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ORIGINAL RESEARCH

Splenic Metabolic Activity Predicts Risk of Future Cardiovascular Events

Demonstration of a Cardiosplenic Axis in Humans

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ABSTRACT

OBJECTIVES This study sought to determine whether splenic activation after acute coronary syndrome (ACS) is linked to leukocyte proinflammatory remodeling and whether splenic activity independently predicts the risk of cardiovascular disease (CVD) events.

BACKGROUND Pre-clinical data suggest the existence of a cardiosplenic axis, wherein activation of hematopoietic tissues (notably in the spleen) results in liberation of proinflammatory leukocytes and accelerated atherosclerotic inflammation. However, it is presently unknown whether a cardiosplenic axis exists in humans and whether splenic activation relates to CVD risk.

METHODS ^{18}F-fluorodeoxyglucose (^{18}FDG)-positron emission tomography (PET) imaging was performed in 508 individuals across 2 studies. In the first study, we performed FDG-PET imaging in 22 patients with recent ACS and 22 control subjects. FDG uptake was measured in spleen and arterial wall, whereas proinflammatory gene expression of circulating leukocytes was assessed by quantitative real-time polymerase chain reaction. In a second study, we examined the relationship between splenic tissue FDG uptake with subsequent CVD events during follow-up (median 4 years) in 464 patients who previously had undergone FDG-PET imaging.

RESULTS Splenic activity increased after ACS and was significantly associated with multiple indices of inflammation: 1) up-regulated gene expression of proinflammatory leukocytes; 2) increased C-reactive protein; and 3) increased arterial wall inflammation (FDG uptake). Moreover, in the second study, splenic activity (greater than or equal to the median) was associated with an increased risk of CVD events (hazard ratio [HR]: 3.3; 95% confidence interval [CI]: 1.5 to 7.3; $p = 0.003$), which remained significant after adjustment for CVD risk factors (HR: 2.26; 95% CI: 1.01 to 5.06; $p = 0.04$) and for arterial FDG uptake (HR: 2.68; 95% CI: 1.5 to 7.4; $p = 0.02$).

CONCLUSIONS Our findings demonstrate increased splenic metabolic activity after ACS and its association with proinflammatory remodeling of circulating leukocytes. Moreover, we observed that metabolic activity of the spleen independently predicted risk of subsequent CVD events. Collectively, these findings provide evidence of a cardiosplenic axis in humans similar to that shown in pre-clinical studies. (J Am Coll Cardiol Img 2015;8:121–30) © 2015 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome

BM = bone marrow

CI = confidence interval

CRP = C-reactive protein

CT = computed tomography

CVD = cardiovascular disease

FDG = 18-fluorodeoxyglucose

FRS = Framingham Risk Score

HR = hazard ratio

PET = positron emission tomography

SAT = subcutaneous adipose tissue

SUV = standardized uptake value

TBR = target-to-background ratio

TLR = toll-like receptor

Patients remain at an increased risk for recurrent cardiovascular disease (CVD) events in the weeks to months after an acute coronary syndrome (ACS) (1,2); however, the pathophysiological basis for this increased risk remains unclear. Preclinical studies have shown that proliferation of monocyte progenitors and proinflammatory activation of monocytes within the hematopoietic tissues (i.e., bone marrow [BM] and spleen) may play an important role in accelerating atherosclerosis after myocardial infarction (3,4). Pre-clinical studies demonstrated that after myocardial infarction in mice, monocyte progenitor cells departed BM niches, which resulted in amplified extramedullary monocytopoiesis (3). The observation of activation of the inflammatory cell milieu and the migration of proinflammatory monocytes from spleen to heart in animal models of heart failure (5) have given rise to the concept of a cardiosplenic axis. Recently, the concept of a cardiosplenic axis has been extended to stable atherosclerosis in murine models as well (6); however, it is presently unknown whether such an axis exists in humans.

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Imaging with ^{18}F-fluorodeoxyglucose-positron emission tomography (FDG-PET) provides a noninvasive measure of tissue glycolysis (7) and is used clinically for the evaluation of tumors (8) and infectious foci (9). The biological basis for FDG accumulation within mononuclear inflammatory cells lies in the fact that macrophages have a high metabolic rate (10), especially after proinflammatory activation (11,12), and hence avidly accumulate FDG (13). Additionally, cellular accumulation of FDG is increased in rapidly proliferating cells (14). Because in animal models of myocardial infarction, splenic activation is marked by proliferation of monocyte progenitor cells and proinflammatory activation of monocytes (3,4), we sought to evaluate splenic activation in humans using FDG PET/computed tomography (CT) imaging. Furthermore, we investigated whether splenic metabolic activity relates to proinflammatory gene expression of circulating leukocytes.

In the first study, we tested the hypothesis that the metabolic activity of hematopoietic tissues (i.e., BM and spleen) occurs in humans with recent ACS. In the second study, and in a separate population, we investigated whether the metabolic activity of these hematopoietic tissues predicts the risk of subsequent CVD events. To do so, we evaluated BM and splenic metabolic activity, by FDG uptake, in a group of individuals with no known atherosclerotic disease who had undergone clinically indicated FDG-PET/CT scans and for whom clinical follow-up data were available. We then assessed whether the baseline hematopoietic tissue FDG signal correlated with arterial wall inflammation and independently predicted the subsequent development of incident CVD events.

METHODS

OVERALL STUDY SCHEMA. We performed 2 separate studies (**Figure 1**). The first study, the ACS study, was designed to test the hypothesis that metabolic activity of the hematopoietic tissues of BM and spleen was more prominent after ACS and was associated with levels of serum proinflammatory biomarkers, proinflammatory gene expression of circulating leukocytes, and arterial wall inflammation. To test these hypotheses, 44 patients were prospectively recruited at Massachusetts General Hospital. FDG-PET/CT imaging was performed in all subjects, and FDG uptake was assessed in the BM, spleen, arterial wall, and control tissues. Additionally, serum biomarker assays and quantitative real-time polymerase chain reaction of proinflammatory gene expression in circulating leukocytes was performed.

