Osteoporosis in nontuberculous mycobacterial pulmonary disease: a cross-sectional study

Hiromu Tanaka1†, Takanori Asakura1*†, Shoji Suzuki1, Satoshi Okamori1, Tatsuya Kusumoto1, Takunori Ogawa1, Shunsuke Uno2, Atsuho Morita1, Ho Lee1, Ho Namkoong2, Hirofumi Kamata1, Yasunori Sato3, Yoshifumi Uwamino4, Tomoyasu Nishimura5, Makoto Ishii1, Koichi Fukunaga1 and Naoki Hasegawa2

Abstract

Background: Since nontuberculous mycobacterial pulmonary disease (NTM-PD) is common in middle-aged/elderly slender women at risk of osteoporosis, we hypothesized that NTM-PD could be associated with osteoporosis. The study aimed to evaluate the prevalence of osteoporosis in patients with NTM-PD compared with that in the general population and determine the factors associated with osteoporosis in the subjects, including the serum estradiol (E2) and 25-hydroxyvitamin D (25OHD) levels.

Methods: We have recruited 228 consecutive adult patients with NTM-PD from a prospective cohort study at the Keio University Hospital, who had no history of osteoporosis or osteoporosis-associated bone fracture but underwent dual-energy X-ray absorptiometry-based bone mineral density (BMD) evaluation from August 2017–September 2019. The E2 and 25OHD levels were measured in 165 patients with available stored serum samples. We performed multivariable logistic regression analyses for osteopenia and osteoporosis.

Results: Osteoporosis (T-score ≤ −2.5) and osteopenia (T-score −1 to −2.5) were diagnosed in 35.1% and 36.8% of patients with NTM-PD, respectively. Compared with the general population, the proportion of osteoporosis was significantly higher in 50–59-, 60–69-, and 70–79-year-old women with NTM-PD. Multivariable analysis revealed that older age (adjusted odds ratio [aOR] for 1-year increase = 1.12; 95% confidence interval [CI] = 1.07–1.18), female sex (aOR = 36.3; 95% CI = 7.57–174), lower BMI (aOR for 1 kg/m2 decrease = 1.37; 95% CI = 1.14–1.65), and chronic Pseudomonas aeruginosa (PA) infection (aOR = 6.70; 95% CI = 1.07–41.8) were independently associated with osteoporosis. Additionally, multivariable analysis in 165 patients whose serum E2 and 25OHD levels were measured showed that both low E2 levels (< 10 pg/mL) and lower 25OHD levels were independently associated with osteoporosis.

Conclusions: Middle-aged/elderly women with NTM-PD have a higher prevalence of osteoporosis than the general population. BMD screening should be considered in NTM-PD, especially in older females with severe diseases such as chronic PA infection and lower BMI, and low serum E2 and 25OHD levels.

†Hiromu Tanaka and Takanori Asakura have contributed equally.

*Correspondence: takanori.asakura@keio.jp

1 Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan

Full list of author information is available at the end of the article

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Background

Nontuberculous mycobacterial (NTM) pulmonary disease (NTM-PD) is the most common NTM infection, and its incidence and prevalence have increased worldwide. A Japanese epidemiological study reported that there were 14.7 estimated cases of NTM-PD per 100,000 person-years in 2014 [1]. The characteristics of patients with NTM-PD differ between Europe and Japan; there are more males and a higher frequency of chronic obstructive pulmonary disease complications in Europe compared with that in Japan [2–4]. The progression of NTM-PD leads to decreased lung function, decreased health-related quality of life, and a poorer prognosis [5–7]. Progressive NTM-PD requires prolonged multidrug antimicrobial therapy; however, macrolide resistance and high recurrence rate may make the management difficult [8]. Thus, as NTM-PD often presents as a chronic disease, physicians should pay attention to comorbidities while managing it.

NTM-PD commonly occurs in middle-aged/elderly, slender, and postmenopausal women [9], and this population also presents with risk factors for osteoporosis, which is a systemic skeletal disease leading to increased bone fragility and fracture risk [10]. Our prior study showed that low serum estradiol (E2) levels were strongly associated with Mycobacterium avium complex (MAC) pulmonary disease (MAC-PD) [11]. Another study showed that NTM-PD is associated with severe vitamin D deficiency [12], which can lead to osteoporosis. Moreover, previous studies on chronic respiratory diseases, such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis, and bronchiectasis without NTM infection, have shown to be associated with osteoporosis [13–15]. Some chronic lung diseases, especially those that cause structural destruction of the lungs, such as idiopathic pulmonary fibrosis [14] and bronchiectasis [15], have been reported to be associated with osteoporosis [16]. Patients with COPD are also reported to be at a higher risk of osteoporosis and fractures compared with age- and sex-matched patients without COPD [17]. Therefore, we hypothesized that NTM-PD could be associated with osteoporosis, especially when associated with low serum E2 and 25-hydroxyvitamin D (25OHD) levels.

Methods

Study design and patients

A cross-sectional study was conducted using the registry of a prospective cohort study that included patients with NTM-PD at Keio University Hospital [20–23]. NTM-PD was diagnosed based on the 2007 American Thoracic Society /Infectious Disease Society of America (ATS/IDSA) guidelines [8]. The Keio University Hospital ethics review board approved our study protocol (#20110267), and we obtained written informed consent from all eligible patients before their inclusion in the study. Figure 1 shows the enrollment process. Between August 2017 and September 2019, dual-energy X-ray absorptiometry (DXA)-based BMD was evaluated in 228 consecutive patients with NTM-PD, who had no history of osteoporosis or osteoporosis-associated bone fracture. The proportion of osteoporosis in the 228 patients was compared with that in the general Japanese population. Five patients did not undergo pulmonary function test (PFT), or multi-detector-row computed tomography (CT) within 4 months before and after DXA measurements. Therefore, we included 223 patients for the cross-sectional analysis.

