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# Relationship of Serum Inflammatory Biomarkers With Plaque Inflammation Assessed by FDG PET/CT

## The dal-PLAQUE Study

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**OBJECTIVES** This study sought to longitudinally investigate the relationship between a broad spectrum of serum inflammatory biomarkers and plaque inflammation assessed by $^{18}$F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT).

**BACKGROUND** Both plaque inflammation and serum biomarkers of inflammation are associated with atherothrombotic events; however, the relationship between them is unclear.

**METHODS** We conducted a post hoc analysis of the dal-PLAQUE (A Randomized Placebo-Controlled Study of the Effect of RO4607381 on Progression or Regression of Atherosclerotic Plaque in Patients With Coronary Heart Disease [CHD] Including Patients With Other CHD Risk Factors), a randomized, placebo-controlled study of dalcetrapib, a cholesteryl ester transfer protein inhibitor, in 130 patients with coronary heart disease, or coronary heart disease risk equivalents on stable lipid-lowering therapy. Baseline and change after 3-month follow-up in inflammatory biomarker levels and baseline and change after 3-month follow-up in aorta and carotid $^{18}$F-FDG PET/CT (mean maximum target-to-background ratio of the most diseased segment [$TBR_{mds}$]) were analyzed.

**RESULTS** Baseline myeloperoxidase positively correlated with baseline carotid $TBR_{mds}$ (rho = 0.25, p = 0.02). This correlation remained at the 3-month follow-up and was independent of traditional cardiovascular disease risk factors. Baseline lipoprotein-associated phospholipase $A_2$ mass correlated with aorta $TBR_{mds}$ (rho = 0.21, p = 0.03). However, this correlation disappeared at the 3-month follow-up and was not independent of cardiovascular disease risk factors. There was no association between change from baseline in myeloperoxidase or lipoprotein-associated phospholipase $A_2$ mass and change from baseline in aorta and carotid $TBR_{mds}$. Baseline and change from baseline in high sensitivity C-reactive protein, interleukin 6, soluble P-selectin, soluble E-selectin, soluble intracellular adhesion molecule 1, soluble vascular cell adhesion molecule 1, and matrix-metalloproteinase 3 and 9 did not correlate with baseline or change from baseline in carotid or aorta $TBR_{mds}$.

**CONCLUSIONS** Our data show that, in patients with coronary heart disease or at high risk of coronary heart disease on stable lipid-lowering therapy, circulating myeloperoxidase levels are associated with carotid plaque inflammation. (A Randomized, Placebo-controlled Study of the Effect of RO4607381 on Progression or Regression of Atherosclerotic Plaque in Patients With Coronary Heart Disease [CHD] Including Patients With Other CHD Risk Factors [dal-PLAQUE]; NCT00655473) (J Am Coll Cardiol Img 2013;6:1087–94) © 2013 by the American College of Cardiology Foundation

Inflammation is increasingly being recognized as a pivotal feature of atherosclerosis and a potential target for therapy (1). Pathology studies have shown that the immediate site of plaque rupture contains a high concentration of inflammatory cells (2). In subjects who die of acute myocardial infarction, plaques throughout the coronary arteries are diffusely infiltrated by inflammatory cells, in contrast to patients who die of other causes, suggesting a generally inflamed state of the vasculature in patients with acute coronary syndromes (3). Also, various serum inflammatory biomarkers have been identified as independent predictors of future atherothrombotic events (4–13). Thus, both plaque inflammation and serum biomarkers of inflammation are associated with atherothrombotic events; however, little is known about the relationship between the two.