The second study, the clinical outcomes study, was conducted in a separate population and was designed to test the hypothesis that hematopoietic tissue (BM and spleen) metabolic activity was associated with arterial wall inflammation and independently predicted the subsequent risk of incident CVD events. To

Boston, Massachusetts; and the ‡‡Division of Cardiology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. Funding for the first study (ACS Study) was provided by Genentech, Inc., and BioInvent International AB. No funding from any source was provided for the second study (Clinical Outcomes Study). Dr. Lehrer-Graiwer is an employee of and owns stock in Global Blood Therapeutics. At the time this study was conducted, Dr. Korsgren was employed by BioInvent International AB, Sweden. Dr. Fredrickson is an employee of and a shareholder in Genentech/Roche. Dr. Baruch is an employee of Genentech/Roche. Dr. Tawakol has received grant support from Genentech. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Emami and Singh are joint first authors. George Beller, MD, has served as Guest Editor for this paper.

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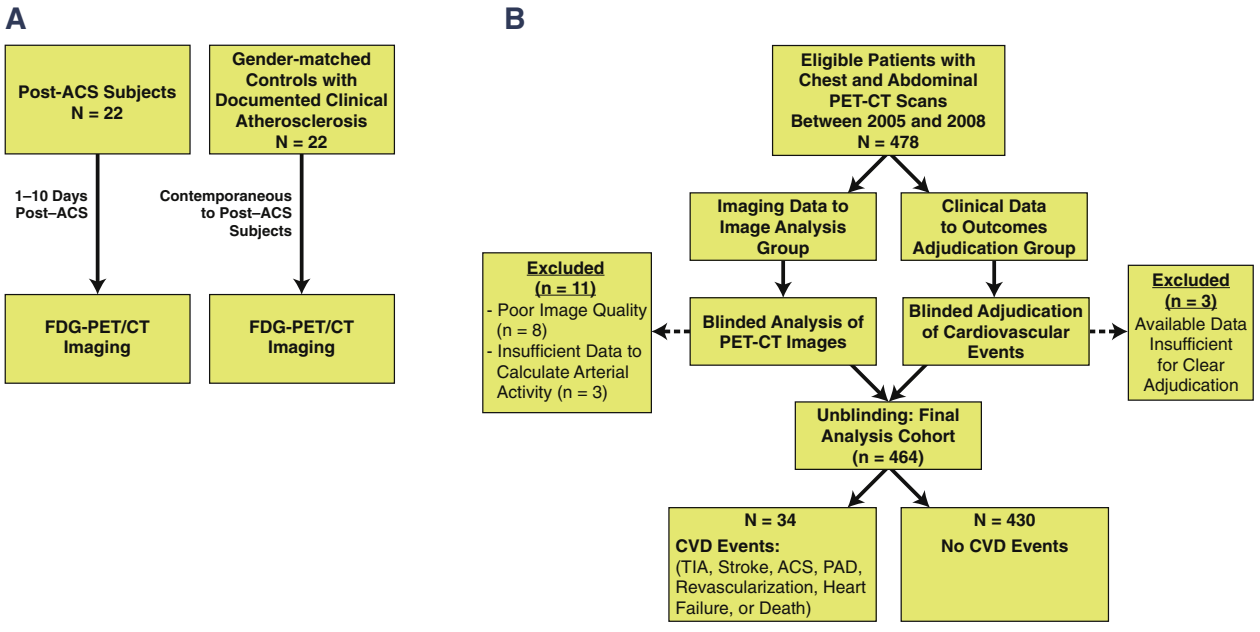
FIGURE 1 Schematics of Studies



In the first study **(A)**, 22 post-acute coronary syndrome (ACS) patients underwent ^{18}F-fluorodeoxyglucose-positron emission tomography/computed tomography (FDG-PET/CT) imaging within 10 days of their event. We compared bone marrow, splenic, and arterial activity in these patients with 22 sex-matched control subjects. In the second study **(B)**, we studied 464 subjects who had undergone FDG-PET/CT imaging for various clinical indications and assessed whether bone marrow and splenic activity were associated with future cardiovascular disease (CVD) events. PAD = peripheral artery disease; TIA = transient ischemic attack.

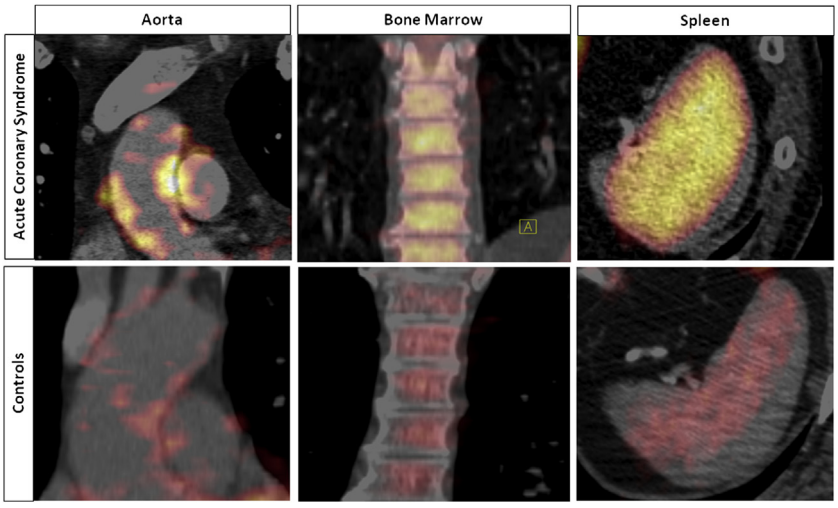
test these hypotheses, we evaluated BM and splenic activity (using FDG PET/CT imaging) in 464 individuals consecutively identified from a database of patients who had undergone clinically indicated FDG-PET/CT scans. Thereafter, the development of incident CVD events was adjudicated by blinded cardiologists, and the relationship between hematopoietic tissue activity and subsequent CVD risk was assessed.

