamada S, Imanishi Y, Tsuchida T, Ishimura E, Nishizawa Y (2008) Association of increased active PTH(1-84) fraction with decreased GFR and serum Ca in predialysis CRF patients: modulation by serum 25-OH-D (in eng). Osteoporos Int 19:709-16
10. Yoshimura N, Muraki S, Oka H, Iidaka T, Kodama R, Kawaguchi H, Nakamura K, Tanaka S, Akune T (2017) Is osteoporosis a predictor for future sarcopenia or vice

versa? Four-year observations between the second and third ROAD study surveys. Osteoporos Int 28:189-99 doi:10.1007/s00198-016-3823-0

11. Foley RN, Wang C, Ishani A, Collins AJ, Murray AM (2007) Kidney function and sarcopenia in the United States general population: NHANES III. Am J Nephrol 27:279-86 doi:10.1159/000101827
12. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A, Collaborators developing the Japanese equation for estimated GFR (2009) Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 53:982-92 doi:10.1053/j.ajkd.2008.12.034
13. National Kidney F (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 39:S1-266
14. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, Heilberg IP (2008) Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clin J Am Soc Nephrol 3:348-54 doi:10.2215/CJN.02870707
15. Dharnidharka VR, Kwon C, Stevens G (2002) Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis 40:221-6 doi:10.1053/ajkd.2002.34487
16. Kaji H, Yamauchi M, Yamaguchi T, Shigematsu T, Sugimoto T (2010) Mild renal dysfunction is a risk factor for a decrease in bone mineral density and vertebral fractures in Japanese postmenopausal women. J Clin Endocrinol Metab 95:4635-42 doi:10.1210/jc.2010-0099
17. Nickolas TL, McMahon DJ, Shane E (2006) Relationship between moderate to severe kidney disease and hip fracture in the United States. J Am Soc Nephrol 17:3223-32 doi:10.1681/ASN.2005111194
18. Orimo H, Nakamura T, Hosoi T, Iki M, Uenishi K, Endo N, Ohta H, Shiraki M, Sugimoto T, Suzuki T, Soen S, Nishizawa Y, Hagino H, Fukunaga M, Fujiwara S (2012) Japanese 2011 guidelines for prevention and treatment of osteoporosis--executive summary. Arch Osteoporos 7:3-20 doi:10.1007/s11657-012-0109-9
19. Yamada Y, Noborisaka Y, Ishizaki M, Yamazaki M, Honda R, Yokoyama H, Kakuma T (2015) Different association of cigarette smoking with GFR estimated from serum creatinine and that from serum cystatin C in the general population. Clin Exp Nephrol 19:669-77 doi:10.1007/s10157-014-1058-y
20. Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, Kihara S, Kinoshita M, Maruyama C, Ohta T, Okamura T, Yamashita S,

Yokode M, Yokote K, Japan Atherosclerosis S (2013) Executive summary of the Japan Atherosclerosis Society (JAS) guidelines for the diagnosis and prevention of atherosclerotic cardiovascular diseases in Japan -2012 version. J Atheroscler Thromb 20:517-23

21. Kanis JA, Oden A, Johnell O, Jonsson B, de Laet C, Dawson A (2001) The burden of osteoporotic fractures: a method for setting intervention thresholds. Osteoporos Int 12:417-27 doi:10.1007/s001980170112
22. Horio M, Imai E, Yasuda Y, Watanabe T, Matsuo S, Collaborators Developing the Japanese Equation for Estimated GFR (2013) GFR estimation using standardized serum cystatin C in Japan. Am J Kidney Dis 61:197-203 doi:10.1053/j.ajkd.2012.07.007
23. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A (2009) Revised equations for estimated GFR from serum creatinine in Japan (in eng). Am J Kidney Dis 53:982-92
24. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G (2005) Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 67:2089-100 doi:10.1111/j.1523-1755.2005.00365.x
25. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW et al. (2014) Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. J Am Med Dir Assoc 15:95-101 doi:10.1016/j.jamda.2013.11.025
26. Inaba M, Kurajoh M, Okuno S, Imanishi Y, Yamada S, Mori K, Ishimura E, Yamakawa T, Nishizawa Y (2010) Poor muscle quality rather than reduced lean body mass is responsible for the lower serum creatinine level in hemodialysis patients with diabetes mellitus. Clin Nephrol 74:266-72
27. Masson I, Maillard N, Tack I, Thibaudin L, Dubourg L, Delanaye P, Cavalier E, Bonneau C, Kamar N, Morelon E, Moranne O, Alamartine E, Mariat C (2013) GFR estimation using standardized cystatin C in kidney transplant recipients. Am J Kidney Dis 61:279-84 doi:10.1053/j.ajkd.2012.09.010
28. Kim JK, Choi SR, Choi MJ, Kim SG, Lee YK, Noh JW, Kim HJ, Song YR (2014) Prevalence of and factors associated with sarcopenia in elderly patients with end-stage renal disease. Clin Nutr 33:64-8 doi:10.1016/j.clnu.2013.04.002
29. Fahal IH (2014) Uraemic sarcopenia: aetiology and implications. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 29:1655-65 doi:10.1093/ndt/gft070

