

Acute and long-term effect of percutaneous coronary intervention on serially-measured oxidative, inflammatory, and coagulation biomarkers in patients with stable angina

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Abstract To derive insights into the temporal changes in oxidative, inflammatory and coagulation biomarkers in patients with stable angina undergoing percutaneous coronary intervention (PCI). PCI is associated with a variety of biochemical and mechanical stresses to the vessel wall. Oxidized phospholipids are present on plasminogen (OxPL-PLG) and potentiate fibrinolysis in vitro. We recently showed that OxPL-PLG increase following acute myocardial infarction, suggesting that they are involved in atherothrombosis. Plasma samples were collected before, immediately after, 6 and 24 h, 3 and 7 days, and 1, 3, and 6 months after PCI in 125 patients with stable angina undergoing uncomplicated PCI. Plasminogen levels, OxPL-PLG, and an array of 16 oxidative,

inflammatory and coagulation biomarkers were measured with established assays. OxPL-PLG and plasminogen declined significantly immediately post-PCI, rebounded to baseline, peaked at 3 days and slowly returned to baseline by 6 months ($p < 0.0001$ by ANOVA). The temporal trends to maximal peak in biomarkers were as follows: immediately post PCI: OxPL-apoB and lipoprotein (a); Day 1—the inflammatory biomarker IL-6; Day 3—CRP and coagulation biomarkers OxPL-PLG, plasminogen and tissue plasminogen activity; Day 3 to 7—plasminogen activator inhibitor activity, and complement factor H binding to malondialdehyde-LDL and MDA-LDL IgG; Day 7–30 MDA-LDL IgM, CuOxLDL IgM, and ApoB-IC IgM and IgG; >30 days uPA activity, uPA antigen, CuOxLDL IgG and peptide mimotope to MDA-LDL. Most of the biomarkers trended to baseline by 6 months. PCI results in a specific, temporal sequence of changes in plasma biomarkers. These observations provide insights into the effects of iatrogenic barotrauma and plaque disruption during PCI and suggest avenues of investigation to explain complications of PCI and development of targeted therapies to enhance procedural success.

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Introduction

Percutaneous coronary intervention (PCI) is often associated with release and embolization of plaque contents into the microcirculation [1, 2]. Studies have documented that vascular injury induced by PCI is associated with pro-inflammatory responses that affect immediate and long-term

outcomes [3]. Inflammation plays an important role in the pathogenesis of atherosclerosis, and arterial injury caused by PCI results in further downstream release of several inflammatory components [4, 5]. A variety of pro-inflammatory oxidation-specific epitopes (OSEs) are generated during the evolution of early atherosclerotic lesions that transition to ruptured plaques in patients with sudden death [2, 5, 6]. Furthermore, oxidized phospholipids (OxPLs) are released from symptomatic, stenotic lesions in patients undergoing PCI or carotid and peripheral interventions, as detected by mass spectroscopy of captured material in distal protection devices [7, 8, 9]. Finally, plasma biomarkers of oxidized lipids, as measured by circulating levels of OxPL on apoB-100 particles (OxPL-apoB), are significant predictors of the presence and extent of carotid and femoral atherosclerosis, development of new lesions, and increased risk of cardiovascular events, including myocardial infarction, stroke and peripheral arterial disease [10].

We recently showed that OxPLs are present on plasminogen (OxPL-PLG), which shares high homology with apolipoprotein (a) [apo(a)]. Lipoprotein (a) [Lp(a)] is composed of apo(a) covalently bound to apolipoprotein B-100. In addition to OxPL on Lp(a), OxPL on plasminogen represent a distinct second pool of plasma circulating OxPL and have different pathophysiological consequences [11]. For example, plasminogen enriched with OxPL is associated with enhanced fibrinolysis compared to plasminogen lacking OxPL. This would correlate with less propensity to develop thrombosis, as opposed to the pro-thrombotic and pro-atherogenic properties of Lp(a). Furthermore, OxPL-PLG levels increase following acute myocardial infarction (AMI), suggesting that they are involved in atherothrombosis and may influence outcome after PCI in AMI.

The aim of this study was to assess changes in acute and long-term effect of PCI on serially-measured oxidative, inflammatory, and coagulation biomarkers in patients with stable angina (SA). We assessed serial changes in OxPL-PLG in patients with SA and evaluated the temporal relationship of a variety of biomarkers measured in the same patients serially prior to and up to 6 months following PCI.

Methods

Study population

This is a biomarker substudy of previously described set of studies derived from the same cohort of patients [7, 9, 12]. Venous blood in EDTA was obtained before, immediately after, 6 and 24 h, 3 and 7 days, and 1, 3, and 6 months after PCI. The current analysis is based on 125 of 159 subjects

with available serial plasma samples for measurement of additional biomarkers. Stable patients with successful PCI of a single de novo lesion in a native coronary artery were enrolled in this single-center prospective study. Exclusion criteria included recent (<2 weeks) unstable angina (defined as rest pain with ST-segment changes) or myocardial infarction (MI), bypass graft lesions, total occlusions, and concurrent illnesses (cancer or chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease). Patients with an unsuccessful procedure (residual diameter stenosis >50 %) or abrupt occlusion within the first 7 days were excluded from additional analyses. None of the patients had undergone recent (<1 year) interventions of other lesions. PCI was performed according to standard techniques. The study was approved by the hospital research ethics board, and all patients gave informed consent [12].

A control group of 41 patients undergoing diagnostic angiography with heparin administration and in whom blood was obtained before and immediately after the procedure was also included.

