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Inadequate response to treatment reveals persistent osteoclast bone resorption in osteoporotic patients

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Short Title: high bone resorption in resistance to osteoporotic treatment

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Abstract

Several drugs are able to reduce fracture risk in osteoporotic patients. Incident fractures occur despite good adherence to treatment. Inadequate response has been found related to high serum bone biomarkers of bone turnover. We here aimed to analyze bone microarchitecture and cellular profiles of inadequate responders. We retrospectively analyzed bone biopsies from patients with major fractures despite long-term treatment (inadequate responder [IR] n=31) in comparison to patients with untreated osteoporosis (U-OP, n=31) and controls without osteoporosis (Ctrl, n=16). Bone samples were analyzed by histomorphometry and micro-computed tomography. Clinical and bone turnover markers and bone mineral density were assessed. As compared with U-OP patients, IRs were older (mean age 69.7 ± 8.8 vs 63.3 ± 9.3 years, $p=0.007$) and had lower mean hip bone mineral density (0.685 ± 0.116 vs 0.786 ± 0.093 g/cm², $p=0.019$ and T-score (-2.3 ± 0.769 vs -1.6 ± 0.900 , $p=0.032$). BV/TV was lower for IRs than U-OP patients and Ctrl (13.9±3.8% vs 15.2±5.1 and 17.6±5.2%, $p=0.044$) as was trabecular thickness (145.6 ± 23.1 vs 160.5 ± 22.7 and 153.7 ± 21.4 μm, $p=0.033$). Mean structure model index was lower for IRs than U-OP patients (1.9 ± 0.806 vs 2.4 ± 0.687 , $p=0.042$) and osteoclast number was higher for IRs than U-OP patients and Ctrl (0.721 ± 0.611 vs 0.394 ± 0.393 and 0.199 ± 0.071 mm⁻², $p<0.001$). The mean Obl.S/BS was lower for IRs than U-OP patients and Ctrl (1.2 ± 1.3 vs 1.9 ± 1.4 and 3.0 ± 0.638 mm⁻², $p<0.0001$), and the mean number of labelled surfaces was lower for IRs than U-OP patients (51.6% vs 87%, $p=0.002$). Cortical parameters did not significantly differ. We show an imbalance of bone remodeling in favor of bone resorption in IRs. The persistence of high bone resorption suggests insufficient inhibition of bone resorption that could explain the incident fractures with anti-osteoporotic drug use. Adaptation to treatment should be considered to inhibit bone resorption and prevent further fractures.

1. Introduction

Osteoporosis is a skeletal disorder characterized by decreased bone mass and deterioration of bone micro-architecture, leading to increased bone fragility and susceptibility to fracture [1]. These conditions are associated with significant morbidity and mortality. Several treatments are available, that have been shown to reduce fracture risk. The reduction of fracture ranges from 30% to 70% for vertebral fractures, 40% to 50% for hip fractures and 15% to 20% for non-vertebral fractures depending on the therapeutic agent [2]. Clinical trials and observational studies have highlighted that patients with prevalent fractures will sustain new fractures during osteoporosis therapy. This so-called resistance to treatment is defined by a failure to reduce fracture incidence with anti-fracture therapies. The monitoring of response to treatment is based on changes in bone mineral density (BMD) and bone turnover markers. However, these are imperfect surrogates for anti-fracture efficacy. Indeed, up to one half of patients with incident fractures have baseline BMD above the World Health Organization diagnostic threshold for osteoporosis [3]. In addition, bone turnover can be used to monitor adherence when measured at baseline and during treatment, in particular when non-compliance or drug discontinuation is suspected [4]. Resistance to treatment remains a clinical challenge. The definition of non-response or inadequate response to osteoporosis therapy in individual patients lacks consensus. Inadequate response may be defined as the incidence of major insufficiency fracture after at least 1 year of treatment, multiple minor insufficiency fracture or a significant decrease in BMD in patients with fractures [5, 6, 7, 8] despite documented adherence to treatment. The level of serum alkaline phosphatase and current smoking could predict inadequate response to osteoporotic treatment [9].

Another hypothesis of recurrent fracture is based on bone microarchitecture, a major component of bone quality and strength. This microarchitecture could be measured by peripheral micro-computed tomography (μ CT), although so far dedicated to research

purposes. Microarchitecture could also be measured in bone biopsies, although these are rarely performed because of the invasive nature. In addition to 3-D quantification of the cortical and trabecular bone structure by μ CT, histomorphometric analysis of bone biopsies allows analyzing the rate of bone remodeling and cell activity, thereby providing additional clues for bone fragility [10]. Bone biopsies also allow analyzing the cellular mechanism underlying the response to treatment. Indeed, inhibitors of bone resorption reduce both bone resorption and formation on the basis of the osteoclast – osteoblast coupling. In osteoporotic individuals treated with Alendronate in a clinical trial, unpaired bone biopsies showed that Alendronate impairs more the bone formation than the bone resorption, which suggest that bisphosphonates impair the expansion of osteoprogenitors, thereby alleviating the initiation of bone formation [11]. In contrast, bone biopsies of osteoporotic patients treated with Denosumab revealed a continuous bone gain through modeling-based bone formation, even in a context of maximal inhibition of bone resorption [12]. These apparent controversial results highlight the various mechanism of action of inhibitors of bone resorption and their consequences in bone formation. However, these reports do not address the potential non-response to anti-osteoporotic drugs. To our knowledge, no study has characterized bone profiles in individuals with inadequate response to osteoporotic therapy observed in a real-life setting. Therefore, the aim of this study was to analyze the cellular and microarchitecture profiles from a bone biobank to gain insight into resistance to treatment and to propose management strategies in non-responders to treatment.

