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Bone Density Loss Is Associated With Blood Cell Counts

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ABSTRACT

Hematopoiesis depends on a supportive microenvironment. Preclinical studies in mice have demonstrated that osteoblasts influence the development of blood cells, particularly erythrocytes, B lymphocytes, and neutrophils. However, it is unknown whether osteoblast numbers or function impact blood cell counts in humans. We tested the hypothesis that men with low BMD or greater BMD loss have decreased circulating erythrocytes and lymphocytes and increased myeloid cells. We performed a cross-sectional analysis and prospective analysis in the Osteoporotic Fractures in Men (MrOS) study, a multisite longitudinal cohort study. A total of 2571 community-dwelling men (≥65 years) who were able to walk without assistance, did not have a hip replacement or fracture, and had complete blood counts (CBCs) at the third study visit were analyzed. Multivariable (MV)-adjusted logistic regression estimated odds of white blood cell (WBC) subtypes (highest and lowest quintile versus middle), and anemia (clinically defined) associated with BMD by DXA scan (at visit 3), annualized percent BMD change (baseline to visit 3), and high BMD loss (>0.5%/year, from baseline to visit 3) at the femoral neck (FN) and total hip (TH). MV-adjusted models included age, BMI, cancer history, smoking status, alcohol intake, corticosteroid use, self-reported health, thiazide use, and physical activity. At visit 3 greater TH BMD loss (per 1 SD) was associated with increased odds of anemia, high neutrophils, and low lymphocytes. Annualized BMD loss of >0.5% was associated with increased odds of anemia, high neutrophils, and low lymphocytes. Similar results were observed for FN BMD regarding anemia and lymphocytes. We conclude that community-dwelling older men with declining hip BMD over about 7 years had increased risks of anemia, lower lymphocyte count, and higher neutrophil count, consistent with preclinical studies. Bone health and hematopoiesis may have greater interdependency than previously recognized. © 2016 American Society for Bone and Mineral Research.

KEY WORDS: DXA; ANALYSIS/QUANTITATION OF BONE; OSTEOPOROSIS; DISEASES AND DISORDERS OF/RELATED TO BONE; OSTEOIMMUNOLOGY; BONE INTERACTORS

Introduction

Accumulating molecular and clinical research evidence suggests that hematopoiesis and bone metabolism are interconnected. In the bone marrow, the production of blood cells depends upon a supportive microenvironment of hematopoietic and nonhematopoietic cells.[1] Nonhematopoietic stromal cells include bone-forming osteoblasts and their precursors, which influence the differentiation of hematopoietic stem cells (HSCs) into mature hematopoietic lineages.[2,3]

Disorders of hematopoiesis, such as sickle cell anemia and thalassemia, can affect the skeletal system. Patients with sickle cell anemia have increased rates of skeletal complications, including osteopenia, osteoporosis, fractures, avascular necrosis, vertebral bone deformities, and bone and joint pain.[4] Thalassemia, a disease of defective hemoglobin production and impaired erythropoiesis, is associated with chronic bone marrow hyperplasia, decreased bone mineral density (BMD), and increased risk of fractures.[5] In Italy, women with anemia had lower trabecular and cortical bone density than women without anemia, based on peripheral quantitative computed tomography (pQCT) near the time of blood draw; men with anemia also had reduced cortical bone density.[6] In the Women's Health Initiative, postmenopausal women with anemia had a 38% to 81% increased risk of incident fracture at any skeletal site, with the highest risk being at the hip, which is composed mainly of cortical bone, after controlling for multiple covariates.[7]

Perturbations in bone metabolism can influence hematopoiesis. In preclinical studies, osteoblasts and their precursors have been implicated in erythroid, myeloid, and lymphoid development.[8–15] Signaling mediated through regulators of osteoblast function such as the parathyroid hormone receptor or Gsα, a stimulatory G protein α subunit, in osteoblast lineage cells regulate numbers of HSCs, B lymphocytes, and neutrophils in mice.[11,14–16] Interactions between osteoblasts and hematopoietic cells in humans have been relatively unexplored. We recently reported increases in circulating HSCs in postmenopausal women with osteoporosis during administration of a

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recombinant parathyroid hormone analog (teriparatide); one of the first demonstrations that bone-targeting medications can influence the human hematopoietic niche.[17]

These studies support the possibility of a clinically relevant relationship between bone health and blood homeostasis. We tested the specific a priori hypotheses that low bone mass and loss of BMD, assessed by dual-energy X-ray absorptiometry (DXA), are associated with altered numbers of circulating blood cells, specifically white blood cell (WBC) subtypes and hemoglobin, in a well-characterized longitudinal cohort of older, relatively healthy men. We hypothesized that low BMD and greater bone loss would relate to decreased erythrocytes and lymphocytes and increased cells of myeloid lineage. We also assessed the relationship of bone mass and BMD with platelet count in exploratory analyses.