**ABBREVIATIONS AND ACRONYMS**

**$^{18}$F-FDG PET/CT** = fluorodeoxyglucose F 18 positron emission tomography/computed tomography

**hsCRP** = high-sensitivity C-reactive protein

**IL-6** = interleukin 6

**Lp-PLA$_2$** = lipoprotein-associated phospholipase A$_2$

**MMP** = matrix metalloproteinase

**MPO** = myeloperoxidase

**sE-selectin** = soluble E-selectin

**sICAM** = soluble intracellular adhesion molecule

**sP-selectin** = soluble P-selectin

**sVCAM** = soluble vascular cell adhesion molecule

**TBR$_{mds}$** = target-to-background ratio of the most diseased segment

Recent advances in the development of noninvasive imaging techniques have enabled quantification of vessel wall inflammation with $^{18}$F-fluorodeoxyglucose positron emission tomography/computed tomography ($^{18}$F-FDG PET/CT). Inflammatory cells have high metabolic activity, especially when they are activated, which markedly enhances their $^{18}$F-FDG uptake against background tissues (14). Studies in experimental atherosclerotic rabbit models, as well as human endarterectomy studies, have shown that $^{18}$F-FDG accumulation in plaque correlates with plaque macrophage content (15,16). Some studies have previously investigated the relationship between serum biomarkers of inflammation and vessel wall $^{18}$F-FDG PET/CT; however, these studies either included a small number of patients or lacked longitudinal follow-up, and their results are contradictory (17–21).

In the current study, we therefore longitudinally investigated the relationship between a broad panel of serum biomarkers of inflammation with aorta and carotid $^{18}$F-FDG PET/CT through post hoc analyses in the dal-PLAQUE (A Randomized Placebo-Controlled Study of the Effect of RO4607381 on Progression or Regression of Atherosclerotic Plaque in Patients With Coronary Heart Disease [CHD] Including Patients With Other CHD Risk Factors) study (22).

## METHODS

The design and study methods of the dal-PLAQUE study have been described previously (22). In summary, dal-PLAQUE was a phase-2b double-blind, randomized, placebo-controlled, multicenter study to investigate the effect of dalcetrapib, a cholesteryl ester transfer protein inhibitor, on vessel wall inflammation assessed by $^{18}$F-FDG PET/CT. Participants were adults with either previous known coronary heart disease or at high risk of coronary heart disease with triglyceride concentrations ≤400 mg/dl, low-density lipoprotein cholesterol concentrations <100 mg/dl (or on maximum tolerated doses of statins), and carotid or aortic arterial wall (target) to background (blood) ratio (TBR) of 1.6 or higher. Details of $^{18}$F-FDG PET/CT imaging procedures and analyses have been published previously (22). For the current analyses, we used baseline values and change after 3 months of follow-up of mean maximum TBR of the most diseased segment (TBR$_{mds}$).

We examined inflammatory biomarker data for baseline and change from baseline after 3-month follow-up. Inflammatory biomarker assays were performed by Medpace Reference Laboratories (Medpace Inc., Cincinnati, Ohio). Serum high-sensitivity C-reactive protein (hsCRP) levels were assessed by immunonephelometry using a BNII analyzer (Dade

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Behring, Deerfield, Illinois). Serum levels of interleukin (IL)-6, soluble P-selectin (sP-selectin), and soluble E-selectin (sE-selectin) were determined using Millipore immunodetection kits and the Luminex system (Millipore, Billerica, Massachusetts). Plasma levels of soluble intracellular adhesion molecule (sICAM)-1 and soluble vascular cell adhesion molecule (sVCAM)-1, and serum levels of matrix metalloproteinase (MMP)-3 and -9 were determined using analyte-specific sandwich enzyme-linked immunosorbent assay kits from R&D systems (Minneapolis, Minnesota) and a Tecan Sunrise microplate reader (Tecan Group Ltd., Männedorf, Switzerland). Myeloperoxidase (MPO) levels in heparin plasma were determined using the Prognostix CardioMPO enzyme-linked immunosorbent assay kit (Cleveland Heart Lab, Cleveland, Ohio). Lipoprotein-associated phospholipase $A_2$ (Lp-$PLA_2$) mass was assessed using enzyme-linked immunosorbent assay with PLAC Test reagents (diaDexus, San Francisco, California) at Berkeley HeartLab (Alameda, California).

**Statistical methods.** All continuous variables are expressed as mean ± SD and categorical data are expressed as absolute numbers and percentages. Pearson correlation coefficients and Student *t* tests were used to assess the association between aorta and carotid $TBR_{mds}$ and traditional cardiovascular disease risk factors (age, male sex, body mass index, current smoking, hypertension, diabetes mellitus, history of coronary heart disease, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides). Spearman rhos were used to assess the association between baseline and change from baseline in serum inflammatory biomarkers and baseline and change from baseline in $TBR_{mds}$. Student *t* tests were used to compare $TBR_{mds}$ in the highest and lowest quartiles of MPO and Lp-$PLA_2$ mass. Multivariate linear regression analyses were used to compare $TBR_{mds}$ in the highest and lowest quartiles of MPO and Lp-$PLA_2$ mass, adjusting for traditional cardiovascular disease risk factors. $TBR_{mds}$ was the response variable, and the highest and lowest quartiles of MPO and Lp-$PLA_2$ mass and traditional cardiovascular disease risk factors were the explanatory variables. All statistical analyses were performed using IBM SPSS statistical package (version 19.0, Armonk, New York).