We used stored serum samples previously obtained from the “NTM Biomarker Study” in the Keio University Hospital (#20170181) to investigate the association of E2 and 25OHD with osteopenia/osteoporosis [11]. Available serum samples within 3 months at the time when the patients underwent DXA measurements were included and analyzed. Stored serum samples were available for 173 patients with NTM-PD. Eight patients with a history of oophorectomy or previous hormone replacement therapy were excluded to eliminate any possible medical effects. Finally, serum E2 and 25OHD were measured in 165 patients.
Assessment of clinical parameters
We obtained patient demographic data, including age, sex, body mass index (BMI), disease duration, smoking history, underlying pulmonary and non-pulmonary diseases, current pharmacological treatment, treatment status for NTM-PD, and sputum smear and culture results of the previous year. Immunosuppressive or biological agents were defined as oral corticosteroids, calcineurin inhibitors, methotrexate, or any biological agents. We obtained patient data regarding chronic *Pseudomonas aeruginosa* (PA) infection as PA infection is known as a poor prognostic factor for bronchiectasis [24]. Chronic PA infection was defined as PA isolated from sputum culture on two or more occasions ≥3 months apart in any 1-year period in the past [20].

PFT was performed in a stable condition using an electronic spirometer (Chestac-9800 or HI-801; Chest M.I., Tokyo, Japan) according to the ATS/ERS recommendations [25]. PFT included the assessment of forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV₁). The radiographic CT patterns were categorized as nodular/bronchiectatic (NB), fibrocavitary (FC), NB + FC, or unclassified [26]. The severity of bronchiectasis was determined using the modified Reiff score [27]. The number of lobes involved (including the lingua, total six) and the degree of dilatation (tubular = 1, varicose = 2, and cystic = 3) were calculated with scores ranging from 0 to 18. Only the completed 6-min walk distance (6MWD), one of the important parameters associated with health-related quality of life in MAC-PD [28], was analyzed.

DXA measurements
DXA measurements of BMD were performed at the hip and lumbar spine using a Hologic 4500A Discovery bone densitometer (HOLOGIC, Bedford, MA, USA). Osteoporosis was diagnosed based on the lowest T-score of these locations and World Health Organization criteria.
T score \( \geq -1 \), T score \(-1 \) to \(-2.5 \), T score \( \leq -2.5 \) were defined as normal BMD, osteopenia, and osteoporosis, respectively.

**Serum E2 and 25OHD levels**

The serum E2 and 25OHD levels were measured using a chemiluminescent immunoassay (CLIA) and electrochemiluminescent immunoassay, respectively. Since the serum E2 level under the limit of detection for CLIA was \(< 10 \) pg/mL, and because this level was associated with low BMD in postmenopausal women [30], low serum E2 levels were defined as \(< 10 \) pg/mL. Serum 25OHD levels were divided into normal (\(\geq 30 \) ng/ml), insufficiency (\(< 30 \) ng/ml and \(\geq 20 \) ng/ml), and deficiency (\(< 20 \) ng/ml) [31].

**The general Japanese population**

To compare the prevalence of osteoporosis in patients with NTM-PD in our study with that of the general population, we used data from the study by Yoshimura et al. [32] and calculated the proportion of osteoporosis in each age strata (\(\leq 39, 40–49, 50–59, 60–69, 70–79, \geq 80 \) years). Our study used T score \(\leq -2.5 \) for the diagnosis of osteoporosis, but the study by Yoshimura et al. [32] used a value of less than 70% of the Young Adult Mean (YAM). Another report (using T score \(\leq -2.5 \) in the same population) showed similar or rather a slightly lower rate of osteoporosis [33], but the raw data based on age and sex were not available to us. Additionally, another report showed that the T score and the YAM criteria were almost equivalent [34]. Therefore, the difference in criteria did not affect the results of the comparison between the two populations.

**Statistical analysis**

We compared the prevalence of osteoporosis between patients with NTM-PD and the Japanese general population by Fisher’s exact probability test. To identify the factors associated with osteopenia/osteoporosis, we first checked multicollinearity in all the variables using Spearman’s correlation. We then performed multivariable logistic regression analyses using a backward elimination method based on the corrected Akaike information criterion (AICc) [35], starting with a model that included age, sex, BMI, smoking history, underlying pulmonary disease, connective tissue diseases, NTM-PD duration, treatment history of NTM-PD, acid-fast bacilli (AFB) smear positivity, NTM species (MAC or non-MAC), chronic PA infection, cavitary lesions, modified Reiff score, and % predicted FEV1 (%FEV1). In 165 NTM-PD patients whose E2 and 25OHD were measured, these variables were added for the multivariable analysis in the same manner. P-values were two-tailed, and \(P < 0.05 \) was considered statistically significant. Data were analyzed using the JMP 14 program (SAS Institute Japan Ltd, Tokyo, Japan). A graph of existing data was made with GraphPad Prism 8 (GraphPad Software, San Diego, California).

**Results**

**Comparison with the general population**

A total of 228 patients were included: 64 patients with normal BMD (28.1%), 84 with osteopenia (36.8%), and 80 with osteoporosis (35.1%). Figure 2 and Additional File 1: Figure S1 shows the comparison of the percentage of osteoporosis between the standard population [32] and that in female and male patients with NTM-PD, stratified by age group, respectively. The comorbidity was significantly higher in women with NTM-PD at age groups 50–59 years (18.8 vs. 4.8%, \(P = 0.010 \)), 60–69 years (46.6 vs. 22.2%, \(P < 0.001 \)), and 70–79 years (54.4 vs. 42.9%, \(P < 0.001 \)).