Subjects and Methods

Study population

The Osteoporotic Fractures in Men (MrOS) study is a prospective observational study of community-dwelling older men recruited from six sites across the United States (Birmingham, AL; Minneapolis, MN; Palo Alto, CA; Monongahela Valley near Pittsburgh, PA; Portland, OR; and San Diego, CA) as described.[18,19] The Institutional Review Board at each site approved the study and all participants provided written informed consent. In brief, eligible men were 65 years or older, able to walk without assistance, and without bilateral hip replacement surgery. Initially, 5994 men were enrolled from March 2000 to April 2002 and completed most baseline measurements. Of these men, 4681 completed the third clinic visit between March 2007 and March 2009, with a mean of 6.9 years between visit 1 and visit 3. A subset of 3636 men had complete blood counts (CBC), including WBC count with differential counts of WBC subtypes measured at visit 3. Two men with extreme total WBC values (over 50,000 total WBC count) were excluded from all analyses. Men with prevalent fracture (defined as self-reported fractures after age 50 years but prior to MrOS study initiation as well as self-reported and adjudicated incident fractures from MrOS visit 1 to visit 3) at visit 3 ($n = 1023$) were excluded because they were felt to be a different and heterogeneous group from those without fracture, due to altered bone remodeling and different bone health treatments, potentially affecting results. Of the remaining 2611 men that were included in descriptive analysis, 25 men did not have DXA at visit 3. Therefore, 2586 men at visit 3 comprised the analytic study population (Fig. 1). Four men were excluded from neutrophil analysis only due to blood counts suggestive of acute illness (total WBC over 17,000 with preponderance of neutrophils).

Pertinent participant characteristics

In MrOS, demographic information, medical history, and tobacco and alcohol use were ascertained at baseline and follow-up visits using standardized questionnaires and in-person participant interviews. The Physical Activity Scale for the Elderly (PASE) questionnaire was used to assess physical activity.[20] At each visit, weight (kg) was measured on balance beams or digital scales while standing height (cm) was measured on Harpenden stadiometers, then used to calculate body mass index (BMI; kg/m^2). Medications were brought in by participants and verified by study staff. All medications recorded by the clinics were stored in an electronic medications inventory database (San Francisco Coordinating Center, San Francisco, CA, USA). Each medication was matched to its ingredient(s) based on the Iowa Drug Information Service (IDIS) Drug Vocabulary (College of Pharmacy, University of Iowa, Iowa City, IA, USA).[21]

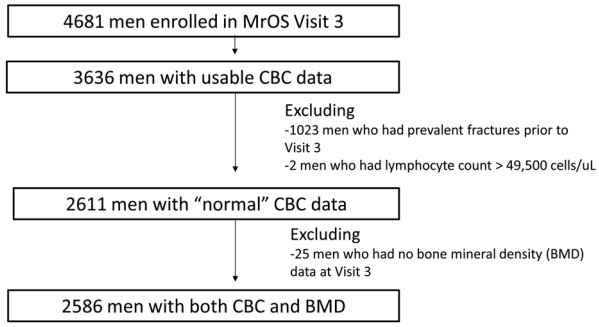

Fig. 1. Participant flowchart for the analytical cohort.

BMD

BMD was measured at baseline and visit 3 at the total hip (TH), femoral neck (FN), and lumbar spine (LS) using DXA on Hologic QDR 4500-W densitometers (Hologic Inc., Waltham, MA, USA). A central quality-control laboratory, certification of DXA operators, and standardized procedures for scanning were used to ensure reproducibility of DXA measurements at all six clinical sites.[19]

CBC

Fasting blood samples were collected at visit 3. CBCs and WBC subtypes were performed at local Quest Diagnostic laboratories for five study sites and Stanford Outreach for one site. Measurements were standardized across laboratories and included hemoglobin concentration and counts of platelet, total WBC, and WBC subtypes: neutrophils, lymphocytes, and monocytes. We defined anemia as hemoglobin less than 12 g/dL and thrombocytopenia (or low platelet count) as less than 150,000/μL platelets. Each of the WBC subtypes were categorized by quintiles: "low" (1st, or lowest, quintile), "middle" (middle 2nd through 4th quintiles), and "high" (highest quintile), based on their corresponding distributions in the entire analytic study population.

Statistical analyses

The analytic study baseline for all analyses was visit 3 in MrOS. We compared pertinent participant characteristics and BMD at three skeletal sites (FN, TH, and LS), according to counts of WBC subtypes, hemoglobin concentration, and platelet count, using ANOVA F-test for continuous variables, chi-square test for dichotomous variables, and Mantel-Haenszel test for three-category variables. Participant characteristics included age, BMI, use of: testosterone, corticosteroids, hydrochlorothiazide, and bone medications, cancer history (including nonmelanoma skin cancer), rheumatoid arthritis history, self-reported health, activity score, alcohol intake, and smoking status.

Initially, we evaluated cross-sectionally at visit 3 the odds of an altered number of a hematopoietic cell type in association with BMD at the FN, TH, and LS. We conducted two multivariable (MV) logistic regression models to estimate the odds ratio (OR) for

each of the following: anemia, thrombocytopenia, and low and high counts of each WBC subtype, associated with 1 SD lower BMD value. The first model adjusted for age and study site, the 2nd was the "full" MV-adjusted model adjusted for the potential confounders or putative risk factors for bone loss: age, study site, BMI, smoking status, alcohol use, physical activity, cancer history, self-reported health, corticosteroid use, and thiazide use.