2. Methods

2.1 Study design

We retrospectively analyzed bone biopsies collected from 2009 to 2019 from 3 tertiary osteoporosis academic centers to which patients were referred because of non-response to

therapy. The indication for bone biopsies was inadequate response (IR; n=31) defined by the occurrence of a new osteoporotic fracture after at least 1 year of osteoporotic treatment. The second group of bone biopsies involved osteoporotic patients who had never received any bone treatment and were called untreated osteoporotic patients (U-OP; n= 31); 14 biopsies were performed during the same period (2009 to 2019) to fully rule out secondary causes and 17 biopsies were obtained from patients with at least one vertebral fracture who were part of a previous reported study [13]. None of the 31 patients of the U-OP group had received anti-osteoporotic drugs at the time of the biopsy. For the IR and U-OP groups, demographic, morphologic, clinical risk factors for fracture, bone biomarkers and treatment sequence were collected. A historical collection of iliac crest bone biopsies was used as controls (Ctrls; n=16). These were obtained from non-osteoporotic, non-treated women with bone biopsy performed to obtain reference values of bone indices [14]. All bone biopsies were taken at the trans iliac site and for the control group trans iliac biopsies were collected during surgery for knee osteoarthritis in women without any digestive, renal or endocrine disorders. In this historical group, no woman had spinal vertebrae observed on systematic profile radiography. Biological tests were performed at the time of the bone biopsy collection for ruling out secondary causes but no biomarkers of bone remodeling.

2.2 *BMD and bone biomarker collection*

BMD measurement of the IR and U-OP groups involved dual energy X-ray absorptiometry at the total hip and lumbar spine (L2-L4) with Lunar or Hologic devices. There was no cross-calibration, so results are reported as T-scores, as recommended in clinical practice as well as in Z-score. Although showed, BMD were not comparable within devices. Vertebral fractures were recorded with lateral radiographs. At the time of the first management, secondary causes of osteoporosis were excluded according to serum levels of total calcium, phosphorus, and 25OH vitamin D; electrophoresis of proteins C-reactive protein, thyroid-stimulating hormone,

and serum creatinine. Serum bone biomarkers were measured in the morning in fasting conditions, including alkaline phosphatase levels (ALP; Atellica CH Alkaline Phosphatase ALP-2c, France), parathyroid hormone levels (PTH; Atellica IM PTH, Siemens, Centaur XP, Siemens and PTH kit assay liaison (Diasorin, France), (Chimiluminescence Liaison, Diasorin, France) and C-telopeptides (CTX; ELISA IDS and ELISA Roche, France). Etiologic tests were also performed for Ctrl and U-OP groups without treatment who had the biopsy before 2009 but not the biomarkers that were not routinely performed at this time.

2.3 Bone biopsy

After double labelling with tetracycline (14 and 13 days, then 3 and 2 days before biopsy), trans-iliac bone biopsies were performed under local anesthesia with a 7.5-mm internal-diameter Bordier trephine, 2 cm below the iliac crest and 2 cm behind the antero-superior iliac crest. Trans-iliac bone biopsy was performed within the 6 months after the fracture in both U-OP and IR groups and during knee arthritis surgery for Ctrl. Specimens were fixed in ethanol, dehydrated in xylene at 4°C, then embedded without demineralization in polymethylmethacrylate. Sections 5 µm thick were cut by using an SM 2500S or a Polycut S microtome (Leica, Germany) and stained with Toluidine blue. A single-blinded reader (C.M) measured static bone histomorphometry of 2 distant sections using an objective eyepiece Zeiss Intergrate plate I1 and a semiautomatic image analyzer (Leitz, Germany). Measurements were performed on total cancellous and cortical bone. Dynamic parameters were measured in the same sections using dedicated Histolab 7.6.0 software (Microvision, Les Ulis, France). Bone formation rate was calculated by Mineral Apposition Rate (MAR) multiplied by the length of double labelled surfaces. The histomorphometric parameters were measured and expressed in accordance with the Guidelines of the Histomorphometry Nomenclature Committee of the American Society for Bone and Mineral Research [15].

2.4 Microarchitectural analysis of bone biopsy cores

Microarchitectural analysis by μ -CT analysis was performed in the same block than histology. The whole bone specimens were scanned by using a SkyScan 1272 scanner (Bruker micro-CT, Kontich, Belgium) with v1.1.17 acquisition software. The source voltage and current were 90kV and 111 μ A. All scans were obtained at 180° with voxel size 6 μ m. To remove soft radiographs, a 0.5-mm-thick aluminum filter was placed in front of the x-ray source. The angular rotation step was fixed at 0.5°, with frame averaging 2 and acquisition time per frame of about 1200 ms. Reference images without specimens were obtained before each acquisition for flat field correction. The SkyScan volumetric NRecon reconstruction software (v1.7.3.0) was used to reconstruct cross-section slices from acquired μ CT angular projections through the object. Image processing and analysis involved using Bruker CTscan v1.18.8, giving size and microarchitecture bone parameters. We measured the ratio of trabecular bone volume to total volume (BV/TV), trabecular separation (Tb.Sp mm), trabecular thickness (Tb.Th mm), trabecular number (Tb.N mm^{-1}), structure model index (SMI), connectivity density (μm^{-1}), trabecular BMD ($\text{g}\cdot\text{cm}^{-3}$), cortical thickness (Ct.Th μm), total porosity (Ct.Por.Tot%) and cortical BMD ($\text{g}\cdot\text{cm}^{-3}$).

