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CLINICAL RESEARCH
Vascular biology and medicine

Bone marrow activation in response to metabolic syndrome and early atherosclerosis

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Abstract

Aims Experimental studies suggest that increased bone marrow (BM) activity is involved in the association between cardiovascular risk factors and inflammation in atherosclerosis. However, human data to support this association are sparse. The purpose was to study the association between cardiovascular risk factors, BM activation, and subclinical atherosclerosis.

Methods and results Whole body vascular ^{18}F-fluorodeoxyglucose positron emission tomography/magnetic resonance imaging (^{18}F-FDG PET/MRI) was performed in 745 apparently healthy individuals [median age 50.5 (46.8–53.6) years, 83.8% men] from the Progression of Early Subclinical Atherosclerosis (PESA) study. Bone marrow activation (defined as BM ^{18}F-FDG uptake above the median maximal standardized uptake value) was assessed in the lumbar vertebrae (L3–L4). Systemic inflammation was indexed from circulating biomarkers. Early atherosclerosis was evaluated by arterial metabolic activity by ^{18}F-FDG uptake in five vascular territories. Late atherosclerosis was evaluated by fully formed plaques on MRI. Subjects with BM activation were more frequently men (87.6 vs. 80.0%, $P = 0.005$) and more frequently had metabolic syndrome (MetS) (22.2 vs. 6.7%, $P < 0.001$). Bone marrow activation was significantly associated with all MetS components. Bone marrow activation was also associated with increased haematopoiesis—characterized by significantly elevated leucocyte (mainly neutrophil and monocytes) and erythrocyte counts—and with markers of systemic inflammation including high-sensitivity C-reactive protein, ferritin, fibrinogen, P-selectin, and vascular cell adhesion molecule-1. The associations between BM activation and MetS (and its components) and increased erythropoiesis were maintained in the subgroup of participants with no systemic inflammation. Bone marrow activation was significantly associated with high arterial metabolic activity (^{18}F-FDG uptake). The co-occurrence of BM activation and arterial ^{18}F-FDG uptake was associated with more advanced atherosclerosis (i.e. plaque presence and burden).

Conclusion In apparently healthy individuals, BM ^{18}F-FDG uptake is associated with MetS and its components, even in the absence of systemic inflammation, and with elevated counts of circulating leucocytes. Bone marrow activation is associated with early atherosclerosis, characterized by high arterial metabolic activity. Bone marrow activation appears to be an early phenomenon in atherosclerosis development.
[Progression of Early Subclinical Atherosclerosis (PESA); NCT01410318].

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Key question

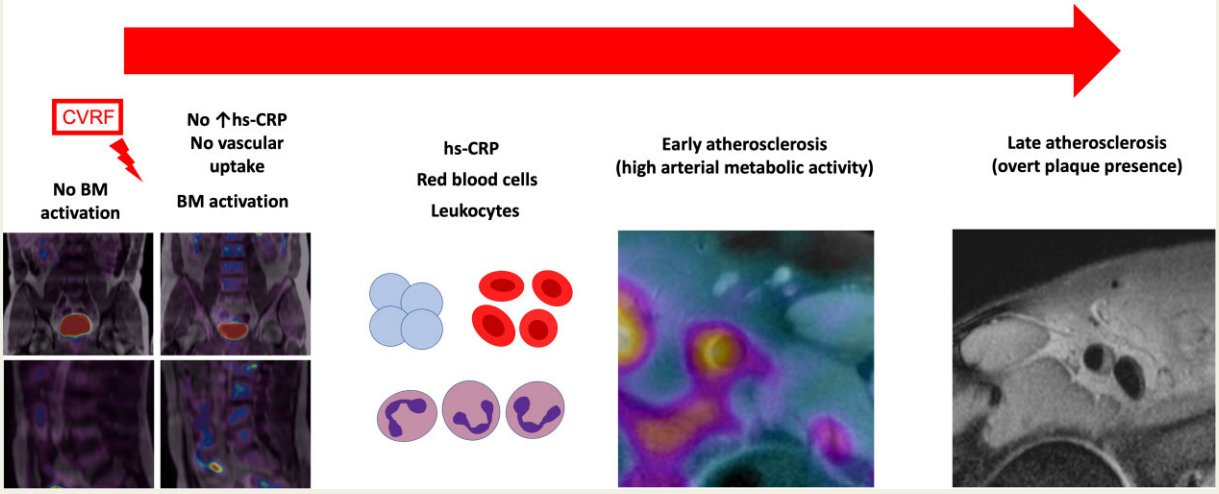
Experimental studies suggest that increased bone marrow activity is involved in the association between cardiovascular risk factors, vascular inflammation, and atherosclerosis. However, data in humans are sparse.

Key finding

Bone marrow ^{18}F-fluorodeoxyglucose uptake is associated with metabolic syndrome and its components, even in the absence of systemic inflammation. Bone marrow activation is associated with early atherosclerosis, characterized by high arterial metabolic activity.

Take-home message

Bone marrow activation appears to be an early phenomenon in atherosclerosis development.



Structured Graphical Abstract The hypothesis of the natural history of the inflammatory process involving the atherosclerotic plaque formation. Bone marrow (BM) is implicated in the atherosclerotic process long before the appearance of acute cardiovascular events. Cardiovascular risk factors trigger BM activation, initially in the absence of systemic inflammation. As BM activation progresses, it is accompanied by an increase in haematopoietic progenitor cells and an associated increase in inflammatory markers. The next step in the process is arterial inflammation, leading to an increase in atherosclerotic burden.

Keywords Subclinical atherosclerosis • Metabolic syndrome • Bone marrow • PET/MRI

Introduction

The association between inflammation and atherosclerosis is well established,[1] and mechanistic studies have demonstrated that inflammation is an essential mediator of all stages of atherosclerosis, from initiation to progression and the development of thrombotic complications.[2,3] Circulating immune cells play a critical role in the build-up of atherosclerotic plaques by adhering to activated endothelium and infiltrating the arterial wall to become lesional cells.[4] This association has led to the study of various anti-inflammatory therapies in the last years, with encouraging results that justify the use of some of them such as low-dose colchicine in selected, high-risk patients.[5]

The bone marrow (BM) is the primary site of haematopoiesis, and the proliferation and migration of haematopoietic progenitors are regulated by various physiological and pathological stimuli.[1,2] After an acute cardiovascular event, BM is activated by sympathetic signalling, triggering an increased haematopoiesis, and the release of progenitor cells that activate spleen production of monocytes, aggravating atherosclerosis progression.[6–9] Experimental studies suggest that increased BM haematopoietic activity may be a central link between cardiometabolic risk factors and exacerbated inflammation in atherosclerosis. In mice, hypercholesterolaemia and low HDL-cholesterol levels associated with elevated haematopoietic activity with increased monocytosis and neutrophilia.[10,11] Moreover, murine models of obesity present marked monocytosis and neutrophilia, associated with BM myeloid progenitor proliferation and expansion.[12,13] Diabetes mellitus has also been associated with increased circulating neutrophils and monocytes, reflecting the expansion of BM myeloid progenitors.[14,15] Hypertension, driven by an overactive sympathetic activation, deteriorates haematopoietic cell niche in the BM which can contribute to atherosclerosis.[16] In humans, it has been suggested that chronic stress accelerates haematopoiesis, giving rise to higher levels of inflammatory cells that might contribute to the atherosclerotic process.[17] In addition, haematopoietic stem cell division rates are increased in subjects with atherosclerosis,[18] and it has been suggested that the haematopoietic system might be chronically affected in these subjects.[19]

Despite the extensive pre-clinical data, human data to support the association between BM haematopoietic activation and cardiovascular risk factors are sparse.

In some tissues, the high metabolic activity can be detected by imaging techniques such as ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) imaging. This technique has been used to characterize BM activation after acute coronary syndrome,[20,21] based on a higher ^{18}F-FDG uptake related to an increased glucose consumption due to cellular proliferation, and vascular inflammation,[20–22] which correlates with macrophage density as measured by histology and immunohistochemistry.[23–25] Moreover, an association between amygdalar activity as evaluated by ^{18}F-FDG PET/CT and cardiovascular disease events has been suggested to be mediated by increased BM activation.[26] Hybrid ^{18}F-FDG PET/magnetic resonance imaging (PET/MRI) is an accurate method for the detection of early atherosclerosis (characterized by high arterial metabolic activity) and for later stages of the disease (plaque detection), in asymptomatic middle-aged individuals.[27] Here, we studied BM activation, detected by ^{18}F-FDG PET/MRI in apparently healthy middle-aged individuals, and its association with cardiovascular risk factors and subclinical atherosclerosis.

Materials and methods

Study population

The study population consisted of participants in the PESA study [Progression of Early Subclinical Atherosclerosis—CNIC–Santander; (PESA) NCT01410318][28] who underwent whole body ^{18}F-FDG PET/MRI.[27] PESA is an observational prospective cohort study of 4184 asymptomatic employees at Santander Bank in Madrid. Participants were aged 40–54 years at enrolment (June 2010–February 2014). Exclusion criteria were previous cardiovascular disease, any condition reducing life expectancy or affecting study adherence, morbid obesity [body mass index (BMI) $\geq$ 40 kg/m^2], or chronic kidney disease (estimated glomerular filtration rate $<$60 mL/min/1.73 m^2). The main goal of the PESA study is to characterize atherosclerosis initiation and progression by means of serial multi-territory, multimodality non-invasive imaging,[29] and paired biological sampling. A subgroup of PESA participants showing atherosclerosis on baseline vascular ultrasound, defined as being in the highest plaque tertile on vascular ultrasound and/or having any coronary artery calcification on CT, underwent baseline whole body ^{18}F-FDG PET/MRI study to characterize arterial metabolic activity.[27] Cardiovascular risk factors were assessed prospectively at enrolment using the 10-year risk algorithm based on Pooled Cohort Equations.[30] Risk bands of $<$5, 5 to $<$7.5, and $\geq$7.5% were defined as low, intermediate, and high risk, respectively.[31] A fasting blood test included blood count and biochemistry with the determination of systemic inflammation parameters.[28] Blood count included leucocytes and their components (including neutrophils, lymphocytes, monocytes, eosinophils, and basophils), red blood cell count, haemoglobin, haematocrit, red blood cell width, and platelets. Leucocytosis was defined as a white blood cell count $>10.5 \times 10^3$ cells/μL. Inflammation parameters included high-sensitivity C-reactive protein (hs-CRP), ferritin, erythrocyte sedimentation rate, fibrinogen, P-selectin, and vascular cell adhesion molecule-1 (VCAM-1). Insulin levels were also measured. Insulin resistance was measured by HOmeostatic Model Assessment for Insulin Resistance (HOMA-IR), calculated as [(fasting plasma glucose level $\times$ fasting insulin level)/405].[32] In this study, metabolic syndrome (MetS) was defined when a participant met at least three of the following conditions: central obesity (waist circumference $\geq$88 cm in women and $\geq$102 cm in men);[33] elevated plasma triglycerides ($\geq$150 mg/dL); low plasma HDL-cholesterol ($<$40 mg/dL in men or $<$50 mg/dL in women); elevated fasting plasma glucose ($\geq$100 mg/dL); and high blood pressure (systolic $\geq$130 mmHg and/or diastolic $\geq$85 mmHg).[34] Dyslipidaemia was defined as total cholesterol $\geq$240 mg/dL, LDL-cholesterol $\geq$160 mg/dL, HDL-cholesterol $<$40 mg/dL, or use of lipid-lowering drugs.

