Case Report: Postmortem bone histomorphometric analysis in a 38-year-old Japanese man with immobilization osteoporosis because of orthostatic hypotension related to amyloid light chain amyloidosis [version 1; peer review: 1 approved with reservations]

Masaki Hatano, Izuru Kitajima, Masaki Nakamura, Kazuya Isawa, Tatsuya Suwabe, Junichi Hoshino, Keiichi Kinowaki, Kenichi Ohashi, Naoki Sawa, Seizo Yamamoto, Yoshifumi Ubara

1Department of Orthopaedic Surgery, Toranomon Hospital, 1-3-1 Kajigaya, Takatu-ku, Kawasaki-shi, Kanagawa, 213-8587, Japan
2Department of Nephrology Center, Toranomon Hospital, Kajigaya, 1-3-1, Takatsu, Kawasaki, Kanagawa, 212-0015, Japan
3Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Minato-ku, Tokyo, 105-8470, Japan
4Department of Pathology, Toranomon Hospital, Minato-ku, Tokyo, 105-8470, Japan
5Department of Human Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, 113-8519, Japan

Abstract
We performed a postmortem bone histomorphometric analysis of iliac bone on a 38-year-old man who had been bedridden for the nine months before his death because of orthostatic hypotension and severe malnutrition related to amyloid-κ-light-chain amyloidosis. Cancellous bone volume was greatly decreased, with a trabecular bone volume to total bone volume ratio of 6.77% (normal value, 19.56% ± 5.62%). Trabecular thinning was also apparent, with a trabecular thickness of 78.9 μm (normal value, 131.3 ± 28.1 μm), although the trabecula was still preserved. Cortical bone width was normal, although areas of porosity were clear throughout the cortical bone. Our findings indicate that immobilization-related osteoporosis may be closely associated with loss of cancellous bone.

Keywords
amyloid light chain amyloidosis, bone histomorphometry, bed rest, disuse osteoporosis, immobilization osteoporosis
Background

Immobilization osteoporosis has been reported to occur after a stroke or spinal cord injury, spaceflight, and long-term bed rest. Long-term immobilization or unloading affect both bone formation and bone resorption and lead to bone loss and an increased risk of fracture. Despite decades of intense research, the effects of long-term bed rest on bone cells and the associated structural changes remain unclear. The effects are difficult to estimate because we cannot exclude factors associated with pre-existing disease and aging. Here, we present a postmortem bone histomorphometric analysis of a man who became bedridden approximately nine months before his death because of severe orthostatic hypotension related to amyloid light-chain (AL) amyloidosis.

Case presentation

An autopsy was performed on a 38-year-old Japanese man with AL amyloidosis who died of interstitial pneumonia. The patient developed AL amyloidosis at the age of 37. At that time, he was otherwise healthy and had no family history of fracture.

At age 37, the patient developed abnormal bowel movements, consisting of alternating constipation and diarrhea, which gradually worsened. He also lost his appetite. He developed orthostatic hypotension and consequently had difficulties standing and sitting and became bedridden. Four months after becoming bedridden, he was admitted to our hospital for diagnosis and treatment.

On admission, the patient was 170.0 cm tall, but his weight had decreased from 90 kg to 62 kg over the previous four months. The laboratory data were as follows: serum albumin, 2.2 g/dL; total protein, 4.2 g/dL; urea nitrogen, 10 mg/dL; serum creatinine, 0.50 mg/dL; calcium, 8.3 mg/dL; phosphate, 3.8 mg/dL; alkaline phosphatase, 151 IU/L Japan Society of Clinical Chemistry (JSCC) method; normal range, 117 to 350), and C-reactive protein, 0.1 mg/dL. An immunologic evaluation found that serum immunoglobulin (Ig) G was 509 mg/dL (normal range, 870-1700 mg/dL); IgA, 98.0 mg/dL (normal range, 110-410 mg/dL); and IgM, 22.0 mg/dL (normal range, 35-220 mg/dL). Serum M-protein was not detectable by immunofixation. Urinalysis detected proteinuria (5.5 g/day), and immunofixation electrophoresis showed kappa (κ)-type Bence-Jones protein in the urine. Kidney biopsy and endoscopic examination of the colon biopsy both showed-κ-positive AL-amyloidosis (Figure 1). Examination of the bone marrow revealed 2.8% monoclonal plasma cells (normal value < 10%); however, the patient did not fit the criteria for multiple myeloma because a full skeletal survey did not detect any osteolytic lesions. Thus, primaryκ-type AL amyloidosis was diagnosed.