**Patient characteristics**

Table 1 shows the clinical characteristics of the 223 patients for the cross-sectional analysis, which included 64 patients with normal BMD (28.7%), 82 with osteopenia (36.8%), and 77 with osteoporosis (34.5%). The median age of the 223 patients was 70 years, and 181 patients were female (82.5%). The median duration of NTM-PD was 10 years. The most common causative species for NTM was MAC (204 patients, 91.5%), followed by *M. abscessus* complex (24 patients, 10.8%). Twenty-nine patients (13.0%) had chronic PA infection. The most
| Table 1 Characteristics of the study population |
|-----------------------------------------------|
| All patients (n = 223) | Normal BMD (n = 64) | Osteopenia (n = 82) | Osteoporosis (n = 77) |
|------------------------|---------------------|---------------------|----------------------|
| **Age, years** | 70 (62–76) | 66 (51–76) | 69 (61–75) | 73 (69–78) |
| **Sex, female** | 181 (82.5) | 42 (65.6) | 69 (84.1) | 73 (94.8) |
| **BMI, kg/m²** | 19.3 (17.4–21.4) | 20.2 (19.0–22.5) | 19.2 (17.6–21.1) | 17.9 (16.3–20.1) |
| **Disease duration, years** | 10 (6–15) | 9 (5–14) | 9 (6–14) | 11 (8–17) |
| **Treatment history** | | | | |
| Never treated | 87 (39.0) | 26 (40.6) | 35 (42.7) | 26 (33.8) |
| Previously treated | 25 (11.2) | 9 (14.1) | 8 (9.8) | 8 (10.4) |
| Currently treated | 111 (49.8) | 29 (45.3) | 39 (47.6) | 43 (55.8) |
| **Smoking status** | | | | |
| Not current / Current | 221 (99.1) / 2 (0.9) | 63 (98.4) / 1 (1.6) | 81 (98.8) / 1 (1.2) | 77 (100) / 0 (0) |
| **Underlying pulmonary disease** | 25 (11.2) | 7 (10.9) | 9 (11.0) | 9 (11.7) |
| **History of TB** | 6 (2.7) | 4 (6.3) | 2 (2.5) | 0 (0) |
| **Asma** | 2 (0.9) | 0 (0) | 2 (2.5) | 0 (0) |
| **COPD** | 2 (0.9) | 1 (1.6) | 1 (1.3) | 0 (0) |
| **Interstitial lung disease** | 5 (2.3) | 2 (3.2) | 3 (3.8) | 0 (0) |
| **Comorbidities** | | | | |
| Diabetes Mellitus | 23 (10.3) | 6 (9.4) | 10 (12.2) | 7 (9.1) |
| Chronic kidney disease | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Connective tissue disease | 15 (6.7) | 3 (4.7) | 7 (8.5) | 5 (6.5) |
| **Use of immunosuppressive or biological agents** | 11 (4.9) | 3 (4.7) | 4 (4.9) | 4 (5.2) |
| **Bacterial variables** | | | | |
| NTM species | | | | |
| MAC | 204 (91.5) | 56 (87.5) | 74 (90.2) | 74 (96.1) |
| **M abscessus complex** | 24 (10.8) | 8 (12.5) | 8 (9.8) | 8 (10.4) |
| **M kansasii** | 4 (1.8) | 0 (0) | 2 (2.4) | 2 (2.6) |
| **M fortuitum** | 3 (1.3) | 1 (1.6) | 0 (0) | 2 (2.6) |
| Sputum AFB smear positive† | 82 (36.8) | 19 (29.7) | 26 (31.7) | 37 (48.1) |
| Sputum AFB culture positive† | 120 (53.8) | 35 (54.7) | 43 (52.4) | 42 (54.5) |
| Chronic P aeruginosa infection | 29 (13.0) | 4 (6.3) | 10 (12.2) | 15 (19.5) |
| **Pulmonary function test** | | | | |
| **FVC, L** | 2.52 (2.07–2.95) | 2.97 (2.52–3.50) | 2.55 (2.12–2.91) | 2.12 (1.68–2.59) |
| **FVC, % predicted** | 82.0 (70.8–94.6) | 93.7 (78.7–120) | 80.9 (71.5–91.7) | 76.5 (64.7–86.3) |
| **FEV₁, L** | 1.79 (1.49–2.16) | 2.22 (1.77–2.55) | 1.78 (1.50–2.03) | 1.58 (1.24–1.91) |
| **FEV₁, % predicted** | 71.2 (60.9–85.6) | 79.9 (65.5–104) | 70.0 (63.5–79.1) | 67.9 (55.6–80.0) |
| **FEV₁/FVC** | 72.1 (67.2–78.4) | 73.3 (67.9–78.3) | 70.8 (65.6–76.2) | 72.1 (67.8–78.5) |
| **CT findings** | | | | |
| Radiographic type | | | | |
| **NB** | 182 (81.6) | 48 (75.0) | 67 (84.8) | 64 (83.1) |
| **FC** | 5 (2.2) | 2 (3.1) | 2 (2.5) | 1 (1.3) |
| **NB + FC** | 17 (7.6) | 4 (6.3) | 4 (5.1) | 9 (11.7) |
| **Unclassified** | 19 (8.5) | 10 (15.6) | 6 (7.6) | 3 (3.9) |
| Presence of cavitary lesion | 47 (21.1) | 8 (12.5) | 15 (18.3) | 24 (31.2) |
| Modified Reiff score | 4 (2–6) | 3 (2–4) | 3 (2–5) | 5 (3–6) |
| Number of affected lobes | 4 (3–5) | 3 (2–4) | 4 (3–5) | 4 (4–6) |
| Six-min walk distance, m‡ | 450 (402–500) | 465 (428–515) | 450 (415–498) | 415 (355–480) |

Abbreviations: AFB Acid-fast bacilli, BMI Body mass index, COPD Chronic obstructive pulmonary disease, FC Fibrocavitary, FEV Forced expiratory volume, FVC Forced vital capacity, MAC Mycobacterium avium complex, NB Nodular/bronchiectatic, NTM Nontuberculous mycobacteria, TB Tuberculosis

† There are no patients with chronic kidney disease
‡ Bacterial status within the previous 1 year
§ Six-minute walk test was performed on 201 patients (61; normal BMD, 73; osteopenia, 67; osteoporosis)
common radiographic type was NB type (182 patients, 81.6%).

