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### Permalink

<https://escholarship.org/uc/item/6vc6s54r>

### Journal

Texas Heart Institute Journal, 43(2)

### ISSN

0730-2347

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### Publication Date

2016-04-01

### DOI

10.14503/thij-15-5053

Peer reviewed

# Longitudinal Changes in Vascular Risk Markers and Mortality Rates

among a Latino Population with Hypertension

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**Key words:** Biomarkers/analysis; cardiovascular diseases/epidemiology/ethnology/prevention & control; Hispanic Americans/ethnology; models, statistical; phospholipases A2; predictive value of tests; pulse-wave analysis/methods; risk assessment; risk factors; socioeconomic factors

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This work was supported by National Heart, Lung, and Blood Institute grant U01 HL079160 and by Health Resources and Services Administration Administrative Unit grant HP00054 5 D12.

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Vascular markers such as pulse-wave velocity and carotid intima-media thickness (CIMT) might improve the prediction of incident cardiovascular disease beyond traditional risk factors. These vascular markers have not been well characterized in minority populations and might be more useful than inflammatory biomarkers. We conducted a prospective, longitudinal cohort study among hypertensive patients in an urban safety-net hospital. We evaluated inflammatory biomarkers, arterial pulse-wave velocity, and carotid intima-media thickness at baseline, 1 year, and 2 years. The primary outcome variable was CIMT. Generalized linear mixed-effects models were used to evaluate associations between CIMT and predictive variables accounting for the correlation of multiple measurements within subjects over time. For our secondary outcome, we used administrative and National Death Index data to determine all-cause death, and univariate relationships were evaluated.

Among 175 subjects, 117 were Latino (67%) and 117 were female (67%). Pulse-wave velocity and CIMT regressed over time (both  $P < 0.001$ ) and were highly correlated ( $P < 0.001$ ). Only pulse-wave velocity ( $P = 0.002$ ) and total cholesterol ( $P = 0.03$ ) were associated with CIMT in time-varying covariate analysis. At a median follow-up period of 80 months, 17 of 175 subjects had died (10%). Higher baseline CIMT and pulse-wave velocity were associated with increased mortality rates (both  $P < 0.01$ ). No serum inflammatory marker was significantly correlated with longitudinal changes in CIMT or death. In conclusion, both arterial stiffness and preclinical carotid atherosclerosis were associated with increased mortality rates and might be useful risk-stratification markers among this minority population. (**Tex Heart Inst J 2016;43(2):131-6**)

The search for novel clinical markers in order to improve the prediction of cardiovascular disease (CVD) beyond traditional risk factors is an ongoing area of research. Whereas risk factors such as tobacco abuse, hyperlipidemia, and diabetes mellitus are currently used to calculate global CVD risk,<sup>1</sup> they are not equally predictive in all subgroups of race<sup>2</sup> and sex,<sup>3</sup> and they are even less reliable predictors of noncoronary vascular disease, which is more prevalent in nonwhite populations.<sup>2</sup> This might be particularly relevant among Latinos, who are among the fastest-growing minority groups, expanding at 4 times the rate of the rest of the United States' population.<sup>4</sup>

Although circulating inflammatory biomarkers such as high-sensitivity C-reactive protein (hs-CRP) have been shown to improve CVD risk discrimination, validation cohorts have included just 1% Latino subjects.<sup>5</sup> In addition, elevation in these circulating biomarkers varies significantly with race and ethnicity,<sup>6</sup> so their diagnostic usefulness in the Latino population remains unknown. Recently, the use of physiologic tools such as pulse-wave velocity (PWV) for estimating vascular stiffness has been shown to improve the accuracy of risk stratification. Specifically, a meta-analysis of more than 17,000 patients suggests that the addition of arterial PWV to traditional 10-year CVD risk calculation improves the net reclassification index.<sup>7</sup> Notably, the results of this study showed improved risk categorization to the greatest degree among younger individuals and those labeled as having intermediate global risk. Reclassifying intermediate-risk patients as high- or low-risk, when measures of arterial stiffness are taken into account, therefore has potentially important implications for initiating or withholding therapeutic interventions. The role of arterial stiffness in improving CVD prediction is promising; however, to date, it has not been adequately evaluated among populations of lower socioeconomic status.