Table 1. Clinical characteristics of patients with and without history of osteoporotic fracture

	Osteoporotic fracture (+) (n=211)	Osteoporotic fracture (-) (n=344)	p value
Age, years	78.0±7.2	76.0±6.9	0.001
Body mass index, kg/m^2	23.3±3.1	22.8±2.6	0.078
Diabetes mellitus, n (%)	27 (12.8)	30 (8.7)	0.125
Hypertension, n (%)	119 (56.4)	175 (50.9)	0.205
Dyslipidemia, n (%)	141 (66.8)	246 (71.5)	0.243
Alcohol drinker, n (%)	3 (1.4)	12 (3.5)	0.145
Smoking habit, n (%)	7 (3.3)	18 (5.2)	0.291
Parental history of femoral neck fracture, n (%)	21 (10.0)	29 (8.4)	0.543
Ln (physical exercise per week +1), hours	1.12±1.00	1.34±0.95	0.013
Albuminuria, n (%)	53 (25.1)	86 (25.0)	0.975
eGFRcr, ml/min/1.73 m^2	64.7±16.3	67.4±15.4	0.049
eGFRcys, ml/min/1.73 m^2	62.9±17.8	69.2±17.0	<0.001
CKDcr, n (%)	82 (38.9)	99 (28.8)	0.014
CKDcys, n (%)	92 (43.6)	100 (29.1)	<0.001
eGFRcys/eGFRcr ratio	0.98±0.17	1.04±0.20	<0.001

Data are presented as the mean ± standard deviation or number (%) for dichotomous variables.

Physical exercise per week was natural logarithm-transformed (ln) to achieve a normal distribution. P values are shown for comparisons of the mean values between the groups (unrepeated t-test) or percentages (chi-square test).

Abbreviations: eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; cr, creatinine; cys, Cystatin C

Table 2. Multivariate logistic regression analysis of factors associated with osteoporotic fracture in all patients

Variables	Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Age (per 1year)	1.042 (1.014-1.070)	0.003	1.038 (1.009-1.069)	0.011	1.020 (0.987-1.054)	0.247	1.018 (0.984-1.052)	0.305	1.031 (1.002-1.061)	0.039
Body mass index (per 1kg/m^2)	1.060 (0.993-1.131)	0.082	1.060 (0.993-1.131)	0.082	1.049 (0.982-1.121)	0.156	1.044 (0.976-1.116)	0.208	1.047 (0.980-1.119)	0.172
Diabetes mellitus (absence = 0, presence = 1)	1.416 (0.790-2.538)	0.243	1.409 (0.786-2.528)	0.250	1.312 (0.729-2.361)	0.364	1.282 (0.713-2.307)	0.407	1.330 (0.741-2.389)	0.339
Parent fractured hip (absence = 0, presence = 1)	1.427 (0.775-2.627)	0.253	1.440 (0.782-2.655)	0.242	1.429 (0.773-2.639)	0.255	1.397 (0.755-2.586)	0.288	1.359 (0.736-2.510)	0.328
Albuminuria (absence = 0, presence = 1)	0.800 (0.523-1.221)	0.301	0.802 (0.525-1.225)	0.307	0.767 (0.500-1.176)	0.224	0.747 (0.486-1.149)	0.184	0.755 (0.492-1.160)	0.199
Ln (physical exercise +1) (per week)	0.823 (0.686-0.988)	0.037	0.823 (0.686-0.988)	0.037	0.831 (0.692-0.999)	0.049	0.836 (0.695-1.004)	0.056	0.837 (0.697-1.006)	0.058
eGFRcr (per 10 ml/min/1.73 m^2)			0.961 (0.851-1.086)	0.527			1.098 (0.932-1.293)	0.265		
eGFRcys (per 10 ml/min/1.73 m^2)					0.861 (0.752-0.985	0.029	0.803 (0.669-0.964)	0.019		
eGFRcys/eGFRcr ratio (per 0.1)									0.894 (0.801-0.997)	0.043

Model 1 included age, BMI, physical activity, presence of diabetes, hypertension, dyslipidemia, alcohol drinking habit, current smoking habit, albuminuria, and parental history of femoral neck fracture as covariates. In other models, eGFRcr (Model 2), eGFRcys (Model 3), eGFRcr and eGFRcys (Model 4), and eGFRcys/eGFRcr ratio (Model 5) were added to model 1.