In the comparison of patients with NTM-PD among the normal BMD, osteopenia, and osteoporosis groups, it was found that osteoporosis patients were older, predominantly females, and had a lower BMI. The PFT results revealed that FVC and FEV₁ were lower in the osteopenia and osteoporosis group compared with those in the normal BMD group. Furthermore, the PFT results in the osteoporosis group were lower than those in the osteopenia group. The proportion of cavitary lesions, the modified Reiff score, and the number of affected lung lobes were higher in the osteoporosis group compared with the normal BMD group (Fig. 3A–C). The 6MWD was lower in the osteoporosis group compared with the normal BMD and osteopenia group but was comparable between the normal BMD and osteopenia groups (Fig. 3D).

Additional File 1: Table S1 shows a comparison of the characteristics of the 165 patients with NTM-PD whose serum E₂ and 25OHD levels were measured. The distribution of these patients is as follows: normal BMD (n = 45, 27.3%), osteopenia (n = 63, 38.2%), and osteoporosis (n = 57, 34.5%) groups. The comparisons among the three groups are almost consistent with Table 1. Although the serum E₂ levels in all male participants were ≥ 10 pg/mL, those in most female subjects were < 10 pg/mL (50–59-year-old, 75.0%; 60–69-year-old, 77.5%; 70–79-year-old, 88.9%; 80–89-year-old, 70.0%), except for those in 40–49-year-old women (Fig. 3E). Low serum E₂ (defined as < 10 pg/mL) was associated with low T-score (Fig. 3F) with a higher proportion of osteopenia and osteoporosis groups, compared with the normal BMD group (Fig. 3G and Additional File 1: Figure S2). Although the comparisons of 25OHD, stratified by serum 25OHD levels, were comparable (Additional File 1: Figure S3), the serum 25OHD level as a continuous variable was lower in the osteoporosis group than in the normal BMD group (Fig. 3H).

Factors associated with osteopenia/osteoporosis
Table 2 shows the results of multivariable logistic regression analysis in the 223 patients with NTM-PD. The analysis revealed that age (adjusted odds ratio [aOR], 1.04; 95% confidence interval [CI], 1.01–1.08), female sex (aOR 4.69, 95%CI 1.81–12.1) and chronic PA infection (aOR 4.31, 95%CI 1.08–17.2) were predictors of osteopenia, while age (aOR 1.12, 95%CI 1.07–1.18), female sex (aOR 36.3, 95%CI 7.57–174), lower BMI (aOR for 1 kg/m²

---

**Fig. 3** [A–D] Comparison of the cavitary lesion (A), modified Reiff score (B), number of affected lobes (C), and the 6-min walk distance (D) among the normal bone mineral density (BMD), osteopenia, and osteoporosis groups. **E** Percentage of low serum estradiol (E₂) levels (< 10 pg/mL) stratified by age in female patients with nontuberculous mycobacterial pulmonary disease. **F** T-score compared between low serum E₂ and others. **G** Comparison of low serum E₂ status among normal bone mineral density (BMD), osteopenia, and osteoporosis groups. **H** Serum 25OHD levels represented as a continuous variable among normal bone mineral density (BMD), osteopenia, and osteoporosis groups. **P** < 0.01. ***P** < 0.001
decrease 1.37, 95% CI 1.14–1.65), and chronic PA infection (aOR 6.70, 95% CI 1.07–41.8) were factors associated with osteoporosis.

Table 3 shows the results of multivariate analysis in the 165 patients with NTM-PD whose serum E2 and 25OHD levels were measured. Low serum E2 (aOR 4.54, 95% CI 1.97–10.5) was the only predictor of osteopenia, while age (aOR 1.12, 95% CI 1.05–1.19), lower BMI (aOR for 1 kg/m² decrease: 1.26, 95% CI 1.02–1.57), lower % FEV1 (aOR for 1% decrease: 1.04, 95% CI 1.01–1.07), low serum E2 (< 10 pg/mL)(aOR 3.29, 95% CI 1.01–10.7), and lower serum 25OHD (aOR for 1 ng/mL decrease: 1.12, 95% CI 1.03–1.23) were associated with osteoporosis.

**Discussion**

Our study showed that 35.1% and 36.8% of patients with NTM-PD were newly diagnosed with osteoporosis and osteopenia, respectively, indicating that BMD loss in many patients with NTM-PD was unrecognized in clinical practice. Additionally, the proportion of osteopenia in female patients with NTM-PD in the age groups 50–59, 60–69, and 70–79 years was significantly higher than that in the general population. Furthermore, factors including age, being female, lower BMI, and chronic PA infection were associated with osteoporosis. Our multivariable analysis revealed a significant association of age, being female and chronic PA infection with osteopenia.

Finally, our multivariable analysis of patients whose serum E2 and 25OHD levels were measured revealed significant associations of both low serum E2 and 25OHD with osteoporosis. There are potentially a large number of patients with NTM-PD with untreated osteoporosis, and treatment may prevent decreased mobility and lung function due to fractures and falls. Bone density screening can improve the long-term prognosis and quality of life of patients with NTM-PD by increasing the intervention for undiagnosed osteoporosis.

