ORIGINAL RESEARCH

BASIC AND TRANSLATIONAL RESEARCH

Intraplaque Myeloperoxidase Activity as Biomarker of Unstable Atheroma and Adverse Clinical Outcomes in Human Atherosclerosis

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ABSTRACT

BACKGROUND The detection of unstable atherosclerosis remains elusive. Intraplaque myeloperoxidase (MPO) activity causes plaque destabilization in preclinical models, holding promise for clinical translation as a novel imaging biomarker.

OBJECTIVES The purpose of this study was to assess whether MPO activity is greater in unstable human plaques, how this relates to cardiovascular events and current/emerging non-invasive imaging techniques.

METHODS Thirty-one carotid endarterectomy specimens and 12 coronary trees were collected. MPO activity was determined in 88 individual samples through the conversion of hydroethidine to the MPO-specific adduct 2-chloroethidium and compared with macroscopic validation, histology, clinical outcomes, and computed tomography–derived high and low attenuation plaques and perivascular adipose tissue. Non-parametric statistical analysis utilizing Mann-Whitney U and Kruskal-Wallis tests for univariate and group comparisons were performed.

RESULTS Unstable compared with stable plaque had higher MPO activity (carotid endarterectomy: n = 26, 4.2 ± 3.1 vs 0.2 ± 0.3 nmol/mgp; $P < 0.0001$; coronary: n = 17, 0.6 ± 0.5 vs 0.001 ± 0.003 nmol/mgp; $P = 0.0006$). Asymptomatic, stroke-free patients had lower MPO activity compared to those with symptoms or ipsilateral stroke (n = 12, 3.7 ± 2.1 vs 0.1 ± 0.2 nmol/mgp; $P = 0.002$). Computed tomography–determined plaque attenuation did not differentiate MPO activity (n = 30, 0.1 ± 0.1 vs 0.2 ± 0.3 nmol/mgp; $P = 0.23$) and MPO activity was not found in perivascular adipose tissue.

CONCLUSIONS MPO is active within unstable human plaques and correlates with symptomatic carotid disease and stroke, yet current imaging parameters do not identify plaques with active MPO. As intraplaque MPO activity can be imaged non-invasively through novel molecular imaging probes, ongoing investigations into its utility as a diagnostic tool for high-risk atherosclerosis is warranted. (JACC Adv 2023;2:100310) © 2023 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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ABBREVIATIONS AND ACRONYMS

2-Cl-E⁺ = 2-chloro-ethidium

CEA = carotid endarterectomy

CRP = C-reactive protein

CT = computed tomography

CTCA = computed tomography coronary angiography

LAP = low attenuation plaque

MPO = myeloperoxidase

PVAT = perivascular adipose tissue

Myeloperoxidase (MPO) is implicated in chronic inflammatory diseases including atherosclerosis.[1,2] MPO generates highly reactive hypochlorite as part of the innate immune response against phagocytosed pathogens. However, via degranulation, apoptosis, or extracellular traps, up to 30% of cellular MPO can be released into the extracellular space,[3,4] where it causes deleterious tissue injury and has been linked to atherogenesis, plaque destabilization, erosion, and rupture.[5-7]

Immunohistochemical studies indicate increased MPO protein-expressing macrophages and hypochlorite-modified proteins in ruptured and eroded human plaques as well as atheromatous lesions.[2,8] However, the association between plaque stability and direct/quantitative measures of intraplaque MPO activity in humans remains unknown. In an animal model of plaque instability, MPO activity was higher in unstable than stable lesions, and genetic or pharmacological blockade of MPO activity attenuated the formation of unstable plaque, thus implicating MPO as causal to plaque destabilization.[9]

Currently there remains limited means to detect and treat high-risk atherosclerosis,[10] with most measures representing surrogates of plaque instability or disease activity. Available molecular probes such as ¹⁸F-fluorodeoxyglucose and 68-gallium dotatate have technical challenges related to myocardial spillover and remain pathologically non-specific to plaque destabilization. Thus, despite overwhelming evidence that plaque composition and biological activity are the most accurate determinants of acute events,[11,12] the major qualifier for escalation of therapy and intervention remains the degree of luminal stenosis—a parameter that has failed to accurately predict cardiovascular events.[13,14]

Computed tomography coronary angiography (CTCA) remains the most widely available non-invasive imaging tool to detect atherosclerosis. Utilizing data sets that correlate histology with clinical outcomes resulted in the development of determinants of stable and higher risk plaques.[15,16]