Measurement of plasminogen, oxidized phospholipids on plasminogen (OxPL-PLG) and complement factor H binding to MDA-LDL

Enzyme linked immunosorbent assay (ELISA) to determine plasminogen and OxPL-PLG plasma levels (Fig. 1) for each time point was performed as previously described in detail [11]. A serial dilution of purified human plasminogen (Meridian, Saco, ME) was used to generate a standard curve to measure plasminogen levels. The OxPL-PLG assay represents OxPL covalently bound to plasminogen as recognized by murine monoclonal antibody E06 that binds to the PC headgroup of oxidized but not normal phospholipids. In the first description of this assay, data were initially reported in relative light units per 100 ms (RLU/100 ms) [11]. In the current study we now report OxPL-PLG levels as nanomolar (nmol/L or nM) OxPL based on a standard curve of phosphocholine (PC) equivalents, to facilitate comparison of absolute OxPL-PLG levels across studies. The standard curve is generated by plating known concentrations of PC-modified bovine serum albumin (PC-BSA), which has approximately 16 mol of PC/mole of BSA (Biotech Technologies, Novato, California) and is recognized by murine monoclonal antibody E06. Biotin-E06 is then added to detect the number of moles of PC in the linear range of the assay measurements and is measured in RLU. This standard curve is then used to convert the RLU derived from wells containing individual human samples to OxPL equivalents. This new method is reported as nanomoles of PC found on OxPL per liter of plasma (i.e., nanomolar or nM) for each

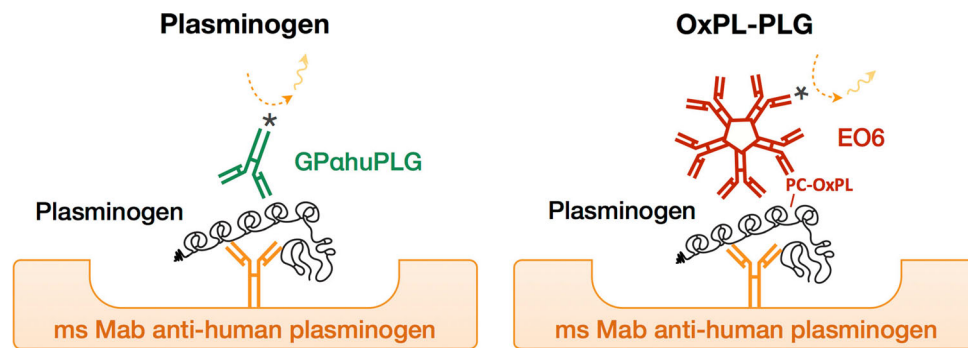


Fig. 1 Plasminogen and OxPL-PLG chemiluminescence assays. The figure displays the methodology of the plasminogen and OxPL-PLG chemiluminescence assays. *ms* mouse, *Mab* monoclonal antibody, *PC* phosphocholine, *OxPL* oxidized phospholipid, *GPzhuPLG* polyclonal

sample. Because each mole of OxPL has 1 PC headgroup recognized by EO6, this can be reported as nM PC-OxPL, as previously described for measurement of OxPL on apolipoprotein B-100 (OxPL-apoB) [13–16]. We present the OxPL-PLG data as both RLU and nM PC-OxPL to facilitate comparison with prior work.

Complement factor H (CFH) binds the oxidation-specific epitope malondialdehyde, as in modified low-density lipoprotein (MDA-LDL) and is enriched in human vulnerable plaques [17]. As such, it functions as an innate immune protein to protect against MDA-mediated oxidative stress [18]. CFH binding to MDA-LDL (noted as CFH-MDA-LDL) was measured by ELISA as recently described [17]. Microtiter well plates (Thermo scientific, Rochester, NY) were incubated overnight at 4 °C with MDA-LDL (50 μ l at 5 μ g/ml), produced from purified human low-density lipoprotein (LDL) as described [7, 17]. Wells were washed with PBS, blocked with 1 % BSA/TBS at 100 μ l/well for 45 min at room temperature. After further washing, human plasma (50 μ L at 1:200 dilution) was added to each well for 75 min. After another wash cycle, biotinylated guinea pig anti-human CFH antibody (50 μ l at 0.25 μ g/ml) was added for 1 h. Wells were washed again, and alkaline phosphatase-conjugated NeutrAvidin (Thermo Fisher Scientific Inc., Rockford, IL) diluted at 1:10,000 was added at 50 μ l/well for 1 h. After washing with TBS, 50 % Lumi-Phos 530 (Lumigen Inc. Southfield, MI) was added at 25 μ l/well for 75 min with light shielding. The plates were read by chemiluminescence detector and reported in RLU/100 ms.

Reproducibility of individual plasma levels was verified by analyzing OxPL-apoB and comparing them with values from an earlier measurement, as well as by comparison of plasminogen, OxPL-PLG, and CFH-MDA-LDL plasma levels from samples that underwent repeated thaw-freeze cycles. Coefficient of variability is 4–10 %, within-person 5-year reproducibility of frozen samples has been shown to be high ($r = 0.78$) and higher than LDL-C and HDL-C;

guinea pig anti-human plasminogen IgG-antibody, EO6 IgM-Mab, asterisk indicates the biotin-neutravidin-alkaline phosphatase complex. Chemiluminescence was measured in relative light units (RLU) at 440 nm for 100 ms

[19, 20]. Oxidative biomarker levels are stable over 24 h on ice (intraclass correlation coefficient 0.96) as well as frozen samples stored under long term conditions, including freeze–thaw cycles [14, 19, 20, 21].