The study protocol was approved by the institutional review board, and all participants provided written informed consent.

Hybrid positron emission tomography/magnetic resonance imaging acquisition protocol and image analysis

The vascular PET/MRI protocol has been published previously.[27] In brief, the protocol included high-resolution black-blood MRI of the carotid, iliac, and femoral arteries and co-registered PET/MRI of the carotid arteries, thoracic aorta, infrarenal abdominal aorta, iliac, and femoral arteries. Ilio-femoral MRI attenuation maps and lower-body PET were acquired at the start of the protocol. Magnetic resonance imaging data were analysed with VP Diagnostics software version 2.1.0 (Seattle, WA, USA) and PET/MRI data were analysed with Philips Fusion Viewer version 2.0 (Philips Healthcare).

As previously described, attenuation correction used MRI attenuation maps with a three-class tissue (soft tissue, lung, and air) validated segmentation technique.[35] A transverse 575 mm field of view was used, and images were generated with a voxel size of 4.0 mm $\times$ 4.0 mm $\times$ 4.0 mm. Specific templates for PET images were added to the attenuation maps to correct for attenuation effects of the scanner bed. For abdominal region, the coil has a minimal attenuation and was not included in the attenuation maps. Similarly, not corrected PET data were used to extend the attenuation maps beyond the magnetic resonance field of view limits and this information was integrated in attenuation correction maps for the final reconstruction.[36]

A total of six vascular territories per participant were analysed in MRI: left/right carotid arteries, left/right iliac arteries, and left/right femoral arteries. The presence, number, and plaque volumes were defined for each territory, and recorded as a surrogate of total plaque burden for each individual.

Lumbar vertebrae L3 and L4 were analysed in fused PET/MRI images.[21] Quantitative ^{18}F-FDG uptake was measured in multiple slices in the coronal axis by drawing 3D regions of interest encompassing the contour of each vertebra, excluding the cortical bone. The maximal standardized uptake value (SUVmax) was calculated in these regions of interest (calculated as decay-corrected tissue radioactivity divided by body weight and injected dose).[37] For each participant, BM SUVmax was calculated as the mean SUVmax of the lumbar vertebrae (L3 and L4). Bone marrow activation was defined as BM SUVmax above the median value (1.9).

Statistical analysis

Normally distributed continuous variables are expressed as mean $\pm$ SD, whereas non-normally distributed variables are expressed as median (Q1–Q3). The distribution of continuous variables was assessed with graphical methods. Categorical variables are expressed as *n* (%). Differences between BM activation were assessed by Student's

t-test or Wilcoxon signed-rank test and χ^2 or Fisher exact test, for continuous and categorical variables, respectively, as appropriate. Linear trends across groups according to quintiles of ^{18}F-FDG uptake were evaluated with an extension of the non-parametric Wilcoxon rank-sum test. For multivariate analysis, ordinal logistic regression models were performed. To evaluate the associations of BM activation in the presence of confounders, Model 1 (adjusting for age and sex) and Model 2 (adjusting for age, sex, glucose levels before PET/MRI, smoking, haemoglobin, and hs-CRP) were created. To evaluate the association between BM activation in the presence of vascular uptake associations and plaque volume (mm^3) (0 and tertiles), several models were generated: Model 1 (qualitative), adjusting for age, sex, hypertension, dyslipidaemia, diabetes, smoking, family history of cardiovascular disease, and obesity; Model 2 (quantitative), adjusting for age, sex, systolic blood pressure, diastolic blood pressure, LDL-cholesterol, HDL-cholesterol, diabetes, smoking, dyslipidaemia treatment, family history of cardiovascular disease, and BMI; and Model 3, which is Model 1 but excluding dyslipidaemia treatment.

For all endpoints, differences were considered statistically significant at *P*-values < 0.05. Statistical analyses were performed using Stata software version 15 (StataCorp, College Station, TX, USA).

Results

A total of 946 PESA participants underwent whole body ^{18}F-FDG PET/MRI at baseline. The mean ^{18}F-FDG dose was 292.3 $\pm$ 11.1 MBq, and the radiation exposure was 5.6 $\pm$ 0.2 mSv. The mean start time after ^{18}F-FDG injection was 106 $\pm$ 15 min for lower-body PET and 132.9 $\pm$ 19.9 min for upper-body PET. Reasons for non-completion were physical intolerance in upper-body studies (8 PETs and 51 MRIs), technical issues with MRI attenuation maps (97 initial PETs), and poor image quality (70 iliac MRIs). Complete ^{18}F-FDG PET/MRI studies were available for 755 (79.8%) participants, and lumbar vertebrae BM images were of good quality for 745 participants (78.8% of the total sample who underwent PET/MRI); these participants constituted the population for the present study.

Baseline characteristics of subjects in relation to bone marrow activation

The median (Q1–Q3) age was 50.5 years (46.8–53.6) and 83.8% were men. Baseline characteristics are represented in *Table 1*. Participants with BM activation (*Figure 1*) were more frequently men (87.6 vs. 80% in those subjects without BM activation, $P = 0.005$) and more frequently had MetS (22.2 vs. 6.7%, $P < 0.001$). Bone marrow activation showed a significant association with central obesity (41.1 vs. 10.7%, $P < 0.001$), hypertension (23.5 vs. 14.9%, $P = 0.003$), higher plasma triglyceride levels (105.5 vs. 87 mg/dL, $P < 0.001$), lower HDL-cholesterol (44.5 vs. 48.4 mg/dL, $P < 0.001$), and higher fasting glucose (93 vs. 89 mg/dL, $P < 0.001$) (*Figure 2*). The BM activation and non-activation groups showed no differences in age, family history of cardiovascular disease, smoking, or total and LDL-cholesterol. Bone marrow activation group had higher levels of glycated haemoglobin (HbA1c, 5.5 vs. 5.4%, $P = 0.007$), and insulin resistance measured by HOMA-IR (1.7 vs. 1.1%, $P < 0.001$). Insulin levels were significantly increased in the group with BM activation (7 vs. 4.5 μU/mL, $P < 0.001$). Bone marrow activation was associated with significantly higher numbers of leucocytes (6.00×10^3 vs. 5.77×10^3 cells/μL in the group without BM activation, $P = 0.027$), especially neutrophils (3.4×10^3 vs. 3.2×10^3 cells/μL, $P = 0.029$) and red blood cell counts (4.9×10^6 vs. 4.8×10^6 cells/μL, $P < 0.001$). The BM activation group also showed significant elevation of the systemic inflammation markers, including hs-CRP (0.13 vs. 0.08 mg/dL, $P < 0.001$), ferritin (138.6 vs. 107.6 ng/dL, $P = 0.001$), fibrinogen (268.6 vs. 260.8 mg/dL, $P = 0.03$), P-selectin (139.7 vs. 129.2 ng/dL, $P = 0.004$), VCAM-1 (686.3 vs. 623.4 ng/mL, $P = 0.025$), and red blood cell distribution width (14.7 vs. 14.6%, $P = 0.055$). The main between-group differences in baseline characteristics are summarized in *Figure 2*.

To explore the association between the degree of BM activation and participant characteristics, we divided the population into BM-uptake quintiles according to SUVmax. The characteristics of each BM-uptake subgroup are presented in *Table 2*. The higher the degree of BM activation (i.e. the higher the BM-uptake quintile), the higher the percentage of men and the more frequent the presence of central obesity. The same pattern of increase across quintiles was observed for the frequency of MetS; hypertension; low HDL; and elevated fasting glucose, HbA1c, and HOMA-IR.

The increase in BM activation was also associated with elevated numbers of leucocytes (mainly neutrophils, with an increased neutrophil to lymphocyte ratio), and red blood cells, with numbers increasing progressively across quintiles. The distribution of relevant participant characteristics stratified by BM-uptake quintile is shown in *Figure 3*. The circulating systemic inflammation markers, hs-CRP, ferritin, fibrinogen, P-selectin, and VCAM-1 also steadily increased across BM-uptake quintiles (*Table 2*).

When adjusted for Model 1 (age and sex) and Model 2 (age, sex, glucose levels before PET/MRI, smoking, haemoglobin, and hs-CRP), BM activation remained significantly associated with MetS and its components, insulin levels, hs-CRP, leucocytes, and arterial uptake (*Figure 4*).

When BM uptake was evaluated as a continuous variable, MetS and its components (particularly central obesity) and the presence of arterial uptake had the largest effect size on BM activation (see Supplementary material online, *Table S1*); this was consistent with the analysis of BM-uptake quintiles.

Association between bone marrow activation and early atherosclerosis

Arterial ^{18}F-FDG uptake is a surrogate for high vascular metabolic activity due to macrophage accumulation, the precursor of atherosclerosis.[27,38] Bone marrow activation was significantly associated with the presence of vascular ^{18}F-FDG uptake (60 vs. 36%, $P < 0.001$). Participants with BM activation also had more sites of vascular ^{18}F-FDG uptakes, and higher degree of uptake (vascular SUVmax) (*Tables 1* and *2* and *Figure 5*).

We next explored the association between BM activation and vascular ^{18}F-FDG uptake. Participants with BM activation and vascular ^{18}F-FDG uptake tended to be older (51.2 vs. 49.4 years among those with BM activation and no vascular uptake, $P < 0.001$) and more frequently presented with MetS (26.9 vs. 15%, $P = 0.007$) (*Table 3*). The co-occurrence of BM activation and vascular ^{18}F-FDG uptake

Table 1 Study population characteristics stratified by bone marrow activation (above or below-median ^{18}F-fluorodeoxyglucose uptake)