Abbreviations: eGFR, estimated glomerular filtration rate; cr, creatinine; cys, Cystatin C; OR, odds ratio; CI, confidence interval

Table 3. eGFRcys, eGFRcr, and eGFRcys/eGFRcr ratio and osteoporotic fracture in patients with and without CKDcys

	With CKDcys			Without CKDcys		
	Osteoporotic fracture (+) (n=92)	Osteoporotic fracture (-) (n=100)	p value	Osteoporotic fracture (+) (n=119)	Osteoporotic fracture (-) (n=244)	p value
eGFRcr, ml/min/1.73 m^2	53.8±14.5	53.0±10.8	0.638	73.0±12.3	73.3±12.8	0.858
eGFRcys, ml/min/1.73 m^2	46.6±9.9	49.3±8.6	0.044	75.5±10.8	77.3±12.2	0.170
eGFRcys /eGFRcr ratio	0.89±0.15	0.95±0.17	0.009	1.05±0.16	1.08±0.20	0.219

Data are presented as the mean ± standard deviation. P values are shown for comparisons of the mean values of 2 groups (unrepeated t-test).

Abbreviations: eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; cr, creatinine; cys, Cystatin C

Table 4. Osteoporotic fracture and eGFRcys/eGFRcr ratio in patients with and without CKDcys

	eGFRcys/eGFRcr ratio <1.0	eGFRcys/eGFRcr ratio ≥1.0	p value
Osteoporotic fracture in patients with CKDcys, n (%)	74 (53.2)	18 (34.0)	0.017
Osteoporotic fracture in patients without CKDcys, n (%)	48 (34.3)	71 (31.8)	0.629

P values are shown for comparisons of percentages (chi-square test).

Abbreviations: eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; cr, creatinine; cys, Cystatin C

Table 5. Multivariate logistic regression analysis of factors associated with osteoporotic fracture in patients with CKDcys

Variables	Model 1		Model 2		Model 3		Model 4		Model 5	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Age (per 1 year)	1.074 (1.008-1.143)	0.026	1.076 (1.010-1.147)	0.023	1.061 (0.995-1.131)	0.071	1.051 (0.985-1.123)	0.134	1.060 (0.994-1.131)	0.075
Body mass index (per 1 kg/m^2)	1.132 (1.017-1.261)	0.024	1.130 (1.015-1.259)	0.026	1.129 (1.013-1.259)	0.029	1.113 (0.997-1.242)	0.056	1.116 (1.001-1.244)	0.047
Diabetes mellitus (absence = 0, presence = 1)	1.597 (0.682-3.737)	0.281	1.609 (0.685-3.782)	0.275	1.612 (0.689-3.774)	0.271	1.675 (0.705-3.982)	0.243	1.634 (0.690-3.870)	0.264
Parent fractured hip (absence = 0, presence = 1)	2.077 (0.652-6.610)	0.216	2.111 (0.660-6.748)	0.208	1.989 (0.621-6.374)	0.247	1.959 (0.599-6.406)	0.266	2.010 (0.622-6.497)	0.243
Albuminuria (absence = 0, presence = 1)	1.070 (0.572-2.004)	0.831	1.081 (0.577-2.027)	0.807	0.999 (0.527-1.894)	0.997	0.945 (0.492-1.815)	0.864	1.003 (0.530-1.901)	0.992
Ln (physical exercise +1) (per week)	0.937 (0.680-1.291)	0.693	0.925 (0.670-1.277)	0.636	0.967 (0.699-1.337)	0.839	0.956 (0.688-1.329)	0.790	0.936 (0.677-1.294)	0.687
eGFRcr (per 10 ml/min/1.73 m^2)			1.105 (0.861-1.419)	0.431			1.478 (1.049-2.083)	0.026		
eGFRcys (per 10 ml/min/1.73 m^2)					0.765 (0.537-1.090)	0.138	0.531 (0.327-0.863)	0.011		
eGFRcys/eGFRcr ratio (per 0.1)									0.802 (0.655-0.982)	0.032

Model 1 included age, BMI, physical activity, presence of diabetes, hypertension, dyslipidemia, alcohol drinking habit, current smoking habit, albuminuria, and parental history of femoral neck fracture as covariates. In other models, eGFRcr (Model 2), eGFRcys (Model 3), eGFRcr and eGFRcys (Model 4), and eGFRcys/eGFRcr ratio (Model 5) were added to model 1.