Coagulation, oxidation, and inflammation biomarkers

For a comprehensive comparison of temporal changes in biomarkers, plasminogen, OxPL-PLG, and CFH-MDA-LDL plasma levels were put in temporal perspective to additional coagulation, oxidation, and inflammation biomarkers that were measured previously from this cohort [7, 9, 12]. Coagulation biomarkers measured consisted of urokinase (uPA) and tissue plasminogen activator (tPA) activity, uPA antigen, and plasminogen activator inhibitor type 1 (PAI-1) activity as previously reported [9, 12]. Indirect markers of oxidation-specific epitopes such as IgG and IgM autoantibodies to MDA-LDL and copper oxidized LDL (CuOxLDL), and peptide mimotopes that reflect the 3-dimensional structure of MDA have been reported [9, 13, 22, 23]. Inflammatory biomarkers included interleukin-6 (IL-6) and C-reactive protein (CRP) as previously reported [2, 23]. IgM and IgG immune complexes with apolipoprotein B (apoB-IC IgM and IgG) were not previously published.

Statistical analyses

For grouped numerical values a mean value \pm standard deviation (mean \pm SD) was calculated. Analysis of quantitative parameters within groups was performed with the Student *t* test, and temporal changes among groups with repeated measures one-way ANOVA with the Bonferroni post-test for multiple comparisons. Data presented in the text and tables are mean \pm SD and in figures the mean value \pm standard error of the mean (mean \pm SEM).

Table 1 Patient and lesion characteristics

Characteristics	N = 125 (%)
Age (years)	56.3 ± 9.9
Gender, female	27 (21.6)
CCS class	
CCS 1	12 (9.6)
CCS 2	59 (47.2)
CCS 3	51 (40.8)
CCS 4	3 (2.4)
Cardiovascular risk factors	
Diabetes mellitus	26 (20.8)
Hypertension	60 (48.0)
Smoking	51 (40.8)
Previous MI	35 (28.0)
Medication	
Heparin	33 (34.4)
Nitroglycerine	71 (79.8)
Beta-blockers	90 (72.0)
Ca-channel blockers	71 (56.8)
Vessel undergoing PCI	
Left anterior descending artery	72 (57.6)
Left circumflex artery	25 (20.0)
Right coronary artery	28 (22.4)
AHA/ACC lesion type	
A	43 (34.4)
B1	49 (39.2)
B2	26 (20.8)
C	7 (5.6)
Lesion appearance	
Eccentric	39 (31.2)
Ulceration	10 (8.0)
Vessel wall irregular	23 (18.4)
Lesion length (mm)	
Pre PCI	9.21 ± 3.13
Post PCI	7.62 ± 3.13
At 6 months	9.36 ± 4.10
Vessel diameter (mm)	
Pre PCI	2.79 ± 0.54
Post PCI	2.94 ± 0.55
At 6 months	2.93 ± 0.57
Lesion diameter (mm)	
Pre PCI	0.86 ± 0.32
Post PCI	2.13 ± 0.67
At 6 months	1.72 ± 0.74
Diameter stenosis (%)	
Pre PCI	69.10 ± 10.13
Post PCI	28.45 ± 12.73
At 6 months	41.67 ± 21.04
Procedure, Stent	39 (31.2)
Restenosis at 6 months	42 (33.6)

Table 2 Baseline and follow-up plasma levels of plasminogen, OxPL-PLG, and CFH-MDA-LDL

	Pre	Post	6 h	24 h	3 d	1 w	1 m	3 m	6 m	p value
Plasminogen, RLU										
Mean ± SD	47,790 ± 8707	41,957 ± 7753	45,849 ± 9193	48,464 ± 10,158	51,263 ± 9345	50,784 ± 8261	48,971 ± 8570	46,875 ± 9513	47,218 ± 9385	<0.0001
Plasminogen (mg/dl)										
Mean ± SD	20.8 ± 4.6	17.7 ± 4.1	19.6 ± 5.3	21.0 ± 5.9	22.7 ± 5.0	22.4 ± 4.4	21.4 ± 4.6	20.3 ± 5.1	20.5 ± 5.0	<0.0001
OxPL-PLG, RLU										
Mean ± SD	23,203 ± 7932	19,046 ± 7936	23,134 ± 9897	23,754 ± 9234	25,124 ± 7913	24,969 ± 8478	24,347 ± 7960	23,216 ± 8434	22,327 ± 8308	<0.0001
OxPL-PLG, nM PC										
Mean ± SD	229 ± 93	180 ± 93	228 ± 116	235 ± 109	252 ± 93	250 ± 100	242 ± 94	229 ± 99	219 ± 98	<0.0001
CFH-MDA-LDL, RLU										
Mean ± SD	31,369 ± 9722	27,193 ± 8590	30,701 ± 10,656	32,222 ± 10,828	32,426 ± 10,851	32,105 ± 10,359	31,513 ± 10,603	31,738 ± 10,714	31,464 ± 10,009	<0.0001