	Total population ($n = 745$)	No bone marrow activation ($n = 375$)	Bone marrow activation[a] ($n = 370$)	*P*-value
Age, years	50.5 (46.8–53.6)	50.5 (47.0–53.8)	50.5 (46.7–53.5)	0.961
Men	624 (83.8)	300 (80.0)	324 (87.6)	0.005
Metabolic syndrome and components				
Metabolic syndrome	107 (14.4)	25 (6.7)	82 (22.2)	<0.001
Central obesity	192 (25.8)	40 (10.7)	152 (41.1)	<0.001
Triglycerides, mg/dL	98 (72–131)	87 (65–121)	105 (77–140)	<0.001
HDL-C, mg/dL	46.5 ± 11.4	48.4 ± 11.6	44.5 ± 10.7	<0.001
Fasting glucose, mg/dL	91 (85–97)	89 (84–94)	93 (87–99)	<0.001
SBP, mmHg	120.5 ± 12.3	118.7 ± 11.6	122.3 ± 12.6	<0.001
DBP, mmHg	75.3 ± 9.1	73.7 ± 8.3	76.9 ± 9.5	<0.001
Other cardiovascular risk factors				
Family history of CV disease, *n* (%)	154 (20.7)	73 (19.5)	81 (21.9)	0.414
Current smoking (%)	197 (26.9)	111 (30.1)	86 (23.8)	0.054
Hypertension	143 (19.2)	56 (14.9)	87 (23.5)	0.003
Dyslipidaemia	440 (59.1)	203 (54.1)	237 (64.1)	0.006
Diabetes	34 (4.6)	13 (3.5)	21 (5.7)	0.149
BMI, kg/m^2	27.2 ± 3.5	25.5 ± 2.8	28.8 ± 3.2	<0.001
Weight, kg	81.4 ± 13.3	75.5 ± 11.3	87.4 ± 12.4	<0.001
Waist circumference, cm	93.8 ± 10.8	89.0 ± 9.6	98.7 ± 9.7	<0.001
Treatment				
Antihypertensive therapy	100 (13.4)	40 (10.7)	60 (16.2)	0.026
Lipid-lowering therapy	113 (15.2)	50 (13.3)	63 (17.0)	0.160
Antidiabetic therapy	28 (3.8)	11 (2.9)	17 (4.6)	0.233
Biochemistry				
Total cholesterol, mg/dL	208.3 ± 33.5	207.6 ± 32.3	209.0 ± 34.7	0.592
LDL-C, mg/dL	139.9 ± 30.1	139.1 ± 28.8	140.6 ± 31.4	0.488
HbA1c, %	5.5 (5.2–5.7)	5.4 (5.2–5.7)	5.5 (5.3–5.7)	0.007
HOMA-IR, %	1.3 (0.9–2.1)	1.1 (0.7–1.7)	1.7 (1.0–2.5)	<0.001
Insulin, μU/mL	5.7 (3.9–8.2)	4.5 (3.4–6.8)	7.0 (5.0–10.3)	<0.001
Inflammatory markers				
hs-CRP, mg/dL	0.11 (0.06–0.19)	0.08 (0.05–0.16)	0.13 (0.07–0.23)	<0.001
Ferritin, ng/mL[b]	120.8 (63.0–204.0)	107.6 (56.4–194.1)	138.6 (74.8–214.1)	0.001
Erythrocyte sedimentation rate (1 h), mm	5 (4–8)	5 (4–7)	6 (4–8)	0.175
Fibrinogen, mg/dL	265.4 (236.5–295.1)	260.8 (234.5–294.6)	268.6 (239.6–299.2)	0.030
P-selectin, ng/mL	134.7 (106.7–166.2)	129.2 (104.5–160.6)	139.7 (109.9–177.0)	0.004
Vascular cell adhesion molecule-1, ng/mL	651.1 (519.6–820.0)	623.4 (509.8–791.9)	686.3 (540.6–849.0)	0.025
Blood count				
Leucocytes, 10^3 cells/μL	5.87 (4.99–7.05)	5.77 (4.94–6.95)	6.00 (5.15–7.13)	0.027

Continued

Table 1 *Continued*

	Total population ($n = 745$)	No bone marrow activation ($n = 375$)	Bone marrow activation[a] ($n = 370$)	*P*-value
Leucocytosis (>10.5 × 10^3 cells/μL)	17 (2.3)	8 (2.1)	9 (2.4)	0.785
Red blood cell count, 10^6 cells/μL	4.84 (4.59–5.08)	4.78 (4.51–5.03)	4.89 (4.64–5.12)	<0.001
Red cell distribution width, %	14.6 (14.0–15.2)	14.6 (14.0–15.1)	14.7 (14.0–15.2)	0.055
Haemoglobin, g/dL	15.0 (14.3–15.7)	14.9 (14.1–15.6)	15.1 (14.4–15.8)	<0.001
Haematocrit, %	44.2 (41.9–46.3)	43.9 (41.6–46.0)	44.5 (42.5–46.5)	0.002
Platelet count, 10^3 cells/μL	225 (198–257)	226 (199–256)	224 (194–258)	0.686
Segmented neutrophils, 10^3 cells/μL	3.31 (2.73–4.23)	3.21 (2.66–4.11)	3.40 (2.78–4.36)	0.029
Lymphocytes, 10^3 cells/μL	1.86 (1.56–2.22)	1.84 (1.55–2.20)	1.88 (1.57–2.22)	0.465
Monocytes, 10^3 cells/μL	0.41 (0.34–0.52)	0.41 (0.33–0.51)	0.42 (0.34–0.52)	0.201
Eosinophils, 10^3 cells/μL	0.13 (0.08–0.20)	0.12 (0.08–0.20)	0.13 (0.08–0.21)	0.446
Basophils, 10^3 cells/μL	0.05 (0.03–0.07)	0.05 (0.03–0.07)	0.05 (0.03–0.06)	0.689
Neutrophil to lymphocyte ratio	1.79 (1.43–2.30)	1.75 (1.41–2.24)	1.83 (1.46–2.36)	0.178
SCORE risk score				
Low (<1%)	459 (64.2)	245 (67.1)	214 (61.1)	0.095
Intermediate (1–5%)	254 (35.5)	120 (32.9)	134 (38.3)	0.131
High (>5%)	2 (0.3)	0 (0.0)	2 (0.6)	0.148
ASCVD risk score				
Low	369 (61.7)	207 (66.1)	162 (56.8)	0.020
Intermediate	109 (18.2)	50 (16.0)	59 (20.7)	0.135
High	120 (20.1)	56 (17.9)	64 (22.5)	0.164
Arterial uptake (^{18}F-FDG)				
Presence of uptake	358 (48.1)	135 (36.0)	223 (60.3)	<0.001
Number of uptakes	0 (0–2)	0 (0–1)	1 (0–2)	<0.001
SUVmax arterial uptake	1.38 (1.26–1.52)	1.30 (1.20–1.41)	1.46 (1.37–1.59)	<0.001
Plaques by magnetic resonance				
Plaque presence	671 (90.1)	337 (89.9)	334 (90.3)	0.854
Number of plaques	3 (2–5)	3 (1–5)	3 (2–5)	0.842
Global plaque burden	365.6 (175.6–706.9)	348.5 (164.9–681.0)	375.8 (192.4–751.2)	0.313

Data are presented as *n* (%) or median (Q1–Q3).
[a]Bone marrow activation was defined when the mean BM SUVmax was above the median value (SUVmax 1.9).
[b]Measured in 622 of 745 individuals.

showed a significant association with central obesity (48.9 vs. 29.3% in participants with BM activation without vascular ^{18}F-FDG uptake, $P < 0.001$), smoking (28.6 vs. 16.2%, $P = 0.007$), higher total cholesterol (211.9 vs. 204.5 mg/dL, $P = 0.045$), lower HDL-cholesterol (43.1 vs. 46.7 mg/dL, $P = 0.001$), higher triglyceride (112 vs. 92 mg/dL, $P < 0.001$), and higher fasting glucose levels (94 vs. 92 mg/dL, $P = 0.013$), as well as with lower rates of individuals assigned to a low-risk group in the SCORE algorithm. There was no between-group difference in age, family history of cardiovascular disease, and LDL-cholesterol. The co-occurrence of BM activation and vascular ^{18}F-FDG uptake was associated with elevated levels of HbA1c (5.5 vs. 5.4%, $P = 0.042$), plasma insulin (7.6 vs. 5.8 μU/mL, $P < 0.001$) and insulin resistance measured by HOMA-IR (1.9 vs. 1.4%, $P < 0.001$). Co-occurring BM activation and vascular ^{18}F-FDG uptake were associated with significantly elevated numbers of leucocytes (6.22 × 10^3 vs. 5.83 × 10^3 cells/μL in the BM activation group without vascular ^{18}F-FDG uptake, $P = 0.023$), especially monocytes (0.43 × 10^3 vs. 0.40 × 10^3 cells/μL, $P = 0.007$) and of red blood cells (4.91 × 10^6 vs. 4.86 × 10^6 cells/μL, $P = 0.038$). This trend was accompanied by significantly elevated markers of systemic inflammation in the BM activation plus vascular ^{18}F-FDG uptake group, including hs-CRP (0.15 vs. 0.11 mg/dL, $P < 0.001$).

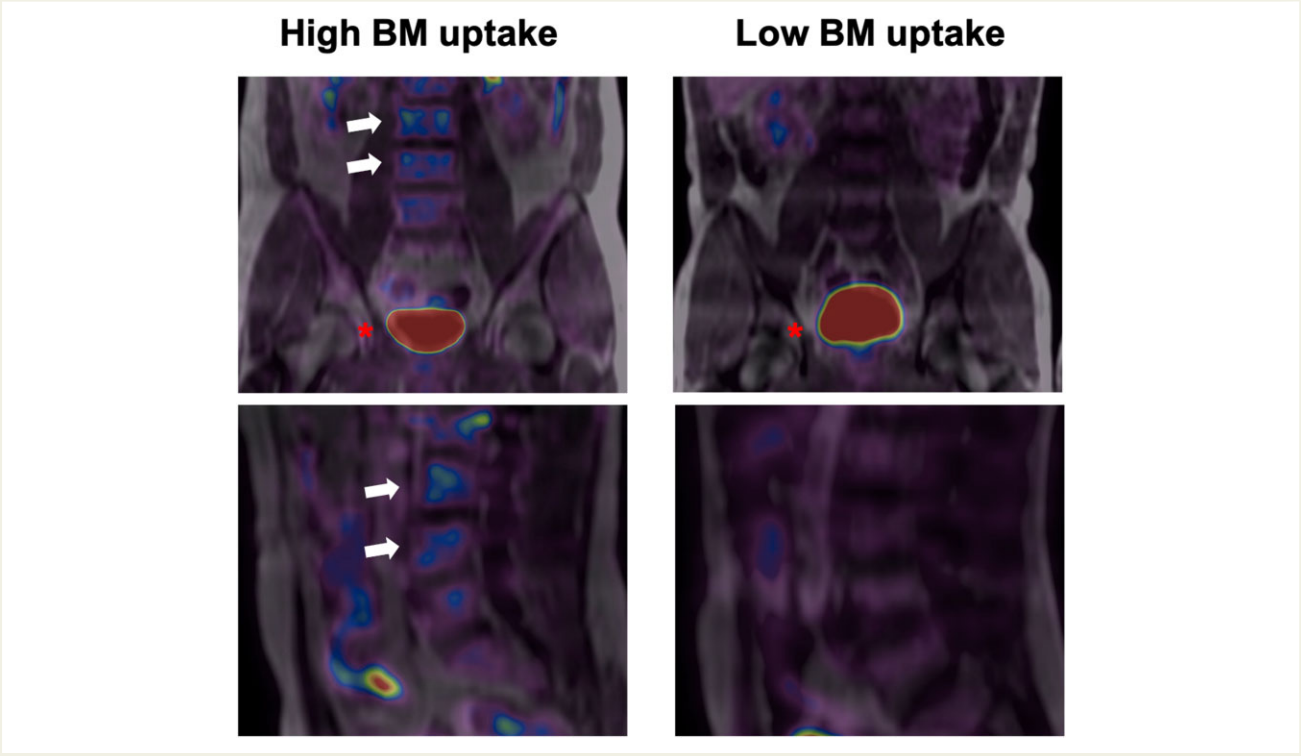


Figure 1 Bone marrow uptake. Representative baseline ^{18}F-fluorodeoxyglucose positron emission tomography/magnetic resonance imaging scans from participants. The left panel shows fused ^{18}F-fluorodeoxyglucose positron emission tomography/magnetic resonance imaging in coronal (upper) and sagittal (lower) views. L3 and L4 vertebrae (white arrows) present high ^{18}F-fluorodeoxyglucose uptake (visualized in blue). The right panel shows the same coronal and sagittal views; ^{18}F-fluorodeoxyglucose uptake is not visualized. The bladder is visualized in red in both upper panels (red asterisk).