Broadly speaking, CTCA-derived high-risk plaques demonstrate low attenuation and can have microcalcifications and/or positive remodeling, whereas plaques with attenuation of ≥1,000 HU appear less likely to cause major adverse events.[17] These "1K" plaques are heavily calcified and do not result in higher morbidity and mortality irrespective of stenotic grade when compared to non-obstructive, high-risk plaques.[18] Nevertheless, CTCA cannot assess biological activity, and here novel molecular probes have shown promise, though are not commonly utilized because of limitations in their predictive value.[10] To compensate for CTCAs inability to assess pathobiological activity, recent interests have turned to examining perivascular adipose tissue (PVAT) attenuation as a determinant of arterial inflammation,[19] with high fat attenuation indices appearing to correlate to future cardiac events.[20]
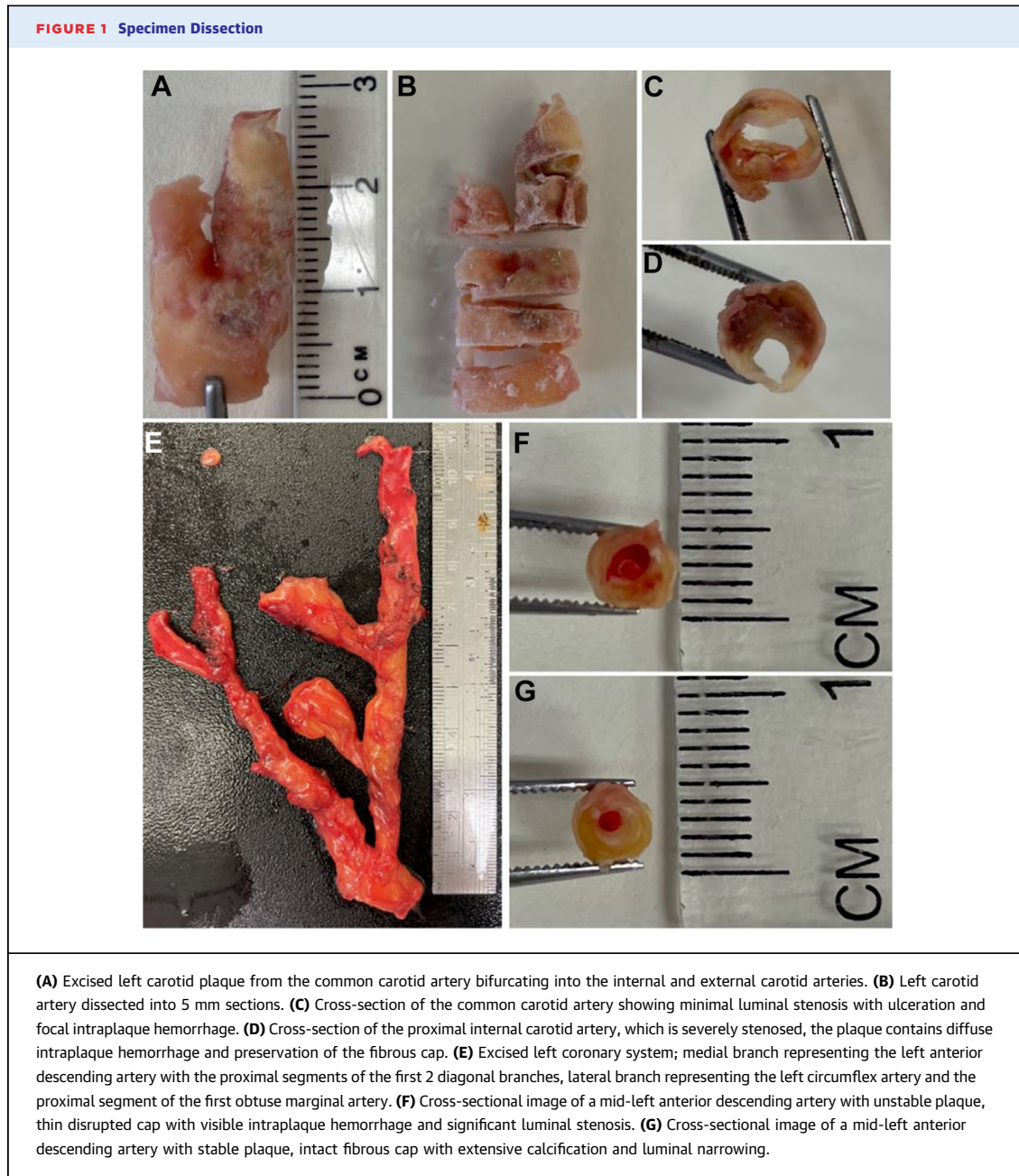
We hypothesized that MPO activity is increased in unstable human coronary and carotid plaques,[21] making it a potential plaque-based biomarker of high-risk atherosclerosis. Additionally, we questioned whether current and emerging non-invasive imaging modalities utilized for the detection of vulnerable atherosclerosis and vascular inflammation could reliably reflect intraplaque MPO activity.

METHODS

RECRUITMENT, TISSUE COLLECTION, AND MACROSCOPIC PLAQUE ASSESSMENT.

Tissues were collected from participants at St. Vincent's Hospital, Sydney, Australia between February 2020 and November 2021. Carotid endarterectomy (CEA) specimens were procured from 31 patients with symptomatic or asymptomatic severe carotid artery disease. Blood samples were collected prior to surgery for biochemical analysis and plasma MPO quantification by enzyme-linked immunoadsorbent assay.