p value indicates significance level of ANOVA test

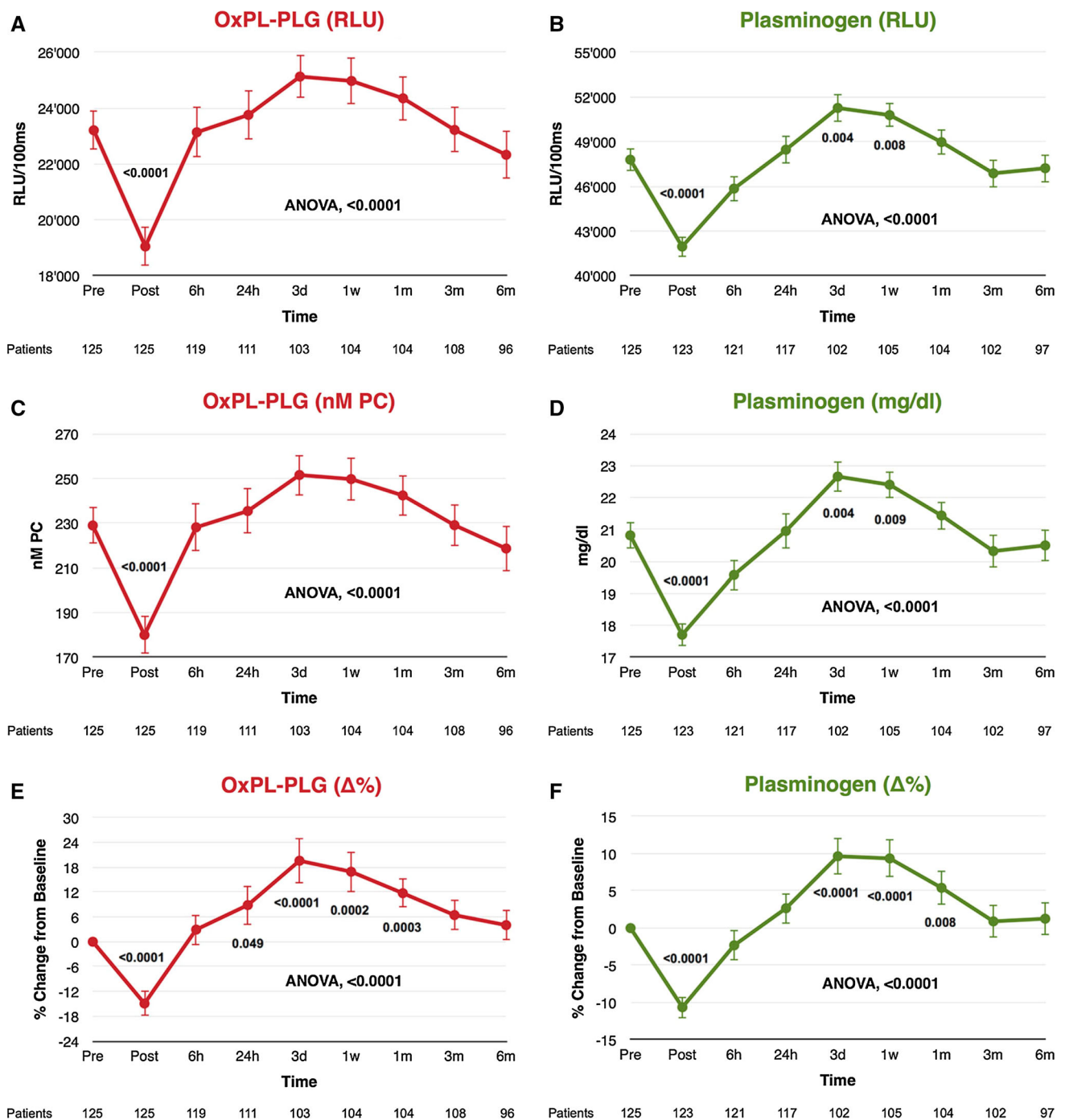


Fig. 2 Plasma levels of plasminogen and OxPL-PLG post PCI. OxPL-PLG and plasminogen (a, b) expressed in relative light units per 100 ms (RLU), nM PC of OxPL-PLG and mg/dl of plasminogen (c, d), and mean percent change from baseline values (e, f). The curves were analyzed by ANOVA. Significant differences were found

between pre-post, pre-3 d, pre-1 w, and pre-1 m values for plasminogen; and between pre-post, pre-24 h, pre-3 d, pre-1 w, and pre-1 m values for OxPL-PLG by Student's *t* test. Absolute values are given in Table 1. *h* hours, *d* days, *w* week, *m* month(s)

Results

Patient and lesion related characteristics are reported in Table 1 and represent typical characteristics of patients with stable angina, with the exception of relatively low use of stent placement reflecting the era of patient recruitment.

Temporal changes in plasminogen and OxPL-PLG

Temporal changes in absolute values of plasminogen and OxPL-PLG are shown in Table 2. Mean plasminogen plasma levels at baseline were 20.8 ± 4.6 mg/dl and changed significantly (ANOVA, $p < 0.0001$) following

