Peripheral Arterial Tonometry for Risk Stratification in Men With Coronary Artery Disease

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ABSTRACT

Background: Coronary artery disease (CAD) risk is not fully revealed by traditional risk factors. Identification of a simple, noninvasive tool that allows for detection of high-risk CAD patients and can be applied in large populations and clinical settings would prove valuable.

Hypothesis: We sought to test the hypothesis that peripheral arterial tonometry (PAT) would be associated with residual risk in men with CAD.

Methods: In this study, finger PAT was used to measure pulse wave amplitude (PWA) during reactive hyperemia (RH) and taken as a measure of microvascular endothelial function in 42 men with stable CAD and well controlled low-density lipoprotein cholesterol (LDL-C) levels. Plasma levels of high-sensitivity C-reactive protein (hs-CRP) and lipoprotein-associated phospholipase A2 (Lp-PLA_2) were measured and used to reclassify men into high-risk (elevated hs-CRP and Lp-PLA_2), moderate-risk (either elevated hs-CRP or Lp-PLA_2), or low-risk (low hs-CRP and Lp-PLA_2) groups.

Results: PWA-RH was significantly lower in the high-risk group (1.3 ± 0.04) compared to the moderate-risk (1.6 ± 0.07, $P < 0.05$) and low-risk (2.0 ± 0.1, $P < 0.05$) groups. According to binary logistic regression, PWA-RH was a significant predictor of high-risk status among men with CAD ($P < 0.05$).

Conclusion: Measurement of peripheral microvascular endothelial function with PAT may be able to distinguish high-risk men from moderate- and low-risk men with stable CAD and well-controlled LDL-C levels and thus aid in residual risk stratification in this at risk cohort.

Introduction

Patients with stable coronary artery disease (CAD) have a high-risk of subsequent cardiovascular (CV) events and mortality. Currently, cardiovascular risk reduction approaches in patients with CAD are focused on lipid management, in particular reducing circulating levels of low-density lipoprotein cholesterol (LDL-C).[1] However, lowering LDL-C alone does not confer adequate risk reduction for many patients with CAD.[1] Both primary and secondary prevention trials indicate that 60% to 70% of major CV events are not prevented with current therapeutic strategies[2] and traditional CV risk factors fail to explain almost 50% of CAD morbidity and mortality.[3] Therefore, identification of a simple, noninvasive prognostic tool that improves detection/stratification of high-risk patients and can be applied in large populations and/or clinical settings would prove invaluable.

Persistent impairment of endothelial function despite optimized therapy to reduce traditional atherosclerotic risk factors has an adverse impact on outcome in CAD patients.[4,5] Use of finger peripheral arterial tonometry (PAT) to measure pulse wave amplitude (PWA) during reactive hyperemia (RH) is currently being investigated as a non-invasive measure of peripheral microvascular endothelial function.[6,7] This measure correlates with coronary and conduit artery endothelial function,[6–8] is dependent on nitric oxide synthesis,[9] and can be improved by therapies known to improve endothelial function.[10–12] PWA-RH is associated with CV risk factors[6,7,13] and is predictive of future CV events.[14,15] Whether PAT can aid with residual risk stratification in men with CAD and well-controlled LDL-C levels remains unknown. Hence the purpose of this study was to examine the clinical utility of PWA-RH for risk stratification in this cohort.

Methods

A total of 42 men with CAD (defined by the presence of ischemia or infarction on single-photon emission computed tomographic nuclear myocardial perfusion imaging or >50% stenosis of an epicardial coronary artery by angiography) were enrolled in this study. All men had statin-controlled LDL-C levels <100 mg/dL. Exclusion criteria included: myocardial infarction or unstable angina pectoris within the previous 3 months; a history of congestive heart failure or left ventricular ejection fraction <40%; known severe valvular

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Table 1. Patient Characteristics