CEA specimens were obtained at the time of excision and purged of intra and extraluminal blood using phosphate buffered saline. Tissues were photographed intact then dissected into 5 mm sections and rephotographed (Figures 1A to 1D). Each CEA section
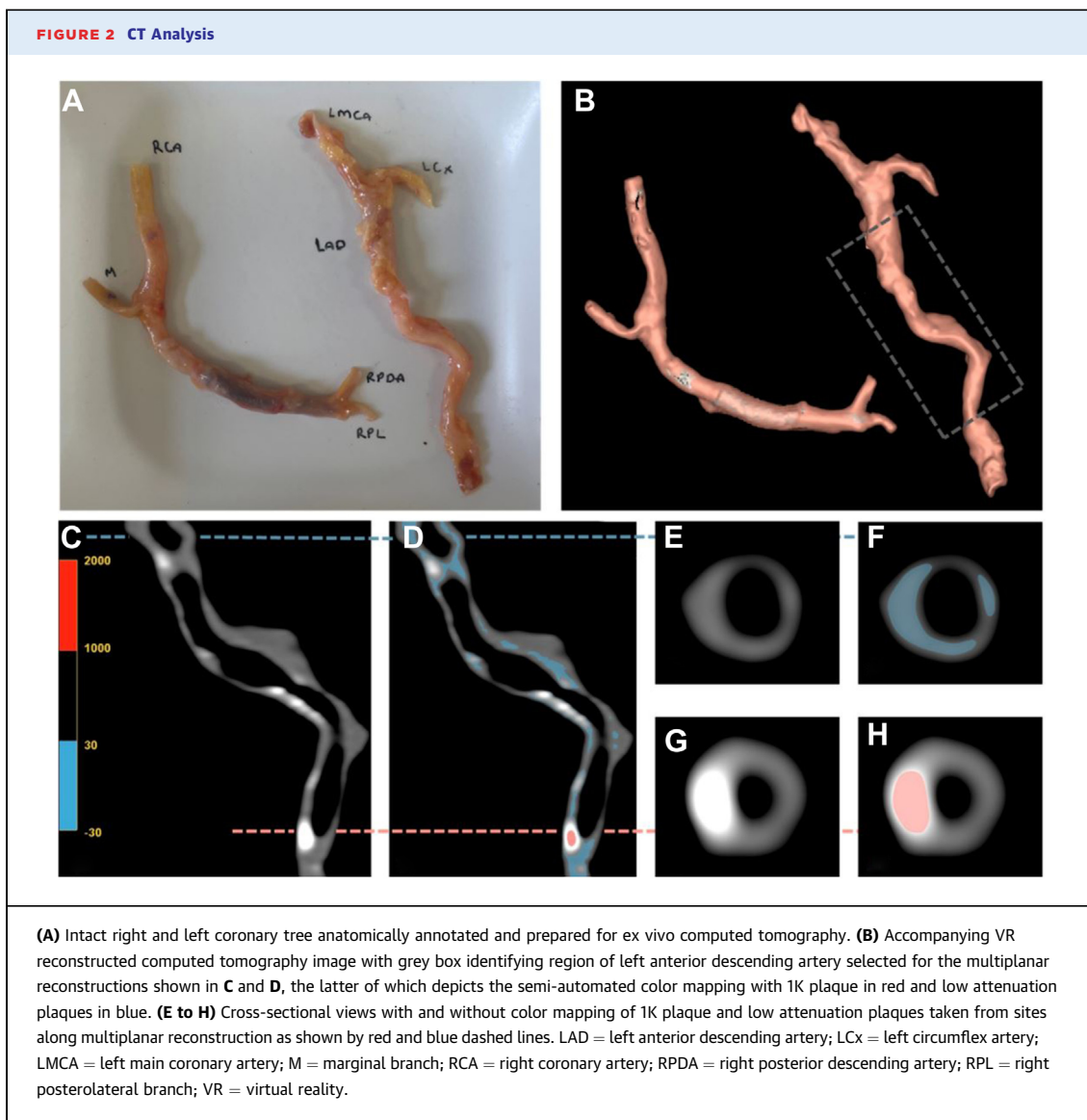
FIGURE 1 Specimen Dissection

(A) Excised left carotid plaque from the common carotid artery bifurcating into the internal and external carotid arteries. (B) Left carotid artery dissected into 5 mm sections. (C) Cross-section of the common carotid artery showing minimal luminal stenosis with ulceration and focal intraplaque hemorrhage. (D) Cross-section of the proximal internal carotid artery, which is severely stenosed, the plaque contains diffuse intraplaque hemorrhage and preservation of the fibrous cap. (E) Excised left coronary system; medial branch representing the left anterior descending artery with the proximal segments of the first 2 diagonal branches, lateral branch representing the left circumflex artery and the proximal segment of the first obtuse marginal artery. (F) Cross-sectional image of a mid-left anterior descending artery with unstable plaque, thin disrupted cap with visible intraplaque hemorrhage and significant luminal stenosis. (G) Cross-sectional image of a mid-left anterior descending artery with stable plaque, intact fibrous cap with extensive calcification and luminal narrowing.

was inspected macroscopically by 2 blinded assessors for features of unstable or stable atherosclerosis, utilizing determinants derived from the American Heart Association's consensus group's histological classification of atherosclerosis (Supplemental Figure 1).[22] Where discordance between macroscopic plaque assessment occurred, plaques were examined together to reach a final decision on plaque phenotype. Once assessed, each section was bisected: one-half undergoing histological assessment to confirm

plaque type and the other half being analyzed for MPO activity.

Cardiac tissue was procured at the time of heart transplantation from 12 recipients with ischemic cardiomyopathy, on average 14 months after any acute coronary syndrome. Within an hour of cardiac explant, the coronary tree was dissected, purged of intraluminal blood using phosphate buffered saline, and then photographed intact (Figure 1E) before undergoing ex vivo computed tomography (CT) as

FIGURE 2 **CT Analysis**

(A) Intact right and left coronary tree anatomically annotated and prepared for ex vivo computed tomography. **(B)** Accompanying VR reconstructed computed tomography image with grey box identifying region of left anterior descending artery selected for the multiplanar reconstructions shown in **C** and **D**, the latter of which depicts the semi-automated color mapping with 1K plaque in red and low attenuation plaques in blue. **(E to H)** Cross-sectional views with and without color mapping of 1K plaque and low attenuation plaques taken from sites along multiplanar reconstruction as shown by red and blue dashed lines. LAD = left anterior descending artery; LCx = left circumflex artery; LMCA = left main coronary artery; M = marginal branch; RCA = right coronary artery; RPDA = right posterior descending artery; RPL = right posterolateral branch; VR = virtual reality.

described below or dissection for MPO activity and histological validation. For histological assessment, individual coronary arteries were cut into 5 mm sections (**Figures 1F and 1G**). Where atheroma was identified macroscopically, the plaque type was assessed, and the section then bisected for histological validation and MPO activity respectively as described for CEA sections. Lesion-free sections were histologically confirmed and set aside for MPO activity determination.

HISTOLOGY AND PLAQUE PHENOTYPING. Histological assessment and plaque phenotyping was performed as described in the Supplemental Methods by a histologist blinded to macroscopic tissue assessment, clinical history, and MPO activity.

EX VIVO CT FOR THE DETERMINATION OF HIGH AND LOW ATTENUATION PLAQUES. To differentiate high (1K) and low attenuation plaques (LAPs), intact ex vivo coronary trees were imaged using a 64-detector row CT scanner (Discovery CT750; GE Healthcare CT) (**Figure 2**), as described in Supplemental Methods.

MPO ACTIVITY. MPO activity was determined blinded from clinical history and plaque phenotype using the MPO-specific chlorination of the synthetic compound hydroethidine to 2-chloroethidium (2-Cl-E$^+$),[23] as described in Supplemental Methods. All samples were analyzed in triplicate and data corrected for recovery of internal standard, with 'MPO activity' expressed as nmol 2-Cl-E$^+$ per mg protein.