2.5 Statistical analysis

Data are presented as mean \pm SD and number (percentage) for categorical variables. To compare quantitative variables among the 3 groups (Ctrl, U-OP, IR), we used ANOVA (in case of homoscedasticity, Bartlett's test after Shapiro-Wilk normality test) followed by Dunnett's test in case of global $p < 0.05$ or Kruskal-Wallis test followed by Steel-Dwass-Critchlow-Fligner test otherwise. The IR group was the reference group for comparing the Ctrl and U-OP groups. When comparing quantitative variables for 2 groups, Student t test was used, with log transformation for variables without normal distribution. In case of non-homoscedasticity, Mann Whitney test was used. Chi-square test was used for categorical

variables or Fisher exact test as appropriate. All tests were two-sided, with significance level fixed at 0.05. Data analysis involved using R v3.5.1 (The R Foundation for Statistical Computing) and XLSTAT v2020.1.3 (Addinsoft, XLSTAT statistical and data analysis solution, Paris, France; <https://www.xlstat.com>).

3. Results

3.1. Clinical parameters

Characteristics of groups are in Table 1. Most patients were women: 100% in the Ctrl group, 87% in the U-OP group, and 90% in the IR group. Mean age was significantly higher in the IR group than Ctrl and U-OP patients ($p=0.02$ and $p=0.036$, respectively). Body mass index did not significantly differ among groups, nor did tobacco use, alcohol intake, history of endocrine diseases, corticosteroids use or family history of fracture. All patients were ambulant at the time of the recurrent fractures as well as the controls.

For the IR group, fractures occurred after mean treatment duration of 8.1 ± 5.1 years. Fractures were major osteoporotic fractures, affecting mainly axial skeleton (77.4% for U-OP patients and 45.16% for IRs). At the time of the bone biopsy, IR patients were receiving oral bisphosphonates ($n=17$), intravenous bisphosphonate ($n=6$), strontium ranelate ($n=2$), raloxifene ($n=2$), denosumab ($n=1$) and teriparatide ($n=3$). None of the patients experienced Romosozumab. IR and U-OP groups did not differ in BMD or T-score at the lumbar spine ($p=0.125$ and 0.178 , respectively). However, at the total hip, BMD and T-scores were significantly lower for IRs than U-OP patients ($p=0.019$ and $p=0.032$, respectively). Z-scores were not statistically different between groups. Circulating bone biochemical markers PTH, ALP, osteocalcin and crosslaps CTX did not differ between IR and U-OP groups, except for serum total calcium level, which was significantly lower for IRs than U-OP patients ($p=0.012$).

3.2. Histomorphometry indices

Histomorphometric analysis of bone biopsies is shown in Table 2. BV/TV was significantly lower for IRs than Ctrl (p<0.0001) but not U-OP patients despite lower values. The osteoblast surface/bone surface (Obl.S/BS) was significantly lower for IRs than U-OP patients (p<0.0001) and Ctrl (p<0.001). IR and U-OP groups did not differ in double-labelled surfaces, total labelled surfaces or MAR. However, the presence of labelling along the bone surface was significantly lower for IRs than U-OP patients (p=0.002). The osteoclast number (N.Oc/TV) was significantly higher for IRs than U-OP patients (p<0.001), but the higher Oc.S/BS did not reach significance (p=0.115). Finally, Ct.Th did not significantly differ between the IR and U-OP groups (p=0.44). We found no association between age and any of the histomorphometric parameters (Figure 1), despite large variations in U-OP and IR groups.

3.3. Microarchitectural indices

Results of the parameters of bone microarchitecture measured in 3D μ CT are in Figure 2. Analysis of trabecular indices revealed a significantly lower BV/TV in the IR than Ctrl group (p=0.031) but not U-OP group. Tb.Th was lower in the IR than U-OP group (p=0.033) as was SMI (p=0.044). The groups did not significantly differ in Tb.Sp (p=0.538), Tb.N (p=0.066), connectivity density (p=0.553) or trabecular BMD (p=0.056). Analysis of cortical bone showed no difference among the groups in Ct.Th (p=0.33), Ct.Po (p=0.64) or cortical BMD (p=0.62) (Figure 3).

4. Discussion

Here, we analyzed the histological and microarchitecture profiles in bone biopsies of patients in whom new fractures occurred during treatment to reduce fracture risk. Indeed, IRs patients were older than Ctrl and U-OP patients in relation with the treatment duration after the diagnosis and initiation of first treatment. Here, inadequate response to treatment was

associated with increased number of osteoclasts, which suggests the persistence of high bone resorption. The lower hip BMD illustrates that the treatment was not able to restore proper level of BMD. This was not observed at the spine, likely not interpretable because of the prevalence of vertebral fractures.

High bone resorption is associated with an increase in bone fragility illustrated by the occurrence of fractures [16]. Although bone biopsies were taken upon various treatments, most treatments in the IR group were anti-resorbing agents. The treatment or sequence of treatment at the time of the biopsy was various, including with 3 patients on teriparatide, the only anabolic drug used in this cohort, but their parameters were not different than the others. Therefore, these findings may indicate the variable sensitivity of osteoclasts to anti-resorbing agents [17] in addition to the variability of response to treatment among osteoporotic patients. A significantly higher osteoclast number was observed in IR than U-OP group, but the higher osteoclast surface did not reach significance despite a trend towards. This might be related to the osteoclast size, in particular osteoclasts maybe as numerous but smaller in IR patients. In addition, treatment may have affected the resorbing activity. Treatment could have inhibited less the pit-forming osteoclasts rather than the trench-forming osteoclasts in contrast to previous report observed in osteoclast cultures [17], and also because treatment was insufficient to inhibit osteoclasts that become more aggressive with age [18]. Finally, there was a large heterogeneity in response to zoledronate in terms of size in osteoclast derived from women donors, which was not observed here in bone biopsies. Recently, in vitro osteoclast maturation and resorption capacity was found highly variable in control individuals but depended on age. Indeed, age, older donors and individuals with a long delay of menopause are factors associated with a high capacity to form osteoclasts in relation to the DNA methylation levels of dendritic cell transmembrane protein and cathepsin K, 2 key markers of osteoclast maturation and function [18]. Moreover, the in vitro response to

zoledronic acid in donors was variable and lower in osteoclasts with high protein levels of cathepsin K [17]. Therefore, our group of IRs may have had lower susceptibility to treatment **that could not be assessed in terms of biological functions**. This finding also raises the issue of the dose adjustment rather than “one-dose-fits-all”, some patients requiring high doses to repress bone resorption. Finally, anabolic agents should be discussed when incident fractures occur during treatment.