STUDY SUBJECTS. Study 1: ACS Study. Forty-four subjects were prospectively recruited into this study. Twenty-two people with ACS (ACS group) and 22 without recent ACS (control group) were recruited (Figure 1A).

The ACS group was identified according to prespecified criteria. Eligible patients were adults (age 38 to 69 years) with recent ACS (defined as myocardial infarction or unstable angina documented by electrocardiogram, cardiac enzymes, or angiogram) who were clinically stable and able to undergo an FDG-PET/CT scan within 10 days of the incident CVD event. The control group consisted of 22 people with documented clinical atherosclerotic disease who were sex-matched to the ACS group. The control group underwent contemporaneous FDG-PET/CT imaging and was selected among male or female subjects, 35 to 80 years of age, with documented clinical atherosclerotic disease (i.e., coronary artery disease, peripheral arterial disease, or carotid disease) but no history of ACS within the previous 6 months. Exclusion criteria for both groups included systemic chronic inflammatory conditions, type I diabetes mellitus or fasting plasma glucose >175 mg/dl, presence of severe heart failure or severe left ventricular dysfunction, or significant radiation exposure within the preceding 12 months. Additionally, individuals were excluded from participation if they had recent evidence of cardiogenic shock, sustained ventricular tachyarrhythmia, or hypoxemia (defined as O_2 saturation <90% or requirement for >2 l/min supplemental oxygen to maintain O_2 saturation >90%).

Study 2: Clinical Outcomes Study. Using a retrospective study design, 464 people who underwent prior PET/CT imaging at the Massachusetts General Hospital between 2005 and 2008 were included in this analysis (Figure 1B). We included everyone with PET/CT images of chest and abdomen who met the following pre-defined inclusion criteria: 1) either absence of prior cancer diagnosis or remission from cancer at the time of PET imaging and throughout

FIGURE 2 Increased Bone Marrow, Spleen, and Arterial FDG Uptake After ACS



Coronal views of aorta **(left)** and bone marrow **(middle)** and axial view of spleen **(right)**. ^{18}F-fluorodeoxyglucose (FDG) uptake was significantly increased in the arterial wall (aorta), bone marrow, and spleen in patients with acute coronary syndrome (ACS) versus control subjects.

the follow-up period; 2) age ≥30 years; 3) no prior history of CVD; and 4) absence of acute or chronic inflammatory or autoimmune disease (on the basis of documented medical history) or use of chronic anti-inflammatory therapy. Subjects were required to have at least 3 clinical visit notes (spanning ≥1 year) to ensure availability of sufficient clinical data and to determine clinical status at the time of PET imaging.

FDG-PET/CT IMAGING. FDG-PET/CT imaging was performed using previously reported reproducible and validated approaches (15). Briefly, ^{18}F-FDG was administered intravenously (approximately 10 mCi for a 70-kg patient) after an overnight fast, and imaging was performed 90 min after FDG injection with PET/CT.

IMAGE ANALYSIS. While investigators were blinded to all clinical and temporal data, images were analyzed at a central core laboratory at Massachusetts General Hospital using Leonardo TrueD software (Siemens, Forchheim, Germany). Arterial inflammation was measured within pre-defined sections of the 3 target vessels (right and left carotid artery and aortic wall) using previously validated methods (Online Appendix) (15,16). BM FDG uptake was measured by placing a region of interest over axial sections of individual vertebrae from T1 to L5. The maximum standardized uptake value (SUVmax) for each vertebra was recorded, and BM activity was calculated as the mean of SUVmax of all vertebrae in the imaging field. Similarly, splenic FDG uptake was assessed by placing regions of interest in 3 orthogonal planes (axial, sagittal, and coronal planes) (Online Figure 1). SUVmax was recorded in each plane, and splenic activity was calculated as the mean of SUVmax values of the 3 planes. Intrareader reproducibility for measurement of BM FDG uptake was determined as intraclass correlation coefficient of 0.99 ($p < 0.001$), and percent variance was calculated as 2.2 ± 2.1%. Reproducibility for splenic FDG uptake measurement was determined as intraclass correlation coefficient of 0.96 ($p < 0.001$), and percent variance was calculated as 4.7 ± 3.8%. For evaluations of the correlation of FDG uptake between tissues (within patients), we additionally used uncorrected SUVs to avoid the contribution that a common background value would make to the relationship.

GENE EXPRESSION ASSAYS. Inflammatory gene expression was assessed by quantitative real-time polymerase chain reaction of peripheral blood leukocyte proinflammatory mRNA. (Please see the Online Appendix for details about assessment of inflammatory gene expression.)

ADJUDICATION OF CVD EVENTS. Events were clinically adjudicated by 2 cardiologists who were blinded to all imaging data. Using clinically available records, incident CVD events included ischemic stroke or transient ischemic attack, ACS, revascularization (coronary, carotid, or peripheral), unstable angina, heart failure, or CVD death. (Please see the Online Appendix for additional details.)

STATISTICAL ANALYSIS. Descriptive data are presented as mean ± SD for continuous parametric variables, median (interquartile range) for continuous nonparametric data, and frequency with proportions for nominal variables as appropriate. An independent samples Student *t* test was used for cross-sectional comparison of normally distributed continuous variables (such as BM, spleen, and arterial FDG uptake) between the ACS and control groups. A Mann-Whitney *U* test was used for the similar analyses of continuous variables without normal distribution (such as C-reactive protein [CRP]). Fisher exact test was performed for comparison of dichotomous variables. Pearson correlation coefficient (R) was used to assess correlations between continuous variables once normal distribution was verified, and Spearman ρ was reported as correlation coefficient for non-normally distributed variables. For comparison of means, 95% confidence intervals (CIs) are provided. Kaplan-Meier estimates of the proportions of patients free from CVD events were stratified separately, by median FDG uptake in spleen and BM (SUVmax). Cox proportional hazards regression was used to calculate hazard ratios (HRs) and 95% CIs. Framingham Risk Score (FRS) was not normally distributed among the study subjects, so we converted it to an ordinal variable (low [<10], intermediate [10 to 20], and high risk [>20]) and used the ordinal variable for the analyses. Two-tailed p values are reported and statistical significance was defined as $p < 0.05$. All statistical analyses were performed with SPSS version 22 (IBM, Armonk, New York).