TABLE 1 Cohort Characteristics		
	Carotid Cohort (n = 31)	Cardiac Cohort (n = 12)
Age, y	72 ± 7.7	59 ± 9.2
Sex		
Male	24 (77)	10 (83)
Female	7 (23)	2 (17)
Ethnicity		
Caucasian	29 (94)	6 (50)
Aboriginal & Torres Strait Islander	1 (3)	1 (8)
Asian	1 (3)	3 (25)
Arabic	0 (0)	1 (8)
African	0 (0)	1 (8)
Body mass index, kg/m^2	28 ± 6.3	26 ± 5.4
Comorbidities		
Hypertension	25 (81)	7 (58)
Hypercholesterolemia	23 (74)	10 (83)
Diabetes	12 (39)	4 (33)
Active smoker	8 (26)	1 (8)
Pharmacotherapy		
Statin	23 (74)	10 (83)
Antiplatelet	28 (90)	12 (100)
Aspirin	25 (81)	12 (100)
Clopidogrel	12 (39)	6 (50)
Other	0 (0)	1 (10)
Anticoagulant	8 (26)	6 (50)
Heparin	3 (10)	3 (25)
LMWH	2 (6)	0 (0)
DOAC	3 (10)	0 (0)
Warfarin	0 (0)	3 (25)
Biochemistry		
Total cholesterol, mmol/L	3.9 ± 1.1	3.7 ± 1.2
LDL, mmol/L	2.1 ± 0.9	1.8 ± 1.2
HDL, mmol/L	1.2 ± 0.4	1.2 ± 0.2
Triglycerides, mmol/L	1.5 ± 0.7	2 ± 1.2
CRP, mg/L	5.6 ± 4.6	13 ± 25.3
Lipoprotein-a, mg/L	516 ± 653.5	502 ± 246.2
Creatinine, μmol/L	84 ± 33.7	100 ± 59.9
MPO, ng/mL	220 ± 114.1	-
Surgical site & indication		
Right CEA	16 (52)	-
Left CEA	15 (48)	-
Symptomatic	12 (39)	-
CVA on neuroimaging	9 (29)	-
Time since last ACS, months	-	15 ± 17

Continued in the next column

TABLE 1 Continued		
	Carotid Cohort (n = 31)	Cardiac Cohort (n = 12)
Prior coronary interventions		11 (92)
PCI	-	7 (58)
CABG	-	7 (58)
Cardiac tissue procurement		
Coronary territories collected	-	(n = 46)
Average per explant	-	4 ± 0.7
Territories		
LMCA	-	11 (24)
LAD	-	12 (26)
LCx	-	9 (20)
RCA	-	11 (24)
PDA	-	3 (7)

Values are mean ± SD or n (%).

ACS = acute coronary syndrome; CABG = coronary artery bypass graft; CEA = carotid endarterectomy; DOAC = direct oral anticoagulant; HDL = high-density lipoprotein; LAD = left anterior descending artery; LCx = left circumflex artery; LDL = low-density lipoprotein; LMCA = left main coronary artery; LMWH = low molecular weight heparin; MPO = myeloperoxidase; n = number; PCI = percutaneous coronary intervention; PDA = posterior descending artery; RCA = right coronary artery.

STATISTICAL ANALYSIS. GraphPad Prism statistical software (version 9.0.1) was used for data analysis. For MPO activity determination, coronary and carotid plaques were categorized as stable and unstable. Carotid plaques were additionally grouped by the presence of neurological symptoms or established ipsilateral stroke on neuroimaging of the donor irrespective of plaque phenotype. Coronary plaques determined by ex vivo CT were dichotomized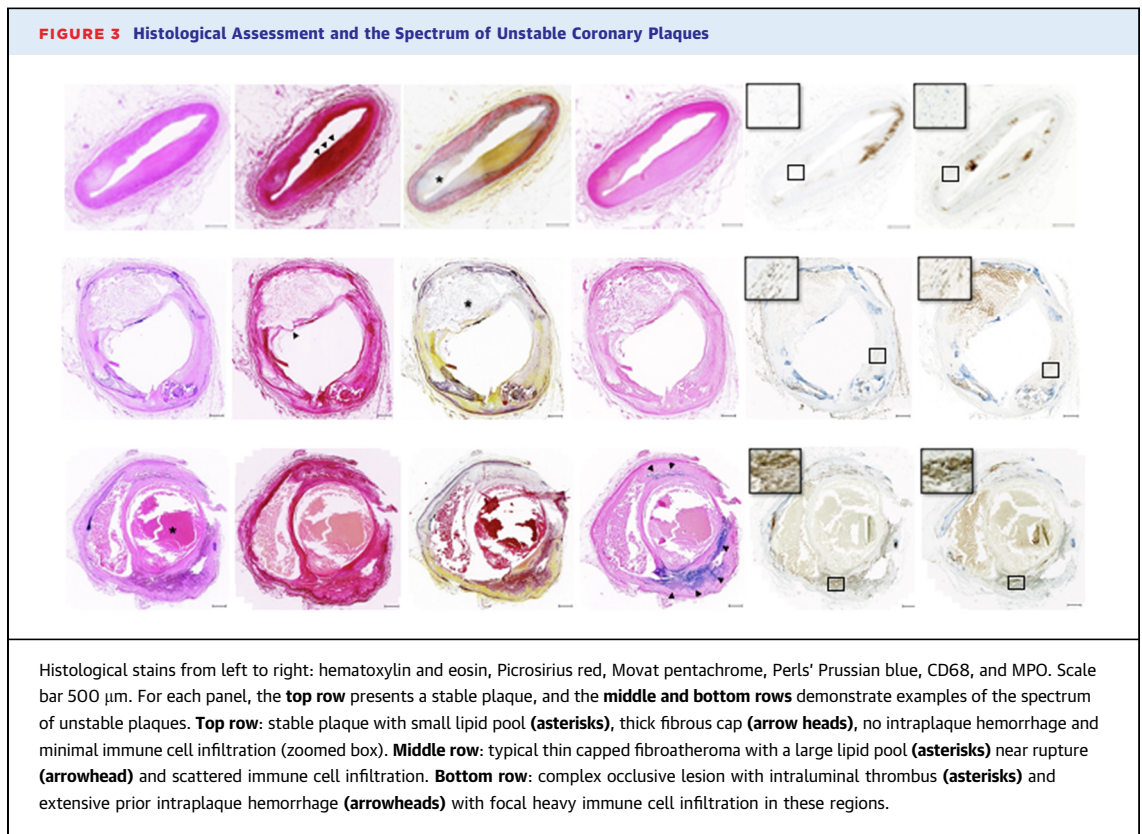 as high (1K) or LAP. Data were first assessed for normality using the D'Agostino and Pearson test. Descriptive statistics were used to characterize demographics and non-continuous variables of the cohort. For continuous variables, simple univariate non-parametric statistics were performed utilizing Mann-Whitney U tests for pairwise comparisons and Kruskal-Wallis tests for groups. Cohen's kappa coefficient was used to identify the degree of accuracy and reliability of interobserver as well as macroscopic to histologic assessment. Simple linear regression analysis was used to correlate plasma MPO and C-reactive protein (CRP) levels with MPO activity. Statistical significance was defined at a 2-tailed P value of <0.05.

