# Is Osteoporosis Vector-Borne or a Complication of Treatment?

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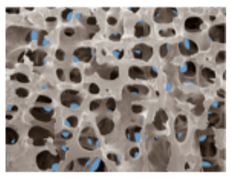
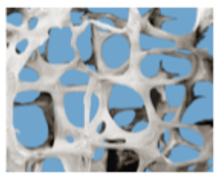
## INTRODUCTION:

Bone is a complex organ with diverse anatomic and physiological functions. The skeleton supports the body, protects vulnerable organs, and makes movement possible. Bone serves as the main reservoir for regulation and storage of calcium and other key minerals critical to homeostasis. In the bone marrow, the hematopoietic and bone remodeling systems share the same microenvironment—together with T cells that migrate from the periphery. Modern bone-targeted pharmacological treatments have reduced the morbidity and mortality of osteoporosis, yet the pathogenesis of the illness remains idiopathic.

The goal of this report was to generate hypotheses, based on the clinical and laboratory findings of two patients who developed severe osteoporosis while undergoing treatment for vector borne illnesses.

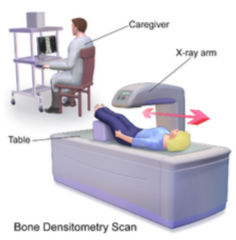




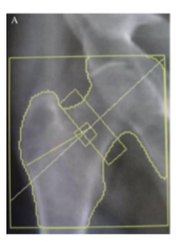
## METHODS:

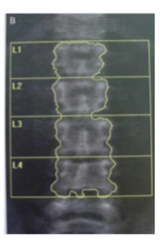
Bone Mineral Density Testing by dual-energy x-ray absorptiometry (DXA) is an enhanced form of X-ray technology. DXA is the established standard for measuring density at the hip and spine, shown as grams of mineral/square cm (g/cm$^2$). Results are expressed as T and Z scores.

- T score is the standard deviation from the mean of a young adult reference population of the same sex and race as the patient.
- Z score compares the patient to an age, sex, ethnicity-matched reference population[1].



Bone Densitometry Scan






Rapid infectious disease identification by next-generation DNA sequencing (RIDI). 16S gene sequences are highly conserved in bacteria, and 18S sequences are highly conserved in protozoa, fungi and other eukaryotes. The RIDI informatics strategy inputs the results of next generation sequencing of clinical samples to identify high probability sequence matches, using the NCBI database and a RIDI-specific database. The system computes the probability of a match at the level of species and genera[2].

Stool analysis by microbial culturomics. Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF) is a proteomic method for identifying bacteria and yeast from stool culture. The stool sample is cultured in a high yield culture system that supports the growth of anaerobes and other fastidious organisms. MALDI-TOF identifies the unique ribosomal protein fingerprints of microorganisms. This spectra is then individually compared to a reference database of enteric organisms and underreported pathogens allowing identification and quantitation of bacteria and yeast present in the stool culture[3,4].

Genetics of vitamin K metabolism. Variants of VKORC1 are associated with lower levels of circulating phylloquinone, and increased sensitivity to warfarin. Also, the percentage of undercarboxylated osteocalcin is increased in variants of GGCX[5]. Therefore, genetic profiling was performed on saliva collected using a kit obtained from 23 and Me®. Raw data was downloaded to the Promethease – SNPedia site, which generates a searchable database of personal snps, with links to source reference materials. We searched both patients' databases for evidence of abnormal snps for VKORC1 (Vitamin K Epoxide Reductase), GGCX (Gamma-glutamyl carboxylase), and NQO1 (NADPH dehydrogenase quinone).