## RESULTS

This study included 130 patients. Patient characteristics have been previously published (22). Eligible serum inflammatory biomarker data at baseline and 3-month follow-up were obtained in 128 and 125 patients, respectively. Eligible aorta $^{18}$F-FDG PET/CT data at baseline and 3-month follow-up were obtained in 105 and 104 patients, respectively. Eligible carotid $^{18}$F-FDG PET/CT data at baseline and 3-month follow-up were obtained in 87 and 50 patients, respectively.

Concerning the relationship between $^{18}$F-FDG PET/CT and traditional cardiovascular risk factors, baseline aorta $TBR_{mds}$ correlated with baseline low-density lipoprotein cholesterol ($r = 0.29$, $p = 0.04$), and a higher baseline carotid $TBR_{mds}$ was found in male subjects (1.75 ± 0.23 vs. 2.08 ± 0.44, $p = 0.004$). Higher increase from baseline aorta $TBR_{mds}$ was found in hypertensive subjects and nonsmokers (−0.46 ± 0.40 vs. −0.12 ± 0.41, $p = 0.02$; and −0.15 ± 0.42 vs. −0.51 ± 0.31, $p = 0.05$, respectively), and higher increase from baseline in carotid $TBR_{mds}$ was associated with higher age ($r = 0.49$, $p = 0.01$).

**Serum inflammatory biomarkers and $^{18}$F-FDG PET/CT imaging.** At baseline, MPO levels were associated with carotid $TBR_{mds}$ (Table 1). When baseline MPO levels were divided into quartiles, subjects in the highest MPO quartile had significantly higher carotid $TBR_{mds}$ values than did those in the lowest MPO quartile (Fig. 1). This relation remained present at 3-month follow-up and was independent of cardiovascular disease risk factors. There was no association between change from baseline in MPO and change from baseline in aorta and carotid $TBR_{mds}$.

At baseline, Lp-$PLA_2$ mass levels were associated with aorta $TBR_{mds}$ (Table 1). However, when baseline Lp-$PLA_2$ mass levels were divided into quartiles, there was no significant difference between subjects in the highest quartile versus in the lowest Lp-$PLA_2$ mass quartile (Fig. 2).

The other baseline serum biomarkers of inflammation were not associated with baseline or change from baseline in aorta and carotid $TBR_{mds}$ (Tables 1 and 2). Similarly, changes from baseline in serum biomarkers of inflammation were not associated with changes from baseline in aorta and carotid $TBR_{mds}$ (Table 3).

The only observed differences in change from baseline in serum inflammatory biomarkers between the dalcetrapib and placebo group (data not shown) were an increase in IL-6 (2.15 ± 7.83 vs. −0.28 ± 3.41, $p = 0.03$) and Lp-$PLA_2$ mass (10.7 ± 40.2 vs. −2.79 ± 27.5, $p = 0.03$), and a decrease in sP-selectin (0.55 ± 12.8 vs. 5.14 ± 12.0, $p = 0.04$) in the dalcetrapib versus placebo group after 3-month follow-up.

**Table 1. Spearman Rhos to Assess the Correlation Between Baseline Inflammatory Biomarkers and Baseline Aorta and Carotid $TBR_{mds}$ Assessed by $^{18}$F-FDG PET/CT**

| | Aorta $TBR_{mds}$ | | Carotid $TBR_{mds}$ | |
|---|---|---|---|---|
| | Rho | p Value | Rho | p Value |
| High-sensitivity C-reactive protein | 0.07 | 0.51 | 0.05 | 0.64 |
| Lipoprotein-associated phospholipase $A_2$ mass | 0.21 | 0.03* | 0.11 | 0.32 |
| Interleukin-6 | −0.05 | 0.60 | 0.03 | 0.77 |
| Soluble P-selectin | 0.17 | 0.09 | 0.14 | 0.20 |
| Soluble E-selectin | −0.13 | 0.18 | −0.10 | 0.36 |
| Soluble intracellular adhesion molecule-1 | −0.02 | 0.81 | −0.18 | 0.11 |
| Soluble vascular cell adhesion molecule-1 | 0.03 | 0.80 | −0.15 | 0.18 |
| Matrix metalloproteinase-3 | 0.02 | 0.85 | −0.03 | 0.76 |
| Matrix metalloproteinase-9 | 0.16 | 0.10 | 0.20 | 0.06 |
| Myeloperoxidase | 0.19 | 0.06 | 0.25 | 0.02* |