ETHICS, DATA, AND RESOURCE ACCESS. The Human Research Ethics Committee of St Vincent's Hospital, Darlinghurst, Australia approved this study that conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Anonymized datasets, analyzing methods and study materials can be made available to other researchers for the purpose of reproducing the results or replicating procedures by contacting the corresponding author.

RESULTS

The demographics and clinical features of the cohorts are presented in Table 1. Participants were predominantly older Caucasian males on optimal medical therapy, with lipid profiles within guideline directed ranges. Approximately 40% of the carotid cohort had symptomatic disease, with over a quarter having had

FIGURE 3   Histological Assessment and the Spectrum of Unstable Coronary Plaques

Histological stains from left to right: hematoxylin and eosin, Picrosirius red, Movat pentachrome, Perls' Prussian blue, CD68, and MPO. Scale bar 500 μm. For each panel, the **top row** presents a stable plaque, and the **middle and bottom rows** demonstrate examples of the spectrum of unstable plaques. **Top row**: stable plaque with small lipid pool **(asterisks)**, thick fibrous cap **(arrow heads)**, no intraplaque hemorrhage and minimal immune cell infiltration (zoomed box). **Middle row**: typical thin capped fibroatheroma with a large lipid pool **(asterisks)** near rupture **(arrowhead)** and scattered immune cell infiltration. **Bottom row**: complex occlusive lesion with intraluminal thrombus **(asterisks)** and extensive prior intraplaque hemorrhage **(arrowheads)** with focal heavy immune cell infiltration in these regions.

an ipsilateral cerebrovascular infarction on presurgical neuroimaging. Of the 12 coronary trees procured, a total of 47 individual plaques plus 7 lesion-free segments were prepared for MPO activity quantification, histology, or CT.

VALIDATION OF MACROSCOPIC ASSESSMENT OF PLAQUE PHENOTYPE. The utility and performance of macroscopic plaque assessment between CEA and coronary tissue is presented in Supplemental Table 1. Following macroscopic assessment of the first 16 CEA specimens, we observed a >90% correlation between macroscopic assessment and histology with excellent interobserver (κ 0.88) and histological agreement (κ 0.88). Therefore, macroscopic assessment alone was used for plaque phenotyping of the remaining 10 (38%) plaques. A lower interobserver concordance (κ 0.55) was achieved with macroscopic assessment of coronary plaques, and thus all coronary samples underwent histological assessment to definitively determine plaque phenotype.

PLAQUE PHENOTYPES. Representative histological sections of stable and unstable plaques from carotid (Supplemental Figure 2) and coronary tissues (Figure 3) depict the spectrum of plaques analyzed. Broadly speaking, unstable plaques fell into 2 main

sub-types: typical thin-capped fibroatheroma (Figure 3 middle row) or complex mature lesions with multiple foci of prior destabilization and thrombosis (Figure 3 bottom row).

MPO ACTIVITY BY PLAQUE PHENOTYPE. MPO activity of CEA plaques defined as stable and unstable are shown in Figure 4A. Unstable plaques had significantly higher MPO activity compared with stable plaques (n = 26, 4.2 ± 3.1 vs 0.2 ± 0.3 nmol/mgp; $P < 0.0001$). This significance was demonstrable in both macroscopically and histologically defined stable and unstable plaques (Supplemental Figure 3). Similar results were seen in coronary segments containing unstable compared with stable plaque and lesion free tissues (n = 24, 0.6 ± 0.4 vs 0.001 ± 0.003 and 0.007 ± 0.002 nmol/mgp; $P = 0.0002$) (Figure 4B). In contrast, there was no difference in the content of the superoxide-specific adduct of hydroethidine, ie, 2-hydroxy-ethidium, between unstable and stable CEA (Figure 4C) and coronary plaques (Figure 4D).

MPO activity was higher in unstable CEA (Figure 4A) segments compared with unstable coronary tissues (Figure 4B). This may be explained partly by the presence of non-plaque constituents, ie, arterial media and adventitia, in coronary but not

carotid samples. To validate this, we determined MPO activity in n = 4 separate human coronary atherectomy samples. This revealed comparable 2-Cl-E$^+$ abundance per mgp (*P* = 0.30) (Supplemental Figure 4), suggesting that unstable plaques in human coronary and carotid arteries have comparably increased MPO activity compared with their corresponding stable plaques.