Using carotid intima-media thickness (CIMT) as a surrogate marker for preclinical atherosclerosis, we have previously shown that PWV was independently associated with CIMT in a group of at-risk patients in a safety-net system.<sup>8</sup> However, neither hs-CRP nor lipoprotein-associated phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>) was associated with preclinical atherosclerosis in that cross-sectional study. In the present study, we sought to determine the relationship between PWV, inflammatory biomarkers, and CIMT longitudinally over 2 years and evaluate their association with subsequent all-cause death in this cohort.

## Patients and Methods

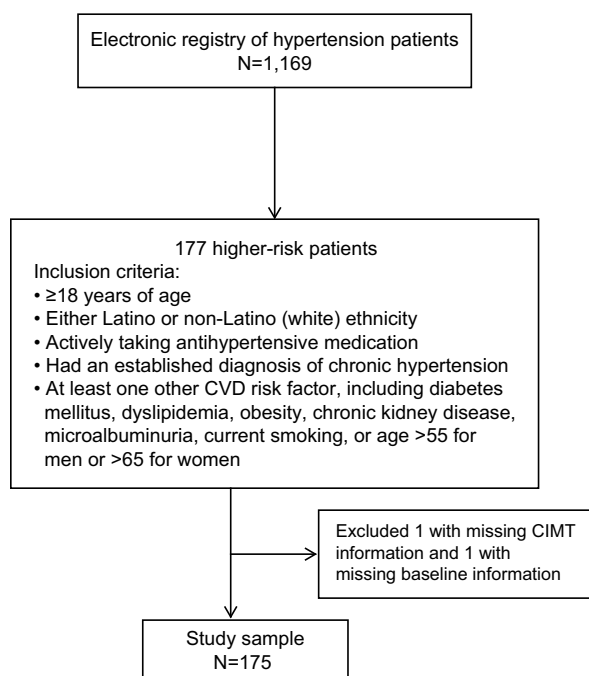
The study sample consisted of 175 subjects recruited from an electronic registry of hypertensive patients at Denver Health, an integrated urban safety-net health system.<sup>9,10</sup> Data from the registry were supplemented with chart review and patient self-reporting during an enrollment interview. Patients were eligible for participation if they were ≥18 years of age, of either Latino or non-Latino (white) ethnicity, and were actively taking antihypertensive medication or had an established diagnosis of hypertension. Subjects had to have at least one other CVD risk factor, including diabetes mellitus, dyslipidemia, obesity, chronic kidney disease, microalbuminuria, current smoking, or age >55 for men or >65 for women. Patients were excluded if they had CVD (history of myocardial infarction, prior percutaneous or surgical coronary revascularization, stroke, cerebrovascular revascularization, or peripheral arterial disease). Additional exclusion criteria were valvular heart disease, end-stage renal disease, collagen-vascular disease, active substance abuse, or a projected life expectancy of <12 months. Figure 1 shows a summary of participant selection and inclusion. Noninvasive vascular structure and function markers, as well as inflammatory biomarkers, were obtained at baseline, 1 year, and 2 years. Studies were performed in compliance with human-studies guidelines at our institution and with U.S. Food and Drug Administration guidelines. After the study was explained to them, all participants provided written informed consent. The local Institutional Review Board approved the protocol.

We asked patients to refrain from eating, drinking, and smoking on the night before their venous plasma samples were drawn. Their hs-CRP and Lp-PLA<sub>2</sub> levels were measured, the latter because of its purported specificity for inflammation localized to atherosclerotic plaque, including in the carotid arteries.<sup>11-14</sup> Levels of Lp-PLA<sub>2</sub> mass and activity were evaluated with use of the PLAC<sup>®</sup> dual monoclonal antibody immunoassay (Dadexus Inc.; South San Francisco, Calif).<sup>14</sup> Samples were measured on-site with use of the Dimension Vista<sup>®</sup> Flex<sup>®</sup> hs-CRP assay (Siemens Healthcare Diagnostics