The persistently high osteoclast parameter values in IRs raised the question of adherence to treatment. Here, 10 patients among 31 were receiving a parenteral treatment in which adherence and anti-osteoporotic effect is usually better than with oral treatment [4]. Monitoring the adherence to treatment could have been assessment by CTX levels [4], but baseline levels were not available in every patient in this retrospective study. Although not significant, the mean CTX level was reduced by almost 40% in IRs versus U-OP patients. The lack of significant reduction in bone resorption marker values might be related to the variable suppression effect of anti-resorbing drugs [19], the use of different kits over time, the delay between the last therapeutic intake and the measure that might affect the CTX levels. In addition, serum markers were measured within the first 6 months after the incident fractures in IR patients and explained the non-significant reduction [20]. Conversely, mean serum calcium level was significantly lower in the IR than U-OP group, which **illustrates the good adherence** to anti-resorbing drugs that are known to decrease total calcium levels. Together, all these elements indicate that inadequate response to conventional treatment was not due to a poor adherence to treatment but to the persistence of bone resorption.

Conversely, bone biopsies revealed a lower mean BV/TV, Tb.Th and osteoblast surface in IRs versus U-OP patients, which indicates low bone formation. We observed a lack of labelled surfaces in IRs versus U-OP patients as previously reported with antiresorptive drugs. Indeed,

the lack of labelling may be common in treated patients because these drugs alter matrix deposition [21]. The imbalance in bone formation to resorption also showed that high or even maintained osteoclast function facilitates the perforation of preferentially thin trabecula [22] as also shown here by a poor connectivity and SMI. The latter parameter revealed the changes from trabeculae plate-like to rod-like distribution that occurs in osteoporosis and aging [23]. Indeed, the low SMI in IRs indicates that the treatment reversed the alteration of the microarchitecture, restoring the rod-like to plaque-like distribution related to age. Indeed, BMD measured in biopsies of IR patients was similar at the cortical bone despite long-term treatment. This finding would be explained by the cross-sectional design of the study that included unpaired biopsies, and the basal level was not known.

To our knowledge, this is the first study describing the histological profile of IRs by using a biobank of bone biopsies, compared to an untreated osteoporotic population and to control subjects without osteoporosis. Here, we could not compare the bone biopsies of IRs patients to those of osteoporotic patients with adequate response in order to confirm whether inadequate response is due to the persistence of high bone resorption, or related to the activity of bone cells, a failure of microarchitecture or to other parameters such as bone strength. Unfortunately, bone biopsies of responders were not available because biopsies were not required in patients who responded properly in the real life setting, those being available mainly in therapeutic clinical trials and are often limited to a small number of samples. Another limitation is the retrospective design that did not allow analyzing the longitudinal changes in serum marker values and bone structure. Here, we did not assess the mechanical competence, a major determinant of bone fragility. We failed to analyze the rate of falls that could have promoted the occurrence of fractures despite adequate response to treatment. Indeed, incident fracture could be precipitated by falls despite mild difference in terms of response in activity of bone cells. This hypothesis could not be ruled out although in our serie,

the majority of incident fractures occurred at appendicular rather than at the peripheral skeleton in which the role of falls is more predominant. Finally, the large range of treatment used is associated with heterogeneity in the IR group and could be a potential confounder. Therefore, our study needs to be confirmed in a larger number of bone biopsies and include the above discussed parameters.

In conclusion, we show that inadequate response to treatment is associated with persistent bone resorption and low bone formation despite adequate compliance with anti-osteoporotic drugs. These findings suggest that insufficient treatment may require a dose adjustment to repress bone resorption.

Statement of Ethics

This study was conducted using the data obtained from the usual standard of care of our institution. Patients were informed that the data could be used for research purposes and their agreement was collected. There were no additional tests designed for the study as this was a retrospective study.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

BL: designed the study, performed the experiments, analyzed the data and wrote the manuscript.

AO: performed the statistical analysis and revised the manuscript.

CM: performed the histology.

TFB and SF: analyzed the data and revised the manuscript

PF and CC: provide the bone biopsies, analyzed the data and revised the manuscript.

JLB: performed the bone biopsies and revised the manuscript.

MCS: designed the experiments, analyzed the data and wrote the manuscript.

All the authors approved the final version of the manuscript.

References

1. Conference C. Osteoporosis prevention, diagnosis, and therapy. NIH Consens. Statement. 2000;17(1):1–45.
2. Zhou H, Dempster DW. Lessons from Bone Histomorphometry on the Mechanisms of Action of Osteoporosis Drugs [Internet]. Fourth Edi. Osteoporos. Fourth Ed. Elsevier; 2013. Available from: <http://dx.doi.org/10.1016/B978-0-12-415853-5.00076-5>
3. Compston, Juliet E; McClung, Michael R; Leslie, William D (2019). Osteoporosis. *The Lancet*, 393(10169), 364–376. doi:10.1016/S0140-6736(18)32112-3
4. Fontalis A, Eastell R. The challenge of long-term adherence: The role of bone turnover markers in monitoring bisphosphonate treatment of osteoporosis. *Bone* [Internet]. Elsevier Inc.; 2020 Jul 1;136.
5. Adami S, Isaia G, Luisetto G, Minisola S, Sinigaglia L, Gentilella R, Agnusdei D, Iori N, Nuti R. Fracture incidence and characterization in patients on osteoporosis treatment: The ICARO study. *J. Bone Miner. Res.* 2006;21(10):1565–70.
6. Díez-Pérez A, Adachi JD, Adami S, Anderson FA, Boonen S, Chapurlat R, Compston JE, Cooper C, Gehlbach SH, Greenspan SL, Hooven FH, Lacroix AZ, Nieves JW, Coen Netelenbos J, Pfeilschifter J, Rossini M, Roux C, Saag KG, Silverman S, Siris ES, Wyman A, Rushton-Smith SK, Watts NB. Risk factors for treatment failure with antiosteoporosis medication: The global longitudinal study of osteoporosis in women (GLOW). *J. Bone Miner. Res.* 2014;29(1):260–7.
7. Diez-Perez A, Adachi JD, Agnusdei D, Bilezikian JP, Compston JE, Cummings SR, Eastell R, Eriksen EF, Gonzalez-Macias J, Liberman UA, Wahl DA, Seeman E, Kanis JA, Cooper C. Treatment failure in osteoporosis. *Osteoporos. Int.* 2012;23(12):2769–74.
8. Díez-Pérez A, Olmos JM, Nogués X, Sosa M, Díaz-Curiel M, Pérez-Castrillón JL,