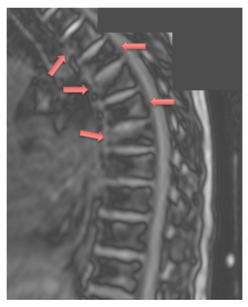
Bone metabolic markers. International authorities recommend that a circulating marker of bone formation (propeptide type 1 collagen, P1NP) and bone resorption (serum C-terminal telopeptide of type 1 Collagen, s-CTX) be used to allow monitoring of treatment of osteoporosis. Due to diurnal and mealtime variation, P1NP and s-CTX are measured fasting in the morning. Both cases were treated with the bone anabolic agent teriparatide (Forteo®). Doubling of P1NP indicates adequate therapeutic response.

Osteocalcin is the major noncollagenous protein of bone matrix, synthesized by osteoblasts. Levels of osteocalcin reflect rates of bone formation, and would be expected to increase with increased osteoblastic activity. Osteocalcin is measured by LabCorp using an enzyme-linked immunosorbent assay (ELISA), based on an antibody that detects the N-terminal stable region of the 49 amino acid protein[6].

Undercarboxylated osteocalcin (Glu-OC) is an index of the carboxylation status of osteocalcin, and indirectly a clinical measure of vitamin K activity. Fully functional osteocalcin carries three carboxylated glutamic acid residues (Gla) at positions 17, 21, and 24. The tertiary structure of Glu-OC differs from Gla-OC. Therefore specific monoclonal antibodies have been developed that distinguish the conformation of Gla-OC from Glu-OC[7]. Genova Diagnostic Lab uses a commercial ELISA kit from Takara Bio, featuring a well-characterized antibody against Gla-OC[8].

## RESULTS:

### Case 1

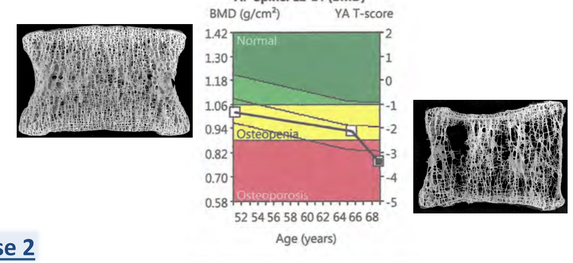


A 69-year-old woman with late previously untreated Borreliosis (blood culture[9], and ispot[10] positive), and Bartonellosis (IFA positive), had received 27 months of oral antibiotics, followed by 5 months of intravenous antibiotics, when she developed severe back pain. An MRI showed vertebral compression fractures.

Kyphoplasty of two vertebrae was performed for relief of pain. A sample of bone obtained at kyphoplasty was submitted for histology and culture. Histology showed woven bone, fibrosis and normal bone marrow elements consistent with fracture. A routine culture was recorded as "no growth." A sample of bone was submitted for next-generation rapid sequencing metagenomics (RIDI) to determine the presence of 16S (prokaryotic) and 18S (eukaryotic) RNA[2]. No bacterial sequences were identified, but 18S RNA sequences matched published organisms in GenBank, characterized at the genus level as *Funneliformis*.

Bone densitometry showed a T score of -3.7 at the lumbar spine, a precipitous decrease from previous measures[1]. She began treatment with teraparatide (Forteo®) 20 µg SQ daily. See Table for bone formation marker response to Forteo®. During the three years prior to fracture, plasma 25-OH vitamin D ranged from 37 to 51 ng/mL. Undercarboxylated osteocalcin, measured after 6 months of therapy was markedly elevated[8].

### Bone Mineral Density



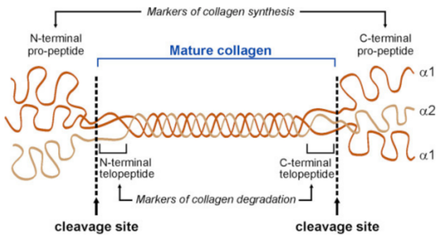

### Case 2

A 62-year-old man with late previously untreated Borreliosis (blood culture positive[9]) had received 24 months of oral antibiotics. Based on his wife's history and some back pain (1/3 of vertebral fractures are subclinical.) bone densitometry was performed. A **T score of -3.1** was demonstrated. Whole blood metagenomics by RIDI failed to show 16S RNA, but 18S RNA sequences revealed a *Funneliformis* genus. He began treatment with teriparatide (Forteo®) 20 µg SQ daily. See table for bone formation marker response to Forteo®. During treatment for Lyme, 25-OH vitamin D ranged from 40 to 53 ng/mL. Undercarboxylated osteocalcin was in the 3rd of 5 quintiles.