Utilizing linear regression analysis, intraplaque MPO activity neither correlated with plasma concentrations of MPO protein nor CRP (R^2 <0.20) (Supplemental Figure 5).

MPO ACTIVITY BY CLINICAL PRESENTATION. The presence of ipsilateral stroke on neuroimaging or clinical symptoms (stroke/transient ischemic attack) at the time of CEA correlated with higher MPO activity compared to those who were asymptomatic without neuroimaging demonstrating stroke (n = 12, 3.7 ± 2.1 vs 0.1 ± 0.2 nmol/mgp; *P* = 0.002) (**Figure 4**). These findings remained significant when separating clinical endpoints based on neuroimaging (n = 10, 3.3 ± 2.1 vs 0.1 ± 0.2 nmol/mgp; *P* = 0.004) or symptoms (n = 10, 3.6 ± 2.2 vs 0.1 ± 0.2 nmol/mgp; *P* = 0.009).

MPO ACTIVITY BY CT-DERIVED PLAQUE ATTENUATION AND PVAT ASSESSMENT. The ex vivo CT cohort characteristics and mean plaque volumes are presented in Supplemental Table 2. Twelve 1K and 18 LAP were collected and assessed for MPO activity. Utilizing CT determinants of high and LAPs, no apparent difference in MPO activity was seen (n = 30, 0.1 ± 0.1 vs 0.2 ± 0.3 nmol/mgp; *P* = 0.23) (Supplemental Figure 6). None of the 8 PVAT specimens analyzed were found to contain enzymatically active MPO (Supplemental Figure 6).

DISCUSSION

We sought to assess whether MPO was more active in atherosclerotic plaques at risk of rupture, thrombosis, and cardiovascular events. We show that MPO activity is higher in histologically defined unstable CEA and coronary plaques compared with stable plaques and plaque-free arteries. In contrast, no difference in the superoxide-specific adduct 2-hydroxy-ethidium was seen between unstable and stable plaques, suggesting the increased MPO activity in unstable plaques was due to increased MPO activity rather than an increase in endogenous hydrogen peroxide derived from superoxide. Moreover, stable and plaque-free tissue had little to no detectable MPO activity, implying that enzymatically active MPO may be a hallmark of vulnerable and destabilized atherosclerotic plaques. Our data are the first to show this

FIGURE 4 MPO Activity in Human Carotid and Coronary Plaque by Histological Phenotype and Clinical Perspective
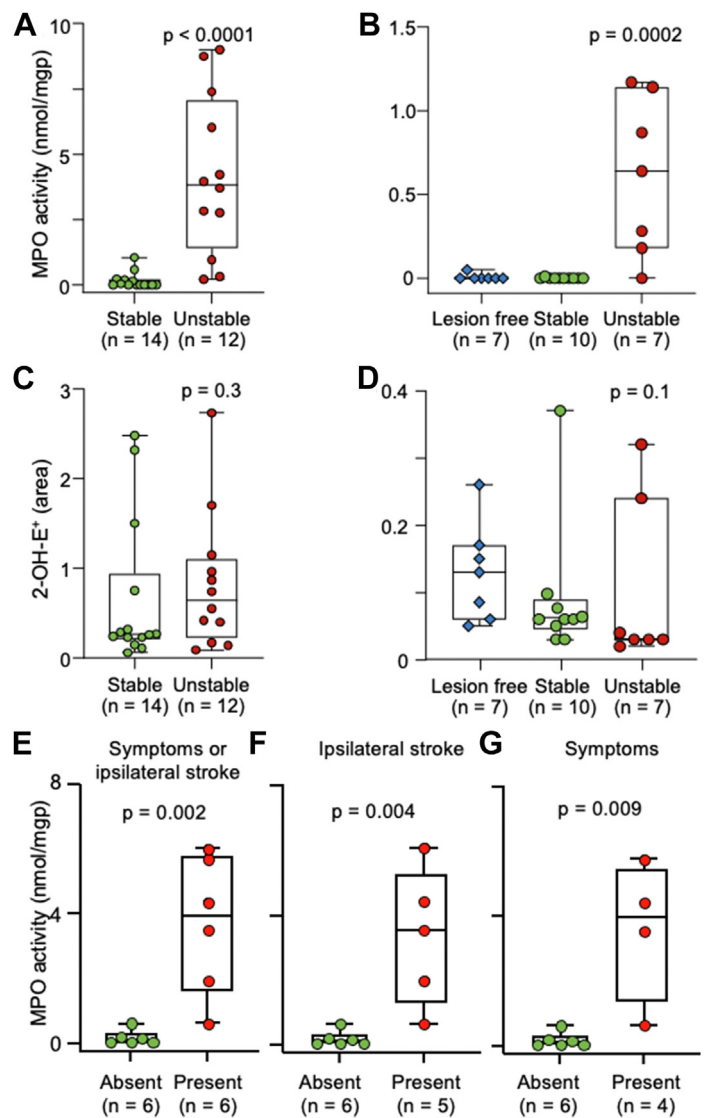
(A) Carotid endarterectomy myeloperoxidase activity (2-chloro-ethidium in nmol per mgp corrected for recovery of deuterated 2-chloro-ethidium) grouped by plaque phenotype, with significantly higher myeloperoxidase activity in unstable compared with stable plaques. **(B)** Coronary myeloperoxidase activity grouped by plaque phenotypes and lesion free tissue, with significantly higher myeloperoxidase activity in unstable plaques compared with stable plaques and lesion-free tissue. **(C)** 2-Hydroxy-ethidium corrected for internal standard (deuterated 2-chloro-ethidium) and standardized per mgp grouped by carotid endarterectomy plaque phenotype, with no significant differences observed. **(D)** 2-Hydroxy-ethidium corrected to internal standard and standardized per mgp grouped by plaque phenotypes and lesion free coronary segments, with no significant differences observed. **(E)** Significantly higher myeloperoxidase activity in carotid endarterectomy specimens of subjects with neurological symptoms or neuroimaging demonstrating ipsilateral stroke compared with those who are asymptomatic without ipsilateral stroke on neuroimaging. This difference remained significant when dividing clinical end points into neuroimaging or symptom-based outcomes. **(F)** Significantly higher myeloperoxidase activity in carotid endarterectomy specimens of subjects demonstrating ipsilateral stroke by neuroimaging. **(G)** Significantly higher myeloperoxidase activity in carotid endarterectomy of subjects with neurological symptoms (stroke or transient ischemic attack). 2-OH-E$^+$ = 2-hydroxy-ethidium; MPO = myeloperoxidase.