To our knowledge, this is the first study revealing the prevalence of osteopenia/osteoporosis in patients with NTM-PD in a clinical setting, especially the higher proportion of osteoporosis in female patients with NTM-PD at 50–79 years. Importantly, our study showed that more than one third of patients with NTM-PD were underdiagnosed with osteoporosis. The risk factors for osteoporosis or bone fracture typically include age, female sex, low body weight, smoking, excessive alcohol intake, and glucocorticoid use [36]. BMI and glucocorticoid use, but not age and sex, have been associated with lower BMD in lung transplantation candidates [37]. Importantly, NTM-PD is more likely to occur in patients with these diseases that involve structural destruction of the lungs [16]. Our study revealed a higher prevalence of osteoporosis in female patients with NTM-PD at the stratified

**Table 2** Multivariable logistic regression analysis of factors associated with osteopenia and osteoporosis in 223 NTM-PD patients

| Characteristics          | Osteopenia | Osteoporosis |
|--------------------------|------------|--------------|
|                          | aOR        | 95% CI       | P value | aOR        | 95% CI       | P value |
| Age, years               | 1.04       | 1.01–1.08    | 0.014   | 1.12       | 1.07–1.18    | <0.001  |
| Sex, female              | 4.69       | 1.81–12.1    | 0.001   | 36.3       | 7.57–174     | <0.001  |
| Lower BMI, 1 kg/m² decrease | 1.12     | 0.99–1.26    | 0.079   | 1.37       | 1.14–1.65    | <0.001  |
| Chronic PA infection     | 4.31       | 1.08–17.2    | 0.039   | 6.70       | 1.07–41.8    | 0.042   |
| Presence of cavitary lesion | –        | –             | –       | 2.39       | 0.74–7.72    | 0.145   |

**Table 3** Multivariable logistic regression analysis in 165 NTM-PD patients whose serum E2 and 25OHD were measured

| Characteristics          | Osteopenia | Osteoporosis |
|--------------------------|------------|--------------|
|                          | aOR        | 95% CI       | P value | aOR        | 95% CI       | P value |
| Age, years               | –          | –             | –       | 1.12       | 1.05–1.19    | <0.001  |
| Lower BMI, 1 kg/m² decrease | –        | –             | –       | 1.26       | 1.02–1.57    | 0.032   |
| Chronic PA infection     | 3.49       | 0.61–19.9    | 0.159   | –          | –             | –       |
| Lower FEV1%predicted, 1% decrease | –        | –             | –       | 1.04       | 1.01–1.07    | 0.008   |
| Low E2 (< 10 pg/mL)      | 4.54       | 1.97–10.5    | <0.001  | 3.29       | 1.01–10.7    | 0.048   |
| Lower 25OHD, 1 ng/mL decrease | –        | –             | –       | 1.12       | 1.03–1.23    | 0.011   |

Abbreviations: aOR Adjusted odds ratio, BMI Body mass index, CI Confidence interval, NTM-PD Nontuberculous mycobacterial pulmonary disease, PA P. aeruginosa

Abbreviations: 25OHD 25-hydroxyvitamin D, aOR Adjusted odds ratio, BMI Body mass index, CI Confidence interval, E2 Estradiol, FEV1 Forced expiratory volume in 1 s, NTM-PD Nontuberculous mycobacterial pulmonary disease, PA P. aeruginosa
comparison of age and sex, indicating that other factors besides age and sex were also associated with osteoporosis. Notably, the present study also revealed that lower serum E2 levels were associated with osteopenia and osteoporosis. The onset of osteoporosis is more common in the postmenopausal period with rapid depletion of estrogen, around the age of 50 years, because estrogen deficiency causes increased bone resorption and decreases bone density [38]. Further, in postmenopausal women, female hormone depletion has also been associated with a low BMI [39], which is also a major risk factor for osteoporosis and bone fracture [40]. Therefore, female hormone deficiency causes BMD loss directly and through decreased BMI. One of the NTM-PD subsets has been classically described as the Lady Windermere syndrome, as it occurs commonly in thin middle-aged/elderly women [41]. A previous report has shown that BMI and body fat are significantly lower in patients with NTM-PD [42]. Our previous study on postmenopausal women aged ≤ 65 years revealed that low serum E2 levels were strongly associated with MAC-PD, although, the menopausal age in both control and MAC-PD groups was around 50 years [11]. In addition, another study showed that median/mean serum E2 levels in normal postmenopausal Japanese women are more than 10 pg/mL [11, 43], while in our study, more than 70% of patients over 50 years had low serum E2 levels. A study in mice suggested that E2 may have a protective role against intracellular mycobacterial infection [44]. Additionally, low BMI has been associated with the development and severity of NTM-PD [45]. Taken together, female hormones and a low BMI are strongly associated with the development of NTM-PD, as well as with osteoporosis, and may have affected the high comorbidity of osteoporosis in the current study.

Our study revealed that chronic PA infection was associated with osteopenia and osteoporosis in addition to previously described factors such as age, sex, lower BMI, and lower serum E2 levels. Chronic PA infection has been associated with increased disease severity in adult bronchiectasis patients [24] and MAC-PD [20]. Our study showed that the osteoporosis group had a higher modified Reiff score, number of affected lobes, and lower lung function and 6MWD, all of which imply that this group contains has severe NTM-PD, causing immobilization. Thus, chronic PA might be associated with osteoporosis. Another possible cause is a negative pathophysiological effect of PA infection on osteoporosis. Basic studies have suggested that various proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and receptor activator of NF-κB ligand (RANKL), which are released during infection, may promote the development and activity of osteoclasts [46]. A study on cystic fibrosis patients primarily colonized with PA showed that infective exacerbation induced an increased production of potential osteoclast precursors in the peripheral blood, which may increase bone resorption, contributing to BMD loss [47]. Although the direct effect of PA infection is still unclear, we should consider screening patients with severe NTM-PD, especially those complicated with chronic PA infection, for osteoporosis.