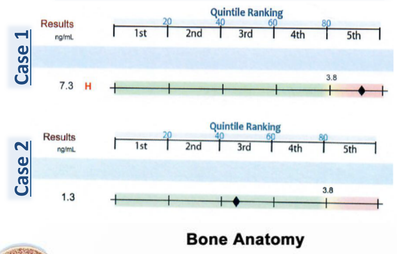
### Genetics of Vitamin K Metabolism

| Gene | Chromo-some # | Reference snp cluster ID | *Case 1* | *Case 2* | Interpretation |
|---|---|---|---|---|---|
| Vitamin K epoxide reductase – VKORC1 | 16 | rs8050894 | **C;C** | C;G | Homozygote for C shows increased coumadin sensitivity |
| | 16 | rs7294 | **G;G** | A;G | Phylloquinone level 30% > G homozygote than heterozygote |
| | 16 | rs9934438 | G:G | A;T | T allele has increased risk of aortic calcification |
| Gamma glutamyl carboxylase – GGCX | 2 | rs7568458 | A;T | A;T | Heterozygotes have lower carboxylated osteocalcin than homozygotes |
| | 2 | rs10187424 | C;T | C;C | |
| NADPH dehydrogenase quinone – NQO1 | 16 | rs1131341 | C;C | C;T | T allele shows lower enzyme activity |

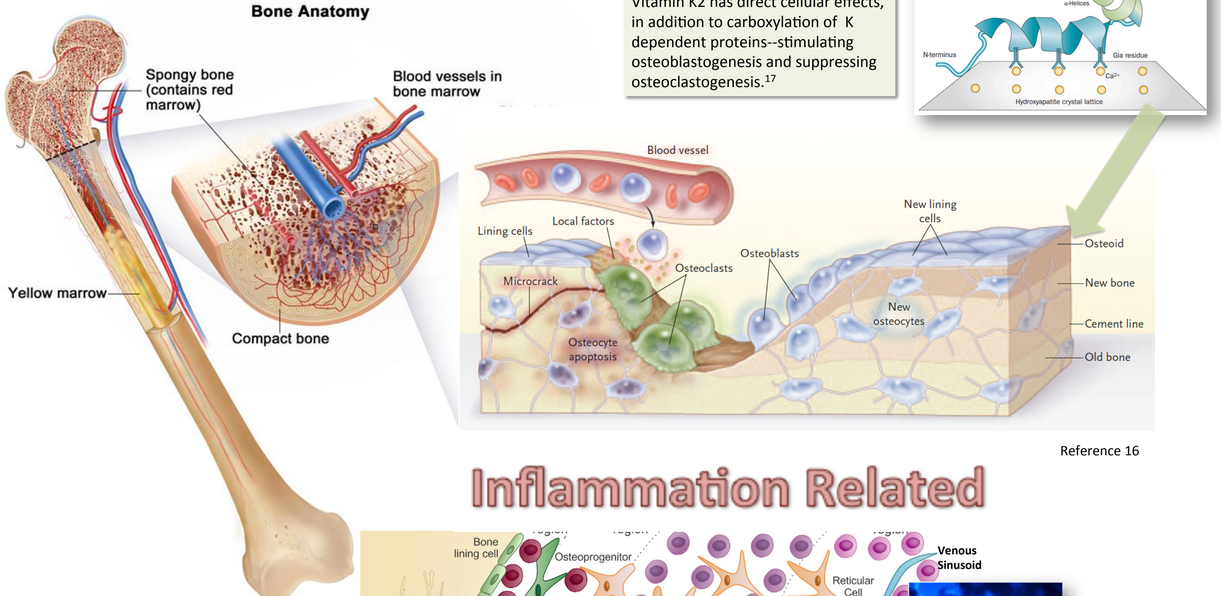
| Bone Metabolic Markers | Start Forteo | 3 months | 6 months |
|---|---|---|---|
| *Case 1* | | | |
| **Bone Formation (Osteoblast markers)** | | | |
| Osteocalcin (postmenopausal range, 9.4 – 47.4 ng/mL | 12 | 32 | 54 |
| Propeptide type 1 collagen (postmenopausal, 16 - 96 µg/mL) | 96 | 144 | 188 |
| **Bone Resorption (Osteoclast marker)** | | | |
| c-telopeptide (postmenopausal 40-465 pg/mL) | 1249 | 1587 | 1816 |
| *Case 2* | | | |
| **Osteoblast Markers** | | | |
| Osteocalcin (male normal range, 3.2 - 39.6 ng/mL | 17.2 | | 42.9 |
| Propeptide type 1 collagen (male 22 - 87 µg/mL) | 46 | 124 | |
| **Osteoclast Markers** | | | |
| c-telopeptide (male 115 - 748 pg/mL) | 642 | | 1226 |