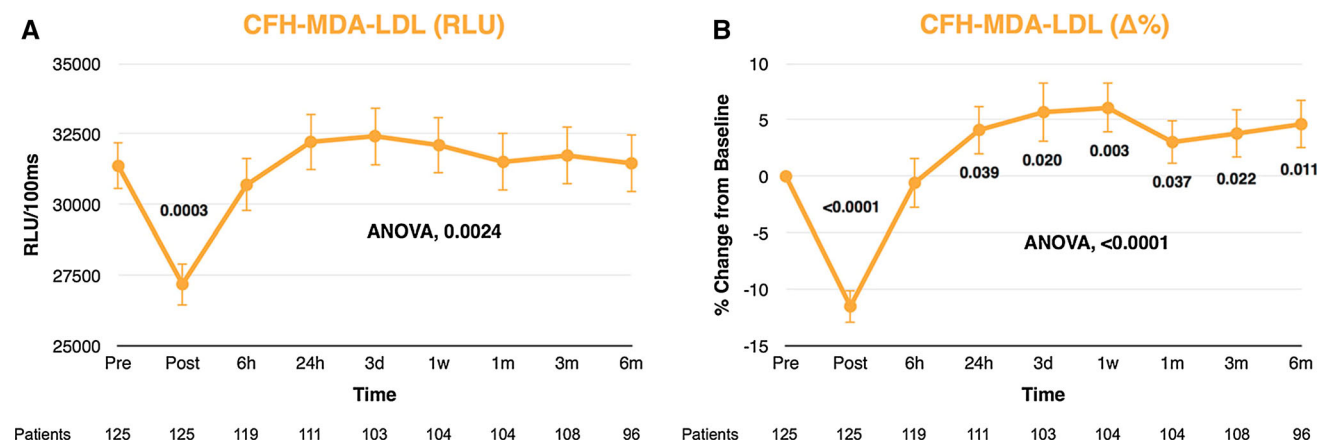


Fig. 3 CFH-MDA-LDL Plasma Levels post PCI. CFH-MDA-LDL levels (a) expressed as RLU/100 ms and (b) as mean percent change from baseline values. The curves were analyzed by ANOVA. Significant differences were found between pre-post values for

PCI. When expressed as mean percent change from baseline, plasminogen values decreased $-10.6 \pm 16.2\%$ ($p < 0.0001$) immediately post-PCI, increased $9.7 \pm 25.4\%$ ($p < 0.0001$) at 3 days and returned to baseline values ($1.3 \pm 22.3\%$, $p = 0.528$) at 6 months (Fig. 2).

Mean OxPL-PLG plasma levels at baseline were 229 ± 93 nM PC-OxPL and changed significantly (ANOVA, $p < 0.0001$) post-PCI. OxPL-PLG changes from baseline were overall similar to the changes of plasminogen, but the extent was more pronounced ($\sim 1.8\times$). A reduction of $-14.9 \pm 34.2\%$ ($p < 0.0001$) in OxPL-PLG was noted post-PCI with an increase of $19.6 \pm 54.7\%$ ($p < 0.0001$) at 3 days with return to baseline ($4.0 \pm 35.8\%$, $p = 0.210$) by 6 months. Changes in absolute values and as percent change from baseline are shown in Fig. 2.

Complement factor H binding to MDA-LDL

Absolute plasma levels of CFH-MDA-LDL decreased significantly during PCI ($p = 0.0003$), rose back to baseline levels after 6 h, and remained at this level over the follow-up period. The percent change from baseline CFH-MDA-LDL decreased during PCI (-11.5 ± 16.7 , $p < 0.0001$), but then increased significantly after the procedure to a maximum at 3 days ($6.1 \pm 23.2\%$, $p = 0.003$) and remained elevated up to 6 months after the procedure ($4.6 \pm 21.0\%$, $p = 0.011$) (Table 2; Fig. 3).

A second analysis evaluating only subjects with a full complement of blood samples at all 9 timepoints did not show any significant differences in changes in plasminogen, OxPL-PLG or CFH-MDA-LDL (data not shown).

absolute CFH-MDA-LDL levels; and between pre-post, pre-24 h, pre-3 d, pre-1 w, pre-1 m, pre-3 m, and pre-6 m values for CFH-MDA-LDL changes from baseline by Student's *t* test. Absolute values are given in Table 1. *h* hours, *d* days, *w* week, *m* month(s)

Subgroup analysis according to baseline characteristics

Subgroup analyses for gender differences, diabetic patients, previous MI, and restenosis are presented in Fig. 4. OxPL-PLG levels were significantly lower in patients with male gender, diabetes, prior MI and with subsequent restenosis. Plasminogen levels were significantly lower in patients with male gender, diabetes, and prior MI but not with subsequent restenosis. CFH-MDA-LDL levels were not different among subgroups.

Biomarker levels according to other variables such as lesion characteristics and cardiovascular risk factors are provided in a Supplementary Figure. Plasminogen levels were significantly lower in patients without ulcerated plaques, no involvement of the left circumflex artery, no history of smoking, and patients receiving stents. OxPL-PLG levels were significantly lower in patients with hypertension and in patients receiving stents. CFH-MDA-LDL levels were significantly lower in patients with concentric lesions, without involvement of the left circumflex, and in patients receiving stents.

No significant changes pre and post angiography were noted in the control group in any of the above variables (data not shown).

Temporal changes in oxidative, inflammatory and coagulation biomarkers following percutaneous coronary intervention

Figure 5 shows time-dependent changes expressed as percent change from baseline of all biomarkers that were assessed in this cohort. The dashed green line represents the peak levels at the specific timepoints in the graph. The *p*-values indicate significant changes in the trend assessed