Variable	All n = 42	Low-risk n = 14	Moderate-Risk n = 16	High-Risk n = 12
Total cholesterol, mg/dL	141 ± 3	135 ± 7	137 ± 5	147 ± 5
LDL-C, mg/dL	80 ± 3	77 ± 5	77 ± 3	82 ± 4
HDL-C, mg/dL	40 ± 1	39 ± 2	40 ± 2	42 ± 2
Systolic BP, mm Hg	124 ± 2	125 ± 4	126 ± 3	120 ± 1
Diastolic BP, mm Hg	75 ± 1	76 ± 2	77 ± 2	72 ± 2
Diabetes, n (%)	8 (19)	2 (14)	4 (25)	2 (17)
Smoker, n (%)	28 (67)	9 (64)	10 (63)	9 (75)
Framingham risk score (%)	13	13	13	13
hs-CRP, mg/L	3.0 ± 0.4	1.2 ± 0.2	3.4 ± 0.6[a]	4.6 ± 0.4[a]
Lp-PLA_2, ng/dL	279.4 ± 11.7	211.5 ± 12.5	287.0 ± 16.7[a]	348.4 ± 11.4[a,b]
Medication History				
Aspirin, n (%)	36 (86)	14 (100)	12 (75)	10 (83)
β-Blocker, n (%)	35 (83)	10 (71)	14 (88)	11 (92)
Calcium channel blocker, n (%)	8 (19)	1 (10)	4 (25)	3 (25)
ACE inhibitor, n (%)	25 (60)	10 (71)	8 (50)	7 (58)
Nitrates, n (%)	8 (19)	3 (21)	2 (13)	3 (25)
AR blocker, n (%)	6 (14)	1 (7)	3 (18)	2 (17)

Abbreviations: ACE, angiotensin-converting enzyme; AR, angiotensin receptor; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; Lp-PLA_2, lipoprotein-associated phospholipase A_2.
Data are mean ± SEM.
[a] Significantly different from low-risk.
[b] Significantly different from moderate-risk.

heart disease; a triglyceride level >400 mg/dL; uncontrolled hypertension; and liver/renal dysfunction. The protocol was approved by the institutional review board at Tufts Medical Center, and all patients provided informed written consent before enrollment.

The presence or absence of the following CV risk factors was assessed in each patient: male sex; hypertension (systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg or treatment with an antihypertensive agent); diabetes mellitus (fasting glucose levels >126 mg/dL or treatment with an oral hypoglycemic agent); family history of CV disease, and smoking status (having smoked at least 5 cigarettes per d within the prior mo). From this information, a Framingham 2-year recurring risk score was calculated as previously described.[16] Vascular measures were performed with the subject in a supine position in a dimly lit, temperature-controlled room following a 10-minute acclimatization period. Participants were tested in a fasted state (>12 h overnight fast) and asked to refrain from exercise, smoking, and caffeine consumption on the day of testing.

Pulse wave amplitude was assessed before and during reactive hyperemia (RH) by PAT (Endo-PAT2000, Itamar Medical Ltd., Caeserea, Israel) as previously described in detail.[6] Baseline PWA data was collected using plethysmographic finger cuffs, placed on the index finger of both hands, for a period of 5 minutes. An ischemic stimulus was induced by cuff occlusion (inflation of a brachial cuff to a supra-systolic pressure of 200 mm Hg for 5 min) and the PWA-RH index was calculated as the ratio of the average PWA over a 1-minute epoch, starting after cuff release, to the preocclusion baseline PWA.

Fasting blood draws were obtained and stored at −70°C for each subsequent batch analysis. Validated assays for lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and high-sensitivity C-reactive protein (hs-CRP) were performed (diaDexus, San Francisco, CA). Blood lipids were assessed using standard techniques in a clinical laboratory.

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Statistical Analysis

All data are reported as means ± SEM. A priori significance was set at $P < 0.05$. Normality of distribution was assessed using Kolmogorov-Smirnof and Shapiro-Wilk tests. Patients were separated using median values of hs-CRP and Lp-PLA_2 as cut points. Patients with both hs-CRP and Lp-PLA_2 above the group median were taken as high-risk. Patients with either hs-CRP or Lp-PLA_2 above the group median were taken as moderate-risk. Patients with both hs-CRP and Lp-PLA_2 below the group median were taken as low-risk. Analysis of variance was used to compare continuous variables between groups. If a significant group effect was detected, post hoc comparisons were made using independent samples t tests. χ^2 tests were used to compare categorical variables between groups. Pearson's correlation coefficients were used to assess relationships between variables of interest. Binary logistic regression analysis was performed to examine predictors of high risk status in our cohort. Variables entered into the model included traditional cardiovascular risk factors (age, presence/absence of hypertension, presence/absence of diabetes, smoking status, high-density lipoprotein cholesterol, total cholesterol) and PWA-RH.