*Significant correlations.
$^{18}$F-FDG PET/CT = fluorodeoxyglucose F 18 positron emission tomography/computed tomography; $TBR_{mds}$ = mean maximum most diseased segment target-to-background ratio.

## DISCUSSION

The aim of the present post hoc analysis in the dal-PLAQUE study was to determine the relationship between serum inflammatory biomarkers and local vessel wall inflammation quantified by $^{18}$F-FDG PET/CT. We observed a positive correlation between baseline serum MPO and baseline carotid $TBR_{mds}$. This relation remained present at 3-month follow-up and was independent of traditional risk factors. Baseline Lp-$PLA_2$ mass positively correlated with baseline aorta $TBR_{mds}$. However, this relationship disappeared after 3 months of follow-up and was not independent of traditional risk factors.

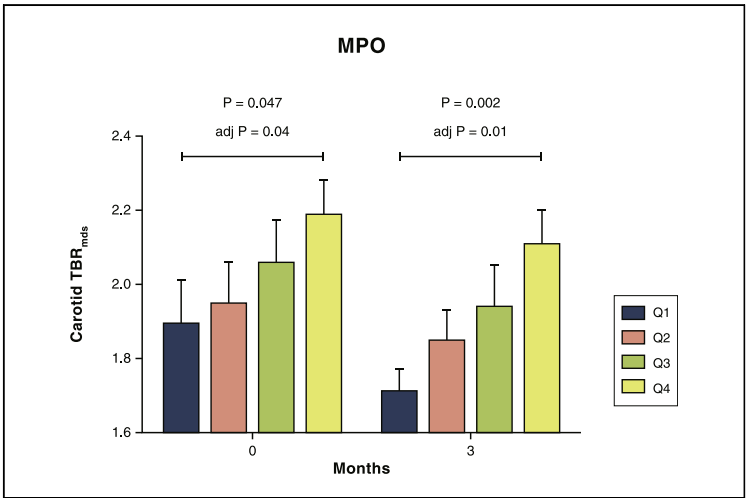


**Figure 1. Carotid $TBR_{mds}$ Values at Baseline and 3-Month Follow-Up for Baseline MPO Quartiles**

Higher baseline myeloperoxidase (MPO) values were associated with higher baseline carotid target-to-background ratio of the most diseased segment ($TBR_{mds}$) values, and this relation remained present at 3-month follow-up. The p values are for the comparison between the lowest and highest quartiles (Q). Adj P = p values adjusted for age, sex, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, hypertension, diabetes mellitus, history of coronary heart disease, and current smoking.

**Traditional risk factors for cardiovascular disease and vessel wall inflammation.** Previous cross-sectional studies have investigated the relationship between traditional risk factors and vessel wall inflammation. In patients who underwent $^{18}$F-FDG PET/CT imaging for cancer screening, carotid $^{18}$F-FDG uptake was associated with waist circumference, hypertension, glucose intolerance, and metabolic syndrome (23). In patients with known coronary artery disease or multiple cardiovascular risk factors, carotid $^{18}$F-FDG uptake was higher in patients with known coronary artery disease and in men (17). In another study including coronary artery disease patients, carotid $^{18}$F-FDG uptake was associated with obesity, increasing age, hypertension, smoking, and male sex (24). A recent study found higher carotid $^{18}$F-FDG uptake in diabetic patients than in nondiabetic patients (25).

The current study—the first to assess the relationship between cardiovascular disease risk factors and change in vessel wall $^{18}$F-FDG uptake longitudinally—corroborates these findings in that we found age and hypertension to be important predictors for increase in vessel wall inflammation. Baseline aorta $TBR_{mds}$ positively correlated with baseline low-density lipoprotein cholesterol, and baseline carotid $TBR_{mds}$ was higher in male subjects. Change from baseline in aorta $TBR_{mds}$ was higher in hypertensive subjects and surprisingly also in nonsmokers. Change from baseline in carotid $TBR_{mds}$ positively correlated with age. However, we did not find a relationship among body mass index, diabetes, and vessel wall inflammation, which were found to be so markedly related in previous studies.