Abbreviations: eGFR, estimated glomerular filtration rate; cr, creatinine; cys, Cystatin C; OR, odds ratio; CI, confidence interval

Figure legends

Figure 1. Relationship between eGFRcr and eGFRcys in CKDcys patients with or without osteoporotic fracture.

The following linear regression equations were used.

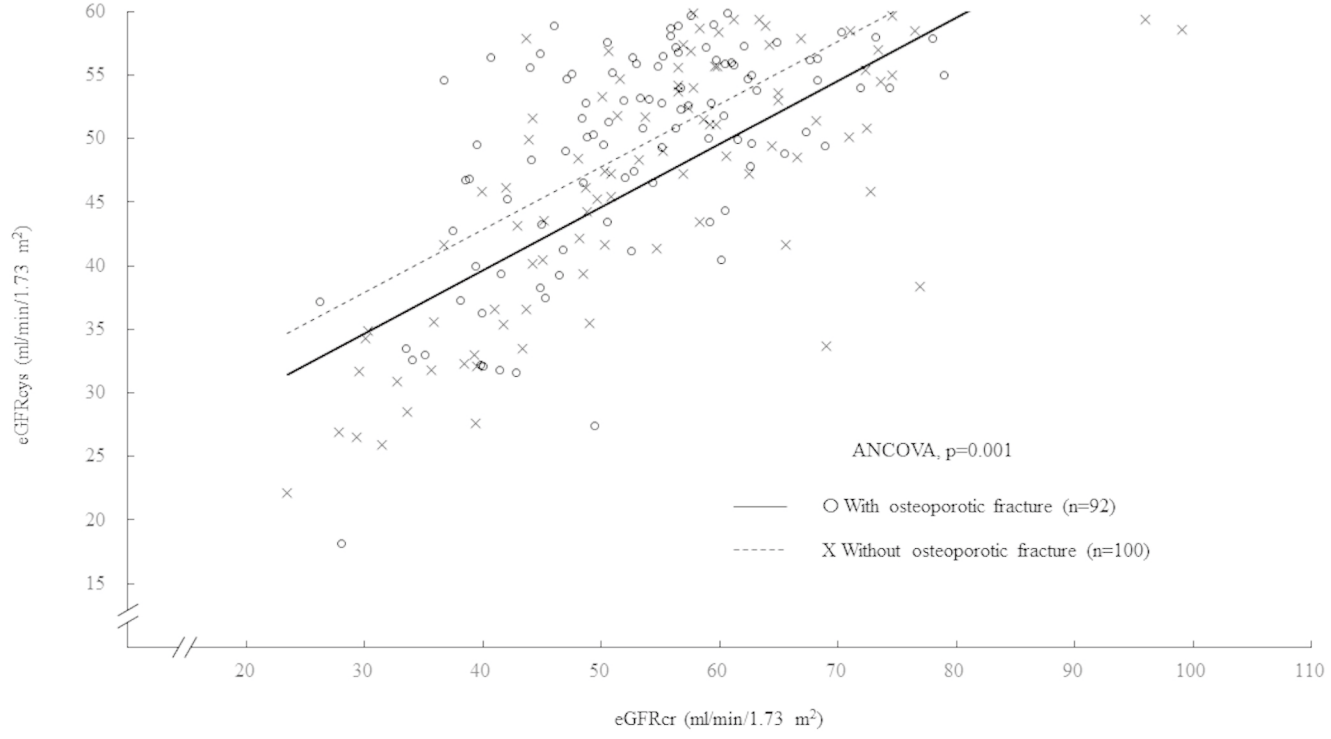
1) Patients with osteoporotic fracture: $y = 19.85 + 0.4963x$, $r=0.725$, $p<0.001$

2) Patients without osteoporotic fracture: $y = 23.12 + 0.492x$, $r=0.624$, $p<0.001$

Analysis of covariance (ANCOVA) indicated that the regression line was significantly different between those with and without osteoporotic fracture ($p=0.001$).

Abbreviations: eGFR, estimated glomerular filtration rate; cr, creatinine; cys, Cystatin C

Figure 1


eGFRcys (ml/min/1.73 m²)
eGFRcr (ml/min/1.73 m²)
ANCOVA, p=0.001
O With osteoporotic fracture (n=92)
X Without osteoporotic fracture (n=100)