Results

Patient ages were 60 ± 1 years old, height = 1.77 ± 0.01 m, and weight = 91.9 ± 2.3 kg. All patients were taking at least 1 antihypertensive agent (range, 1–3 agents; average 2 antihypertensive agents). Patient demographic data according to risk (identified by hs-CRP and Lp-PLA_2) are presented in the Table. When separated according to risk, groups did not differ in age, height, and weight ($P > 0.05$). There were significant group differences in PWA-RH ($P < 0.05$; Figure 1). According to post hoc comparisons, PWA-RH was different in high-risk vs moderate-risk and low-risk patients ($P < 0.05$), and PWA-RH was different in moderate-risk vs low-risk patients ($P < 0.05$). According to binary logistic regression, after controlling for traditional risk factors, PWA-RH was a significant predictor of high-risk status among all patients (β = −27.9, Wald statistic = 5.1, $P < 0.05$). When examining moderate-risk and high-risk patients only, PWA-RH was able to predict high-risk status (β = −7.1, Wald statistic = 5.0, $P < 0.05$). When examining moderate-risk and low-risk patients only, there was a trend for PWA-RH to predict moderate-risk status (β = −1.8, Wald statistic = 3.4, $P = 0.06$). There was a positive association between hs-CRP and Lp-PLA_2 ($r = 0.28$, $P < .05$). There was a significant inverse association between PWA-RH and hs-CRP ($r = −0.37$, $P < 0.05$) and Lp-PLA_2 ($r = −0.54$, $P < 0.05$; Figure 2).

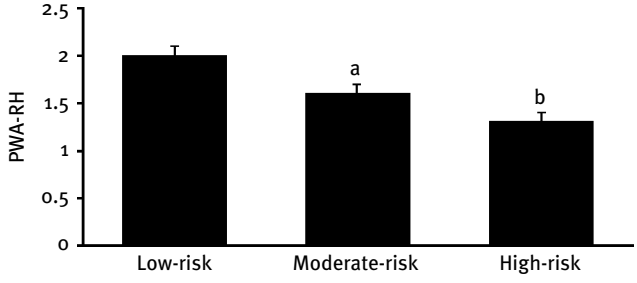

Figure 1. Index of pulse wave amplitude during reactive hyperemia in men with CAD according to CV risk. Data are mean ± SEM.
Abbreviations: CAD, coronary artery disease; CV, cardiovascular; PWA-RH, pulse wave amplitude during reactive hyperemia.
[a] Significantly different from low-risk.
[b] Significantly different from moderate-risk.

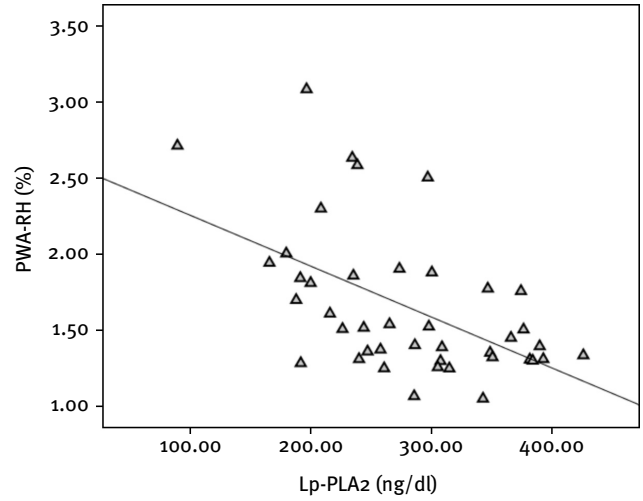

Figure 2. Association between PWA-RH and Lp-PLA_2 in men with CAD.
Abbreviations: CAD, coronary artery disease; Lp-PLA_2, lipoprotein-associated phospholipase A_2; PWA-RH, pulse wave amplitude during reactive hyperemia.

Discussion

Patients with stable CAD have a high risk of subsequent CV events and mortality. However, CV risk appears quite variable as not all patients who have had a CV event are at the same risk of such an event recurring. Moreover, approximately two-thirds of all CV events still occur with statin therapy, signifying that significant residual risk prevails despite well controlled LDL-C.[17] Recently, results from the Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial have highlighted that CV event rates are reduced with statin therapy provided both LDL-C and hs-CRP levels are targeted.[18,19] Patients with "normal" LDL-C (<130 mg/dL) but high hs-CRP are at increased CV risk and hs-CRP reduction via statin therapy reduces risk independent of LDL-C modulation[18,19] suggesting a role for inflammation in residual risk. Lipoprotein-associated

phospholipase A_2 is a marker of vascular inflammation that when combined with hs-CRP may improve prognostic power for CV risk stratification beyond traditional risk factors.[20–25] In the Atherosclerosis Risk In Communities (ARIC) study, patients with elevated hs-CRP and elevated Lp-PLA_2 were 3 times more likely to suffer a coronary event and 11 times more likely to suffer an ischemic stroke compared with individuals with low levels of these inflammatory biomarkers.[26,27] Thus, men with CAD and elevated hs-CRP and Lp-PLA_2 may be a particularly vulnerable, higher risk group.