One explanation is the selected population. One inclusion criterion was triglyceride concentrations ≤400 mg/dl. Serum triglyceride levels are strongly associated with body mass index and diabetes, and therefore this might have obscured the relationship between body mass index and diabetes with vessel wall inflammation. Furthermore, all subjects were on statin therapy with either low-density lipoprotein cholesterol <100 mg/dl or on maximum tolerated doses of statins, which might also have influenced the relationship between traditional cardiovascular disease risk factors and vessel wall inflammation.

**Inflammatory biomarkers and vessel wall inflammation.** Various studies have investigated the relationship between serum biomarkers of inflammation and vessel wall $^{18}$F-FDG uptake. However, ours is the first study to investigate a broad panel of inflammatory biomarkers and their relationship with vessel wall inflammation quantified by $^{18}$F-FDG PET/CT in a longitudinal study.

**CRP AND IL-6.** Both increased CRP and IL-6 are associated with increased cardiovascular disease risk (4,5). Previous studies have shown contradicting results regarding the relationship between vessel wall $^{18}$F-FDG uptake and CRP and IL-6. Yoo et al. (19) observed in 120 healthy individuals that mean and maximum carotid TBR correlated with hsCRP. Yang et al. (20) performed $^{18}$F-FDG PET/CT in 142 nondiabetic subjects without history of cardiovascular disease and showed that carotid mean TBR was higher in subjects with a higher hsCRP (≥2.0 mg/dl) than in subjects with a lower hsCRP (<2.0 mg/dl). On the other hand, a study by Rudd et al. (17) in 33 patients with known coronary artery disease or multiple cardiovascular risk factors found no correlation between carotid and aorta $^{18}$F-FDG uptake and serum CRP, IL-6, and other markers of systemic inflammation, namely IL-10, IL-18, and tumor necrosis factor-alpha (17). Similarly, Wu et al. (18) did not observe a relationship between hsCRP levels and $^{18}$F-FDG PET/CT in patients with carotid stenosis ≥70%. In another study by Wu et al. (21), in 43 statin-naive subjects with clinical evidence of atherosclerosis, mean TBR of various segments of the aorta, and iliofemoral arteries did not correlate with hsCRP. Interestingly, the 2 studies in which CRP correlated with $^{18}$F-FDG PET/CT were both performed in healthy individuals. In contrast, the 4 studies (including our current report) that did not show a relationship among vessel wall inflammation, CRP and IL-6 were all conducted in subjects with atherosclerotic disease.

**LP-PLA2.** Increased serum levels of Lp-PLA$_2$ mass and activity are associated with increased risk of cardiovascular events (8,9). One previous study has investigated the relationship between Lp-PLA$_2$ and vessel wall $^{18}$F-FDG uptake and observed that, in 120 healthy individuals, mean and maximum carotid TBR did not correlate with Lp-PLA$_2$ (19). In the current study, we found a correlation between baseline Lp-PLA$_2$ and baseline aorta TBR$_{mds}$. However, when Lp-PLA$_2$ mass levels were divided into quartiles, there was no significant difference in aorta TBR$_{mds}$ between the highest versus the lowest Lp-PLA$_2$ mass quartile at baseline and 3-month follow-up.