relationship in human subjects, and we do so through the analysis of 73 individual plaques from 43 patients across 2 separate vascular beds relevant to cardiovascular disease burden. Our findings are consistent with preclinical studies that identified intraplaque MPO as causal to plaque destabilization.[9]

In addition, when correlating MPO activity to clinical outcomes we identified that CEA specimens of patients with neurological symptoms or ipsilateral stroke had higher MPO activity compared with those free from these clinical events. This finding suggests that MPO activity may be able to identify culprit lesions in cases of stroke and heart attack and further underscores the potential utility of MPO activity as a diagnostic biomarker of plaque vulnerability and rupture. The novelty of intraplaque MPO activity as a biomarker of plaque instability is further emphasized by the apparent lack of correlation to circulating CRP and MPO protein concentrations.

When examining currently available and novel non-invasive imaging techniques for plaque characterization and arterial inflammation, CTCA-derived parameters for stable and higher risk plaque were not able to reliably differentiate MPO activity, and PVAT did not appear to contain enzymatically active MPO. This suggests that MPO activity may not play a direct role in the inflammatory changes seen in PVAT. As such, diagnostic measures like fat attenuation indices are likely to reflect a different pathobiological process to MPO-driven plaque destabilization.

The inability to differentiate MPO activity by CT-derived plaque attenuation may be due in part to limited spatial resolution and partial volume averaging as well as the reduced sensitivity of LAP as a determinant of vulnerable atherosclerosis. This notion is highlighted in the present study where a trend is seen toward LAP-containing higher MPO activity but many of these plaques contained little to no MPO activity. It is possible that a difference between high and LAP to identify MPO activity may be confirmed by increasing sample size. Nevertheless, when comparing the correlation with gold standard histologic assessment, our data suggest that novel molecular probes that detect MPO activity may assist with enhanced differentiation of stable from unstable atherosclerotic plaque (**Central Illustration**), complimenting established imaging techniques, and assisting with improved management.[10,24]

Molecular imaging tracers have been developed to selectively detect MPO activity including magnetic resonance imaging-[25] and positron emission tomography-based probes.[26] These probes have been used to localize MPO activity and plaque inflammation related metabolism in animal models including the selective enhancement of unstable plaque.[9] The present findings support the preclinical evidence and reinforce the foundations for human trials utilizing MPO-based molecular probes.

Coronary and carotid cohorts in this study represented medically well-managed patients with established cardiovascular disease. Approximately 3 quarters of both cohorts were on statin therapy and lipid profiles were close to target. Additionally, almost all patients were on an antiplatelet regimen. Interestingly, CRP levels were high across the groups, indicating that the cohorts studied may capture those with residual inflammatory risk.[27] Nevertheless, higher plaque MPO activity was still detectable in symptomatic patients and those with vulnerable plaques despite apparent optimization of pharmacotherapy and evidence for statins lowering MPO activity in hypercholesterolemic subjects[28] and circulating MPO protein in patients with acute coronary syndrome.[29] These findings suggest that available treatments may not sufficiently target inflammation reflected by plaque MPO activity and identify specific MPO inhibition as a potential therapeutic pathway to treat at-risk populations (**Central Illustration**). MPO inhibitors have been trialed,[30-32] and clinical phase II trials are currently underway. In animal studies of vulnerable plaque, treatment with a selective MPO inhibitor improved endothelial function, increased cap thickness and plaque stability, and stabilized preexisting unstable plaques without a reduction in circulating immune cells or deleterious side effects.[9,33,34] Together, these data suggest that MPO inhibition may have therapeutic potential.

There are several limitations to this study. Firstly, the MPO activity assay used requires tissue to be homogenized, which likely results in the liberation of intracellular MPO. Therefore, the enzymatic activity determined likely reflects total MPO rather than extracellular MPO exclusively, the latter of which is posited to be deleterious to plaque stability. There are currently no precise ex vivo analytical methods available to detect extracellular MPO activity. Secondly, the histological assessment of plaque was made from tissue sections adjacent to those analyzed for MPO activity, which may limit the precision of the histological validation. Due to the required workup for histology, including formalin fixation, MPO activity could not be assessed from the specific tissue sections analyzed. This issue could potentially be overcome by utilizing mass spectrometry imaging techniques such as matrix-assisted laser desorption ionization or desorption electrospray ionization.