Next, we conducted analogous logistic regression models to estimate the OR for each of the following: anemia, thrombocytopenia, and low and high numbers of each WBC subtype, associated with 1 SD decrease in annualized percent BMD loss at the FN, TH, and LS. We calculated BMD loss (from visit 1 to visit 3) and converted it to an annualized percentage. In addition, we estimated the OR for altered numbers of hematopoietic cell type associated with having "high BMD loss" compared to not having high BMD loss. High BMD loss was defined as an annualized decline in BMD of more than 0.5% from visit 1 to visit 3.

All p values reported were two-sided, and all analyses were performed using SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Participant characteristics at visit 3

Our cohort consisted of 2586 men with a mean age of 78.9 years at visit 3 and a mean follow-up time of 6.8 years since their first study visit. Participant characteristics are shown in Table 1 and Table 2. Participants with anemia, high neutrophil count, thrombocytopenia, or high monocyte count were slightly older and less active than those without. Participants with anemia and high neutrophil count also had slightly shorter height, lower alcohol intake, had a higher frequency of corticosteroid use, and less often had excellent or good self-reported health. Physical activity was lowest in the anemia group. A high proportion of participants with low lymphocytes had a history of cancer (46%), but age, height, physical activity, and corticosteroid use was similar to those who had middle and high lymphocytes. Osteoporosis medication use was $\leq$6% in all groups across the cohort.

TH BMD was lower in participants with anemia (0.93 g/cm^2) and low lymphocytes (0.95 g/cm^2), whereas FN BMD was lower in participants with high neutrophil count (0.77 g/cm^2) than in their respective comparison groups. The mean annualized BMD loss in our cohort was –0.37% at the TH and –0.36% at the FN. Participants with anemia, high neutrophil count, or low lymphocyte count had significantly more BMD loss at the TH (–0.87%, –0.48%, –0.49%, respectively) and FN (–0.75%, –0.51%, –0.51%, respectively), and had a larger proportion with high BMD loss (>0.5% annualized loss) at the TH and FN than those in the comparison groups. A relative minority of participants had coexisting anemia, high neutrophil count, or low lymphocyte count as shown in Fig. 2. TH BMD loss was higher when two of these conditions coexisted. Participants with thrombocytopenia had more BMD loss at the FN than those without (–0.47% versus –0.35%). Overall there was BMD gain of 1.23% at the LS in our cohort. Participants with low monocyte count had lower TH BMD (0.94 g/cm^2) and LS BMD (1.24 g/cm^2) and less LS BMD gain than those with middle and high monocyte counts (1.02% versus 1.25% and 1.38%, respectively).

Risk of altered hematopoietic cell numbers by BMD

Age- and site-adjusted and MV-adjusted associations of absolute BMD values and hematopoietic cell counts are presented in Table 3. Lower BMD at the hip was associated with increased risk for high neutrophil count and low monocyte count. Age- and

Table 1. Characteristics of MrOS Participants by Hgb (Normal Versus Anemia) and Platelet Counts (Normal Versus Thrombocytopenia)

	Hgb ($n = 2611$)			Platelets ($n = 2590$)		
Covariates	Normal ($n = 2442$)	Anemia ($n = 169$)	p	Normal ($n = 2276$)	Thrombocytopenia ($n = 314$)	p
Age (years)	78.8 ± 5.0	81.8 ± 5.5	<0.001	78.8 ± 5.1	79.7 ± 5.1	0.007
BMI (kg/m^2)	27.2 ± 3.7	26.5 ± 4.1	0.024	27.2 ± 3.7	26.9 ± 4.1	0.29
Height (cm)	173.5 ± 6.8	171.7 ± 7.6	0.004	173.3 ± 6.8	173.8 ± 6.9	0.19
History of cancer	922 (38%)	69 (41%)	0.43	857 (38%)	126 (40%)	0.40
Health status excellent/good	2155 (88%)	117 (69%)	<0.001	1990 (88%)	265 (84%)	0.12
Smoking status	45 (2%)	3 (2%)	0.95	41 (2%)	6 (2%)	0.89
Activity score[a]	135.9 ± 67.7	108.6 ± 67.7	<0.001	135.8 ± 68.1	124.9 ± 66.9	0.008
RA	170 (7%)	23 (14%)	0.001	173 (8%)	18 (6%)	0.23
Alcohol intake	1569 (65%)	93 (55%)	0.012	1459 (64%)	192 (62%)	0.36
Corticosteroid use	255 (11%)	29 (17%)	0.007	255 (11%)	23 (7%)	0.037
Thiazide use	492 (20%)	38 (23%)	0.47	475 (21%)	50 (16%)	0.041
Anti-OP use	122 (5%)	7 (4%)	0.62	109 (5%)	19 (6%)	0.33
FN BMD (g/cm^2)	0.78 ± 0.13	0.77 ± 0.14	0.18	0.78 ± 0.13	0.77 ± 0.12	0.24
Total hip BMD (g/cm^2)	0.96 ± 0.14	0.93 ± 0.16	0.006	0.96 ± 0.15	0.95 ± 0.14	0.17
Lumbar spine BMD (g/cm^2)	1.27 ± 0.31	1.31 ± 0.34	0.18	1.28 ± 0.30	1.28 ± 0.35	0.99
FN BMD Δ (g/cm^2)	−0.34 ± 0.86	−0.75 ± 1.08	<0.001	−0.35 ± 0.89	−0.47 ± 0.88	0.027
Total hip BMD Δ (g/cm^2)	−0.33 ± 0.70	−0.87 ± 0.97	<0.001	−0.36 ± 0.72	−0.43 ± 0.79	0.11
LS BMD Δ (g/cm^2)	1.22 ± 1.95	1.45 ± 3.74	0.43	1.22 ± 2.16	1.31 ± 1.73	0.39
FN BMD loss >0.5%	920 (39%)	94 (56%)	<0.001	869 (39%)	136 (45%)	0.06
TH BMD loss >0.5%	801 (34%)	100 (60%)	<0.001	768 (35%)	124 (41%)	0.036
LS BMD loss >0.5%	367 (16%)	30 (19%)	0.26	360 (17%)	35 (18%)	0.037