The novel finding of the present study is that PWA-RH obtained from PAT is able to distinguish high-risk from moderate-risk and low-risk patients in men with stable CAD and well controlled LDL-C levels. Moreover, this technique may be able to distinguish moderate-risk from low-risk patients. Thus, PAT may aid in residual risk stratification in this at risk cohort.

Microvascular endothelial function measured by PAT is reflective of CV risk factor burden and is reduced in patients with documented CAD compared to patients without CAD.[6,7,13,15] In the present study, CV risk (according to traditional risk factor assessment) was similar among men with CAD. However, Lp-PLA_2 and hs-CRP were quite variable, possibly suggesting varying levels of risk. Recent work from Kitta et al have shown that persistent impairment of endothelial function despite optimized therapy to reduce atherosclerotic risk factors has an adverse impact on outcome in CAD patients.[5] Conversely, interventions that improve endothelial function improve clinical outcome.[4,28] Therefore, it has been suggested that measurement of endothelial function may have better predictive value for future CV events than the analysis of traditional risk factors alone.[5] Given the ease of use of PAT in an ambulatory setting,[7] our findings suggest that this technique may have clinical utility as a means of identifying patient subgroups at higher risk for CV events. Subsequently, these patients may require more stringent medical management of traditional and nontraditional risk factors to improve endothelial function and reduce CV risk.

It is currently accepted that inflammation is intimately entwined in the pathogenesis of CAD[29,30] and there is a strong link between inflammation and endothelial dysfunction.[31–35] To our knowledge, this study is the first to note an association between peripheral microvascular endothelial function (measured using PAT) and systemic/vascular inflammation. Therefore, similar to other noninvasive methods of endothelial function assessment,[36,37] PWA-RH obtained from PAT may be a sensitive method for detecting inflammatory burden in men with CAD.

Limitations to this study should be noted. This study has a relatively small sample size with a rather homogenous group of participants. Additional research is needed to examine if findings extend to other cohorts such as women. Residual risk was evaluated using circulating levels of inflammatory biomarkers. Whether said biomarkers are causative or reflective of disease remains to be determined. Finally, this study lacks information on clinical end points and only examines a single surrogate marker (ie, PWA-RH from PAT).

In conclusion, measurement of peripheral microvascular endothelial function with PAT can distinguish high-risk from moderate-risk and low-risk patients in men with stable CAD and well controlled LDL-C and thus aid in residual risk stratification in this at risk cohort. Future research is needed to examine if more aggressive therapy that targets inflammation and endothelial dysfunction can concomitantly improve clinical outcome (ie, reduce residual risk) in patients with CAD.

References

1. Davidson MH, Toth PP. High-density lipoprotein metabolism: potential therapeutic targets. *Am J Cardiol*. 2007;100:n32–40.
2. Bisoendial RJ, Hovingh GK, Levels JH, et al. Restoration of endothelial function by increasing high-density lipoprotein in subjects with isolated low high-density lipoprotein. *Circulation*. 2003;107:2944–2948.
3. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*. 2002;106:3143–3421.
4. Suessenbacher A, Frick M, Alber HF, et al. Association of improvement of brachial artery flow-mediated vasodilation with cardiovascular events. *Vasc Med*. 2006;11:239–244.
5. Kitta Y, Obata JE, Nakamura T, et al. Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease. *J Am Coll Cardiol*. 2009;53:323–330.
6. Kuvin JT, Patel AR, Sliney KA, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J*. 2003;146:168–174.
7. Kuvin JT, Mammen A, Mooney P, et al. Assessment of peripheral vascular endothelial function in the ambulatory setting. *Vasc Med*. 2007;12:13–16.
8. Dhindsa M, Sommerlad SM, Devan AE, et al. Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. *J Appl Physiol*. 2008;105:427–432.
9. Nohria A, Gerhard-Herman M, Creager MA, et al. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol*. 2006;101:545–548.
10. Yeo TW, Lampah DA, Gitawati R, et al. Impaired nitric oxide bioavailability and L-arginine reversible endothelial dysfunction in adults with falciparum malaria. *J Exp Med*. 2007;204:2693–2704.
11. Aversa A, Vitale C, Volterrani M, et al. Chronic administration of sildenafil improves markers of endothelial function in men with type 2 diabetes. *Diabet Med*. 2008;25:37–44.
12. Bonetti PO, Barsness GW, Keelan PC, et al. Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. *J Am Coll Cardiol*. 2003;41:1761–1768.
13. Hamburg NM, Keyes MJ, Larson MG, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation*. 2008;117:2467–2474.
14. Rubinshtein R, Kuvin JT, Soffler M, et al. Assessment of endothelial function by peripheral arterial tonometry predicts cardiovascular events beyond the Framingham Risk Score. *J Am Coll Cardiol*. 2009;53:A457.