RESULTS

STUDY 1: ACS STUDY. Baseline characteristics. There were no significant differences between ACS and control subjects for age or major CVD risk factors except for current smoking ($p = 0.04$). All the people in the control group had documented clinically diagnosed CAD. Baseline patient characteristics and demographics are summarized in Table 1. Additional information on clinical presentations of ACS patients is detailed in Online Table 1.

Hematopoietic tissue metabolic activity is up-regulated after ACS. We observed a significantly higher splenic FDG uptake in individuals with recent ACS (SUV, 2.6 ± 0.6 vs. 2.1 ± 0.3; $p = 0.03$). Similarly, BM FDG uptake was substantially higher in ACS patients than in control subjects (SUV, 2.9 ± 0.5 vs. 2.4 ± 0.6; $p = 0.01$) (Figure 2). In contrast, FDG uptake in control tissues (subcutaneous adipose tissue [SAT] and pectoralis muscles) was not increased in ACS patients compared with control subjects (SAT SUV, 0.16 ± 0.05 vs. 0.17 ± 0.06; $p = 0.57$; pectoralis muscle SUV, 0.41 ± 0.07 vs. 0.43 ± 0.12; $p = 0.53$) (Table 1).

TABLE 1 Baseline Characteristics of ACS Study Subjects

	ACS Patients (n = 22)	Control Subjects (n = 22)	p Value
Age (yrs)	58 ± 8.5	62 ± 10	0.19
Male	16 (72)	16 (72)	0.63
Current smoker	9 (41)	3 (14)	0.04
Diabetes mellitus	4 (18)	3 (14)	0.50
Hypertension	17 (77)	18 (82)	0.50
BMI (kg/m^2)	31.5 ± 5.6	29.2 ± 3.4	0.16
Baseline values (mg/dl)			
Total cholesterol	173.2 ± 38.2	159.8 ± 21.9	0.21
LDL cholesterol	95.1 ± 31.6	92.8 ± 18.5	0.80
HDL cholesterol	42.8 ± 12.7	53.1 ± 16.2	0.03
Triglycerides	174.2 ± 94.6	120.4 ± 42.1	0.04
FDG uptake*			
Bone marrow	2.9 ± 0.5	2.4 ± 0.6	0.01
Spleen	2.6 ± 0.6	2.1 ± 0.3	0.03
Arterial wall	2.7 ± 1.2	2.2 ± 0.6	0.04
Control tissues			
Subcutaneous adipose tissue	0.16 ± 0.05	0.17 ± 0.06	0.57
Pectoralis muscle	0.41 ± 0.07	0.43 ± 0.12	0.53

Values are mean ± SD or n (%). *FDG uptake in bone marrow, spleen, and control tissues is reported as standardized uptake value, whereas arterial FDG uptake is reported as TBR.

ACS = acute coronary syndrome; BMI = body mass index; FDG = ^{18}F-fluorodeoxyglucose; LDL = low-density lipoprotein; HDL = high-density lipoprotein; SUV = standardized uptake value; TBR = target-to-background ratio.

Hematopoietic tissue metabolic activity correlates with CRP and proinflammatory gene expression in leukocytes. Serum CRP concentrations were significantly higher in the ACS group than in control subjects (median [interquartile range]: 7.70 [1.45 to 21.2] vs. 2.47 [1.00 to 4.13]; $p = 0.04$). In addition, CRP correlated with BM FDG uptake ($\rho = 0.62$, $p = 0.002$) and splenic FDG

TABLE 2 Relationship Between Hematopoietic Tissue Activity and Inflammatory Biomarkers

	Bone Marrow FDG Uptake		Spleen FDG Uptake	
	Correlation Coefficient	p Value	Correlation Coefficient	p Value
Serum biomarkers				
CRP	0.62	0.002	0.44	0.04
TNF	0.19	0.46	0.39	0.44
IL-1β	0.43	0.09	−0.37	0.47
Gene expression in leukocytes				
CD36	0.05	0.85	0.51	0.03
MSR-1	0.53	0.02	0.48	0.04
S100A9	0.15	0.54	0.59	0.01
TLR-2	0.19	0.45	0.45	0.06

Bone marrow and spleen FDG uptakes were analyzed as standardized uptake value. Correlation coefficients represent Spearman ρ. Correlations were assessed in the entire population (n = 44) including both post-acute coronary syndrome patients and control subjects. In analyses that were limited to the subgroups, we did not observe significant correlations except for between S100A9 expression and splenic activity in the control group ($\rho = 0.8$, $p = 0.02$).

CRP = C-reactive protein; FDG = ^{18}F-fluorodeoxyglucose; IL = interleukin; MSR = macrophage scavenger receptor; TLR-2 = toll-like receptor 2; TNF = tumor necrosis factor.

uptake ($\rho = 0.44$, $p = 0.04$). There was a strong correlation between CRP and BM FDG uptake ($\rho = 0.66$, $p = 0.02$) in post-ACS patients, whereas no significant correlation was observed in control subjects ($\rho = 0.48$, $p = 0.13$). Furthermore, we observed a significant correlation between hematopoietic tissue FDG uptake and the expression of several genes associated with proinflammatory activation within circulating leukocytes (Table 2).

Relationships with arterial inflammation. As expected, the mean arterial FDG uptake (target to background ratio [TBR]) was greater in patients with ACS than in control subjects (2.7 ± 1.2 vs. 2.2 ± 0.6; $p = 0.04$) (Table 1). Moreover, BM and splenic metabolic activity (SUV) significantly correlated with arterial inflammation (TBR; $r = 0.34$, $p = 0.03$ and $r = 0.46$, $p = 0.01$ for BM and spleen, respectively). Similarly, the uncorrected arterial FDG signal (SUV) also correlated with BM and splenic SUV ($r = 0.67$, $p < 0.001$ and $r = 0.37$, $p = 0.04$ for BM and spleen, respectively). In contrast, FDG uptake in the control tissues did not correlate with FDG uptake in the arterial wall ($r = 0.11$, $p = 0.39$ and $r = 0.20$, $p = 0.10$ for the arterial wall vs. SAT and pectoralis muscle, respectively).