Values are mean ± SD or n (%), as indicated. Definition of anemia: Hgb <12 g/dL; thrombocytopenia: <150 k/μL.
[a]Activity score is based on the Physical Activity Scale for the Elderly (PASE) questionnaire.

Table 2. Characteristics of MrOS Participants by WBC Subtypes

	Neutrophils ($n = 2602$)				Lymphocytes ($n = 2606$)				Monocytes ($n = 2606$)			
Covariates	Low ($n = 522$)	Middle ($n = 1560$)	High ($n = 520$)	p	Low ($n = 518$)	Middle ($n = 1567$)	High ($n = 521$)	p	Low ($n = 519$)	Middle ($n = 1566$)	High ($n = 521$)	p
Age (years)	78.0 ± 4.7	79.0 ± 5.1	79.7 ± 5.2	<0.001	79.3 ± 5.0	78.8 ± 5.1	79.1 ± 5.2	0.12	78.3 ± 4.9	78.8 ± 4.9	80.1 ± 5.6	<0.001
BMI (kg/m^2)	26.4 ± 3.6	27.3 ± 3.8	27.3 ± 3.9	<0.001	26.5 ± 3.8	27.1 ± 3.7	27.7 ± 4.0	<0.001	26.5 ± 3.7	27.2 ± 3.7	27.4 ± 3.9	<0.001
Height (cm)	173.8 ± 7.1	173.4 ± 6.7	172.7 ± 6.9	0.033	173.7 ± 7.1	173.3 ± 6.7	173.3 ± 6.9	0.42	173.7 ± 7.0	173.4 ± 6.7	172.9 ± 6.9	0.18
History of cancer	212 (41%)	597 (38%)	177 (34%)	0.029	237 (46%)	569 (36%)	181 (35%)	<0.001	202 (39%)	602 (38%)	183 (35%)	0.21
Health status: excellent/good	467 (89%)	1375 (88%)	421 (81%)	<0.001	451 (87%)	1371 (88%)	445 (86%)	0.43	456 (88%)	1386 (89%)	425 (82%)	0.003
Smoking status	4 (1%)	22 (1%)	22 (4%)	<0.001	4 (1%)	28 (2%)	16 (3%)	0.006	8 (2%)	25 (2%)	15 (3%)	0.11
Activity score[a]	144 ± 71	135 ± 67	124 ± 68	<0.001	132 ± 66	136 ± 69	131 ± 69	0.25	139 ± 66	136 ± 69	123 ± 67	<0.001
RA	34 (7%)	115 (7%)	44 (9%)	0.23	36 (7%)	123 (8%)	34 (7%)	0.80	36 (7%)	107 (7%)	50 (10%)	0.10
Alcoho1 intake	359 (69%)	l003 (65%)	294 (57%)	<0.001	328 (64%)	1005 (65%)	325 (63%)	0.81	335 (65%)	1002 (64%)	321 (62%)	0.23
Corticosteroid use	42 (8%)	150 (10%)	91 (18%)	<0.001	53 (10%)	178 (11%)	53 (10%)	0.97	42 (8%)	150 (10%)	92 (18%)	<0.001
Thiazide use	91 (18%)	297 (19%)	140 (27%)	<0.001	100 (19%)	316 (20%)	112 (22%)	0.38	84 (16%)	332 (21%)	112 (22%)	0.033
Anti-OP use	30 (6%)	74 (5%)	24 (5%)	0.39	32 (6%)	73 (5%)	23 (4%)	0.19	24 (5%)	77 (5%)	27 (5%)	0.68
FN BMD (g/cm^2)	0.79 ± 0.13	0.79 ± 0.13	0.77 ± 0.13	0.016	0.78 ± 0.13	0.78 ± 0.13	0.79 ± 0.14	0.07	0.77 ± 0.13	0.79 ± 0.13	0.78 ± 0.14	0.17
Total hip BMD (g/cm^2)	0.95 ± 0.14	0.96 ± 0.15	0.95 ± 0.15	0.11	0.95 ± 0.15	0.96 ± 0.14	0.97 ± 0.15	0.047	0.94 ± 0.14	0.96 ± 0.15	0.95 ± 0.15	0.002
Lumbar spine BMD (g/cm^2)	1.27 ± 0.34	1.28 ± 0.30	1.28 ± 0.30	0.70	1.27 ± 0.32	1.27 ± 0.30	1.30 ± 0.32	0.22	1.24 ± 0.29	1.29 ± 0.31	1.29 ± 0.30	0.007
FN BMD Δ (g/cm^2)	−0.32 ± 0.87	−0.34 ± 0.85	−0.51 ± 0.99	<0.001	−0.51 ± 0.89	−0.34 ± 0.87	−0.33 ± 0.92	<0.001	−0.40 ± 0.85	−0.36 ± 0.87	−0.37 ± 0.95	0.64
Total hip BMD Δ (g/cm^2)	−0.32 ± 0.72	−0.34 ± 0.70	−0.48 ± 0.82	<0.001	−0.49 ± 0.80	−0.34 ± 0.70	−0.32 ± 0.75	<0.001	−0.41 ± 0.71	−0.34 ± 0.72	−0.40 ± 0.77	0.06
LS BMD Δ (g/cm^2)	1.23 ± 2.15	1.28 ± 2.15	1.09 ± 1.93	0.24	1.07 ± 2.13	1.25 ± 1.95	1.33 ± 2.50	0.12	1.02 ± 1.85	1.25 ± 1.95	1.38 ± 2.70	0.019
FN BMD loss >0.5%	196 (38%)	592 (39%)	223 (44%)	0.042	240 (48%)	589 (39%)	183 (36%)	<0.001	211 (42%)	605 (40%)	196 (39%)	0.48
TH BMD loss >0.5%	163 (32%)	518 (34%)	217 (43%)	<0.001	211 (42%)	526 (35%)	162 (32%)	<0.001	200 (39%)	507 (33%)	192 (39%)	0.77
LS BMD loss >0.5%	76 (15%)	227 (15%)	92 (19%)	0.10	102 (21%)	224 (15%)	70 (14%)	0.004	89 (18%)	218 (15%)	89 (18%)	0.95