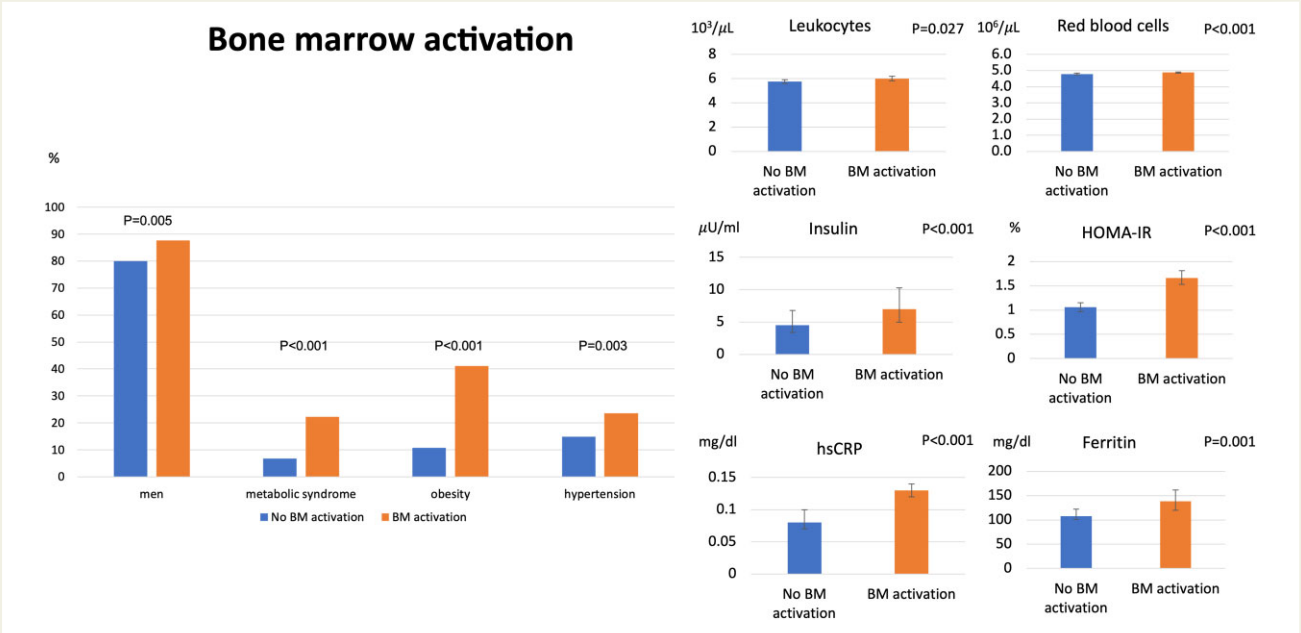


Figure 2 Population with bone marrow activation. Characteristics of participants with baseline bone marrow ^{18}F-fluorodeoxyglucose uptake compared with the group without bone marrow uptake. Participants with bone marrow ^{18}F-fluorodeoxyglucose uptake were more frequently male and had a higher prevalence of metabolic syndrome and its components, central obesity, hypertension, and altered glucose metabolism. The bone marrow uptake group also had higher levels of systemic inflammatory markers (high-sensitivity C-reactive protein and ferritin) and showed higher counts of leucocytes and red blood cells.

Table 2 Study population characteristics stratified by bone marrow activation (^{18}F-fluorodeoxyglucose uptake) quintile

	BM-uptake Q1 ($n = 149$)	BM-uptake Q2 ($n = 149$)	BM-uptake Q3 ($n = 149$)	BM-uptake Q4 ($n = 149$)	BM-uptake Q5 ($n = 149$)	*P* for trend
Mean BM SUVmax	0.57–1.6	1.6–1.81	1.81–2.0	2.0–2.3	2.3–3.8	
Age, years	50.0 (46.7–53.0)	51.7 (47.5–54.3)	49.9 (45.6–52.8)	50.5 (47.0–53.8)	50.9 (47.1–53.8)	0.431
Men	105 (70.5)	128 (85.9)	128 (85.9)	134 (89.9)	129 (86.6)	<0.001
Metabolic syndrome and components						
Metabolic syndrome	7 (4.7)	12 (8.1)	20 (13.4)	18 (12.1)	50 (33.6)	<0.001
Central obesity	11 (7.4)	16 (10.7)	27 (18.1)	40 (26.8)	98 (65.8)	<0.001
Triglycerides, mg/dL	81 (61–113)	96 (72–128)	101 (68–128)	103 (73–133)	107 (88–147)	<0.001
HDL-C, mg/dL	50.8 ± 12.5	46.3 ± 11.3	47.0 ± 10.6	45.5 ± 10.7	42.8 ± 10.2	<0.001
Fasting glucose, mg/dL	87 (83–92)	90 (83–95)	91 (84–98)	93 (88–97)	94 (87–101)	<0.001
SBP, mmHg	117.6 ± 12.4	119.8 ± 11.6	120.5 ± 11.3	121.2 ± 11.8	123.5 ± 13.5	<0.001
DBP, mmHg	73.1 ± 9.1	74.3 ± 8.1	75.1 ± 7.9	75.4 ± 9.2	78.6 ± 10.1	<0.001
Other cardiovascular risk factors						
Family history of CV disease	28 (18.8)	26 (17.4)	30 (20.1)	38 (25.5)	32 (21.5)	0.201
Current smoking	47 (32.2)	45 (30.6)	31 (21.2)	32 (22.1)	42 (28.6)	0.176
Hypertension	21 (14.1)	25 (16.8)	24 (16.1)	30 (20.1)	43 (28.9)	0.001
Dyslipidaemia	67 (45.0)	91 (61.1)	88 (59.1)	98 (65.8)	96 (64.4)	0.001
Diabetes	4 (2.7)	2 (1.3)	11 (7.4)	6 (4.0)	11 (7.4)	0.026
BMI, kg/m^2	24.7 ± 3.0	25.9 ± 2.5	26.7 ± 2.7	28.1 ± 2.7	30.5 ± 3.2	<0.001
Weight, kg	71.9 ± 12.1	77.3 ± 10.1	79.6 ± 10.6	85.2 ± 10.9	93.1 ± 12.0	<0.001
Waist circumference, cm	85.7 ± 10.0	90.7 ± 8.9	92.9 ± 8.5	96.7 ± 8.5	103.0 ± 9.5	<0.001
Treatment						
Antihypertensive therapy	16 (10.7)	15 (10.1)	18 (12.1)	21 (14.1)	30 (20.1)	0.010
Lipid-lowering therapy	14 (9.4)	20 (13.4)	25 (16.8)	32 (21.5)	22 (14.8)	0.043
Antidiabetic therapy	3 (2.0)	1 (0.7)	11 (7.4)	5 (3.4)	8 (5.4)	0.057

Continued

Table 2 *Continued*

	BM-uptake Q1 ($n = 149$)	BM-uptake Q2 ($n = 149$)	BM-uptake Q3 ($n = 149$)	BM-uptake Q4 ($n = 149$)	BM-uptake Q5 ($n = 149$)	*P* for trend
Biochemistry						
Total cholesterol, mg/dL	205.8 ± 30.3	209.3 ± 34.2	210.0 ± 31.2	210.8 ± 36.4	205.6 ± 35.1	0.837
LDL-C, mg/dL	136.4 ± 28.3	141.2 ± 29.9	141.9 ± 27.0	142.3 ± 32.9	137.5 ± 31.8	0.583
HbA1c, %	5.4 (5.2–5.6)	5.4 (5.1–5.6)	5.5 (5.2–5.7)	5.5 (5.2–5.8)	5.5 (5.3–5.7)	0.005
HOMA-IR, %	0.9 (0.6–1.3)	1.2 (0.8–1.9)	1.2 (0.8–1.9)	1.5 (1.0–2.1)	2.0 (1.3–2.9)	<0.001
Insulin, μU/mL	4.0 (2.8–5.8)	5.1 (3.8–7.5)	5.3 (3.8–7.5)	6.3 (4.3–8.6)	8.2 (6.0–11.5)	<0.001
Inflammatory markers						
hs-CRP, mg/dL	0.07 (0.04–0.13)	0.10 (0.05–0.18)	0.10 (0.05–0.20)	0.12 (0.07–0.21)	0.15 (0.08–0.28)	<0.001
Ferritin, ng/mL[a]	90.2 (40.9–152.4)	127.7 (71.3–198.5)	122.4 (69.0–224.6)	137.5 (74.5–200.2)	154.9 (81.6–253.0)	<0.001
Erythrocyte sedimentation rate (1 h), mm	5 (4–8)	5 (4–7)	5 (4–7)	6 (4–8)	6 (4–8)	0.277
Fibrinogen, mg/dL	255.4 (230.1–286.6)	266.2 (236.5–296.1)	265.7 (235.3–294.6)	264.4 (239.9–299.2)	277.2 (249.8–302.8)	0.001
P-selectin, ng/mL	125.0 (102.8–156.1)	130.5 (105.9–166.2)	135.6 (106.9–166.7)	135.3 (106.7–167.2)	144.1 (115.4–180.5)	0.002
VCAM-1, ng/mL	615.3 (503.8–777.6)	625.1 (499.1–790.5)	681.2 (530.4–859.5)	674.0 (518.6–849.0)	680.8 (555.1–821.7)	0.027
Blood count						
Leucocytes, 10^3 cells/μL	5.59 (4.91–6.72)	5.93 (4.95–7.07)	5.80 (4.91–6.75)	5.98 (5.17–7.13)	6.20 (5.35–7.48)	0.001
Leucocytosis (>10.5×10^3 cells/μL)	0 (0)	5 (3.4)	4 (2.7)	4 (2.7)	4 (2.7)	0.225
Red blood cell count, 10^6 cells/μL	4.71 (4.38–4.99)	4.83 (4.60–5.05)	4.85 (4.59–5.05)	4.90 (4.67–5.10)	4.90 (4.65–5.15)	<0.001
Red cell distribution width, %	14.6 (14.0–15.2)	14.6 (14.0–15.2)	14.5 (13.9–15.1)	14.7 (13.9–15.2)	14.8 (14.2–15.2)	0.089
Haemoglobin, g/dL	14.7 (13.8–15.3)	15.1 (14.4–15.7)	15.0 (14.2–15.6)	15.1 (14.6–15.8)	15.2 (14.5–15.8)	<0.001
Haematocrit, %	43.0 (40.9–45.6)	44.5 (41.9–46.4)	44.2 (41.7–46.0)	44.6 (42.9–46.6)	44.6 (42.8–46.7)	<0.001
Platelets, 10^3 cells/μL	228 (199–253)	224 (196–256)	222 (196–259)	227 (200–257)	222 (193–262)	0.831
Segmented neutrophils, 10^3 cell/μL	3.13 (2.60–3.77)	3.34 (2.72–4.26)	3.19 (2.71–3.92)	3.48 (2.61–4.42)	3.48 (2.97–4.50)	0.001
Lymphocytes, 10^3 cells/μL	1.83 (1.62–2.16)	1.86 (1.55–2.17)	1.86 (1.54–2.23)	1.82 (1.55–2.22)	1.91 (1.64–2.28)	0.136
Monocytes, 10^3 cells/μL	0.39 (0.32–0.49)	0.41 (0.33–0.53)	0.41 (0.33–0.50)	0.42 (0.35–0.51)	0.43 (0.35–0.53)	0.019
Eosinophils, 10^3 cells/μL	0.12 (0.08–0.20)	0.12 (0.08–0.20)	0.12 (0.07–0.21)	0.13 (0.08–0.19)	0.14 (0.09–0.23)	0.361
Basophils, 10^3 cells/μL	0.05 (0.03–0.07)	0.05 (0.03–0.06)	0.04 (0.03–0.06)	0.05 (0.03–0.06)	0.05 (0.03–0.07)	0.128
Neutrophil to lymphocyte ratio	1.66 (1.37–2.15)	1.81 (1.46–2.25)	1.74 (1.37–2.30)	1.82 (1.42–2.45)	1.88 (1.51–2.27)	0.048