Clinical course

At admission, the patient’s blood pressure was 100/60 mmHg in the sitting position but decreased to 60/30 mmHg in the standing position. Therefore, the patient remained in bed. Treatment was started with vincristine (0.4 mg/day for 4 days), adriamycin (14 mg/day for 4 days), and dexamethasone (40 mg/day for 12 days). However, five months after hospitalization, the patient suddenly died of interstitial pneumonia. At death, the patient had been bedridden for nine months.

After obtaining consent from the patient’s family, we performed an autopsy. Bone histomorphometric analysis of the right iliac bone was performed at the Ito Bone Science Institute (Niigata, Japan); tetracycline double labeling was not performed.

Bone histomorphometric examination

Cancellous bone was assessed by bone histomorphometry (Table 1). All bone volume markers were decreased compared with the age-matched reference ranges presented in the report by Reccker et al., as follows: trabecular bone volume to total volume, 6.77%; trabecular thickness, 78.9 μm; trabecular unit wall thickness, 21.0 μm.

All bone osteoid markers were also decreased compared with the age-matched reference range, as follows: osteoid volume to total volume ratio, 0.03%; osteoid volume to bone volume ratio, 0.51%; osteoid surface to bone surface ratio, 4.12%; and osteoid thickness, 6.91 μm. The fibrous tissue volume to total volume could not be assessed, and the eroded surface to bone surface was increased to 16.2%.

The cancellous bone volume was greatly decreased, and trabecular thinning was apparent; the trabecula was preserved, but the number of trabecular termini was increased to 52 points and the number of nodes (bifurcated trabecula) was decreased to 12 points (Figure 2a). Trabecula thinning indicated resorption by osteoclasts, and island bones and clubbing trabecula with bilateral termini were noted. We found more empty lacunae, which are characterized by the disappearance of osteocytes, than lacunae containing osteocytes (Figure 2b).

Cortical bone was preserved with cortical bone width of 1.65 mm and 0.78 mm. However, the area of porosity to total cortical bone area ratio, which increases when large pores appear because of enlargement of the bone marrow cavity due
to resorption by osteoclasts, was increased to 37.19% and 22.11% (Figure 2a, Table 1). The number of cortical node structure connecting from the endocortical surface to the cancellous trabecula was decreased (Figures 2a and 2c). More empty lacunae were seen than lacunae containing osteocytes (Figure 2c).

Severe osteoporosis of cancellous bone closely associated with immobilization was diagnosed.

Postmortem Iliac bone of a 46-year-old healthy man.

The standard values of cortical bone have not been reported. Therefore, to put the degree of osteoporosis in our patient into context, we determined the parameters of postmortem iliac bone of a 46-year-old healthy man. The control individual showed abundant cortical bone surrounding the cancellous bone. The cortical bone width was 1.45 mm and 0.67 mm, and the area of porosity area to total cortical bone area ratio was 3.2% and 8.45%.