Values are mean ± SD or n (%), as indicated. Definition of WBC subtypes: neutrophils: low <2705 cells/μL; lymphocytes: low <1080 cells/μL, high >1885 cells/μL; monocytes: low <350 cells/μL, high >607 cells/μL. All cut points were based on quintile distribution with low group as the lowest (1st) quintile, middle group as 2nd–4th quintiles, and high group as the highest (5th) quintile.

[a]Activity score is based on the Physical Activity Scale for the Elderly (PASE) questionnaire.

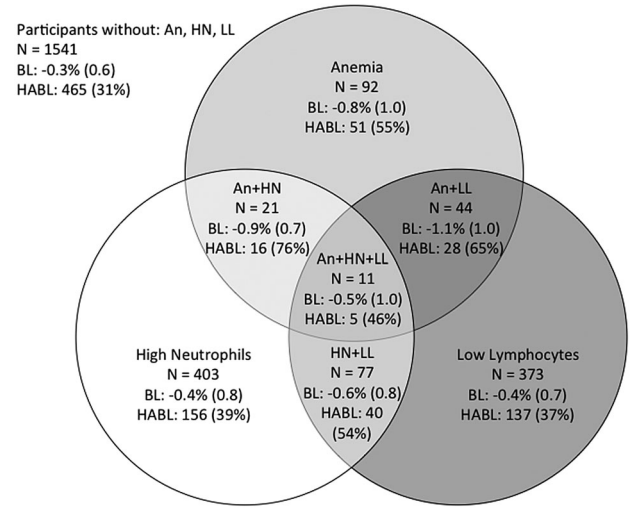

Fig. 2. Bone variables of interest at total hip in selected participant subgroups. The number and characteristics of participants with anemia, high neutrophils, and low lymphocytes and those in which these conditions coexist are shown inside the diagram whereas participants with none of these conditions are shown outside of the diagram. BL = annualized percent bone loss (mean [SD]); HABL = high annual bone loss defined as >0.5% per year (*n* [%]); An = anemia; HN = high neutrophils; LL = low lymphocytes.

site-adjusted analyses revealed that every 1 SD decrease in BMD at the FN was associated with decreased odds of having high lymphocyte count and increased odds of having high neutrophil and low monocyte count, whereas decrease in BMD at the TH was associated with decreased odds of having high lymphocyte count and increased odds of having low monocyte count. However, in MV-adjusted models only the association of monocytes with TH BMD remained significant (OR 1.15; 95% CI, 1.02 to 1.29).

Risk of altered hematopoietic cell counts by change in BMD

Age- and site-adjusted and MV-adjusted associations of annualized percent change in BMD and hematopoietic cell counts are presented in Table 4. Loss of BMD at the FN and TH was associated with anemia, high neutrophils, and low lymphocytes. In separate MV-adjusted models, greater annualized BMD loss (per 1 SD of annualized BMD loss), at the FN was associated with increased odds of anemia (OR 1.33; 95% CI, 1.15 to 1.55), high neutrophil count (OR 1.16; 95% CI, 1.04 to 1.29), and low lymphocyte count (OR 1.21; 95% CI, 1.09 to 1.34). Greater BMD loss at the TH was associated with increased odds of anemia (OR 1.50; 95% CI, 1.30 to 1.73), high neutrophil count (OR 1.13; 95% CI, 1.01 to 1.25), and low lymphocyte count (OR 1.22; 95% CI, 1.09 to 1.35).