TABLE 3 Baseline Characteristics of the Clinical Outcomes Study

	Full Cohort (n = 464)	Individuals Without Subsequent CVD (n = 430)	Individuals With Subsequent CVD (n = 34)	p Value
Age (yrs)	55 (44-46)	54 (44-65)	66 (60-78)	<0.001
Male	201 (43)	186 (43)	15 (44)	0.53
Current smoker	46 (10)	36 (8.5)	10 (29)	0.001
Hypertension	158 (34)	139 (32)	19 (56)	0.006
Diabetes mellitus	41 (9)	34 (8)	7 (20)	0.02
Statin use	89 (19)	75 (17)	14 (41)	0.002
BMI (kg/m^2)	26 (23-31)	26 (23-31)	27 (24-32)	0.33
Dyslipidemia	127 (27)	112 (26)	15 (44)	0.02
Baseline values (mg/dl)				
Total cholesterol	192 ± 45	193 ± 45	184 ± 41	0.32
LDL cholesterol	111 ± 38	112 ± 32	107 ± 34	0.49
HDL cholesterol	56 ± 18	57 ± 19	50 ± 14	0.04
Triglycerides	124 ± 72	121 ± 72	140 ± 71	0.18
FRS*				
<10	186 (82)	168 (85)	18 (60)	0.08
10-20	36 (16)	24 (12)	12 (40)	<0.001
>20	5 (2)	5 (2.5)	0 (0)	0.68
FDG uptake†				
Bone marrow	2.2 (1.9-2.6)	2.2 (1.9-2.6)	2.4 (2.1-2.8)	0.03
Spleen	2.1 (1.8-2.4)	2.1 (1.8-2.3)	2.2 (2.1-2.5)	0.01
Arterial wall	1.9 (1.8-2.2)	1.9 (1.8-2.2)	2.2 (1.9-2.3)	<0.001
Subcutaneous adipose tissue	0.17 (0.14-0.2)	0.16 (0.14-0.2)	0.19 (0.13-0.22)	0.28

Values are mean ± SD, median (interquartile range), or n (%). *A subset of 227 subjects provided all the required data to calculate FRS. †FDG uptake in bone marrow, spleen, and subcutaneous adipose tissue is reported as SUV whereas arterial FDG uptake is reported as TBR.

BMI = body mass index; CVD = cardiovascular disease; FDG = ^{18}F-fluorodeoxyglucose; FRS = Framingham Risk Score; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

Additionally, we observed a significant correlation between arterial inflammation (TBR) and CRP ($\rho = 0.45$, $p = 0.04$). Arterial inflammation (TBR) was associated with mRNA levels of CD16 and toll-like receptor (TLR)-4 in the peripheral leukocytes such that tertiles of mean arterial TBR correlated with mRNA levels of CD16 ($\rho = 0.47$, $p = 0.04$) and TLR-4 ($\rho = 0.49$, $p = 0.03$).

STUDY 2: CLINICAL OUTCOMES STUDY. Baseline characteristics. In the second study, we evaluated 464 participants with follow-up for 6.5 years (median, 4 years). A total of 34 individuals developed CVD events (2 per 100 person-years at risk) during this period. Baseline characteristics and demographics are presented in Table 3. Additional information on baseline cancer and cancer treatment status of "clinical outcome" subjects is detailed in Online Table 2.

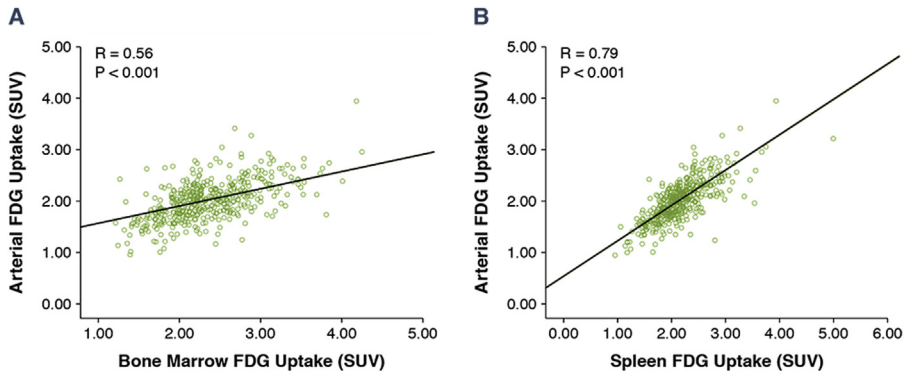
CVD events. The events were characterized as follows: 8 ACS (6 acute myocardial infarction and 2 unstable angina), 8 coronary revascularizations, 7 strokes, 2 transient ischemic attacks, 2 carotid endarterectomies, 4 cases of new-onset angina (1 with and 3 without obstructive disease documented on coronary catheterization), 2 new diagnoses of peripheral arterial disease, 2 peripheral revascularizations secondary to peripheral artery disease, and 1 subsequent death due to acute myocardial infarction. The distribution of events between men and women was not statistically significant (15 men and 19 women, $p = 0.54$).

Hematopoietic tissue activity correlates with arterial inflammation. BM metabolic activity (FDG uptake) significantly correlated with arterial SUV ($r = 0.56$, $p < 0.001$) (Figure 3A) and background-corrected arterial TBR ($r = 0.22$, $p < 0.001$). Similarly, splenic activity significantly correlated with arterial SUV ($r = 0.79$, $p < 0.001$) (Figure 3B) and arterial TBR ($r = 0.23$, $p < 0.001$). Additionally, we observed a strong positive correlation between BM and splenic activation ($r = 0.71$, $p < 0.001$). In contrast, there was no significant correlation between SAT metabolic activity and arterial inflammation ($r = -0.074$, $p = 0.11$).