About 70% patients with NTM-PD showed vitamin D deficiency (< 20 ng/mL), which is similar to the results of a previous Korean study [12]. The lower serum 25OHD was independently associated with osteoporosis in NTM-PD. Vitamin D is essential for calcium absorption and can work importantly as an immunomodulator. Specifically, for mycobacterial infection, vitamin D deficiency was associated with tuberculosis progression [48, 49]. The osteoporosis group had higher severity of NTM-PD, which can induce immobilization and reduced exposure to sunlight can lower serum 25OHD; another possibility is that patients with low serum 25OHD might show disease progression as well as osteoporosis. Although, the causal relationship between serum vitamin D and NTM-PD is unclear, serum 25OHD may aid diagnosis with osteoporosis as well as vitamin D supplementation.

There are several limitations to the current study. First, though the population of NTM-PD at our institution was not significantly different from populations in other studies [50, 51], this is a single-center, cross-sectional study, which limits us to elucidate causal relationships. The paucity of underlying pulmonary diseases other than NTM-PD may also allow us to assess the direct impact of NTM-PD on BMD. Additionally, the general population we used included mountainous and seacoast patients outside of our institute in Tokyo. Therefore, an additional multicenter study would be desirable to avoid patient selection bias. Secondly, the small number of male patients included in the present study warrants additional evaluation. Future studies with a larger number of participants and analyses with gender and age matching are required. Third, the implementation of DXA in this cohort is based on the clinician’s judgment of necessity, which could cause selection bias. We may have also underestimated the prevalence of osteoporosis in the study population because we excluded patients with previously noted osteoporosis. However, this study is retrospective, which is limited as a study design, and requires future prospective studies. Finally, the study did not measure any bone metabolism markers, which may provide more insight into the association and pathophysiology between pulmonary disease and osteoporosis. While we measured serum E2 and 25OHD, this
showed a significant association between serum E2 and BMD loss, further longitudinal studies including these markers are needed to investigate the pathophysiology and effect of intervention including pharmacological treatment.

Conclusions
The findings of our study revealed a higher incidence of osteoporosis in patients with NTM-PD than that in the general population of middle-aged/older women. BMD screening should be considered in patients with NTM-PD, especially when older in age, female, and diagnosed with severe diseases like chronic PA infection and lower BMI. The measurement of serum E2 and 25OHD levels may also be useful to assess the factors associated with osteopenia and osteoporosis in NTM-PD patients.

Abbreviations
25OHD: 25-Hydroxyvitamin D; AFB: Acid fast bacilli; aOR: Adjusted odds ratio; BMD: Bone mineral density; BMI: Body mass index; CI: Confidence interval; COPD: Chronic obstructive pulmonary disease; DXA: Dual-energy X-ray absorptiometry; E2: Estradiol; FC: Fibrocavitary; FEV1: Forced expiratory volume in 1 s; FVC: Forced vital capacity; MAC: Mycobacterium avium Complex; MAC-PD: MAC pulmonary disease; NB: Nodular/bronchietatic; NTM: Nontuberculous mycobacteria; NTM-PD: NTM pulmonary disease; PA: Pseudomonas aeruginosa; PFT: Pulmonary function test; TB: Tuberculosis.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12890-022-01991-3.

Acknowledgements
We thank Shoko Takahashi, Shoko Kashimura, and Kana Misawa (Keio University, Tokyo, Japan) for assistance with data and serum sample collection. We thank Kumiko Matsuzaki (Keio University, Tokyo, Japan) for assistance in obtaining patient consent.

Author contributions
HT, TA, and NH designed the study. HT, TA, SS, SO, TK, TO, AM, and KL collected the data. HT, TA, SU, and NH analyzed and interpreted the data. YS supervised the statistical analyses and the study design. SU, YU, and TN collected serum samples. HT and TA drafted the manuscript, and HK, YS, YU, TN, MI, KF, and NH supervised the manuscript revision. All authors read and approved the final manuscript.

Funding
This work was supported by the Research Program on Emerging and Re-emerging Infectious Disease from the Japan Agency for Medical Research and Development (AMED) (grant number: JP18fk0108043 and JP20fk01080129), JSPS KAKENHI (grant number: 19H03704, 21K08515, 21KK0148).

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations
Ethics approval and consent to participate
This study was registered on the University Hospital Medical Information Network (UMIN00007546) and was approved by the ethics committees of Keio University hospital (No. 20110267). All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
We obtained written, informed consent from all eligible patients before their inclusion in the study.

Competing interests
Hiromu Tanaka, Takanori Asakura, Shoji Suzuki, Satoshi Okamori, Tatsuya Kusumoto, Takunori Ogawa, Shunsuke Uno, Atsuho Morita, Ho Lee, Ho Namkoong, Hirofumi Kamata, Yasunori Sato, Yoshifumi Uwamino, Tomoyasu Nishimura, Makoto Iishi, Koichi Fukunaga, and Naoki Hasegawa declare that they have no conflict of interest.

Author details
1Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, 35 Shinannomachi, Shinjuku, Tokyo 160-8582, Japan.
2Department of Infectious Diseases, Keio University School of Medicine, 35 Shinannomachi, Shinjuku, Tokyo, 160-8582, Japan.
3Department of Preventive Medicine and Public Health, Keio University School of Medicine, 35 Shinannomachi, Shinjuku, Tokyo 160-8582, Japan.
4Department of Laboratory Medicine, Keio University School of Medicine, 35 Shinannomachi, Shinjuku, Tokyo 160-8582, Japan.
5Keio University Health Center, Tokyo, Japan.