Discussion
Immobilization or skeletal unloading, which can occur during spaceflight, hindlimb suspension, and long-term bedrest, are well recognized as causes of loss of bone mass and strength. Both clinical and animal studies have reported on the mechanisms of bone loss in these situations.\textsuperscript{5-10} For example, previous research has examined the effects of tail

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**Figure 1.**
(a): Colon biopsy (PAS stain, light microscopy) (× 100). Amorphous material was noted on small arteries and surrounding tissues in the subserosal layer.
(b): Colon biopsy (Congo red stain, light microscopy) (× 100). Congo red-material was positive for amorphous material.
(c): Kidney biopsy (Congo red stain, light microscopy) (×400). Congo red material was noted in the glomeruli.
(d): Kidney biopsy (κ-stain, immunofluorescence microscopy) (×400). κ-stain was positive for Congo red material.
suspension, tenotomy, or sciatic neurectomy in animal models and of bed rest with a head-down tilt in human studies.\textsuperscript{9,11,13-15}

In animal models, unloading reduces the rate of bone formation because of changes in osteoblast progenitor cell recruitment and defective functioning of differentiated osteoblasts.\textsuperscript{9-11} On the other hand, the effect of immobilization or skeletal unloading has been reported to cause inconsistent changes in osteoclast surface and number.\textsuperscript{9-12}

In a human study, 12 weeks of bed rest led to suppression of the osteoblastic surface in cancellous bone and an increase of bone resorption and eroded surface in both cancellous and cortical bone, although cortical and cancellous bone volume, as measured histomorphometrically, did not change.\textsuperscript{7} This study and another one found that mean cortical and trabecular thickness also did not change after long-term bed rest.\textsuperscript{7,13} Another study found that 120 days of bed rest led to loss of

\textbf{Table 1. Histomorphometric analysis of the iliac crest.} Cancellous bone was assessed by bone histomorphometry. All bone volume markers were decreased compared with the age-matched reference ranges. All bone osteoid markers were also decreased compared with the age-matched reference range. The fibrous tissue volume to total volume could not be assessed, and the eroded surface to bone surface was increased to 16.2%.

| Parameter                          | Ratio or abbreviation | Unit    | Measured value | Normal range |
|------------------------------------|-----------------------|---------|----------------|--------------|
| **Cancellous bone**                |                       |         |                |              |
| Bone volume                        | BV/TV                 | %       | 6.77           | 19.56±5.62   |
| Trabecular thickness               | Tb.Th                 | μm      | 78.9           | 131.3±28.1   |
| Wall thickness                     | W.Th                  | μm      | 21             | 28.29±28.1   |
| **Osteoid**                        |                       |         |                |              |
| Osteoid volume                     | OV/TV                 | %       | 0.03           | 0.36±0.31    |
| Osteoid volume                     | OV/BV                 | %       | 0.51           | 1.2±0.87     |
| Osteoid surface                    | OS/BS                 | %       | 4.12           | 14.0±6.64    |
| Osteoid thickness                  | O.Th                  | μm      | 6.91           | 8.31±1.99    |
| **Resorption**                     |                       |         |                |              |
| Eroded surface                     | ES/BS                 | %       | 16.2           | 3.66±1.69    |
| Osteoclast number                  | N.Oc/BS               | N.mm    | 0.3            |              |
| **Cortical bone**                  |                       |         |                | 46-year old man |
| Total cortical bone area           | Tt.Cr                 | μm\(^2\) | 12,345,066.73  | 17,947,243.64 |
| Periosteal Surface                 | Ps.S                  | μm      | 7,482.79       | 10,103.18    |
| Cortical Width                     | Ct.Wi                 | mm      | 1.65           | 1.45         |
| Porosity Area                      | Po.Ar                 | μm\(^2\) | 4,591,976.31   | 577,882.07   |
| Porosity Area/total cortical bone area | Po.Ar/Tt.Cr   | %       | 37.19          | 3.21         |

In animal models, unloading reduces the rate of bone formation because of changes in osteoblast progenitor cell recruitment and defective functioning of differentiated osteoblasts.\textsuperscript{9-11} On the other hand, the effect of immobilization or skeletal unloading has been reported to cause inconsistent changes in osteoclast surface and number.\textsuperscript{9-12}
cancellous bone volume. However, mean trabecular separation and the node to terminus ratio did not change. The differences in the results of these studies were probably related to differences in their duration, the causes of immobilization, and the age and sex of the participants.