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15. Bonetti PO, Pumper GM, Higano ST, et al. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol*. 2004;44: 2137–2141.
16. D'Agostino RB, Russell MW, Huse DM, et al. Primary and subsequent coronary risk appraisal: new results from the Framingham study. *Am Heart J*. 2000;139:272–281.
17. Fruchart JC, Sacks F, Hermans MP, et al. The Residual Risk Reduction Initiative: a call to action to reduce residual vascular risk in patients with dyslipidemia. *Am J Cardiol*. 2008;102:1K–34K.
18. Ridker PM, Danielson E, Fonseca FA, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. *Lancet*. 2009;373:1175–1182.
19. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195–2207.
20. Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated phospholipase A_2 as a cardiovascular risk marker. *Am J Cardiol*. 2008;101:41F–50F.
21. Lerman A, McConnell JP. Lipoprotein-associated phospholipase A_2: a risk marker or a risk factor? *Am J Cardiol*. 2008;101:11F–22F.
22. Nambi V, Hoogeveen RC, Chambless L, et al. Lipoprotein-associated phospholipase A_2 and high-sensitivity C-reactive protein improve the stratification of ischemic stroke risk in the Atherosclerosis Risk in Communities (ARIC) study. *Stroke*. 2009;40: 376–381.
23. Davidson MH, Corson MA, Alberts MJ, et al. Consensus panel recommendation for incorporating lipoprotein-associated phospholipase A_2 testing into cardiovascular disease risk assessment guidelines. *Am J Cardiol*. 2008;101:51F–57F.
24. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A_2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. *Arterioscler Thromb Vasc Biol*. 2006;26:1586–1593.
25. Koenig W, Khuseyinova N, Lowel H, et al. Lipoprotein-associated phospholipase A_2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. *Circulation*. 2004;110:1903–1908.
26. Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A_2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Arch Intern Med*. 2005;165:2479–2484.
27. Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A_2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation*. 2004;109:837–842.
28. Modena MG, Bonetti L, Coppi F, et al. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol*. 2002;40:505–510.
29. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111:3481–3488.
30. Tousoulis D, Charakida M, Stefanadis C. Endothelial function and inflammation in coronary artery disease. *Postgrad Med J*. 2008;84: 368–371.
31. Clapp BR, Hirschfield GM, Storry C, et al. Inflammation and endothelial function: direct vascular effects of human C-reactive protein on nitric oxide bioavailability. *Circulation*. 2005;111: 1530–1536.
32. Kals J, Kampus P, Kals M, et al. Inflammation and oxidative stress are associated differently with endothelial function and arterial stiffness in healthy subjects and in patients with atherosclerosis. *Scand J Clin Lab Invest*. 2008: 1–8.
33. Liuba P, Aburawi EH, Pesonen E, et al. Residual adverse changes in arterial endothelial function and LDL oxidation after a mild systemic inflammation induced by influenza vaccination. *Ann Med*. 2007;39:392–399.
34. Tousoulis D, Charakida M, Stefanadis C. Endothelial function and inflammation in coronary artery disease. *Heart*. 2006;92: 441–444.
35. Kharbanda RK, Walton B, Allen M, et al. Prevention of inflammation-induced endothelial dysfunction: a novel vasculoprotective action of aspirin. *Circulation*. 2002;105:2600–2604.
36. Vitale C, Cerquetani E, Wajngarten M, et al. In patients with coronary artery disease endothelial function is associated with plasma levels of C-reactive protein and is improved by optimal medical therapy. *Ital Heart J*. 2003;4:627–632.
37. Kang SM, Chung N, Kim JY, et al. Relation of vasodilator response of the brachial artery to inflammatory markers in patients with coronary artery disease. *Echocardiography*. 2002;19:661–667.

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