Risk of accelerated BMD loss with hematopoietic cell counts

The relationship between high BMD loss (>0.5% annualized BMD decline) and hematopoietic cell counts are presented in Table 5. High BMD loss at the FN and TH was associated with increased odds of having anemia, high neutrophil count, and low lymphocyte count. In MV-adjusted models, men with high BMD loss at the FN had an 79% increased odds of anemia (OR 1.79; 95% CI, 1.28 to 2.49) and a 49% increased odds of low

Table 3. Associations of Site-Specific BMD to Hematopoietic Cell Types at Visit 3 ($n = 2571$)

	WBC subtype							
			Neutrophils		Lymphocytes		Monocytes	
	Anemia	Low platelets	Low	High	Low	High	Low	High
Femoral neck								
Age- and site-adjusted	1.02 (0.87–1.20)	1.06 (0.93–1.19)	1.01 (0.91–1.12)	**1.14 (1.03–1.27)**	1.01 (0.91–1.12)	**0.88 (0.80–0.97)**	**1.13 (1.02–1.25)**	0.99 (0.89–1.09)
MV-adjusted[a]	0.92 (0.78–1.09)	1.02 (0.90–1.17)	0.93 (0.83–1.04)	1.12 (1.00–1.25)	0.95 (0.85–1.07)	0.92 (0.83–1.02)	1.05 (0.94–1.18)	0.98 (0.88–1.09)
Total hip								
Age- and site-adjusted	1.13 (0.96–1.33)	1.07 (0.94–1.21)	1.10 (0.99–1.22)	1.08 (0.97–1.20)	1.04 (0.94–1.16)	**0.89 (0.80–0.98)**	**1.23 (1.11–1.37)**	1.01 (0.91–1.12)
MV-adjusted[a]	1.02 (0.86–1.22)	1.02 (0.89–1.17)	1.01 (0.90–1,13)	1.05 (0.93–1.17)	0.99 (0.88–1.10)	0.93 (0.83–1.04)	**1.15 (1.02–1.29)**	1.01 (0.90–1.13)
Lumbar spine								
Age- and site-adjusted	0.89 (0.76–1.04)	1.03 (0.91–1.17)	1.04 (0.94–1.15)	0.98 (0.89–1.09)	1.00 (0.90–1.11)	0.93 (0.84–1.03)	**1.14 (1.02–1.27)**	1.04 (0.94–1.16)
MV-adjusted[a]	0.86 (0.74–1.00)	1.01 (0.89–1.15)	0.99 (0.89–1.10)	0.98 (0.88–1.09)	0.96 (0.86–1.07)	0.96 (0.86–1.07)	1.08 (0.97–1.21)	1.05 (0.94–1.17)

Values are OR (95% CI) per 1 SD decrease of BMD. Bold values, $p < 0.05$.
[a]Adjusted for age, site, BMI, smoking status, alcohol use, physical activity, history of cancer, self-reported health, corticosteroid use, and thiazide use.

Table 4. Associations of Site-Specific Annualized BMD % Change (Visit 1 to Visit 3) to Hematopoietic Cell Types at Visit 3 ($n = 2571$)

			WBC subtype					
			Neutrophils		Lymphocytes		Monocytes	
	Anemia	Low platelets	Low	High	Low	High	Low	High
Femoral neck								
Age- and site-adjusted	**1.45 (1.26–1.68)**	1.13 (1.00–1.27)	1.00 (0.90–1.11)	**1.19 (1.07–1.31)**	**1.22 (1.10–1.35)**	0.97 (0.87–1.07)	1.07 (0.97–1.19)	0.98 (0.89–1.09)
MV-adjusted[a]	**1.33 (1.15–1.55)**	1.09 (0.96–1.23)	0.98 (0.88–1.09)	**1.16 (1.04–1.29)**	**1.21 (1.09–1.34)**	0.98 (0.88–1.09)	1.03 (0.93–1.15)	0.95 (0.85–1.06)
Total hip								
Age- and site-adjusted	**1.60 (1.40–1.83)**	1.07 (0.95–1.21)	1.02 (0.92–1.14)	**1.16 (1.05–1.28)**	**1.23 (1.11–1.36)**	0.96 (0.86–1.07)	**1.14 (1.03–1.26)**	1.03 (0.92–1.14)
MV-adjusted[a]	**1.50 (1.30–1.73)**	1.02 (0.91–1.16)	1.00 (0.89–1.12)	**1.13 (1.01–1.25)**	**1.22 (1.09–1.35)**	0.98 (0.88–1.09)	1.09 (0.98–1.21)	0.99 (0.89–1.10)
Lumbar spine								
Age- and site-adjusted	0.87 (0.76–1.00)	0.98 (0.87–1.10)	1.05 (0.94–1.17)	1.07 (0.95–1.21)	1.05 (0.94–1.18)	0.99 (0.90–1.10)	1.09 (0.97–1.23)	0.99 (0.89–1.09)
MV-adjusted[a]	0.89 (0.77–1.02)	0.97 (0.86–1.09)	1.03 (0.92–1.14)	1.09 (0.97–1.23)	1.03 (0.92–1.15)	1.00 (0.90–1.12)	1.06 (0.94–1.19)	0.99 (0.89–1.10)

Values are OR (95% CI) per 1 SD decrease of annualized BMD % change. Bold values, $p < 0.05$.