- Pérez-Cano R, Muñoz-Torres M, Torrijos A, Jodar E, Del Rio L, Caeiro-Rey JR, Farrerons J, Vila J, Arnaud C, González-Macías J. Risk factors for prediction of inadequate response to antiresorptives. *J. Bone Miner. Res.* 2012;27(4):817–24.
9. Cairoli E, Eller-Vainicher C, Ulivieri FM, Zhukouskaya V V., Palmieri S, Morelli V, Beck-Peccoz P, Chiodini I. Factors associated with bisphosphonate treatment failure in postmenopausal women with primary osteoporosis. *Osteoporos. Int.* 2014;25(4):1401–10.
 10. Chappard D, Retailleau-Gaborit N, Legrand E, Baslé MF, Audran M. Comparison insight bone measurements by histomorphometry and μ CT. *J. Bone Miner. Res.* 2005;20(7):1177–84.
 11. Jensen PR, Andersen TL, Chavassieux P, Roux JP, Delaisse JM. Bisphosphonates impair the onset of bone formation at remodeling sites. *Bone.* 2021 Apr;145:115850.
 12. Dempster, DW; FRMS, ; Chines, A; Bostrom, MP; Nieves, JW; Zhou, H; Chen, L; Pannacciulli, N; Wagman, RB; Cosman, F (2020). Modeling-Based Bone Formation in the Human Femoral Neck in Subjects Treated With Denosumab. *J. Bone Miner. Res.*, jbmr.4006–. doi:10.1002/jbmr.4006
 13. Carasco MG, de Vernejoul MC, Sterkers Y, Morieux C, Kuntz D, Miravet L. Decreased bone formation in osteoporotic patients compared with age-matched controls. *Calcif. Tissue Int.* 1989;44(3):173–5.
 14. de Vernejoul MC, Kuntz D, Miravet L, Goutallier D, Ryckewaert A. Bone histomorphometric reproducibility in normal patients. *Calcif. Tissue Int.* 1981;33(1):369–74.
 15. Dempster DW, Compston JE, Drezner MK, Kanis FHG, John A Malluche, Ott S, Malluche H, M PJM, Recker RR, Parfitt M. Histomorphometry Nomenclature: A 2012 Update of the Report of the ASBMR Histomorphometry Nomenclature David. *J. Bone*

- Miner. Res. [Internet]. 2014;28(1):2–17.
16. Recker RR. Transmenopausal and age-related changes in bone remodeling. *J. Musculoskelet. Neuronal Interact.* 2003;3(4):411–2.
 17. Møller AMJ, Delaisse J, Olesen JB, Bechmann T, Madsen JS, Søe K. Zoledronic Acid Is Not Equally Potent on Osteoclasts Generated From Different Individuals. *JBMR Plus.* 2020;00(00):1–13.
 18. Møller AMJ, Delaissé JM, Olesen JB, Madsen JS, Canto LM, Bechmann T, Rogatto SR, Søe K. Aging and menopause reprogram osteoclast precursors for aggressive bone resorption. *Bone Res.* [Internet]. Springer US; 2020;8(1).
 19. Moreira CA, Dempster DW. Histomorphometric changes following treatment for osteoporosis. *J. Endocrinol. Invest.* Springer International Publishing; 2017;40(9):895–7.
 20. Ivaska KK, Gerdhem P, Åkesson K, Garnero P, Obrant KJ. Effect of fracture on bone turnover markers: A longitudinal study comparing marker levels before and after injury in 113 elderly women. *J. Bone Miner. Res.* 2007;22(8):1155–64.
 21. Recker RR, Kimmel DB, Dempster D, Weinstein RS, Wronski TJ, Burr DB. Issues in modern bone histomorphometry. *Bone* [Internet]. 2011 Nov;49(5):955–64.
 22. Parfitt AM, Mathews CHE, Villanueva AB, Kleerekoper M, Frame B, Rao DS. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. *J. Clin. Invest.* 1983;72(4):1396–409.
 23. Ding M, Hvid I. Quantification of age-related changes in the structure model type and trabecular thickness of human tibial cancellous bone. *Bone.* 2000;26(3):291–5.

Figure Legends

Figure 1: Scatterplots of histomorphometric parameters by age.

Bone formation and resorption parameters were plotted against age. Grey zone is the range of normal controls, Controls (white), untreated osteoporotic patients (grey), and inadequate responders (black).

Figure 2: Trabecular indices for the 3 groups.

Trabecular microstructure of bone crest iliac cores measured by micro-CT in controls (white), untreated osteoporotic patients (grey), and inadequate responders (black).

Statistical tests were used for each parameter: BV/TV, Tb.Th and SMI: ANOVA test followed by Dunnet's test; Tb.Sp and Connectivity: Kruskal-Wallis test followed by Steel-Dwass-Critchlow-Fligner test; Tb. BMD: ANOVA test following a log normalization of parameter; For Tb.Sp and Connectivity, median is reported in the figure.