Continued

Table 2 *Continued*

	BM-uptake Q1 (*n* = 149)	BM-uptake Q2 (*n* = 149)	BM-uptake Q3 (*n* = 149)	BM-uptake Q4 (*n* = 149)	BM-uptake Q5 (*n* = 149)	*P* for trend
SCORE risk score						
Low (<1%)	106 (72.1)	88 (59.5)	102 (73.9)	82 (57.8)	81 (57.9)	0.003
Intermediate (1–5%)	41 (27.9)	60 (40.5)	35 (25.4)	59 (41.5)	59 (42.1)	0.002
High (>5%)	0 (0.0)	0 (0.0)	1 (0.7)	1 (0.7)	0 (0)	0.538
ASCVD risk score						
Low	96 (73.8)	69 (56.1)	84 (70.0)	57 (54.3)	63 (52.5)	0.001
Intermediate	17 (13.1)	24 (19.5)	18 (15.0)	22 (20.9)	28 (23.3)	0.047
High	17 (13.1)	30 (24.4)	18 (15.0)	26 (24.8)	29 (24.2)	0.040
Arterial uptake (^{18}F-FDG)						
Presence of uptake	37 (24.8)	59 (39.6)	73 (49.0)	82 (55.0)	107 (71.8)	<0.001
Number of uptakes	0 (0–0)	0 (0–1)	0 (0–1)	1 (0–2)	1 (0–2)	<0.001
SUVmax arterial uptake	1.26 (1.14–1.32)	1.33 (1.24–1.43)	1.37 (1.27–1.47)	1.41 (1.32–1.51)	1.58 (1.46–1.69)	<0.001
Plaques by magnetic resonance						
Plaque presence	127 (85.2)	136 (91.3)	138 (92.6)	134 (89.9)	136 (91.3)	0.166
Number of plaques	3 (1–5)	3 (2–5)	3 (2–5)	3 (2–5)	3 (2–5)	0.855
Global plaque burden, mm^3	348.5 (164.9–669.9)	362.3 (166.8–699.5)	370.2 (177.8–688.8)	372.5 (175.6–753.5)	371.4 (204.8–753.3)	0.365

Data are presented as *n* (%) or median (Q1–Q3).
[a]Measured in 622 of 745 individuals.

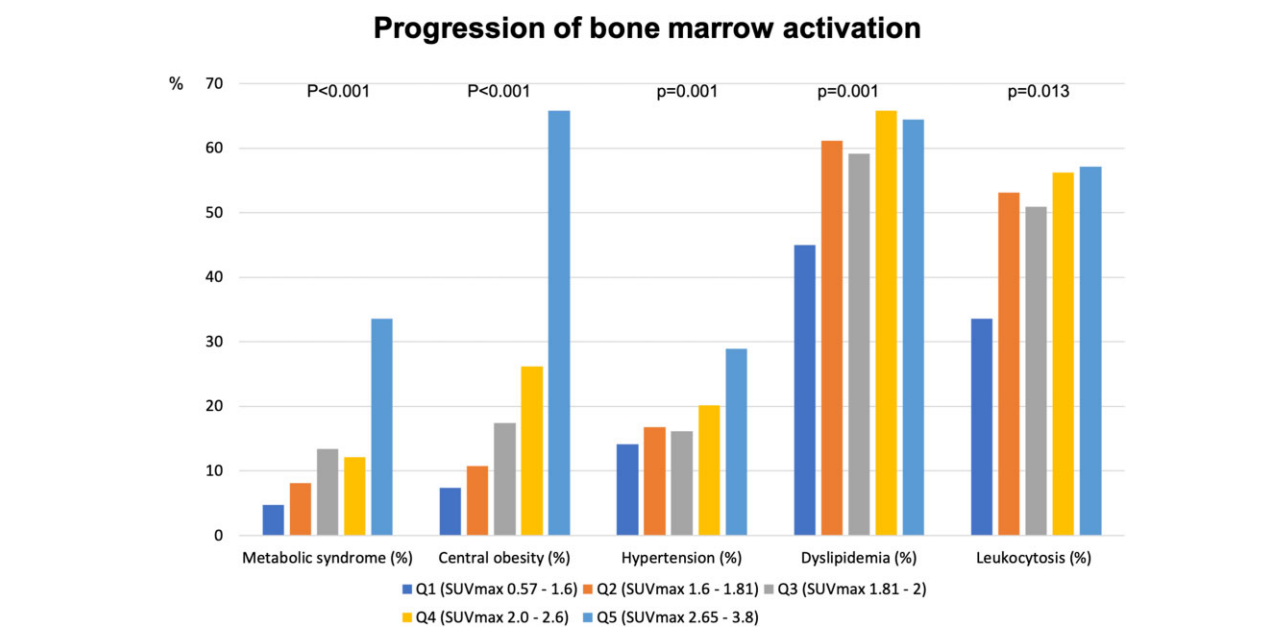


Figure 3 Progression of bone marrow activation. Prevalence of metabolic syndrome, central obesity, hypertension, dyslipidaemia, and leucocytosis stratified by quintiles of bone marrow ^{18}F-fluorodeoxyglucose uptake.

More advanced stages of atherosclerosis are characterized by full-grown plaques. We observed that subjects with BM activation plus vascular ^{18}F-FDG uptake had a higher prevalence of plaques than those with BM activation but without vascular ^{18}F-FDG uptake (95.1 vs. 83%, $P < 0.001$). Similarly, the BM activation plus vascular ^{18}F-FDG uptake group had more plaques (4 vs. 2, $P < 0.001$) and a higher plaque burden (448.4 vs. 284.5 mm^3, $P < 0.001$). These associations remained significant after adjusting for classical risk factors (odds ratio 2.33, 95% confidence interval 1.54–3.52, $P < 0.001$ in the fully adjusted model) (*Figures 6* and *7*). This association was consistent when atherosclerosis was evaluated by 2D and 3D vascular ultrasound; subjects with BM activation plus ^{18}F-FDG uptake had a higher prevalence and number of plaques and higher plaque burden when compared with those with BM activation but without vascular uptake.

Bone marrow activation in the absence of systemic inflammation

To assess whether BM activation occurred as part of a systemic inflammatory reaction, we studied the subgroup of 402 participants showing no systemic inflammation (below-median hs-CRP). The characteristics of this subpopulation stratified by BM activation are summarized in *Table 4*. Participants with BM activation were more frequently male (86.4 vs. 75.8%, $P = 0.009$), and were more frequently positive for MetS (17.9 vs. 3.8%, $P < 0.001$) and its components, hypertension (25.3 vs. 12.9%, $P = 0.001$), and diabetes (7.4 vs. 2.5%, $P = 0.020$). This group also showed higher elevations in fasting glucose (92 vs. 88 mg/dL, $P < 0.001$), and insulin resistance index (HOMA-IR 1.54 vs. 0.97%, $P < 0.001$) and plasma insulin (6.1 vs. 4.3 μU/mL, $P < 0.001$); and presented lower levels of HDL-cholesterol (46.1 vs. 49.7 mg/dL, $P = 0.002$) and higher levels of triglycerides (97 vs. 83 mg/dL, $P = 0.010$). The BM activation group showed a higher elevation of erythropoiesis (red blood cell count 4.90×10^6 vs. 4.76×10^6 cells/μL, $P = 0.001$); however, leucocyte numbers did not differ between inflammation-free participants with and without BM activation. In this subgroup without systemic inflammation, BM activation was significantly associated with higher arterial metabolic activity: more prevalence of vascular ^{18}F-FDG uptake (51.9 vs. 31.2%, $P < 0.001$), and more sites of vascular ^{18}F-FDG uptake and higher vascular SUVmax.

Discussion

The present study analysed BM activation in a population of apparently healthy individuals from the PESA study who underwent whole body ^{18}F-FDG PET/MRI. The main study findings are as follows: (i) BM activation is associated with MetS and its individual components, as well as with elevated numbers of leucocytes, and systemic inflammation; (ii) BM activation is associated with high arterial metabolic activity (high arterial ^{18}F-FDG uptake), a surrogate for macrophage infiltration (i.e. the precursor of atherosclerosis); (iii) the association between BM activation and MetS, and between BM activation and arterial ^{18}F-FDG uptake is maintained even in the absence of systemic inflammation; and (iv) co-occurring BM activation and vascular ^{18}F-FDG uptake was significantly associated with more advanced stages of atherosclerosis (higher plaque prevalence, number of plaques, and plaque burden). To the best of our knowledge, this is the first demonstration of an association between BM activation and metabolic factors linked to atherosclerosis in humans.