In conclusion, we performed postmortem bone histomorphometric analysis of the iliac bone on a 39-year-old man who had been bedridden for nine months before his death because of severe orthostatic hypotension and severe malnutrition related to AL-amyloidosis. The cancellous bone volume was greatly decreased and trabecular thinning was apparent, although the trabecula was preserved. Cortical bone volume was also preserved. Our findings indicate that immobilization-related osteoporosis is associated mainly with bone loss in cancellous bone.

**Limitation**

The significance of this report is limited to the presentation of pathological data showing that the long-term immobilization in the patient with AL amyloidosis leads to severe osteoporosis. However, the patient’s underlying low nutrient condition, which was caused by AL amyloidosis, may have contributed to the bone abnormalities.

![Figure 2. Bone histology.](image)

(a) Low-power field of light microscopy (>20).

The cancellous bone volume was greatly decreased, and trabecular thinning was apparent; however, the trabecula was preserved, although the number of trabecular termini (N.Tm; red) was increased to 52 points and the number of nodes (bifurcated trabecula) (N.Nd; blue) was decreased to 12 points. Compared with the width of cancellous bone, the width of cortical bone was preserved with 1.65 mm and 0.78 mm. The number of cortical node structures connecting from the endocortical surface to the cancellous trabecula was decreased.

(b) (A and B): Trabecula thinning indicated resorption by osteoclasts.

(C) Island bones and clubbing trabecula with bilateral termini were noted.

(D) More empty lacunae, characterized by the disappearance of osteocytes, were seen than lacunae containing osteocytes.

(c) High-power field of cortical bone (>100, 200 or 400).

(A, B and C) Large pores associated with enlargement of the bone marrow cavity due to resorption by osteoclasts.

(D) More empty lacunae were seen than lacunae containing osteocytes.
Figure 2. (continued)
**Consent**

This investigation was conducted in accordance with the Declaration of Helsinki. Before his death, the patient provided written informed consent for publication of this case report.

After his death, the patient’s wife provided written informed consent for publication of this case report and any accompanying images.

**Data availability**

All data underlying the results are available as part of the article and no additional source data are required.

**Acknowledgements**

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**References**

1. Jørgensen L, Crabtree NJ, Reeve J, et al.: Ambulatory level and asymmetrical weight bearing after stroke affects bone loss in the upper and lower part of the femoral neck differently: bone adaptation after decreased mechanical loading. Bone. 2000; 27(3): 701–707. PubMed Abstract | Publisher Full Text

2. Dauty M, Perrouin Verbe B, Maugars Y, et al.: Supraspinal and sublesional bone mineral density in spinal cord injured patients. Bone. 2000; 27(2): 305–309. PubMed Abstract | Publisher Full Text

3. Cirnigliaro CM, Myslinski MJ, La Fountaine MF, et al.: Bone loss at the distal femur and proximal tibia in persons with spinal cord injury: imaging approaches, risk of fracture, and potential treatment options. Osteoporos Int. 2017; 28(3): 747–765. PubMed Abstract | Publisher Full Text

4. Leblanc AD, Schneider VS, Evans HJ, et al.: Bone mineral loss and recovery after 17 weeks of bed rest. J Bone Miner Res. 1990; 5(8): 843–850. PubMed Abstract | Publisher Full Text

5. Rittweger J, Frost HM, Schiessl H, et al.: Muscle atrophy and bone loss after 90 days’ bed rest and the effects of flywheel resistive exercise and pamidronate: results from the LTBER study. Bone. 2005; 36(6): 1019–1029. PubMed Abstract | Publisher Full Text