Figure 3: Cortical indices for the 3 groups.

Cortical microstructure of bone crest iliac cores measured by micro-CT in controls (white), untreated osteoporotic patients (grey), and inadequate responders (black). Following statistical tests were used for each parameter: Ct.Th: Kruskal-Wallis test; Ct.Por: ANOVA test; Ct.BMD: ANOVA test. For Ct.Th, median is reported on the figure.

Figure 1

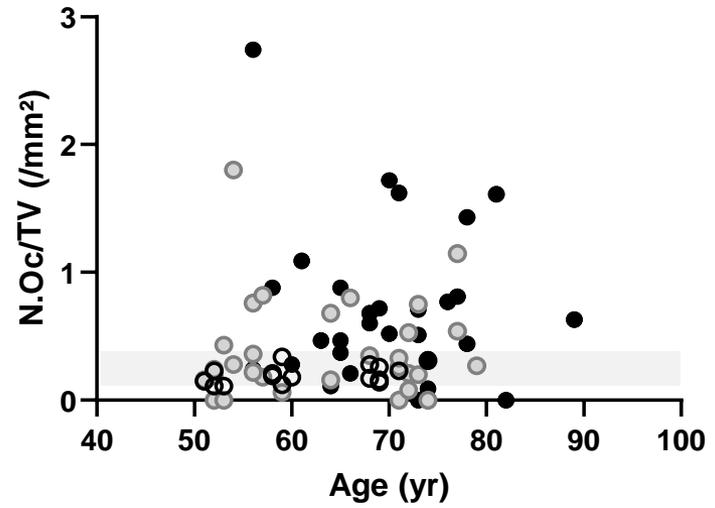
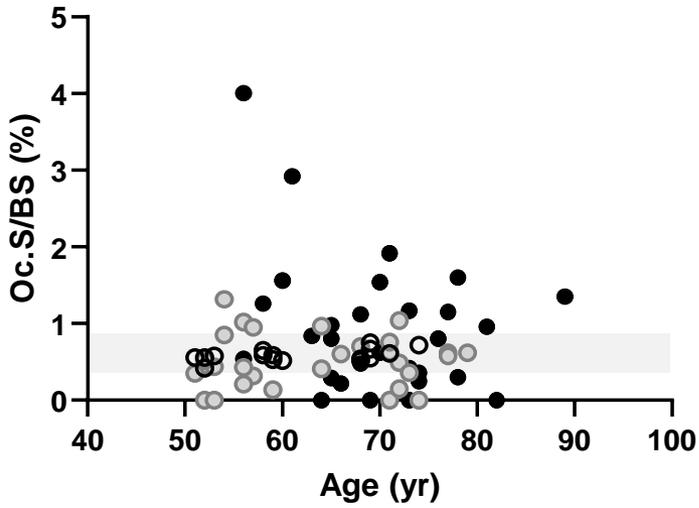
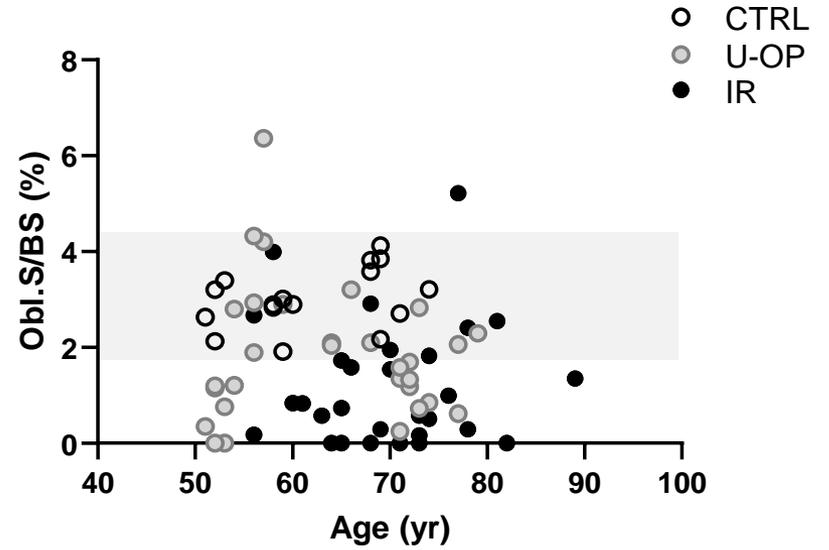
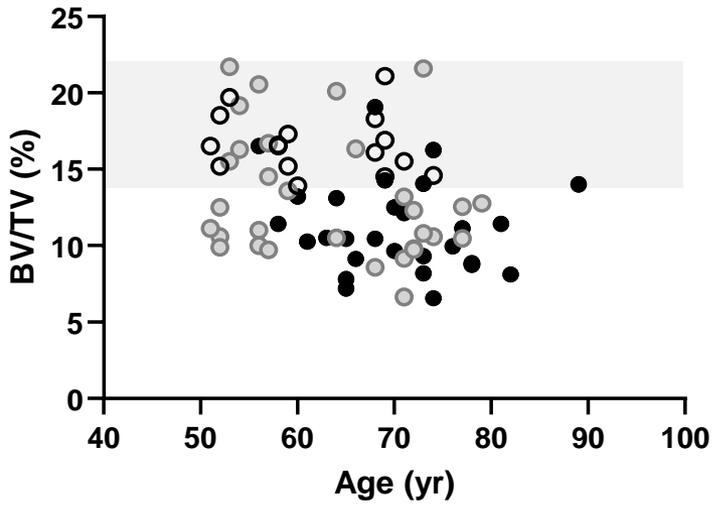