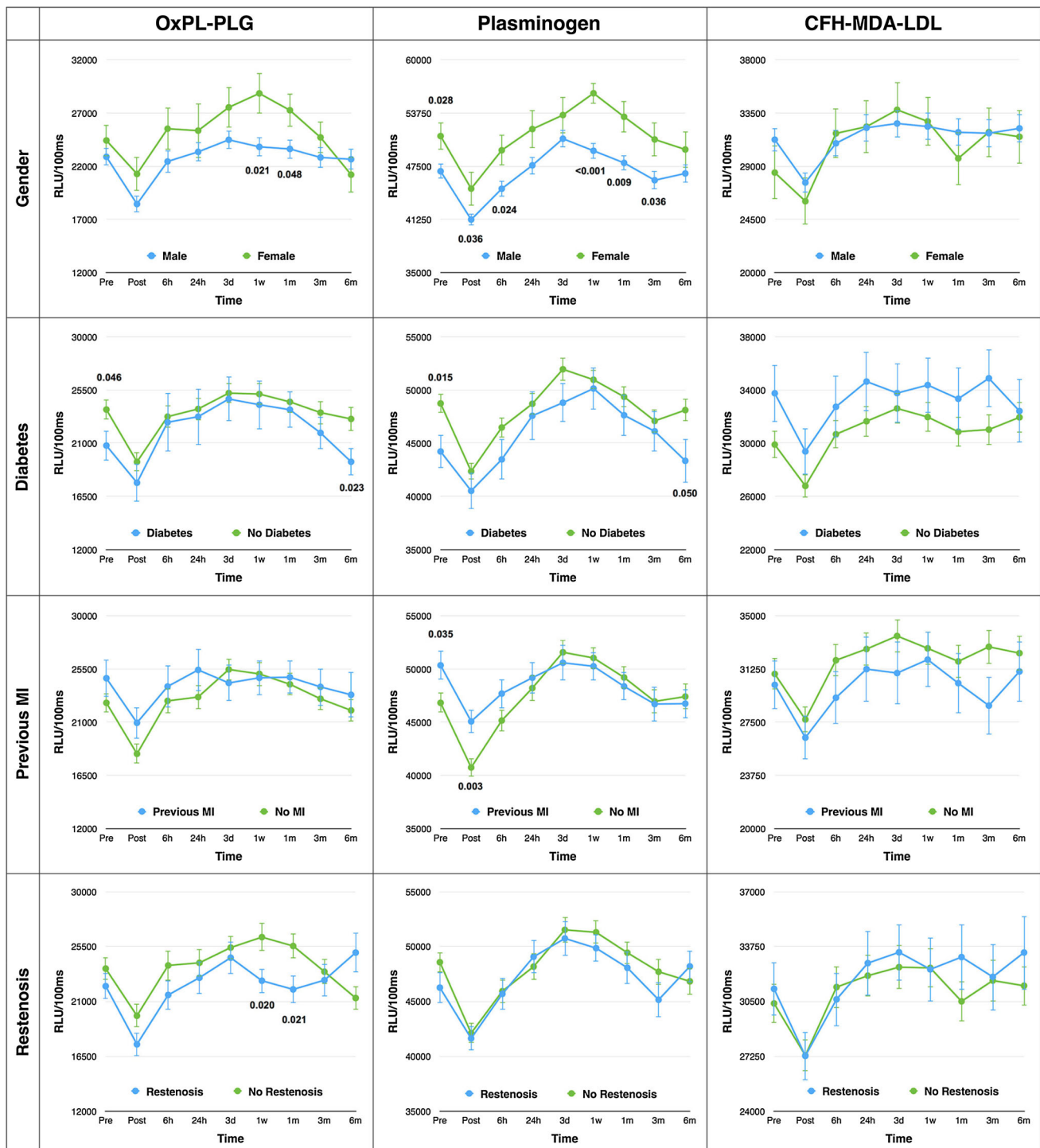


Fig. 4 Subgroup analyses of changes in OxPL-PLG, plasminogen and CFH-MDA-LDL. Relationship of changes in OxPL-PLG, plasminogen, and CFH-MDA-LDL plasma levels in RLU/100 ms to subgroups of gender, diabetes, previous MI and restenosis in patients undergoing PCI. Significant differences between genders were found at 1 w and 1 m for OxPL-PLG levels and at pre, post, 6 h, 1 w, 1 m,

by ANOVA. The earliest biomarker changes were noted in OxPL-apoB and Lp(a) which peaked immediately following PCI, representing approximately 30 min to 2 h of blood

and 3 m for plasminogen levels; in diabetic patients at pre for OxPL-PLG levels and at pre and 6 m for plasminogen levels; in patients with prior MI at pre and post for plasminogen levels; in patients with subsequent restenosis at 1 w and 1 m for OxPL-PLG levels by Student's *t* test. *h* hours, *d* days, *w* week, *m* month(s)

sampling from sheath placement prior to PCI. IL-6 levels peaked at 24 h and C-reactive protein (CRP), which is upregulated by IL-1 signaling, peaked at 3 days post PCI.