Received: 22 November 2021 Accepted: 5 May 2022
Published online: 21 May 2022

References
1. Namkoong H, Kurashima A, Morimoto K, Hoshino Y, Hasegawa N, Ato M, et al. Epidemiology of pulmonary nontuberculous mycobacterial disease. Jpn Emerg Infect Dis. 2016;22:1116–7.
2. Ringshausen FC, Ewen R, Multmeier J, Monga B, Obradovic M, van der Laan R, et al. Predictive modeling of nontuberculous mycobacterial pulmonary disease epidemiology using German health claims data. Int J Infect Dis. 2021;104:398–406.
3. Uno S, Asakura T, Morimoto K, Yoshimura K, Uwamino Y, Nishimura T, et al. Comorbidities associated with nontuberculous mycobacterial disease in Japanese adults: a claims-data analysis. BMC Pulm Med. 2020;20:262.
4. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med. 2015;36:13–34.
5. Park HY, Jeong BH, Chon HR, Jeon K, Daley CL, Koh WJ. Lung function decline according to clinical course in nontuberculous mycobacterial lung disease. Chest. 2016;150:1222–32.
6. Asakura T, Funatsu Y, Iishi M, Namkoong H, Yagi K, Suzuki S, et al. Health-related quality of life is inversely correlated with C-reactive protein and age in Mycobacterium avium complex lung disease: a cross-sectional analysis of 235 patients. Respir Res. 2015;16:145.
7. Morimoto K, Iwai K, Uchimura K, Okumura M, Yoshiyama T, Yoshimori K, et al. A steady increase in nontuberculous mycobacteriosis mortality and estimated prevalence in Japan. Ann Am Thorac Soc. 2014;11:1–8.
8. Daley CL, Iaccarino JM, Lange C, Cambau E, Wallace RJ Jr, Andrejak C, et al. Treatment of nontuberculous mycobacterial pulmonary disease:
an official ATS/ERS/SCCM/IDSA clinical practice guideline. Eur Respir J. 2020;56:2000535.
9. Chan ED, Iseman MD. Scleroderma. Older women appear to be more susceptible to non-tuberculous mycobacterial lung disease. Gend Med. 2010;7:5–18.
10. Organization WH. WHO scientific group on the assessment of osteoporosis at primary health care level. In: Summary meeting report Brussels, Belgium. 2004.
11. Uwamimo Y, Nishimura T, Sato Y, Tamizu E, Asakura T, Uno S, et al. Low serum estradiol levels are related to Mycobacterium avium complex lung disease: a cross-sectional study. BMC Infect Dis. 2019;19:1055.
12. Jean K, Kim SY, Jeong BH, Chang B, Shin SJ, Koh WJ. Severe vitamin D deficiency is associated with non-tuberculous mycobacterial lung disease: a case-control study. Respir Physiol. 2021;189:983–8.
13. Watanabe R, Tanaka T, Aita K, Hayagai M, Homma T, Yokosuka K, et al. Osteoporosis is highly prevalent in Japanese males with chronic obstructive pulmonary disease and is associated with deteriorated pulmonary function. J Bone Miner Metab. 2015;33:392–400.
14. Caffarelli C, Gonnelli S, Tomasi Pitinca MD, Francolini V, Fui A, Bargagli E, et al. Idiopathic pulmonary fibrosis: a rare disease with severe bone fragility. Intern Emerg Med. 2016;11:1087–94.
15. Contreras-Bolivar V, Oliveira G, Porras N, Acosta E, Rubio-Martín E, Tapia-Guererro MJ, et al. Osteopenia and osteoporosis in patients with bronchectasis: association with respiratory parameters, body composition, muscle strength and bone remodeling biomarkers. Sci Rep. 2019;9:14496.
16. Ratnaguru CN, Lutzky VP, Kupz A, Doolan DL, Reid DW, Field M, et al. The rise of non-tuberculous mycobacterial lung disease. Front Immunol. 2020;11:303.
17. Stallberg B, Janson C, Larsson K, Johansson G, Kostikas K, Gruenberger JB, et al. Real-world retrospective cohort study ARCTIC shows burden of comorbidities in Swedish COPD versus non-COPD patients. NPJ Prim Care Respir Med. 2018;28:33.
18. Ringshausen FC, Wagner D, de Roux A, Diel R, Hohmann D, Hickstein LD, et al. Prognosis in adult bronchiectasis. Ann Am Thorac Soc. 2015;12:1602–11.
19. Kamata H, Asakura T, Suzuki S, Namkoong H, Yagi K, Funatsu Y, et al. Impact of chronic Pseudomonas aeruginosa infection on health-related quality of life in Mycobacterium avium complex lung disease. BMC Pulm Med. 2017;17:198.
20. Asakura T, Ishii M, Ishi K, Suzuki S, Namkoong H, Okamoto S, et al. Health-related QOL of elderly patients with pulmonary M. avium complex disease in a university hospital. Int J Tuberc Lung Dis. 2018;22:695–703.
21. Asakura T, Yamada Y, Suzuki S, Namkoong H, Okamoto S, Kusumoto T, et al. Quantitative assessment of erector spinae muscles in patients with Mycobacterium avium complex lung disease. Respir Med. 2018;145:66–72.
22. Asakura T, Kimsukza Y, Nishimura T, Suzuki S, Namkoong H, Masuy S, et al. Serum Krebs von den Lungen-6 level in the disease progression and treatment of Mycobacterium avium complex lung disease. Respirology. 2020;26:112–9.
23. Finch S, McDonnell MJ, Abo-Leyah H, Aliberti S, Chalmers JD. A comprehensive analysis of the impact of Pseudomonas aeruginosa colonization on prognosis in adult bronchectasis. Am Am Thorac Soc. 2015;12:602–11.
24. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26:319–38.
25. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26:319–38.
26. Asakura T, Yamada Y, Namkoong H, Suzuki S, Niijima Y, Kamata H, et al. Impact of cavity and infiltration on pulmonary function and health-related quality of life in pulmonary Mycobacterium avium complex disease: a 3-dimensional computed tomographic analysis. Respir Med. 2017;126:9–16.