Figure 2

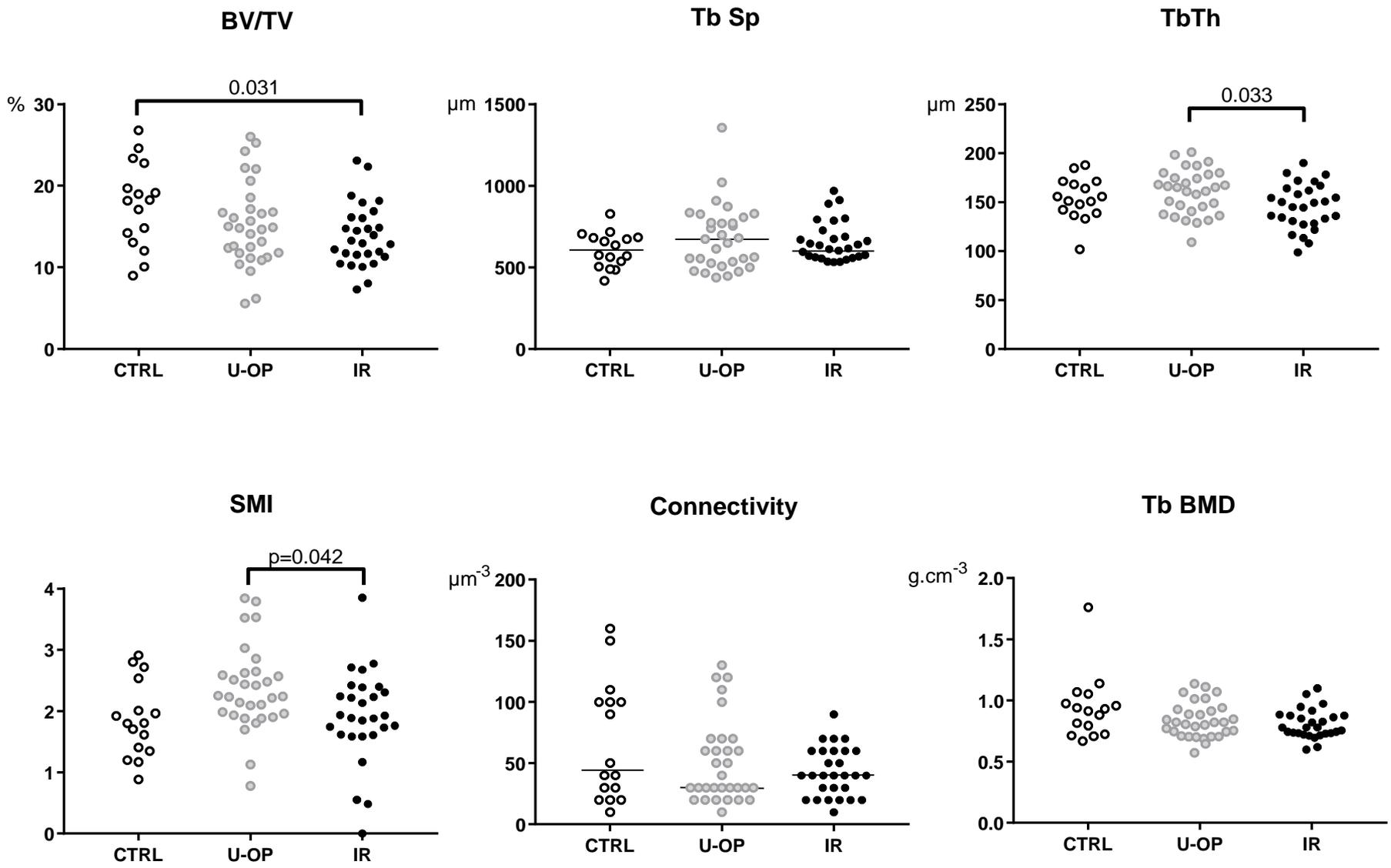
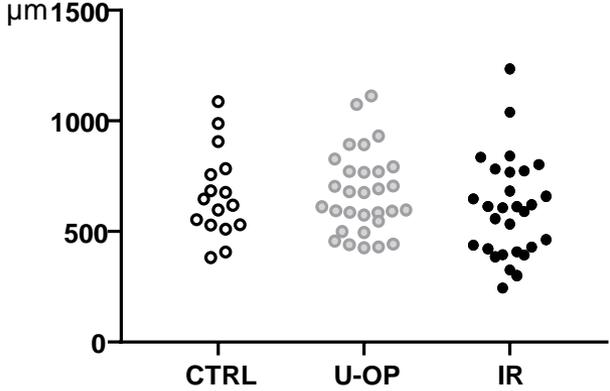
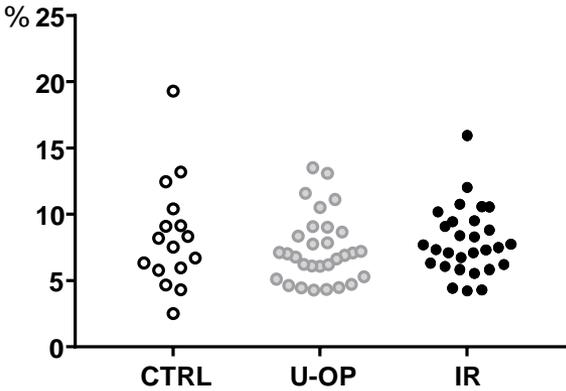