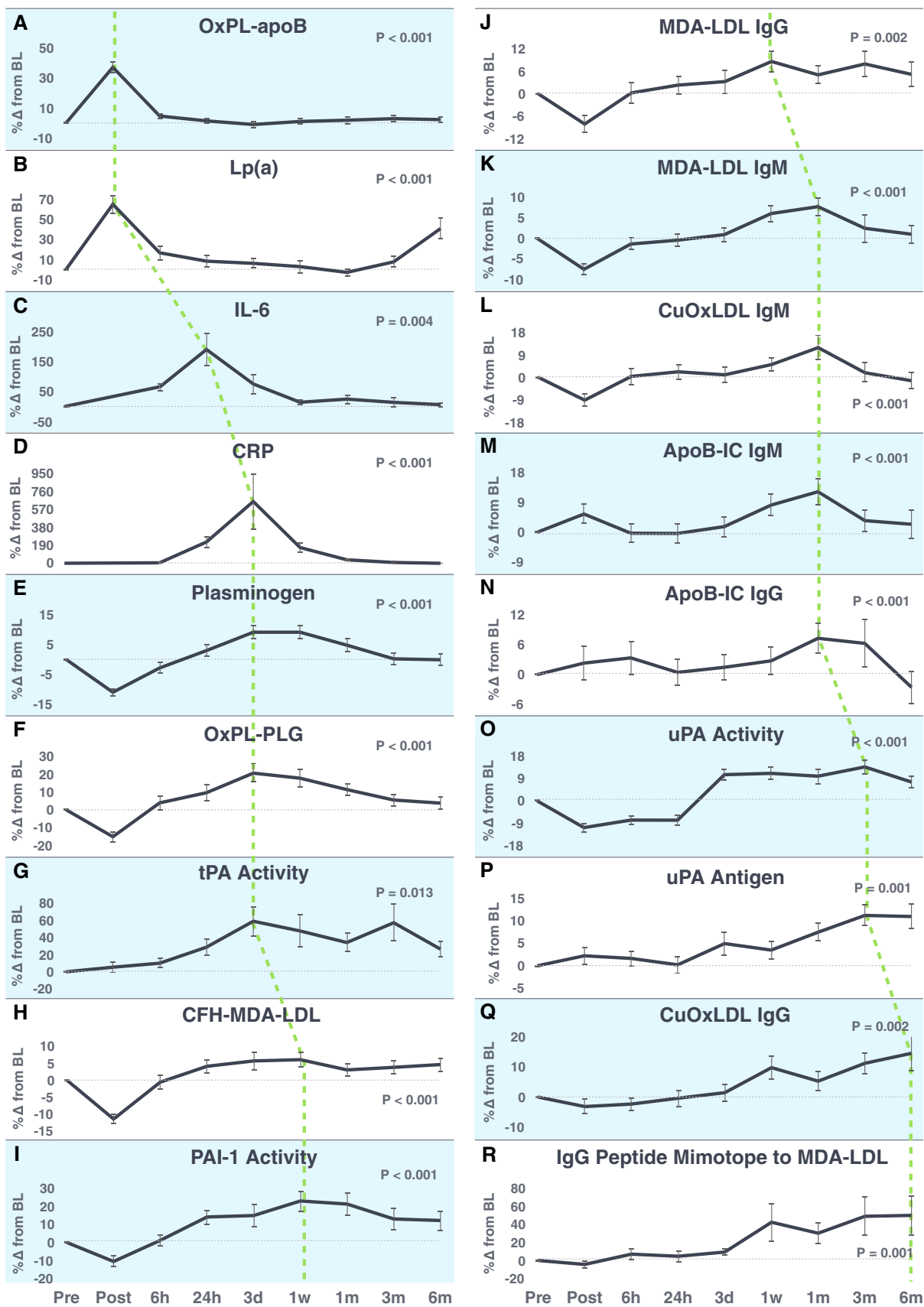


Fig. 5 Time dependence of oxidative, inflammatory and coagulation biomarkers following PCI. **a–r** Show time related changes in mean percent change from baseline values in oxidative, inflammatory and coagulation biomarkers following PCI. Friedman's 2-way analysis of variance by Ranks was used to determine significant changes. $P < 0.05$ was considered statistically significant. The *dashed green line* indicates time dependence of the highest peak of the plasma values. Original data: **a, b, m, n, o, and p** [8]; **c, d** [19]; **k** [18]; **g, h, j, l** [13]. *h* hours, *d* days, *w* week, *m* month(s)

OxPL-PLG and the coagulation factors plasminogen, uPA activity and PAI-1 activity, as well as CFH-MDA-LDL, MDA-LDL IgG and IgM, and CuOxLDL IgM decreased immediately following PCI and then increased above baseline to a maximum at 3 days to 1 month after the procedure. CFH-MDA-LDL, PAI-1 activity, and MDA-LDL IgG peaked between 7 days. MDA-LDL IgM, CuOxLDL IgM, ApoB-IC IgM and IgG peaked at 1 month. uPA antigen and activity peaked at 3 months, and CuOxLDL IgG and peptide mimotopes to MDA-LDL at 6 months post PCI.

Discussion

The present study demonstrates that uncomplicated PCI results in acute decreases in plasminogen and OxPL carried by plasminogen, followed by rebound increase by 24 h. Furthermore, distinct temporal changes were noted in a variety of biomarkers. Oxidative biomarkers and Lp(a), which is a carrier of OxPL, rise first and likely emanate from the treated site as previously shown in material captured from distal protection devices [2, 5, 6]. These biomarkers are followed by changes in inflammatory biomarkers, coagulation factors and finally by indirect measures of oxidized lipoproteins. These observations provide insights into biomarkers reflecting iatrogenic plaque disruption putatively occurring during PCI and suggest analogous avenues of investigation to address temporal pathophysiological relationships of spontaneous plaque disruption.

In an earlier study in the same group of patients and in a second study of acute coronary syndromes [24], we showed acute increases in circulating OxPL-apoB, which primarily reflects OxPL on Lp(a) [7]. We hypothesized that OxPLs, which are preferentially bound to Lp(a), become released from disrupted atherosclerotic lesions. We have recently documented the strong presence of OxPL in progressing and ruptured human atherosclerotic lesions [5]. Furthermore, we have shown that percutaneous coronary, carotid, renal and peripheral arterial interventions result in downstream release of OxPL and oxidized cholesteryl esters [2]. Such vasoactive compounds, along with others such as endothelin-1 and other vasoactive compounds in plaque

debris [25–28], have the ability to promote downstream vasoconstriction and atherothrombosis resulting in no-flow phenomenon, may compromise procedural success, and complicate clinical outcome [29].