The relationship between BM activation and acute cardiovascular events has been described extensively, both in experimental models and in clinical studies. In mice, acute myocardial infarction is followed

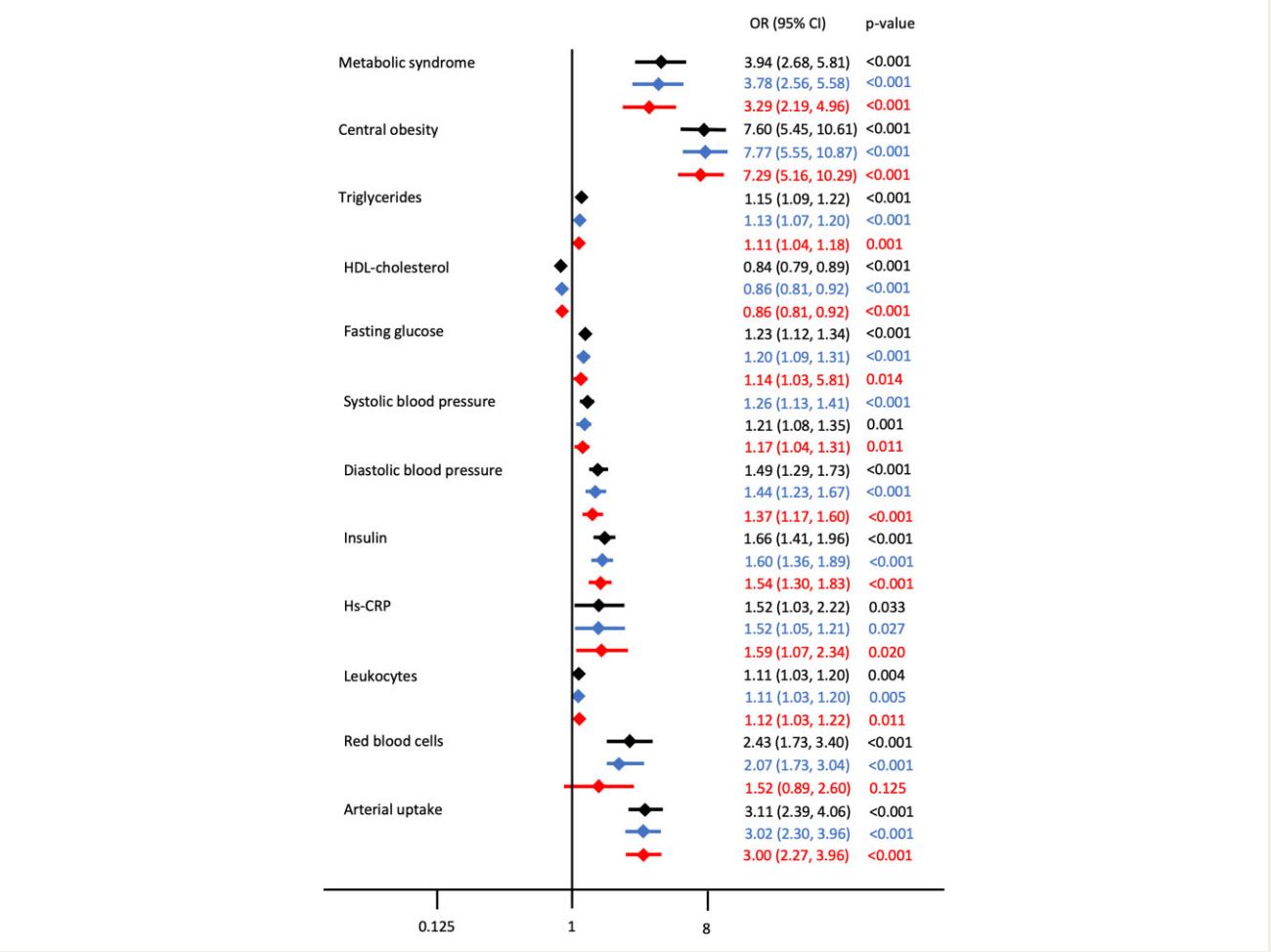


Figure 4 Unadjusted and adjusted associations for different factors with bone marrow activation. Associations between different factors with bone marrow activation, expressed as odds ratio with its 95% confidence interval. The unadjusted estimates are presented in black, the estimates adjusted for Model 1 (adjusted for age and sex) are presented in blue, and the estimates adjusted for Model 2 (adjusted for age, sex, glucose levels before positron emission tomography/magnetic resonance imaging, smoking, haemoglobin, and high-sensitivity C-reactive protein) are presented in red.

by activation of the sympathetic nervous system that increases BM activation signals. These stimuli trigger the release of progenitor cells from the BM and activate monocyte production in the spleen,[6,7] which together with local plaque macrophage proliferation produce a rapid turnover that facilitates atherosclerosis progression.[39]

Experimental studies have demonstrated that increased haematopoietic activity in BM plays a central role in the association between cardiovascular risk factors, vascular inflammation, and atherosclerosis formation. Studies in mice have demonstrated that low HDL-cholesterol levels and hypercholesterolaemia are associated with an increase in BM myelopoietic activity which leads to increased neutrophilia and monocytosis.[10,11] Elevated HDL-cholesterol levels have an anti-atherogenic role based on the suppression of BM myeloid proliferation. In conditions such as obesity, inflamed adipose tissue increases BM haematopoietic cells proliferation, leading to an exacerbated inflammation and associated disease processes.[12,13] Hyperglycaemia and diabetes have been associated with an increased production of inflammatory myeloid cells in the BM, which exacerbate diabetes mellitus-associated complications including atherosclerosis.[14,15] Sympathetic activation present in hypertension has been demonstrated to modulate BM haematopoiesis with the increase of myeloid cells and contributing to atherosclerosis and cardiovascular disease.

However, evidence of the association between BM metabolic activity and cardiovascular risk factors in humans is lacking.[16]

In this PESA subcohort of middle-aged healthy participants, BM activation indexed as lumbar vertebrae ^{18}F-FDG uptake was associated with the presence of MetS and its components, with higher frequencies detected for central obesity, hypertension, low HDL-C, triglycerides, and altered glucose metabolism in these participants. Bone marrow activation was also associated with increased haematopoiesis and systemic inflammation, assessed from circulating hs-CRP, ferritin, fibrinogen, P-selectin, and VCAM-1 levels. Notably, the association between BM activation and MetS was maintained even in the absence of systemic inflammation (*Table 4*). The activation of BM in the presence of MetS even in the absence of systemic inflammation

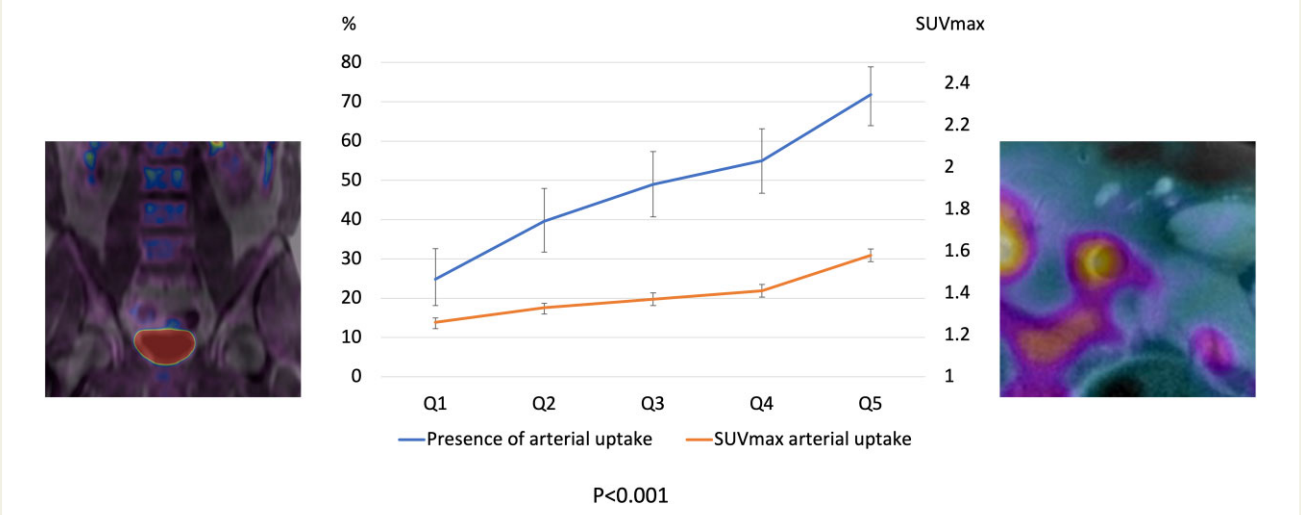


Figure 5 Relationship between bone marrow and vascular ^{18}F-fluorodeoxyglucose uptake. The left and right panels show representative positron emission tomography/magnetic resonance imaging analysis of ^{18}F-fluorodeoxyglucose uptake in lumbar vertebrae and vascular tissue, respectively. The chart shows increases in the presence of vascular uptake (left *Y*-axis) and in vascular-uptake SUVmax (right *Y*-axis) with increasing bone marrow uptake quintile.

Table 3 Characteristics of population with bone marrow activation according to the presence or absence of vascular ^{18}F-fluorodeoxyglucose uptake

	BM activation without vascular uptake ($n = 147$)	BM activation and vascular uptake ($n = 223$)	*P*-value
Age, years	49.4 (45.4–52.9)	51.2 (48.0–54.1)	<0.001
Men	123 (83.7)	201 (90.1)	0.065
Metabolic syndrome and its components			
Metabolic syndrome	22 (15.0)	60 (26.9)	0.007
Central obesity	43 (29.3)	108 (48.9)	<0.001
Triglycerides, mg/dL	92 (68–128)	112 (86–147)	<0.001
HDL-C, mg/dL	46.7 ± 11.7	43.1 ± 9.8	0.001
Fasting glucose, mg/dL	92 (85–98)	94 (88–101)	0.013
SBP, mmHg	120.4 ± 11.4	123.6 ± 13.2	0.018
DBP, mmHg	75.6 ± 8.7	77.8 ± 9.9	0.029
Other cardiovascular risk factors			
Family history of CV disease	29 (19.7)	52 (23.3)	0.414
Current smoking	23 (16.2)	63 (28.6)	0.007
Hypertension	31 (21.1)	56 (25.1)	0.372
Dyslipidaemia	85 (57.8)	152 (68.2)	0.043
Diabetes	6 (4.1)	15 (6.7)	0.282
BMI, kg/m^2	27.9 ± 3.3	29.5 ± 3.0	<0.001
Weight, kg	84.1 ± 12.3	89.5 ± 12.1	<0.001
Waist circumference, cm	95.4 ± 9.3	100.8 ± 9.3	<0.001