BM and splenic activation are associated with subsequent risk of CVD events. Patients with higher BM activity (greater than or equal to the median) had an increased risk for subsequent CVD events (HR: 2.15; 95% CI: 1.05 to 4.41; $p = 0.04$) (Figure 4A). However, in a Cox regression model, the association was no longer significant after adjusting for FRS ($p = 0.12$), statin use ($p = 0.06$), or arterial FDG uptake ($p = 0.14$).

In contrast, splenic FDG uptake (greater than or equal to the median) was associated with an increased risk for CVD events (HR: 3.3; 95% CI: 1.5 to 7.3; $p = 0.003$) (Figure 4B), which remained significant after adjustment for CVD risk factors of age,

FIGURE 3 **Correlation Between FDG Uptake in Bone Marrow, Spleen, and Arterial Wall**



In the clinical outcomes study, a strong correlation between baseline arterial ^{18}F-fluorodeoxyglucose (FDG) uptake with **(A)** bone marrow and **(B)** spleen was observed. SUV = standardized uptake value.

male sex, smoking (HR: 2.25; 95% CI: 1.01 to 5.04; p = 0.04) and also after adjustment for hypertension, diabetes mellitus, and dyslipidemia (HR: 2.76; 95% CI: 1.24 to 6.18; p = 0.01). In 2 additional models, the association between splenic FDG uptake and CVD events remained significant after adjustment for FRS (HR: 2.9; 95% CI: 1.2 to 6.9; p = 0.01) and after adjustment for statin use (HR: 2.8; 95% CI: 1.3 to 6.4; p = 0.01).

The association between higher splenic activity and risk for CVD events remained significant after adjustment for history of prior cancer diagnosis, chemotherapy, and radiotherapy (HR: 3.37; 95% CI: 1.5 to 7.4; p = 0.003), after adjustment for time from chemotherapy to imaging (HR: 2.65; 95% CI: 1.03 to 6.78; p = 0.04), after the exclusion of subjects with a history of lymphoma and hematologic malignancies (HR: 3.4; 95% CI: 1.4 to 8.4; p = 0.008), and after the exclusion of subjects with a prior history of any type of cancer (HR: 5.1; 95% CI: 1.1 to 23.8; p = 0.04). In addition, we observed similar findings after adjustment for arterial TBR (HR: 2.68; 95% CI: 1.5 to 7.4; p = 0.02). In contrast, metabolic activity in the control tissue (SAT) was not associated with risk of cardiovascular events (HR: 1.26; 95% CI: 0.64 to 2.48; p = 0.50) (**Figure 4C**).

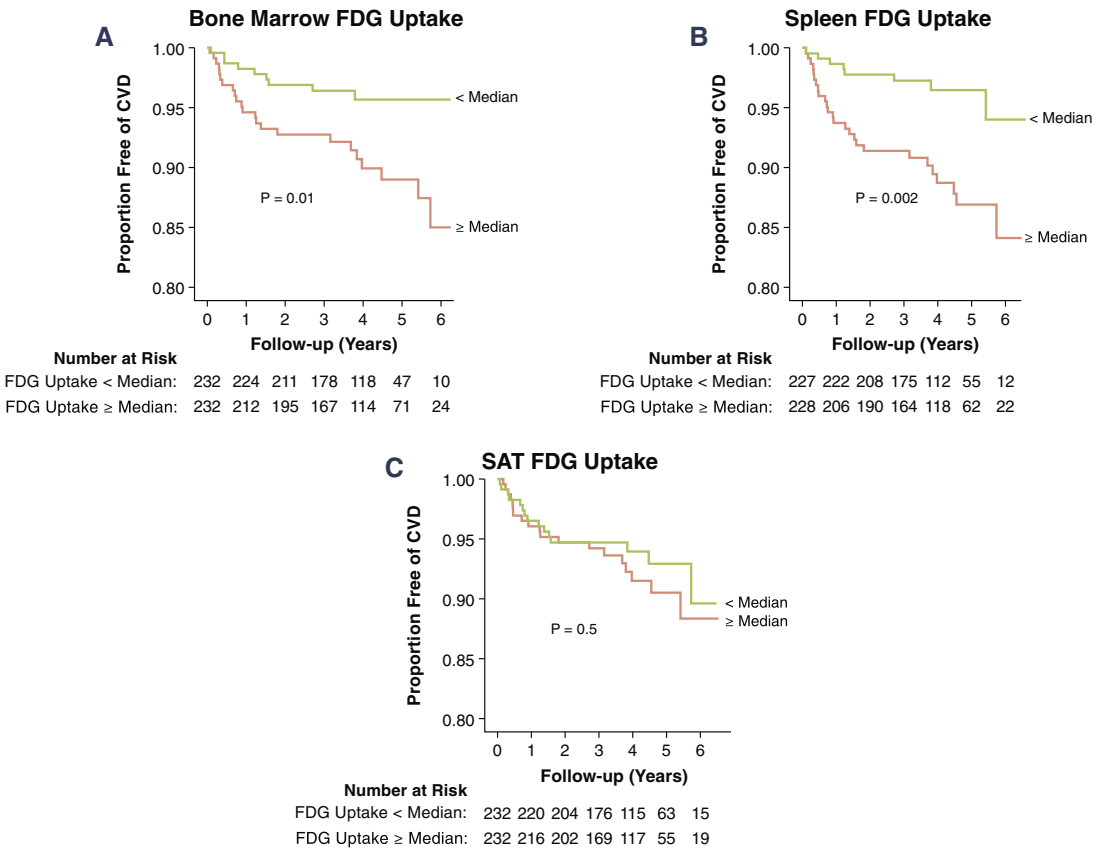
DISCUSSION

We observed that BM and splenic tissue metabolic activity increased substantially after ACS and correlated with CRP level and expression of proinflammatory markers of circulating leukocytes. We also observed, in a separate study group, that splenic tissue activity was independently associated with an increased risk of CVD events in people without known atherosclerosis. These data suggest that in humans, BM and splenic activity are associated with an increase in proinflammatory mediators, which may play a role in increased atherosclerotic plaque inflammation and an elevated risk of subsequent CVD events.