CENTRAL ILLUSTRATION Myeloperoxidase Activity is Higher in Unstable Than Stable Human Carotid and Coronary Atheroma, and Carotid Plaque Myeloperoxidase Activity Correlates With Cardiovascular Events
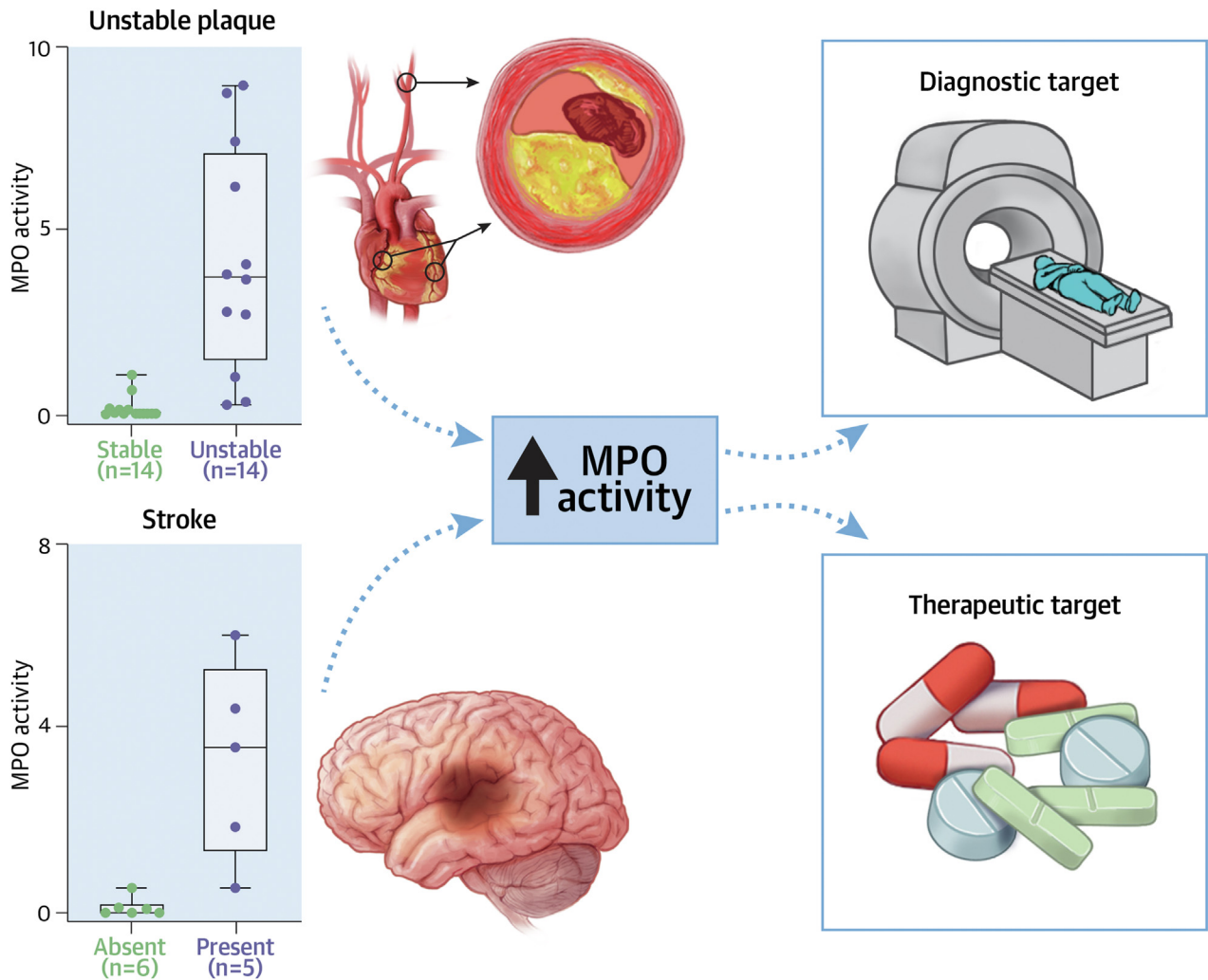
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These findings identify myeloperoxidase as a novel diagnostic and therapeutic target for emerging imaging probes which detect MPO activity and pharmacotherapies which inhibit MPO activity in the management of high-risk atherosclerosis. The **arrow marks** indicate increasing. MPO = myeloperoxidase.

Theoretically, this could allow for histological and MPO activity quantification to be performed on matching tissue sections, though such techniques are yet to be developed.

A further limitation can be deduced from the absence of contrast enhancement used in the ex vivo CT imaging. Increasing concentrations of intraluminal contrast has a direct relationship to plaque attenuation, more so in non-calcified than calcified plaques.[35,36] The nature in which coronary tissues were collected limited the availability of up-to-date pre-explant CTCA and necessitated ex vivo imaging.

Also, no validated ex vivo method for CT-derived contrast-enhanced coronary imaging and plaque characterization exists. Given these constraints, we purposefully only selected plaques with very high and very low attenuation to overcome any signal increase that may have been seen with contrast CT. In addition, our stricter definition of LAP with ex vivo <30 HU is reasonably expected to correspond to <75 HU determined by in vivo CTCA,[35,36] which has been reported to correlate histologically to LAP.[37]

Lastly, there is an observable difference in the range of MPO activity detected between unstable

plaques in CEA samples and coronary segments, with 10-fold greater protein-standardized MPO activity seen in carotid tissue. Analysis of a limited number of coronary atherectomy samples suggested that this can be explained by the fact that CEA samples consist mostly of plaque with some surrounding endothelium, whereas the coronary tissue segments include the plaque as well as the surrounding tunica media and adventitia. This implies that MPO activity in coronary plaque is substantially higher than that in the non-plaque arterial compartment.

CONCLUSIONS

Intraplaque MPO activity is greater in unstable than stable carotid and coronary atheroma and is associated with adverse cardiovascular outcomes. In addition, CT-derived determinants of high-risk plaques do not reliably detect plaques enriched with MPO, and PVAT appears devoid of enzymatically active MPO. These findings highlight a unique role for this inflammatory enzyme and endorses the ongoing exploration and development of MPO activity as a diagnostic tool and therapeutic target for vulnerable atherosclerosis.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: MPO activity is higher in unstable than stable human coronary and carotid plaques, correlates with symptomatic carotid disease and stroke, and current non-invasive imaging techniques do not appear to reliably determine active MPO.

TRANSLATIONAL OUTLOOK 1: As intraplaque MPO activity appears to be a hallmark of unstable human atherosclerosis and can be imaged non-invasively, further investigation into its potential utility as a diagnostic and therapeutic target is warranted.

TRANSLATIONAL OUTLOOK 2: These data are supportive of preclinical data and reinforce the basis of planned Phase I and II clinical trials looking at an MPO-based molecular positron emission tomography probe for the detection of high-risk atherosclerosis.

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APPENDIX For supplemental tables and figures, please see the online version of this paper.