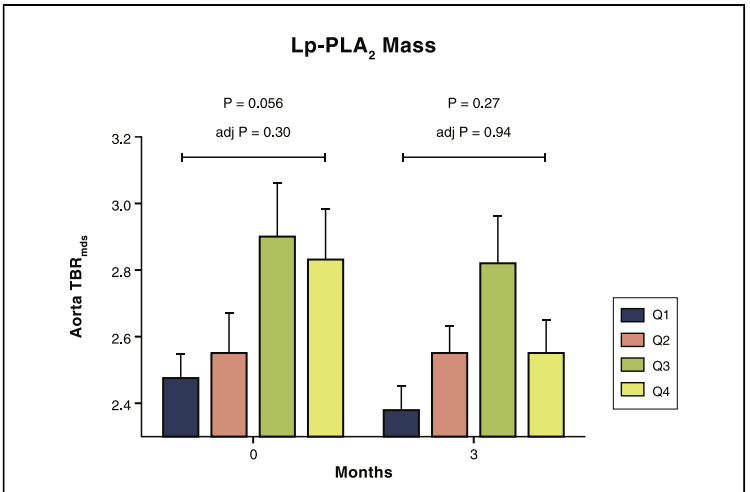


**Figure 2. Aortic TBR$_{mds}$ Values at Baseline and 3-Month Follow-Up for Baseline Lp-PLA$_2$ Mass Quartiles**

Although there is a trend toward higher baseline aorta TBR$_{mds}$ in higher baseline lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) mass quartiles, this relation is not independent of other risk factors and the relation disappears after 3-month follow-up. The p values are for the comparison between the lowest and highest quartiles. Abbreviations as in Figure 1.

**SP-SELECTIN, SE-SELECTIN, SICAM-1, AND SVCAM-1.** Increased serum levels of P-selectin, E-selectin, ICAM-1, and VCAM-1 are predictive of coronary heart disease (10,11). One study performed by Wu et al. (21) investigated the relationship among ICAM-1, VCAM-1, and E-selectin, and mean TBR of various segments of the aorta and iliofemoral arteries in statin-naive subjects with

**Table 2. Spearman Rhos to Assess the Correlation Between Baseline Inflammatory Biomarkers and Change From Baseline in Aorta and Carotid TBR$_{mds}$ Assessed by $^{18}$F-FDG PET/CT**

| | Aorta TBR$_{mds}$ | | Carotid TBR$_{mds}$ | |
|---|---|---|---|---|
| | Rho | p Value | Rho | p Value |
| High-sensitivity C-reactive protein | −0.11 | 0.25 | 0.02 | 0.91 |
| Lipoprotein-associated phospholipase A$_2$ mass | −0.16 | 0.10 | −0.07 | 0.62 |
| Interleukin-6 | 0.16 | 0.11 | 0.07 | 0.63 |
| Soluble P-selectin | −0.17 | 0.09 | −0.16 | 0.28 |
| Soluble E-selectin | 0.00 | 0.98 | 0.05 | 0.75 |
| Soluble intracellular adhesion molecule-1 | −0.14 | 0.16 | 0.14 | 0.32 |
| Soluble vascular cell adhesion molecule-1 | −0.02 | 0.84 | 0.19 | 0.20 |
| Matrix metalloproteinase-3 | 0.01 | 0.96 | 0.16 | 0.26 |
| Matrix metalloproteinase-9 | −0.15 | −0.14 | −0.14 | 0.35 |
| Myeloperoxidase | 0.04 | 0.69 | −0.09 | 0.55 |

Abbreviations as in Table 1.

**Table 3. Spearman Rhos to Assess the Correlation Between Change From Baseline Inflammatory Biomarkers and Change From Baseline in Aorta and Carotid $TBR_{mds}$ Assessed by $^{18}$F-FDG PET/CT**

| | Aorta $TBR_{mds}$ | | Carotid $TBR_{mds}$ | |
|---|---|---|---|---|
| | Rho | p Value | Rho | p Value |
| High-sensitivity C-reactive protein | −0.09 | 0.56 | −0.02 | 0.95 |
| Lipoprotein-associated phospholipase $A_2$ mass | −0.27 | 0.06 | −0.28 | 0.19 |
| Interleukin-6 | 0.08 | 0.59 | 0.12 | 0.59 |
| Soluble P-selectin | −0.03 | 0.83 | −0.11 | 0.61 |
| Soluble E-selectin | −0.03 | 0.86 | −0.10 | 0.64 |
| Soluble intracellular adhesion molecule-1 | −0.03 | 0.85 | −0.21 | 0.33 |
| Soluble vascular cell adhesion molecule-1 | −0.03 | 0.83 | −0.24 | 0.27 |
| Matrix-metalloproteinase-3 | 0.17 | 0.25 | −0.05 | 0.83 |
| Matrix-metalloproteinase-9 | −0.02 | 0.89 | −0.02 | 0.92 |
| Myeloperoxidase | 0.21 | 0.16 | 0.33 | 0.11 |

Abbreviations as in Table 1.

clinical evidence of atherosclerosis. Similar to the results of our analyses, they did not find a correlation between these biomarkers and vessel wall inflammation.