INFLAMMATION IN ATHEROSCLEROSIS. Inflammation plays a central role in the pathogenesis of atherosclerosis and its associated clinical complications (17). The response to acute ischemic injury is believed to trigger a molecular and cellular systemic inflammatory response whereby the recruitment of proinflammatory leukocytes results in deleterious effects on the blood vessel wall (18). Numerous mediators contribute to this process, including chemokines, cytokines, proteases, and adhesion molecules, and their interactions contribute to atherosclerotic plaque instability (19). Although statins appear to reduce vascular risk by favorably modifying this biology (20), a substantial residual risk remains; thus, there exists a strong impetus to further our knowledge of the underlying pathobiology of atherosclerosis (21).

METABOLIC ACTIVITY OF SPLEEN AND PROINFLAMMATORY REMODELING OF LEUKOCYTES. Our data suggest that after ACS, increased metabolic activity of hematopoietic tissue (especially spleen), as assessed by FDG-PET, may represent an early step in a process that results in proinflammatory remodeling of leukocytes (especially monocytes). This concept is supported by our observation that CRP, a marker of systemic inflammation, was strongly correlated to BM and splenic activity. These findings are consistent with those of Kim et al. (22). Moreover, in the current study, we have extended the observations of Kim

FIGURE 4 Proportion Free of CVD Events Stratified by Bone Marrow, Spleen, and SAT Metabolic Activity



Individuals with higher (greater than or equal to median) **(A)** bone marrow activity (^{18}F-fluorodeoxyglucose [FDG] uptake) and **(B)** splenic activity at baseline had an increased risk for cardiovascular disease (CVD) events. Baseline subcutaneous adipose tissue (SAT) activity did not predict CVD events in individuals stratified into higher versus lower FDG values. **(C)** p Value from Kaplan-Meier survival curve was derived from log-rank test.

et al. (22) by providing novel data on the gene expression of proinflammatory leukocytes and by evaluating the association between splenic activity and subsequent CVD events. We showed that the gene expression of circulating proinflammatory monocytes (i.e., MSR-1 [macrophage scavenger receptor-1] [23,24], S100A9 [25], CD36 [24], interleukin-1β and TLR-4 [26]) was significantly correlated with BM and splenic activity. It is notable that proinflammatory gene activation within circulating leukocytes was more closely associated with metabolic activity of the spleen than it was for the BM. Several reasons may account for this observation. First, although the BM is an important and steady source of circulating leukocytes, the spleen acts as a large reservoir and may contribute substantially to the pool of circulating leukocytes after myocardial infarction (27). Second, the spleen has been shown to be an important locus of myeloid cell production in animal models of atherosclerosis and myocardial infarction (3). Hence, the activation state of the spleen (more than the BM) may closely relate to the proinflammatory state of circulating leukocytes, especially after injury or stress. Further studies are warranted to explore the precise underlying mechanisms.

CARDIOSPLENIC AXIS. Both the BM and spleen may be involved in a highly coordinated and dynamic biological interaction with the vascular wall that results in accelerated atherogenesis after ACS in humans. In pre-clinical studies, the role of BM and spleen activity after acute MI is established. Prior pre-clinical studies support our findings in ACS patients, demonstrating a paradigm in which BM- and spleen-derived inflammatory cells provoke vascular inflammation. Additionally, pre-clinical studies have demonstrated that activation of inflammatory

mononuclear cells in spleen plays a central role in progression of heart failure in response to cardiac-derived alarmins, which further emphasizes the presence of interplay between cardiovascular system and spleen-cardiosplenic axis (5). A recent autopsy study of patients after myocardial infarction showed that proinflammatory monocytes were depleted from the BM and spleen but were increased in atherosclerotic plaques, whereas the spleen experienced a pronounced reduction of monocytes, which suggests a prominent role of the spleen in trafficking monocytes to atherosclerotic plaques (28). The results of our clinical outcome study suggest that this interconnection between the spleen and arterial wall may be present in a stable setting as well.

In this study, we confirmed in humans the paradigm-shifting pre-clinical observation (3) that BM and splenic activity occurs after ACS and is associated with increased arterial wall inflammation. It has been shown that apolipoprotein E-deficient mice that lack BM-derived progenitor cells or spleen (3) have a reduction in atherosclerosis. In addition, this study for the first time raises the possibility that residual risk in stable patients might also be related to the spleen-atherosclerotic plaque axis. As such, the results of our study may encourage the development of novel therapeutic agents aimed at modulating the activity of progenitor cells to attenuate the systemic inflammatory milieu and its adverse clinical sequelae. The study also highlights that splenic metabolic activity may represent a novel target of future therapies designed to minimize atherosclerosis progression.

STUDY LIMITATIONS. Because of ethical concerns, BM and splenic biopsy samples were not obtained from patients, which limits histological correlation of the FDG signal. Flow cytometry analysis of peripheral blood leukocytes was not performed, thus limiting evaluation of monocyte subsets. In addition, a large proportion of patients with ACS (41% of the total study population) presented with unstable angina, and many did not have increased levels of cardiac biomarkers. Hence, it is possible that in a population with a greater degree of myocardial injury, BM and splenic activation may be greater than what was observed in the ACS population. In addition, the design of this study precludes assessment of splenic activity before ACS. Given the retrospective nature of the clinical outcomes study and the fact that the study population was drawn from a clinical database that consisted primarily of cancer survivors, the generalizability of the findings may be constrained. Other confounding factors might include history and type of cancer, as well as type of chemotherapy or radiotherapy. Although we tried to minimize the impact of these potential confounders through statistical adjustments, we believe our findings warrant confirmation in prospective studies. Lastly, although we observed compelling interrelationships between BM and splenic activation, leukocyte proinflammatory gene expression, and arterial inflammation, we acknowledge that these relationships do not necessarily indicate causality.