We now extend these observations to the other major carrier of OxPL in plasma, plasminogen. As opposed to OxPL on Lp(a) which are associated with atherothrombosis and increased risk of CVD events, the presence of OxPL on plasminogen potentiates fibrinolysis, which would be a potential benefit during and following PCI [11]. Interestingly, in contrast to increases in OxPL-apoB and Lp(a), plasminogen and OxPL-PLG showed acute decreases post PCI with subsequent rebound above baseline levels. Although this type of clinical study cannot define the mechanisms behind these observations, it does suggest the hypothesis that there may be consumption of plasminogen and its associated phospholipids during PCI that may tip the balance to increased predisposition to clotting by generating a transient pro-thrombotic state. In settings where the balance of pro- and anti-thrombotic pathways is critical, such as in acute coronary syndromes or in lesions with high-risk characteristics, such as those that have ruptured or have visible thrombus, these changes may be associated with higher risk for peri-procedural events. Similar decreases of plasminogen levels have been reported during septic shock, which also may have pro-thrombotic properties [30]. Consistent with these findings, subgroups traditionally noted by higher risk, such as males, patients with prior MI or diabetes, had lower level of OxPL-PLG post PCI.

CFH is the major inhibitor of the alternative pathway of complement activation, binds to MDA of OSEs [17], and plays a role in age-related macular degeneration (AMD) [31]. Association of the single nucleotide polymorphism (SNP) Y402H of the CFH gene with increased cardiovascular risk has been controversial [32–35]. However, complement activation has been shown to be higher in individuals presenting with ACS [36], and recently to be associated with an anti-coagulant role [37]. The biological activity of CFH binding to MDA-LDL decreased by ~12 % post PCI, and there was an increase of ~6 % at 3–7 days post PCI that remained until 6 months. Although the reason cannot be determined from this study, MDA-binding capacity as reflected by CFH binding, may also play a role in the peri-procedural period.

The array of 18 oxidative, inflammatory, and coagulation biomarkers provides new insights into temporal changes of individual constituents before, during, and after atherosclerotic plaque disruption. Based on these observations, one can conclude that material known to be directly present in lesions, such as OxPL and Lp(a) rise first and are accompanied temporally by acute reduction in OxPL-PLG, then followed by a generalized inflammatory

state, and finally by increases in coagulation factors. IL-6 is known to upregulate CRP and plasminogen, and this may explain these later increases [38]. The late rise in indirect measures of markers of OxLDL may reflect the acute plaque disruption and activation of the adaptive immune system in dealing with these released oxidative neoepitopes in the circulation or exposed neoepitopes in the atherosclerotic plaque. These observations provide a fairly comprehensive and unique assessment into changes in a variety of biomarkers reflecting iatrogenic plaque rupture and may be useful in further studies of spontaneous plaque rupture.

Although our group and others have studied individual biomarkers, or even panels of related biomarkers, this study is unique in not only measuring such a comprehensive list of serially measured biomarkers over 6 months. The unique study design allowed an assessment of several distinct pathophysiological pathways to be evaluated simultaneously and assessment of their temporal relationship to PCI.

Limitations

Although this study shows temporal changes in a variety of biomarkers, basic investigations are needed to provide mechanistic insights and outcomes studies are needed to define the clinical implications into these observations. Due to the fact that was an investigator-initiated study without significant study support, and that the patients were derived from a wide geographic area, and that the local caregivers were responsible for procuring blood samples and sending them to the central site, all timepoints could not be obtained in all patients, which may have influenced study findings. However, we saw no significant differences in patients with a full set of samples versus those without. The rate of stent placement was relatively low by current standards, reflecting the era when these patients were recruited. However, we did not find differences in patients undergoing balloon angioplasty versus stent placement, although this analysis may have been underpowered. Finally, statin therapy was not recorded in the database and we cannot assess its effect on these biomarkers.

Clinical implications

The temporal changes in the oxidative, coagulation and inflammatory biomarkers may have clinical implications now and in the future. For example, in a recent study [39], we showed mean baseline levels of plasminogen and OxPL-PLG were lower in acute MI than in the stable CAD, consistent with prior data [11]. However, mean baseline

levels of plasminogen and OxPL-PLG were also lower in atherothrombotic versus non-atherothrombotic MI. These findings may reflect a reduced fibrinolytic capacity associated with an increased risk of atherothrombotic events, and allow differentiation and clinical evaluation of stable CAD from unstable CAD and atherothrombotic MI. In addition, these findings may allow “biotheranostic” applications (i.e. biomarker, therapeutics and diagnostic imaging) in future studies [40]. In the example if OxPL on lipoproteins is the biotheranostic target, it can be measured in the plasma as a biomarker to screen for high risk or diagnose a higher risk patient [2, 41, 42]. If the plasma level is elevated, one can employ nuclear or magnetic resonance imaging to localize the source [43]. Finally one may utilize passive immunization in the form of a therapeutic dose of antibody to treat the source, as has been shown with antibodies E06 [44] and IK17 in experimental models [45, 46].

Conclusion

PCI results in a unique expression and temporal sequence of oxidative, inflammatory and coagulation and immunity related biomarkers that may have clinical implications in plaque disruption and plaque vulnerability.

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Compliance with ethical standards

Disclosures Dr. Tsimikas is a co-inventor and receives royalties for patents owned by the University of California for the clinical use of oxidation-specific antibodies and currently holds a dual appointment at UCSD and as an employee of Ionis Pharmaceuticals.

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