Continued

Table 3 *Continued*

	BM activation without vascular uptake ($n = 147$)	BM activation and vascular uptake ($n = 223$)	*P*-value
Treatment			
Antihypertensive therapy	22 (15.0)	38 (17.0)	0.596
Lipid-lowering therapy	25 (17.0)	38 (17.0)	0.993
Antidiabetic therapy	6 (4.1)	11 (4.9)	0.702
Biochemistry			
Total cholesterol, mg/dL	204.5 ± 31.3	211.9 ± 36.5	0.045
LDL-C, mg/dL	137.1 ± 27.1	143.0 ± 33.7	0.077
HOMA-IR, %	1.4 (1.0–2.1)	1.9 (1.2–2.9)	<0.001
HbA1c, %	5.4 (5.2–5.7)	5.5 (5.3–5.8)	0.042
Insulin, μU/mL	5.8 (4.3–8.0)	7.6 (5.4–11.5)	<0.001
Inflammatory markers			
hs-CRP, mg/dL	0.11 (0.05–0.18)	0.15 (0.08–0.29)	<0.001
Ferritin, ng/mL[a]	131.38 (61.13–207.27)	151.23 (81.63–224.11)	0.390
Erythrocyte sedimentation rate (1 h), mm	5 (4–7)	6 (4–8)	0.084
Fibrinogen, mg/dL	264.4 (237.3–291.5)	273.1 (240.9–302.8)	0.178
P-selectin, ng/mL	139.9 (111.0–177.0)	139.3 (107.7–177.2)	0.981
Vascular cell adhesion molecule-1, ng/mL	645.2 (512.4–861.2)	712.4 (557.1–838.8)	0.379
Blood count			
Leucocytes, 10^3 cells/μL	5.83 (4.99–6.89)	6.22 (5.23–7.33)	0.023
Leucocytosis (>10.5 × 10^3 cells/μL)	2 (1.4)	7 (3.1)	0.277
Red blood cell count, 10^6 cells/μL	4.86 (4.59–5.05)	4.91 (4.68–5.18)	0.038
Red cell distribution width, %	14.6 (14.0–15.2)	14.7 (14.0–15.3)	0.130
Haemoglobin, g/dL	15.0 (14.4–15.6)	15.1 (14.5–15.9)	0.107
Haematocrit, %	44.3 (41.6–46.1)	44.7 (42.9–46.7)	0.047
Platelet count, 10^3 cells/μL	228 (194–259)	221 (193–258)	0.452
Segmented neutrophils,10^3 cell/μL	3.30 (2.69–4.17)	3.45 (2.86–4.50)	0.072
Lymphocytes,10^3 cells/μL	1.83 (1.55–2.13)	1.92 (1.58–2.29)	0.097
Monocytes, (10^3 cells/μL	0.40 (0.32–0.49)	0.43 (0.36–0.54)	0.007
Eosinophils,10^3 cells/μL	0.12 (0.08–0.19)	0.14 (0.08–0.23)	0.326
Basophils,10^3 cells/μL	0.04 (0.03–0.06)	0.05 (0.03–0.07)	0.085
Neutrophil to lymphocyte ratio	1.79 (1.42–2.36)	1.85 (1.48–2.38)	0.498
SCORE risk score			
Low (<1%)	100 (71.9)	114 (54.0)	0.001
Intermediate (1−5%)	39 (28.1)	95 (45.0)	0.001
High (>5%)	0 (0.0)	2 (1.0)	0.250
ASCVD risk score			
Low	83 (72.8)	79 (46.2)	<0.001
Intermediate	15 (13.2)	44 (25.7)	0.010
High	16 (14.0)	48 (28.1)	0.005

Continued

Table 3 *Continued*

	BM activation without vascular uptake ($n = 147$)	BM activation and vascular uptake ($n = 223$)	*P*-value
Plaques by magnetic resonance			
Plaque presence	122 (83.0)	212 (95.1)	<0.001
Number of plaques	2 (1–4)	4 (2–5)	<0.001
Global plaque burden, mm^3	284.5 (125.2–519.5)	448.4 (229.4–819.0)	<0.001
Plaques by 2D vascular ultrasound			
Plaque presence	117 (81.2)	203 (94.4)	<0.001
Number of plaques	2 (1–4.5)	4 (2–7)	<0.001
Plaques by 3D vascular ultrasound			
Plaque presence	90 (67.2)	180 (87.4)	<0.001
Global plaque burden, mm^3	30.3 (0–128.6)	100.8 (37.8–214.4)	<0.001

Data are presented as *n* (%) or median (Q1–Q3).
[a]Measured in 622 of 745 individuals.

suggests that an association between them exists. Indeed, cardiovascular risk factors, including lifestyle factors, have been shown to contribute to haematopoiesis activation.[40] In line with this finding, we found an association between BM activation and increased haematopoiesis, even in the absence of systemic inflammation (*Table 4*). The increase in leucocyte numbers and red blood cell counts was slight but still significant. Bone marrow activation showed a significant association with high arterial metabolic activity (a precursor of atherosclerosis) indexed by arterial ^{18}F-FDG uptake. These results suggest that BM activation is an early phenomenon occurring in response to MetS that contributes to the early stages of atherosclerosis (*Structured Graphical Abstract*). It has been shown that BM activation causes the release of haematopoietic progenitors into the blood stream as a response to different stimuli including acute cardiovascular events.[6] In the absence of such an event, as in the asymptomatic PESA population studied here, BM activation in response to cardiovascular risk factors triggers the release of haematopoietic progenitors and starts the inflammatory process that leads to atherosclerosis initiation and progression. In this regard, haematopoietic progenitor cells mobilized from the BM during the atherosclerotic process and seeded in the spleen, contribute to leucocyte production.[40]

Metabolic syndrome has been associated with several lipid abnormalities such as elevated triglyceride levels, low HDL-cholesterol levels, and increased proportion of small dense LDL particles, despite optimal LDL-cholesterol levels.[41,42] Consistent with this, in our population, the group with BM activation presented higher triglyceride and lower HDL-cholesterol levels, but there was no association with LDL-cholesterol and total cholesterol levels.

In our population, we have observed a myeloid-bias haematopoiesis in patients with BM activation (*Table 1*). This is consistent with experimental findings, which have demonstrated that cardiovascular risk factors are linked to an increased BM myeloid proliferation.[10–13]

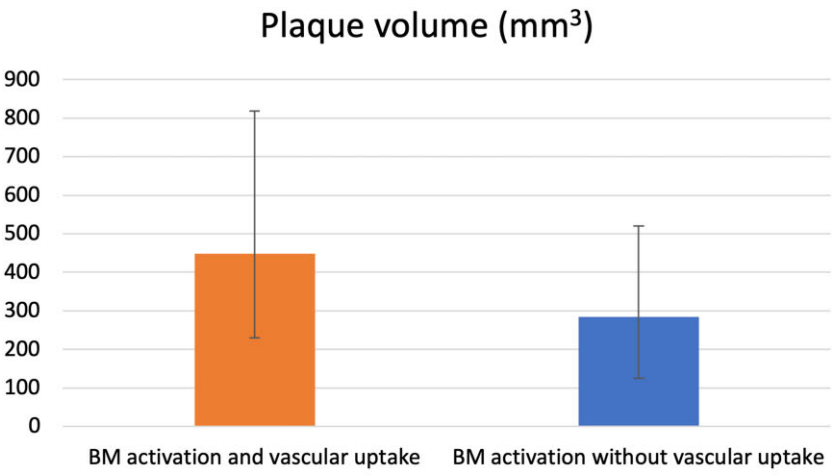
Bone marrow activation is associated with early atherosclerosis, characterized by high arterial metabolic activity (arterial ^{18}F-FDG uptake). Moreover, BM activation in the presence of arterial ^{18}F-FDG uptake is associated with more advanced stages of atherosclerosis, characterized by higher plaque burden, suggesting that high arterial metabolic activity is a prerequisite to trigger atherogenesis.

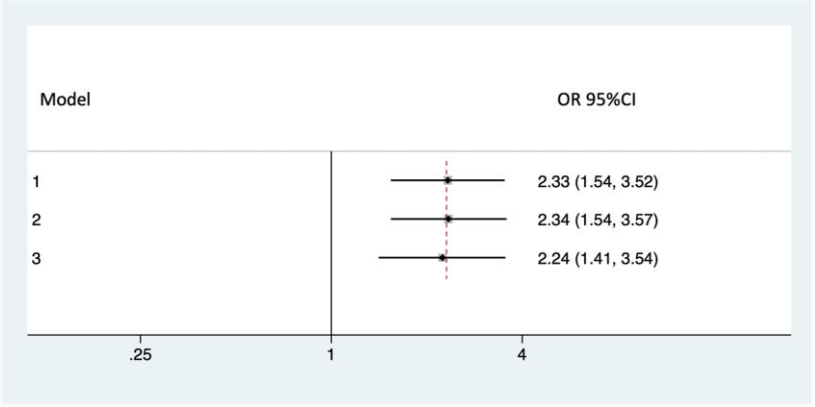
Taken together, our results suggest that, in the presence of cardiovascular risk factors, mainly those associated with MetS, there is an increase in BM metabolic activity that contributes to early atherosclerosis by increasing inflammatory cell proliferation. Further research is needed to define the mechanisms involved in this process in order to identify targets to prevent subclinical atherosclerosis progression and its clinical complications.

Study limitations

The study was subject to selection bias because the subcohort undergoing whole body ^{18}F-FDG PET/MRI was selected from the total PESA cohort based on the presence of subclinical atherosclerosis on vascular ultrasound; this design could also lead to collider bias, which also applies when looking at the non-inflammation subgroup. However, if we had studied participants with no evidence of atherosclerosis, associations might have been even stronger. Moreover, the cross-sectional nature of the study precludes a definite conclusion about causal relationship between risk factors, BM activation, and atherosclerosis. Based on pathology studies, the progression of areas with ^{18}F-FDG uptake to fully grown plaques is thought to be part of the natural history of atherosclerosis, but it has not been formally demonstrated yet.

The availability of data on markers of systemic inflammation allows us to validate that the association between cardiometabolic risk factors and increased BM activity is not simply a reflection of systemic inflammation. Around 20% of participants invited to participate declined enrolment or had MRI contraindications, comparable to similar studies.[43] As previously reported,[27] the first 100 PET





	OR 95% CI	P value
Model 1	2.33 (1.54 ; 3.52)	<0.001
Model 2	2.34 (1.54 ; 3.57)	<0.001
Model 3*	2.24 (1.41 ; 3.54)	0.001

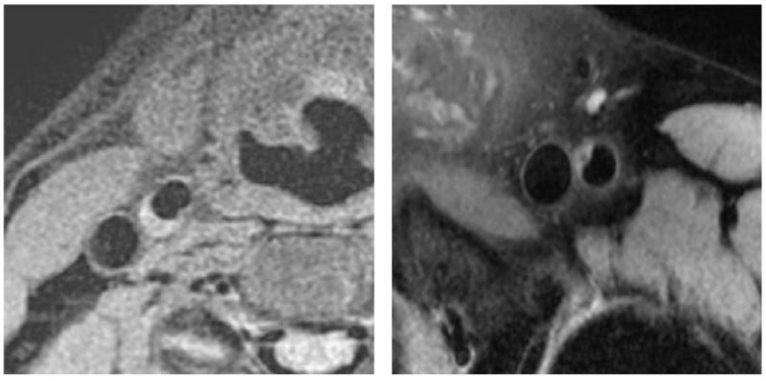


Figure 6 Bone marrow activation in the presence of vascular ^{18}F-fluorodeoxyglucose uptake is associated with higher atherosclerotic plaque volume. Participants with co-occurring bone marrow activation and vascular ^{18}F-fluorodeoxyglucose uptake had a significantly higher plaque burden than those with bone marrow activation but no vascular ^{18}F-fluorodeoxyglucose uptake. The upper panel shows atherosclerotic plaque volume (mm^3) in the group with bone marrow activation and vascular uptake (orange bar) and in the group with bone marrow activation without vascular uptake (blue bar). The mid-panel shows the comparison of adjusted odds ratios and 95% confidence interval for the different models. *In Model 3, 105 participants taking lipid-lowering therapies were eliminated. The lower panel shows representative magnetic resonance images of atherosclerotic plaques.