**MMP-3 AND MMP-9.** Epidemiological studies have shown that increased serum MMP-3 and MMP-9 levels are associated with increased future risk of atherothrombotic events (12,13). Two studies have investigated the relationship among MMP-3, MMP-9, and vessel wall $^{18}$F-FDG uptake. The first study was performed by Rudd et al. (17) in patients with known coronary artery disease or multiple cardiovascular risk factors. They showed a positive correlation among MMP-3 and MMP-9 levels and vessel wall $^{18}$F-FDG uptake. The second study was performed by Wu et al. (21) in statin-naive subjects with clinical evidence of atherosclerosis. All subjects were treated with atorvastatin for 12 weeks. At baseline, mean TBR of various segments of the aorta and iliofemoral arteries correlated with MMP-9. The correlation between $^{18}$F-FDG uptake and MMP-9 was not independent of other risk factors. The decline in mean TBR after statin treatment correlated with the MMP-9 reduction. Our study does not corroborate with both previous studies, because we did not find a relationship among MMP-3, MMP-9, and vessel wall $^{18}$F-FDG uptake.

**MPO.** MPO is a peroxidase identified in atherosclerotic plaques (26) with pro-atherogenic effects that comprise oxidation of LDL (27) and oxidative modification of apolipoprotein A-I, altering its capacity to promote cholesterol efflux (28). Its effects on nitric oxide bioavailability and induction of hypochlorous acids result in endothelial dysfunction (29) and can promote endothelial cell apoptosis and detachment, making plaques prone to rupture (30).

Our study is the first to investigate the relationship between MPO and vessel wall $^{18}$F-FDG uptake. We observed a marked correlation between MPO and carotid $TBR_{mds}$ at baseline. This correlation remained present at 3-month follow-up. Baseline and change in MPO levels were not associated with change in carotid $TBR_{mds}$. Apparently, subjects with higher MPO levels have a constantly higher degree of vessel wall inflammation.

Daugherty et al. (26) investigated the presence of MPO in atherosclerotic lesions. Interestingly, they observed striking colocalization of immunostaining for MPO and macrophages in atherosclerotic plaques. In fact, both were especially prominent in shoulder regions of the plaques. Thus, vessel wall $^{18}$F-FDG uptake correlates with plaque macrophage content (15,16), and plaque macrophage content correlates with plaque MPO content (26). This makes it biologically plausible that MPO levels can show a relationship with vessel wall $^{18}$F-FDG uptake.

**Study limitations.** First, we pooled the data of serum biomarkers of inflammation and $^{18}$F-FDG PET/CT of both the dalcetrapib- and placebo-treated patients. However, we did not observe an effect of dalcetrapib on $TBR_{mds}$ or serum biomarkers of inflammation, except for IL-6, Lp-$PLA_2$, and sP-selectin. Second, MPO was measured in plasma. MPO levels may be related to leukocytes. Leukocytes are a known, strong predictor of cardiovascular disease. Unfortunately, we could not assess cellular MPO, as there are no samples left from the study. Of note, most publications refer to serum MPO levels when predicting cardiovascular disease risk. Third, we investigated a selected population with a high risk of coronary heart disease, all on statin therapy with low-density lipoprotein cholesterol concentrations <100 mg/dl or on maximum tolerated doses of statins. The fact that we investigated a small and limited patient collective (no patients without disease were included) might have obscured the relationship between vessel wall inflammation and circulating markers of inflammation. Fourth, although this is an exploratory post hoc analysis, we did not account for multiple testing, which makes our analysis prone to type 1 error. Furthermore, many inflammatory markers correlate with each other,

making correction for multiple testing too rigorous. Finally, it is unknown if vessel wall inflammation measured by $^{18}$F-FDG PET/CT is predictive for future cardiovascular events. Longitudinal studies are currently ongoing to investigate such a relation (31).

## CONCLUSIONS

Of the broad panel of inflammatory biomarkers evaluated in this longitudinal study, we observed a positive correlation between baseline serum MPO and baseline carotid $TBR_{mds}$. This relation remained present at 3-month follow-up and was independent of traditional cardiovascular disease risk factors. The other inflammatory biomarkers did not correlate with aorta and carotid $TBR_{mds}$. Our data show that, in patients with coronary heart disease or at high risk of coronary heart disease on stable lipid-lowering therapy, circulating MPO levels are associated with carotid plaque inflammation.

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