Figure 3

Ct. Th



Ct. Po



Ct. BMD

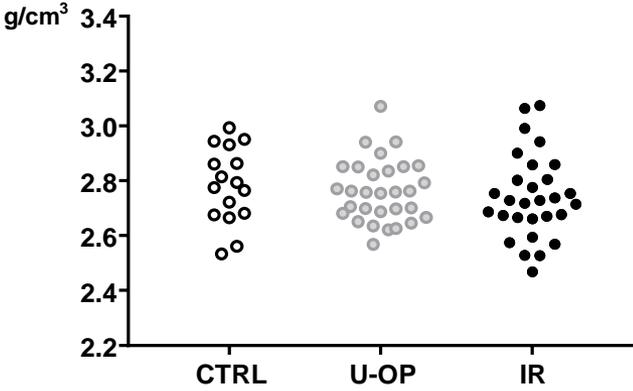


Table 1: Patient characteristics

	Controls n=16	Untreated osteoporotic patients n=31		Inadequate responders n=31		p values
Clinical characteristics						
Women (n)	100% (16)	87% (27)		90% (28)		0.510 ^a
Age (years)	61.9 ± 7.7 ^a	63.3	± 9.3 ^a	69.7	± 8.8	0.007 ^b
BMI (kg/m ²)	NA	25.8	± 6.5	22.7	± 3.5	0.369 ^c
Tobacco	NA					
previous n		0% (0)		6.45% (2)		
current n		9.67% (3)		6.45% (2)		0.288 ^a
Alcohol yes. (n)	NA	3.22% (1)		6.45% (2)		0.600 ^a
Corticosteroids (n)	0% (0)	12.9% (4)		12.9% (4)		0.405 ^a
Family history of fracture (n)	NA	3.22% (1)		19.35% (6)		0.586 ^d
Personal history of fracture (n)	NA	90% (28)		90%(28)		1 ^d
DXA						
Lumbar spine BMD (g/cm ²)	NA	0.921	± 0.192	0.802	± 0.186	0.125 ^e
Lumbar spine T-score	NA	-1.9	± 1.4	-2.5	± 1.562	0.178 ^e
Lumbar Spine Z-Score	NA	-1.1	± 1.4	-1.3	± 1.9	0,288 ^e
Total hip BMD (g/cm ²)	NA	0.786	± 0.093	0.685	± 0.116	0.019 ^e
Total hip T-score	NA	-1.6	± 0.900	-2.3	± 0.769	0.032 ^c
Total hip Z-Score	NA	-0.3	± 1.3	-0.866	± 0.866	0,126 ^e
Biology						
Calcium (mmol/L)	NA	2.4	± 0.1	2.3	± 0.100	0.012 ^e
Phosphore (mmol/L)	NA	1.08	± 0.280	1.2	± 0.169	0.251 ^e
25OH Vitamin D (ng/mL)	NA	40.4	± 18.6	33.9	± 13.3	0.265 ^e
PTH (ng/L)	NA	41.5	± 28.0	34.2	± 22.1	0.404 ^f
ALP (UI/L)	NA	87.2	± 50.5	72.3	± 20.8	0.959 ^c
Osteocalcin (µg/L)	NA	27.3	± 13.7	29.8	± 24.7	0.973 ^f
CTX crosslaps (ng/L)	NA	447.6	± 387.7	266.8	± 228.4	0.489 ^c

BMI: body mass index, BMD: bone mineral density; PTH: parathyroid hormone, ALP alkaline phosphatase; CTX: C-telopeptides

Data are mean ± SD. *:p-value multiple comparison tests: p=0.02 vs controls. p=0.036 vs untreated osteoporotic patients

Other tests were performed comparing untreated osteoporotic patients and Inadequate responders; ^a: Fisher exact test, b: Kruskal Wallis' test, c: MannWhitney test, d: Yates' chi2 corrected

^e: Student's test, f: Student's test log transform

Table 2: Histomorphometry analysis of trabecular and cortical bone

	Controls n=16	Untreated osteoporotic patients n=31	Inadequate responders n=31	
Trabecular parameters				
BV/TV (%)	16.7 ± 2.0 ^α	13.2 ± 4.1	11.2 ± 3.0	<0.0001 ^a
OV/BV (%)	NA	3.2 ± 3.1	2.6 ± 2.5	0.653 ^b
OS/BS (%)	NA	28.2 ± 19.4	23.4 ± 8.6	0.485 ^c
Obl.S/BS (%)	3.0 ± 0.638 ^β	1.9 ± 1.4	1.2 ± 1.3	<0.0001 ^a
Oc.S/BS (%)	0.585 ± 0.082	0.510 ± 0.342	0.947 ± 0.893	0.115 ^d
N.Oc/TV (/mm ²)	0.199 ± 0.071 ^Ω	0.394 ± 0.393	0.721 ± 0.611	<0.001 ^a
MAR (μm/j)	NA	0.556 ± 0.234	0.580 ± 0.207	0.710 ^b
Double labelled surfaces (%)	NA	3.7 ± 3.8	4.1 ± 6.0	0.618 ^f
Total labelled surfaces (%)	NA	6.8 ± 4.5	5.3 ± 3.7	0.256 ^b
Presence of labelling (n)	NA	87% (27)	51.61% (16)	0.002 ^e
BFR/BS (μm ² /μm ³ /d)	NA	1.847 ± 1.760	2.269 ± 3.521	0.753 ^f
Cortical parameters				
Ct.Th (μm)	NA	707 ± 234	802.9 ± 380.4	0.440 ^g

Data are mean ± SD

BV/TV: trabecular bone volume to tissue volume; OV/TV: osteoid volume; OS/BS: osteoid surface/bone surface; Ob.S: osteoblast surface/bone surface; Oc.S/BS: osteoclast surface/bone surface; N.Oc/TV: osteoclast number/tissue volume; BFR/BS: bone formation rate/bone surface; NA, not available

^a: Kruskal-Wallis test followed by Steel-Dwass-Critchlow-Fligner test, ^b: Student test, ^c: Mann Whitney test, ^d: Kruskal-Wallis test

^e: Chi2 test, ^f: Student's t test logtransformed, ^g: unpaired t-test,

p-value multiple comparison tests : ^α inadequate responders to controls (IR-Ctrls): <0.0001, ^β IR-Ctrls: <0.0001, ^Ω IR-Ctrls: <0.001