6. Bikle DD, Sakata T, Halloran BP: The impact of skeletal unloading on bone formation. J Biomech Sci Eng. 2003; 16(2): 45–54. PubMed Abstract

7. Zerwekh JE, Ruml LA, Gottschalk F, et al.: The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. J Bone Miner Res. 1998; 13(10): 1594-1601. PubMed Abstract | Publisher Full Text

8. Recker RR, Kimmel DB, Parfitt MA, et al.: Static and tetracycline-based bone histomorphometric data from 34 normal postmenopausal females. J Bone Miner Res. 1988; 3(2): 133–144. PubMed Abstract | Publisher Full Text

9. Sakai A, Nakamura T: Changes in trabecular bone turnover and bone marrow cell development in tail-suspended mice. Muscle Nerve. 2001; 24(4): 387–392. PubMed Abstract

10. Morey-Holton ER, Globus RK: Hindlimb unloading of growing rats: a model for predicting skeletal changes during space flight. Bone. 1998; 22(5 Suppl): 835–885. PubMed Abstract | Publisher Full Text

11. Machwate M, Zerath E, Holy X, et al.: Systemic administration of transforming growth factor-beta 2 prevents the impaired bone formation and osteopenia induced by unloading in rats. J Clin Invest. 1995; 96(3): 1245–1253. PubMed Abstract | Publisher Full Text | Free Full Text

12. Damrongrungruang T, Kuroda S, Kondo H, et al.: A simple murine model for immobilization osteopenia. Clin Orthop Relat Res. 2004; 425: 244–251. PubMed Abstract | Publisher Full Text

13. Palle S, Vico L, Bourrin S, et al.: Bone tissue response to four-month antithrombotic bedrest: a bone histomorphometric study. Calcif Tissue Int. 1992; 51(3): 189–194. PubMed Abstract | Publisher Full Text

14. Thomsen JS, Morukov BV, Vico L, et al.: Cancellous bone structure of iliac crest biopsies following 370 days of head-down bed rest. Aviat Space Environ Med. 2005; 76(10): 915–922. PubMed Abstract

15. Thomsen JS, Ebbesen EN, Mosekilde L: Relationships between static histomorphometry and bone strength measurements in human iliac crest bone biopsies. Bone. 1998; 22(2): 153–163. PubMed Abstract | Publisher Full Text

16. Teti A, Zallone A: Do osteocytes contribute to bone mineral homeostasis? Osteocytic osteolysis revisited. Bone. 2005; 36(1): 11–16. PubMed Abstract | Publisher Full Text
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Naoki Kondo

Division of Orthopedic Surgery, Department of Regenerative and Transplant Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

In this paper, the authors claim a post-mortem 38-year-old patient showed remarkable decreased parameter-related bone formation in both cancellous and cortical bones. Bone histomorphometric data itself is very relevant and well reported.

Major points

1. I would like to know the significance of the increase of empty lacunae in the cortex for the case. For example, if osteocytes do not function as well, osteocytes cannot cause cortical bone damage and result in decreased cortical bone thickness.

2. I think that bone metabolic marker data are deficient. Please add it if possible.

3. How can amyloidosis affect “immobilization osteoporosis”? In bone tissue, was amyloid protein detected? (Or was it possible technically?) If amyloid deposited in bone tissue and influenced bone turnover directly, this case report would be a more relevant one.

4. I would like to know this case's bone mineral density if the authors measured it.

Minor points

1. I think that the age 39 is not correct in the last paragraph of the Discussion, please correct it.

2. I also think "postmortem Iliac bone of a 46-year-old healthy man" at the 5th line from the end of the Case Presentation is very solitary and not natural. Please delete this sentence.

Is the background of the case's history and progression described in sufficient detail?

Yes

Are enough details provided of any physical examination and diagnostic tests, treatment
given and outcomes?
Partly

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?
Partly

Is the case presented with sufficient detail to be useful for other practitioners?
Yes

**Competing Interests:** No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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