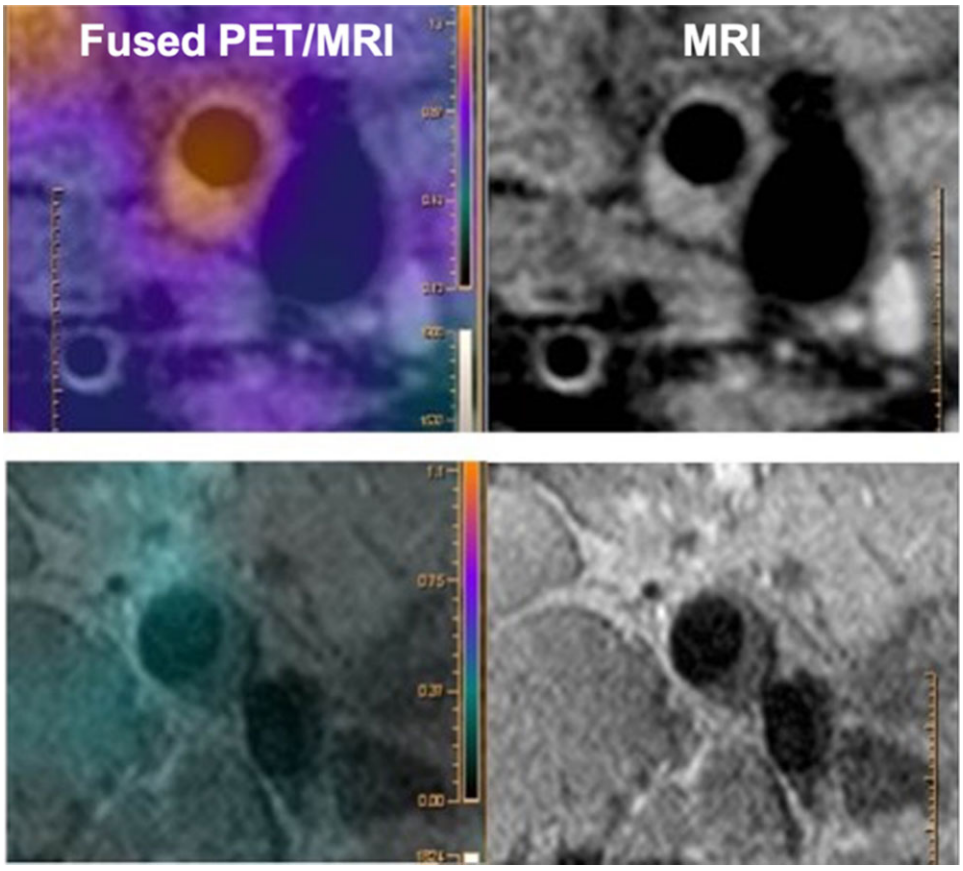


Figure 7 Positron emission tomography/magnetic resonance imaging analysis of the atherosclerotic plaque ^{18}F-fluorodeoxyglucose uptake. The upper row shows a carotid atherosclerotic plaque with ^{18}F-fluorodeoxyglucose uptake. The lower row shows a femoral atherosclerotic plaque without ^{18}F-fluorodeoxyglucose uptake.

Table 4 Bone marrow activation in the subpopulation without systemic inflammation (below-median high-sensitivity C-reactive protein)

	Total ($n = 402$)	No bone marrow uptake CRP < median ($n = 240$)	Bone marrow uptake[b] CRP < median ($n = 162$)	*P*-value
Age, years	50.1 (46.7–53.2)	50.1 (46.2–53.2)	50.3 (46.7–53.2)	0.657
Men	322 (80.1)	182 (75.8)	140 (86.4)	0.009
Metabolic syndrome and its components				
Metabolic syndrome	38 (9.5)	9 (3.8)	29 (17.9)	<0.001
Central obesity	73 (18.2)	17 (7.1)	56 (34.6)	<0.001
Triglycerides, mg/dL	88 (64–118)	83 (62–115)	97 (71–128)	0.010
HDL-C, mg/dL	48.2 ± 11.4	49.7 ± 11.4	46.1 ± 11.1	0.002
Fasting glucose, mg/dL	89 (84–96)	88 (83–94)	92 (85–98)	<0.001
SBP, mmHg	119.4 ± 11.8	117.7 ± 11.3	121.9 ± 12.1	<0.001
DBP, mmHg	74.6 ± 8.9	72.9 ± 8.4	77.2 ± 9.1	<0.001

Continued

Table 4 *Continued*

	Total ($n = 402$)	No bone marrow uptake CRP < median ($n = 240$)	Bone marrow uptake[b] CRP < median ($n = 162$)	*P*-value
Other cardiovascular risk factors				
Family history of CV disease	88 (21.9)	50 (20.8)	38 (23.5)	0.533
Current smoking	92 (23.3)	65 (27.4)	27 (17.1)	0.017
Hypertension	72 (17.9)	31 (12.9)	41 (25.3)	0.001
Dyslipidaemia	218 (54.2)	118 (49.2)	100 (61.7)	0.013
Diabetes	18 (4.5)	6 (2.5)	12 (7.4)	0.020
BMI, kg/m^2	26.4 ± 3.3	25.1 ± 2.8	28.2 ± 3.1	<0.001
Weight, kg	79.0 ± 13.4	74.0 ± 11.4	86.4 ± 12.8	<0.001
Waist circumference, cm	91.5 ± 10.8	87.4 ± 9.5	97.5 ± 9.7	<0.001
Treatment				
Antihypertensive therapy	50 (12.4)	23 (9.6)	27 (16.7)	0.035
Lipid-lowering therapy	59 (14.7)	31 (12.9)	28 (17.3)	0.225
Antidiabetic therapy	15 (3.7)	5 (2.1)	10 (6.2)	0.034
Biochemistry				
Total cholesterol, mg/dL	205.6 ± 33.3	206.2 ± 30.9	204.7 ± 36.6	0.646
LDL-C, mg/dL	137.5 ± 30.2	137.7 ± 28.0	137.2 ± 33.2	0.869
HbA1c, %	5.4 (5.2–5.7)	5.4 (5.2–5.7)	5.4 (5.2–5.7)	0.404
HOMA-IR, %	1.15 (0.77–1.86)	0.97 (0.67–1.52)	1.54 (0.98–2.23)	<0.001
Insulin, μU/mL	5.1 (3.6–7.2)	4.3 (3.2–6.2)	6.1 (4.3–8.2)	<0.001
Inflammatory markers				
hs-CRP, mg/dL	0.06 (0.04–0.08)	0.06 (0.04–0.08)	0.07 (0.04–0.08)	0.080
Ferritin, ng/mL[a]	107.7 (56.5–197.9)	101.7 (46.2–187.5)	122.8 (70.5–227.4)	0.015
Erythrocyte sedimentation rate (1 h), mm	5 (4–6)	5 (4–6)	5 (2–7)	0.311
Fibrinogen, mg/dL	252.0 (229.4–275.4)	252.5 (229.8–276.1)	250.9 (226.6–275.1)	0.926
P-selectin, ng/mL	131.1 (103.4–162.0)	125.8 (103.2–160.2)	139.3 (103.7–168.0)	0.071
Vascular cell adhesion molecule-1, ng/mL	638.3 (512.8–816.6)	620.4 (507.8–788.8)	699.9 (514.4–862.5)	0.113
Blood count				
Leucocytes, 10^3 cells/μL	5.59 (4.86–6.64)	5.55 (4.83–6.73)	5.63 (4.93–6.47)	0.575
Leucocytosis, (>10.5 × 10^3 cells/μL)	2 (0.5)	2 (0.8)	0 (0)	0.244
Red blood cells, 10^6 cells/μL	4.81 (4.51–5.06)	4.76 (4.46–5.02)	4.90 (4.62–5.15)	0.001
Red cell distribution width, %	14.5 (14.0–15.1)	14.5 (14.0–15.0)	14.6 (14.0–15.2)	0.245
Haemoglobin, g/dL	14.9 (14.0–15.6)	14.8 (13.9–15.5)	15.1 (14.2–15.9)	0.002
Haematocrit, %	43.9 (41.6–46.3)	43.5 (41.1–45.8)	44.5 (42.2–46.9)	0.005
Platelets, 10^3 cells/μL	219 (192–255)	224 (196–256)	214 (189–244)	0.074
Segmented neutrophils, 10^3 cell/μL	3.10 (2.56–3.80)	3.09 (2.55–3.88)	3.12 (2.59–3.77)	0.839
Lymphocytes, 10^3 cells/μL	1.83 (1.54–2.17)	1.82 (1.52–2.18)	1.87 (1.55–2.16)	0.471
Monocytes, 10^3 cells/μL	0.39 (0.33–0.48)	0.39 (0.33–0.48)	0.39 (0.33–0.47)	0.678
Eosinophils number (1000 cells/μL)	0.12 (0.08–0.19)	0.11 (0.08–0.19)	0.12 (0.07–0.20)	0.547

Continued

Table 4 *Continued*

	Total ($n = 402$)	No bone marrow uptake CRP < median ($n = 240$)	Bone marrow uptake[b] CRP < median ($n = 162$)	*P*-value
Eosinophils, 10^3 cells/μL	0.04 (0.03–0.06)	0.05 (0.03–0.06)	0.04 (0.02–0.06)	0.094
Neutrophil to lymphocyte ratio	1.68 (1.37–2.10)	1.71 (1.37–2.13)	1.66 (1.36–2.08)	0.431
SCORE risk score				
Low (<1%)	265 (68.6)	165 (70.2)	100 (66.2)	0.410
Intermediate (1–5%)	121 (31.4)	70 (29.8)	51 (33.8)	0.410
High (>5%)	0 (0.0)	0 (0.0)	0 (0.0)	—
ASCVD risk score				
Low	216 (66.9)	139 (69.5)	77 (62.6)	0.201
Intermediate	55 (17.0)	33 (16.5)	22 (17.9)	0.748
High	52 (16.1)	28 (14.0)	24 (19.5)	0.191
Plaques by magnetic resonance				
Plaque presence	357 (88.8)	213 (88.8)	144 (88.9)	0.965
Number of plaques	3 (1–5)	3 (1–5)	3 (1–4)	0.708
Global plaque burden	328.7 (162.4–616.0)	321.6 (150.2–604.3)	347.3 (180.2–628.0)	0.647
Arterial uptake (^{18}F-FDG)				
Presence of uptake	159 (39.5)	75 (31.2)	84 (51.9)	<0.001
Number of uptakes	0 (0–1)	0 (0–1)	1 (0–2)	<0.001
SUVmax arterial uptake	1.33 (1.23–1.45)	1.28 (1.18–1.37)	1.44 (1.32–1.54)	<0.001

Data are presented as *n* (%) or median (Q1–Q3).
[a]Measured in 622 of 745 individuals.
[b]Bone marrow activation was defined when the mean BM SUVmax was above the median value (SUVmax 1.9).

studies could not be used due to inaccuracies in MRI-based attenuation and reconstruction; however, feasibility was almost 100% thereafter.

Conclusions

In middle-aged apparently healthy individuals, BM activation, identified as ^{18}F-FDG uptake, is associated with cardiovascular risk factors, mainly MetS, and its components. This association is present even in the absence of systemic inflammation. Bone marrow activation is associated with early atherosclerosis, characterized by high arterial metabolic activity (^{18}F-FDG uptake).

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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