27. Reff DB, Wells AU, Carr DH, Cole PJ, Hansell DM. CT findings in bronchiectasis: limited value in distinguishing between idiopathic and specific types. AJR Am J Roentgenol. 1995;165:261–7.
28. Yagi K, Asakura T, Namkoong H, Suzuki S, Asami T, Okamoto S, et al. Association between six-minute walk test parameters and the health-related quality of life in patients with pulmonary Mycobacterium avium complex disease. BMC Pulm Med. 2018;18:114.
29. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO study group. In: WHO technical report series. 1994.
30. Ettinger B, Pressman A, Sklair P, Bauer DC, Cauley JA, Cummings SR. Associations between low levels of serum estradiol, bone density, and fractures among elderly women: the study of osteoporotic fractures. J Clin Endocrinol Metab. 1998;83:2239–43.
31. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266–81.
32. Yoshimura N, Muraki S, Oka H, Machida A, En-Yo Y, Yoshida M, et al. Prevalence of knee osteoarthritis, lumbar spondylosis, and osteoporosis in Japanese men and women: the research on osteoarthritis/osteoporosis against disability study. J Bone Miner Metab. 2009;27:620–8.
33. Yoshimura N, Muraki S, Oka H, Kawaguchi H, Nakamura K, Akune T. Cohort profile: research on osteoarthritis/osteoporosis against disability study. Int J Epidemiol. 2010;39:988–95.
34. Soen S, Fukunaga M, Sugimoto T, Sone T, Fujiwara S, Endo N, et al. Diagnostic criteria for primary osteoporosis: year 2012 revision. J Bone Miner Metab. 2013;31:247–57.
35. Burnham KP, Anderson DR. Multimodel inference. Soc Methods Res. 2002;33:261–304.
36. Kanis JA, Oden A, Johansson H, De Laet C, Brown J, et al. The use of clinical risk factors enhances the performance of BMD in the prediction of hip and osteoporotic fractures in men and women. Osteoporos Int. 2007;18:1033–46.
37. Tschopp O, Boehler A, Speich R, Wieder W, Seifert B, Rusi EW, et al. Osteoporosis before lung transplantation: association with low body mass index, but not with underlying disease. Am J Transplant. 2002;2:167–72.
38. Riggs BL. The mechanisms of estrogen regulation of bone resorption. J Clin Invest. 2000;106:1203–4.
39. Oh H, Coburn SB, Matthews CE, Falk RT, LeBlanc ES, Wactawski-Wende J, et al. Anthropometric measures and serum estrogen metabolism in postmenopausal women: the Women’s health initiative observational study. Breast Cancer Res. 2017;19:28.
40. Johansson H, Kanis JA, Oden A, McCloskey E, Chapurlat RD, Christiansen C, Cummings SR, Diez-Perez A, Erismann JA, Fujisawa S, et al. A meta-analysis of the association of fracture risk and body mass index in women. J Bone Miner Res. 2014;29:233–33.
41. Reich JM, Johnson RE. Mycobacterium avium complex pulmonary disease presenting as an isolated lingular or middle lobe pattern. The Lady Winder-mere syndrome. Chest. 1992;101:1605–9.
42. Kartalja M, Ovrsutsky AR, Bryan CL, Pott GB, Fantuzzi G, Thomas J, et al. Patients with nontuberculous mycobacterial lung disease exhibit unique body and immune phenotypes. Am J Respir Crit Care Med. 2013;187:197–205.
43. Nagata C, Shimizu H, Takami R, Hayashi M, Takeda N, Yasuda K. Soy Product intake and serum isoflavonoid and estradiol concentrations in relation to bone mineral density in postmenopausal Japanese women. Osteoporos Int. 2002;13:200–8.
44. Calippe B, Douin-Echinard V, Laffargue M, Laurell H, Rana-Poussine V, Pipy B, et al. Chronic estradiol administration in vivo promotes the proinflam-matory response of macrophages to TLR4 activation: involvement of the phosphatidylinositol 3-kinase pathway. J Immunol. 2008;181:9708–8.
45. Song JH, Kim BS, Kwak N, Han KD, Yim JY. Impact of body mass index on development of nontuberculous mycobacterial pulmonary disease. Eur Respir J. 2020;55:2000454.
46. Xing L, Schwarz EM, Boyce BF. Osteoestrogen precursors, RANKL/RANK, and immunology. J Bone Miner Res. 2005;20:19–29.
47. Sheaf ED, Haworth CS, Gunn E, Bilton D, Scott MA, Compston JE. Osteooste-othesis during infective exacerbations in patients with cystic fibrosis. Am J Respir Crit Care Med. 2006;174:306–11.
48. Gibney KB, MacGregor L, Ledder K, Torresi J, Marshall C, Ebeling PR, et al. Vitamin D deficiency is associated with tuberculosis and latent tuberculo-sis infection in immigrants from sub-Saharan Africa. Clin Infect Dis. 2008;46:443–5.
49. Talat N, Perry S, Parsonnet J, Dawood G, Chaudhry H, et al. Prevalence of tuberculosis progression. Emerg Infect Dis. 2010;16:853–5.
50. Furuchchi K, Morimoto K, Yoshimura T, Tanaka Y, Fujiwara K, Okumura M, et al. Interrelational changes in the epidemiology and clinical features of nontuberculous mycobacterial pulmonary disease and tuberculosis in a referral hospital in Japan. Respir Med. 2019;152:74–80.
51. Aksamit TR, O’Donnell AE, Barker A, Oliver KN, Winthrop KL, Daniels MLA, et al. Adult patients with bronchiectasis: a first look at the US bronchiectasis research registry. Chest. 2017;151:982–92.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.