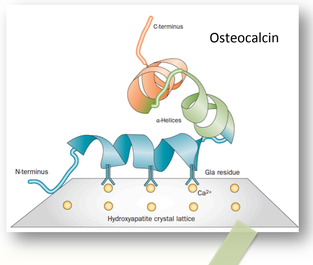

### Undercarboxylated Osteocalcin




## Treatment Related

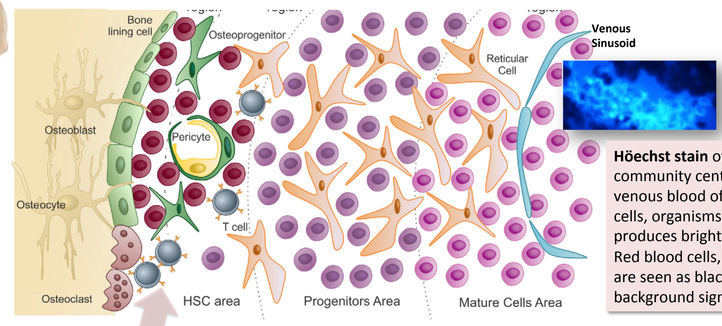



Reference 16

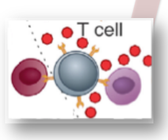



Vitamin K2 has direct cellular effects, in addition to carboxylation of K dependent proteins--stimulating osteoblastogenesis and suppressing osteoclastogenesis.[17]