[a]Adjusted for age, site, BMI, smoking status, alcohol use, physical activity, history of cancer, self-reported health corticosteroid use, and thiazide use.

Table 5. Associations of Site-Specific High BMD Loss (>0.5% Annualized) (Visit 1 to Visit 3) to Hematopoietic Cell Types at Visit 3 ($n = 2571$)

			WBC subtype					
			Neutrophils		Lymphocytes		Monocytes	
	Anemia	Low platelets	Low	High	Low	High	Low	High
Femoral neck								
Age- and site-adjusted	**1.93 (1.40–2.67)**	1.23 (0.96–1.57)	0.99 (0.81–1.23)	1.20 (0.97–1.48)	**1.49 (1.21–1.84)**	0.86 (0.69–1.06)	1.12 (0.91–1.38)	0.92 (0.74–1.13)
MV-adjusted[a]	**1.79 (1.28–2.49)**	1.17 (0.91–1.50)	0.97 (0.78–1.20)	1.16 (0.93–1.43)	**1.49 (1.21–1.84)**	0.88 (0.71–1.09)	1.06 (0.85–1.31)	0.87 (0.70–1.08)
Total hip								
Age- and site-adjusted	**2.37 (1.70–3.30)**	1.23 (0.95–1.58)	0.98 (0.79–1.22)	**1.40 (1.13–1.72)**	**1.38 (1.12–1.71)**	0.86 (0.69–1.07)	**1.38 (1.11–1.70)**	1.12 (0.90–1.39)
MV-adjusted[a]	**2.10 (1.49–2.95)**	1.14 (0.88–1.48)	0.96 (0.76–1.20)	**1.34 (1.08–1.68)**	**1.36 (1.09–1.70)**	0.87 (0.69–1.09)	**1.29 (1.04–1.61)**	1.05 (0.84–1.31)
Lumbar spine								
Age- and site-adjusted	1.23 (0.80–1.91)	0.69 (0.47–1.01)	1.02 (0.76–1.36)	1.22 (0.92–1.61)	**1.37 (1.04–1.80)**	1.00 (0.74–1.35)	1.18 (0.89–1.56)	**1.48 (1.11–1.97)**
MV-adjusted[a]	1.18 (0.75–1.84)	**0.67 (0.45–0.98)**	0.96 (0.71–1.29)	1.23 (0.92–1.64)	1.30 (0.99–1.71)	1.00 (0.74–1.36)	1.10 (0.83–1.46)	**1.43 (1.06–1.91)**

Values are OR (95% CI) per 1 SD decrease of annualized BMD % change. Bold values, $p < 0.05$.

[a]Adjusted for age, site, BMI, smoking status, alcohol use, physical activity, history of cancer, self-reported health, corticosteroid use, and thiazide use.

lymphocyte count (OR 1.49; 95% CI, 1.21 to 1.84), while high BMD loss at the TH was associated with a 2.10-fold increased odds of anemia (OR 2.10; 95% CI, 1.49 to 2.95), a 34% increased odds of high neutrophil count (OR 1.34; 95% CI, 1.08 to 1.68), a 36% increased odds of low lymphocyte count (OR 1.36; 95% CI, 1.09 to 1.70), and a 29% increased odds of low monocyte count (OR 1.29; 95% CI, 1.04 to 1.61). In addition, high BMD loss at the LS was associated with a 33% decreased odds of thrombocytopenia (OR 0.67; 95% CI, 0.45 to 0.98) and a 43% increased odds of high monocyte count (OR 1.43; 95% CI, 1.06 to 1.91) in MV-adjusted models.

Discussion

We evaluated the relationship of BMD, and BMD change, to numbers of various hematopoietic cell types, in a community-based cohort of older men enrolled in a prospective observational study. High BMD loss (defined as >0.5% annual decline) at the hip, a site composed mainly of cortical bone, was associated with a higher risk of anemia. BMD loss at the hip was also associated with low lymphocytes and separately, high neutrophils. To our knowledge, this is the first study to report associations between bone density measurements by DXA and counts of WBC subtypes in humans. Our study is also one of the few to observe associations between bone density and anemia in elderly men. We found that the rate of BMD loss, and less so low absolute BMD, relates to altered hematopoiesis. Further study of the relationship between the skeletal system and hematopoiesis and their reciprocity may have important clinical implications, because hematopoietic cell counts would be simple and relatively inexpensive adjuncts to bone health assessment.

Overall, annualized loss of BMD appeared to correlate better with hematopoietic cell counts than absolute BMD. Absolute bone density is a cross-sectional snapshot of bone health whereas annualized BMD loss measures a trend across time. Further studies are needed to evaluate why the rate of bone density loss may be a better clinical marker of bone health as it relates to hematopoiesis.