CONCLUSIONS

Our findings demonstrate that splenic metabolic activity is increased after ACS and correlates with proinflammatory remodeling of circulating leukocytes. Furthermore, we show for the first time that splenic activity correlates with arterial inflammation and is an independent predictor of the risk of incident CVD events. These observations suggest that the cardiosplenic axis may be clinically relevant in both acute and stable atherosclerotic vascular disease and provide impetus for further study of the mechanisms underlying the link between the hematopoietic tissues (BM and spleen) and progression of atherosclerotic CVDs.

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REFERENCES

1. Rosamond WD, Chambless LE, Folsom AR, et al. Trends in the incidence of myocardial infarction and in mortality due to coronary heart disease, 1987 to 1994. N Engl J Med 1998;339:861–7.

2. Thune JJ, Signorovitch JE, Kober L, et al. Predictors and prognostic impact of recurrent myocardial infarction in patients with left ventricular dysfunction, heart failure, or both following a first myocardial infarction. Eur J Heart Fail 2011;13:148–53.

3. Dutta P, Courties G, Wei Y, et al. Myocardial infarction accelerates atherosclerosis. Nature 2012;487:325–9.

4. Swirski FK, Nahrendorf M, Etzrodt M, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 2009;325:612–6.

5. Ismahil MA, Hamid T, Bansal SS, Patel B, Kingery JR, Prabhu SD. Remodeling of the mononuclear phagocyte network underlies chronic inflammation and disease progression in heart failure: critical importance of the cardiosplenic axis. Circ Res 2014;114:266–82.

6. Robbins CS, Hilgendorf I, Weber GF, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med 2013;19:1166–72.

7. Rudd JH, Narula J, Strauss HW, et al. Imaging atherosclerotic plaque inflammation by fluorodeoxyglucose with positron emission tomography: ready for prime time? J Am Coll Cardiol 2010;55:2527–35.

8. Lardinois D, Weder W, Hany TF, et al. Staging of non-small-cell lung cancer with integrated positron-emission tomography and computed tomography. N Engl J Med 2003;348:2500–7.

9. Jamar F, Buscombe J, Chiti A, et al. EANM/SNMMI guideline for 18F-FDG use in inflammation and infection. J Nucl Med 2013;54:647–58.

10. Garedew A, Henderson SO, Moncada S. Activated macrophages utilize glycolytic ATP to maintain mitochondrial membrane potential and prevent apoptotic cell death. Cell Death Differ 2010;17:1540–50.

11. Rodriguez-Prados J-C, Través PG, Cuenca J, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. J Immunol 2010;185:605–14.

12. Satomi T, Ogawa M, Mori I, et al. Comparison of contrast agents for atherosclerosis imaging using cultured macrophages: FDG versus ultrasmall superparamagnetic iron oxide. J Nucl Med 2013;54:999–1004.

13. Kubota R, Kubota K, Yamada S, Tada M, Ido T, Tamahashi N. Microautoradiographic study for the differentiation of intratumoral macrophages, granulation tissues and cancer cells by the dynamics of fluorine-18-fluorodeoxyglucose uptake. J Nucl Med 1994;35:104–12.

14. Higashi K, Ueda Y, Yagishita M, et al. FDG PET measurement of the proliferative potential of non-small cell lung cancer. J Nucl Med 2000;41:85–92.

15. Tawakol A, Fayad ZA, Mogg R, et al. Intensification of statin therapy results in a rapid reduction in atherosclerotic inflammation: results of a multicenter fluorodeoxyglucose-positron emission tomography/computed tomography feasibility study. J Am Coll Cardiol 2013;62:909–17.

16. Subramanian S, Tawakol A, Burdo TH, et al. Arterial inflammation in patients with HIV. JAMA 2012;308:379–86.

17. Libby P. Inflammation in atherosclerosis. Nature 2002;420:868–74.

18. Kempe S, Kestler H, Lasar A, Wirth T. NF-kappaB control subjects the global pro-inflammatory response in endothelial cells: evidence for the regulation of a pro-atherogenic program. Nucleic Acids Res 2005;33:5308–19.

19. Virmani R, Burke AP, Farb A, Kolodgie FD. Pathology of the unstable plaque. Prog Cardiovasc Dis 2002;44:349–56.

20. Murphy SA, Cannon CP, Wiviott SD, McCabe CH, Braunwald E. Reduction in recurrent cardiovascular events with intensive lipid-lowering statin therapy compared with moderate lipid-lowering statin therapy after acute coronary syndromes from the PROVE IT-TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22) trial. J Am Coll Cardiol 2009;54:2358–62.

21. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011;473:317–25.

22. Kim EJ, Kim S, Seo HS, Kang DO. The metabolic activity of the spleen and bone marrow in patients with acute myocardial infarction evaluated by 18F-FDG PET imaging. Circ Cardiovasc Imaging 2014;7:454–60.

23. Croce K, Gao H, Wang Y, et al. Myeloid-related protein-8/14 is critical for the biological response to vascular injury. Circulation 2009;120:427–36.

24. Piechota M, Banaszewska A, Dudziak J, Slomczynski M, Plewa R. Highly upregulated expression of CD36 and MSR1 in circulating monocytes of patients with acute coronary syndromes. Protein J 2012;31:511–8.

25. Averill MM, Barnhart S, Becker L, et al. S100A9 differentially modifies phenotypic states of neutrophils, macrophages, and dendritic cells: implications for atherosclerosis and adipose tissue inflammation. Circulation 2011;123:1216–26.

26. Blich M, Golan A, Arvatz G, et al. Macrophage activation by heparanase is mediated by TLR-2 and TLR-4 and associates with plaque progression. Arterioscler Thromb Vasc Biol 2013;33:e56–65.

27. Pittet MJ, Nahrendorf M, Swirski FK. The journey from stem cell to macrophage. Ann N Y Acad Sci 2014;1319:1–18. Review.

28. van der Laan AM, Ter Horst EN, Delewi R, et al. Monocyte subset accumulation in the human heart following acute myocardial infarction and the role of the spleen as monocyte reservoir. Eur Heart J 2013;35:376–85.

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APPENDIX For an expanded Methods section and supplemental tables and a figure, please see the online version of this paper.