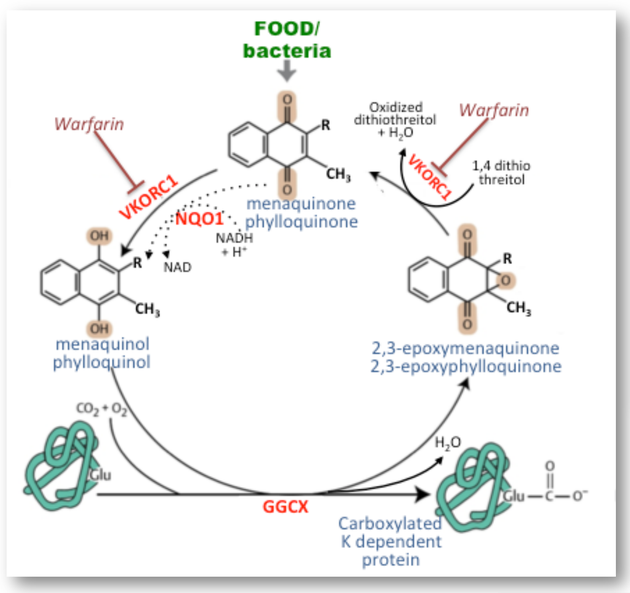
## Inflammation Related

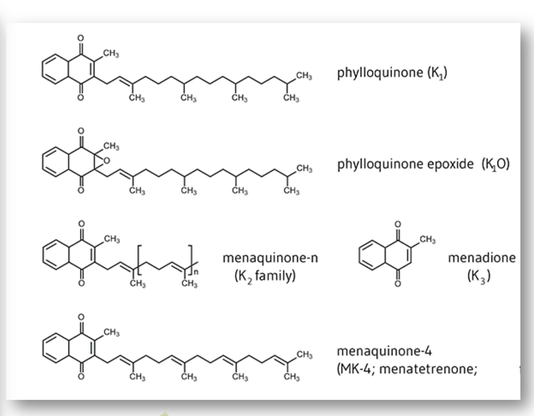


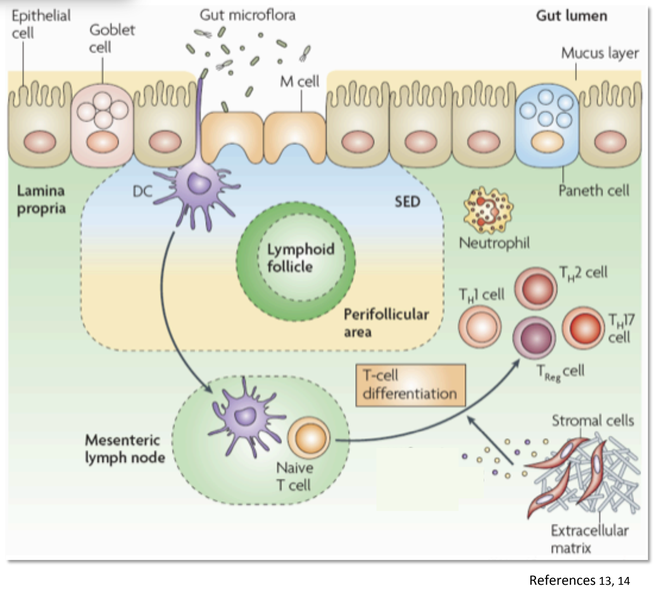

**Höechst stain** of mixed biofilm community centrifuged from the venous blood of ***Case 2***. DNA in cells, organisms and biofilm produces bright blue fluorescence. Red blood cells, containing no DNA, are seen as black marks against the background signal.[18]




T cells travel from the periphery to the bone marrow where they regulate and influence both the hematopoietic and bone remodeling systems. Activated T cells stimulate osteoclast resorption through their production of osteoclast inducing factor, and receptor activator of NFκβ ligand (RANKL).[16]




References 13, 14

## Treatment Related

| Bacteroidetes *Gram negative* | Proteobacteria *Gram negative* | Firmicutes *Gram positive* |
|---|---|---|
| Bacteroides - *MK 9,10,11,12* | Escherichia - *MK 8* | Clostridia - *MK 7* |
| | Salmonella | Bacillus subtilis - *MK 7* |
| | Vibrio | Staphlococcus - *MK 6,7,8* |
| | Helicobacter | Enterococcus - *MK 8,9* |
| | Prevotella - *MK 5,11,12,13* | Lactobacillus - *none* |

All prokaryotes make quinones, including coenzyme Q and respiratory quinones. Menaquinones are associated with the bacterial inner cytoplasmic membrane and function as redox reagents in anaerobic electron transport[11].