We observed that high BMD loss at the hip was associated with a greater than twofold risk of anemia. These observations are consistent with the findings for cortical bone density and anemia in men participating in the InCHIANTI study.[6] Although we did not find that reduced absolute BMD is associated with anemia in cross-sectional analysis, it may be that the DXA scan we used is less sensitive at uncovering differences in bone density than the pQCT used in the Italian study. It is plausible that deteriorating bone would provide a less supportive environment for hematopoiesis, resulting in anemia; however, we cannot categorically determine whether bone is affecting hematopoiesis or vice-versa from the current study. Alternatively, anemia may be a marker for disability and lower muscle strength,[22] which are known to be associated with decreased BMD,[23] perhaps due to decreased mechanical stimuli on bone. In our study, the relationship between BMD loss and anemia persisted despite controlling for physical activity and BMI, which are frequently linked to disability and lower muscle mass,[24–26] but the possibility of residual confounding from decreased muscle strength in participants with anemia cannot be completely excluded.

We also observed that high BMD loss was associated with increased neutrophil counts, decreased monocyte counts, and decreased lymphocyte counts. With aging there is a well-documented increase in myeloid cells accompanied by a decline in lymphocytes. Several mechanisms have been proposed, including inflammation, intrinsic bias of HSCs favoring myeloid over lymphoid lineage commitment, and extrinsic effects of an aging bone marrow microenvironment.[27–30] No previous studies have evaluated counts WBC subtypes and clinical assessments of bone health. WBC subtype counts can be considered measures of inflammation and the inflammatory biomarkers IL-6 and C-reactive protein (CRP) have been associated with lower BMD[31] and bone loss.[32] Older populations are known to have increased comorbidities and multi-morbidity has been associated with IL-6 and CRP levels, specifically in the Midlife in the United States (MIDUS) longitudinal study.[33] One potential explanation for our findings, therefore, could be increased comorbidity with associated increases in inflammation and bone loss. Increasing levels of comorbidity are related to lower self-reported health,[34] which was associated with high bone loss (data not shown), and neutrophil and monocyte counts in our study. We found that high bone loss was associated with high neutrophils and low monocytes, independent of self-reported health. The increase in neutrophils may be related to the chronic inflammation that occurs with aging regardless of general health or comorbidities, but it is unclear why neutrophils and monocytes, which are both myeloid derived cells, have differing relationships with bone loss, although cells of the osteoblastic lineage might affect differentiation of neutrophils and monocytes differently.[35]

In animal models, cells at distinct stages of maturation along the osteoblast lineage interact with specific populations of hematopoietic cells.[35] For example, mesenchymal stem cells (MSCs) constitute a critical niche for HSCs.[13,36–38] In contrast, B lymphocyte and erythrocyte lineages are dependent upon osteoblast progenitors,[8–10,13–15], whereas terminally differentiated osteoblasts and osteocytes influence myelopoiesis.[11] Based on mouse models of altered bone metabolism,[9,11,14,15] we predicted that poor bone health would lead to decreased erythrocytes and lymphocytes and increased cells of myeloid lineage such as neutrophils. Overall, our results are consistent with these predictions. Whether B lymphocytes, erythrocytes, and myeloid cells are supported by cells of the osteoblast lineage at distinct stages of differentiation in humans has not been determined. As noted in Fig. 2, there was relatively little overlap between men with decreased lymphocytes, decreased erythrocytes, or increased myeloid cells. We therefore explored descriptively whether greater BMD loss was more prevalent in men with more than one altered hematopoietic cell count. This hypothesis was not tested further because of limited sample size of men with multiple altered cell lines. However, we suspect that no one unifying mechanism exists to explain the observed associations of BMD loss and hematopoiesis.

Our study has several strengths and limitations. The analyses were performed in a large prospective observational cohort of community-dwelling older men. The cohort was multisite across the United States and well characterized in detail, from extensive data collection using standardized questionnaires, interviews, exams, DXA, blood draws, and assay methods. However, the relevance to other racial/ethnic groups or women is as yet unknown due to the predominantly white (about 90%) and exclusively male cohort. Although there is intraindividual variability in blood counts, the large sample size of the study population should temper this. Because CBC was measured only at visit 3, temporality of the development of altered blood counts and associations between change in BMD and change in

blood cell counts could not be achieved. We did not have information about acute infection or illness at the time of blood draw, and although we did exclude participants who had patterns suggestive of acute illness it is possible that some of the cell populations analyzed were affected in some instances. Performing multiple comparisons can increase the chance findings statistically (false positives), so we were careful not to make conclusions based on findings that were not consistent with a priori hypotheses or were not consistent in our analyses. Our results revealed similar patterns of associations at both hip sites, and agreed with previous literature. Spine BMD generally increased in our study, which could be due to artifacts such as arthritis or aortic calcification; this may have biased our results toward the null, making it hard to detect small differences at this site. We did not have information on the type of anemia or testosterone level at visit 3 for our participants, which would have been informative for that analysis.

In summary, older white men with BMD loss had an increased risk of anemia, low lymphocyte count, or high neutrophil count; the directions of these associations are consistent with findings from animal studies. High BMD loss, particularly at the hip, were more strongly related to these altered blood counts than absolute BMD, suggesting that rate of BMD loss influences hematopoiesis. Future translational studies are warranted to confirm our findings and to further map the bone-blood interaction and potential clinical implications.

Disclosures

All authors state that they have no conflicts of interest.

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