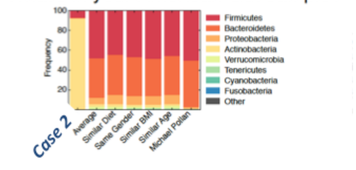
**Evidence for Vitamin K deficiency in humans treated with long-term antibiotics:**

1. Clotting abnormalities in sick children reversed by phylloquinone
2. Post-mortem study of human liver tissue shows markedly decreased content of menaquinone but not phylloquinone[12]

### Stool Microbial Culturomics

| | Expected/ Beneficial flora | Commensal (imbalanced) flora | Dysbiotic flora |
|---|---|---|---|
| *Case 1* | **NG Bacteroides fragilis group**<br>NG Bifidobacterium spp.<br>**NG Eschericia coli**<br>**NG Clostridium spp**<br>2+ Lactobacillus spp<br>4+ Enterococcus spp | 4+ Gamma hemolytic strep | 4+ Enterobacter cloacae<br>3+ E cloacae ESBL |
| *Case 2* | **NG Bacteroides fragilis group**<br>4+ Bifidobacterium spp.<br>**NG Eschericia coli**<br>**NG Clostridium spp**<br>2+ Lactobacillus spp<br>NG Enterococcus spp<br>1+ Candida papsilosis | 2+ Candida glabrata<br>4+ Saccharomyces cerevisiae/boulardii | |

What's in your American Gut sample?




## DISCUSSION:

Accelerated age-related osteoporosis is not surprising in a post-menopausal woman, but is distinctly unusual in a man with no risk factors or family history for fractures. The severity of bone loss experienced by these cases is not explained directly by clinical results.

Nonetheless we postulate that two general mechanisms are implicated:

1. Activation of inflammatory pathways involving bone:
   a. Indolent infection with a *Funneliformis*-like organism of unclear pathogenesis and/or other unidentified vector-borne pathogens.
   b. T cell activation due to gut dysbiosis and/or inflammation associated with chronic systemic infection (Lyme).

2. Altered gut microbiome leading to undercarboxylation of vitamin K dependent proteins (worse in genetically susceptible individuals). Deficient carboxylation caused and contributed to poor bone quality and density in these cases.[22,23]

There are major gaps in basic understanding of the pathophysiology of osteoporosis. Similarly, knowledge of the creation, absorption, bioavailability, tissue-specific metabolism/utilization, excretion, and therapeutic use of vitamin K is woefully incomplete.[24] Rigorous clinical observation, and research studies of the future, may shed more light on these relationships.

## RECOMMENDATIONS:

The National Osteoporosis Foundation recommends obtaining bone densitometry studies on women aged 65 or older, and men 70 or older, or younger adults treated with steroids, with a fracture after age 50, or an inflammatory condition[1]. In patients with chronic vector borne illnesses we suggest:

1.Obtain baseline bone mineral density testing in patients over the age of 50 who are treated for Lyme long-term.

   a. Measure height annually in everyone. A loss of 0.8 inches (2 cm) in a year strongly suggests subclinical vertebral fractures, as does a decline of 1.5 inches (4 cm) from peak young-adult height.

   b. As bone mineral density testing alone cannot indicate the quality of bone, consider pharmacologic osteoporosis therapy for patients over 50 with a fracture history or other inflammatory condition such as Lyme.

   c. For T scores less than -1.5, or declining T scores, begin bone-specific pharmacologic osteoporosis treatment.

2. Assess vitamin K status (undercarboxylated osteocalcin) and consider treating with vitamin K, if patient is NOT taking warfarin. The following metabolites have been administered to humans for periods of months to years without apparent side effects.

*Note: The dietary intake required for full or optimal γ carboxylation of coagulation factors and extra-hepatic gla proteins is not known[25]. A normal diet provides 90-120 µg/d of phylloquinone.*

   a. Phylloquinone up to 1000 µg/day p.o.[26]

   b. Menaquinone 7 (MK7) 360 µg/ day p.o.[22]

   c. Menatetrenone (MK4) 45 mg/day p.o. (in divided doses of 15 mg tid) [23,24]