Products; Marburg, Germany) and were then validated against the Roche Hitachi Modular assay.<sup>15</sup>

All vascular-function measurements were performed in a quiet environment at room temperature. Blood pressure was measured in duplicate with subjects in the supine position, in the nondominant arm. Ankle-brachial PWV was derived from the pulse transit time between and the estimated path length between proximal and distal arterial sites, expressed as cm/s. With use of an HEM-9000AI device (Omron Healthcare, Inc.; Lake Forest, Ill), applanation tonometry was performed at the radial artery to derive the augmentation index (AIx) and central aortic pressure. The AIx was calculated as the difference between the first (ejection) and second (reflected) peaks of the arterial waveform, expressed as a percentage of the pulse pressure, whereby higher AIx values indicated greater vascular stiffness.

Measures of maximal CIMT were obtained by a single ultrasonographer with subjects in the supine position. Longitudinal B-mode images were obtained with the scanner head turned 45° from the area scanned. Gain settings were optimized to acquire far-wall arterial images and to limit echogenicity of the lumen. A Sonos 5500<sup>®</sup> linear array probe (Philips; Best, The Netherlands) was used for image acquisition. Three longitudinal views of both internal carotid arteries (total, 6 CIMT images per subject) were acquired as previously described.<sup>16</sup> The internal carotid artery was defined to include the bulb and the initial 10 mm of vessel distal to



**Fig. 1** Flow chart shows inclusion criteria.

CIMT = carotid intima-media thickness; CVD = cardiovascular disease

separation of the external from internal arteries. High-resolution images were stored digitally and were read offline by interpreters who were blinded to the clinical characteristics of the study participants. Near- and far-wall thicknesses were calculated as the maximum distances between the lines.

Data on deaths were obtained by means of an electronic query of the Denver Health Data Warehouse through September 2014. The Warehouse culls retrospective data from administrative and clinical electronic health-information applications used in the provision of patient care. Additional death data were obtained from the Centers for Disease Control National Death Index (NDI), a centralized dynamic registry of death-record information in the United States on file in the Office of Vital Statistics (available at [www.cdc.gov/nchs/ndi.htm](http://www.cdc.gov/nchs/ndi.htm)). We matched study participants to the NDI by using combinations of name, Social Security number, birthdate, sex, race, marital status, and state of residence. Only those matches considered to be true, on the basis of probabilistic scoring reported by the NDI, were retained. The mean follow-up period was based on 60 months for NDI information and 100 months for Data Warehouse information.

### Statistical Analysis

Mean  $\pm$  SD, median, and range were calculated for all continuous variables. Pulse-wave velocity was normalized after transformation with use of the relationship  $P_{\text{inverse}} = -(1/\text{PWV} - 0.0006) / (0.00011)$ .<sup>17</sup> Longitudinal change in CIMT was the primary outcome variable; a secondary outcome was all-cause death. To account for the longitudinal nature of the CIMT data, generalized PROC MIXED linear mixed-effects models (SAS Institute Inc.; Cary, NC) were used to evaluate the association between CIMT and the independent variables at baseline, 1 year, and 2 years. Baseline age, sex, body mass index, current smoking status, and diabetes mellitus diagnosis, as well as temporal changes in systolic blood pressure, AIx, high-density-lipoprotein (HDL) cholesterol, total cholesterol, Lp-PLA<sub>2</sub> mass, Lp-PLA<sub>2</sub> activity, statin use, and antihypertensive medication use were candidate variables. The  $\chi^2$ , Fisher exact, Mantel-Haenszel  $\chi^2$ , Student *t*, or Wilcoxon rank-sum test was used as appropriate to evaluate univariate relationships between independent variables, temporal changes in CIMT, and death. We did not perform multivariate analyses on death, given the low number of outcomes. For modeling CIMT over time, we included factors with bivariate *P* values  $<0.25$  as candidates in a multivariable model. These were baseline age, sex, longitudinal changes in systolic blood pressure, AIx, HDL and total cholesterol, PWV, and Lp-PLA<sub>2</sub> mass. We then used a backward elimination procedure in which the least significant predictor in the model was removed sequentially. At each step, estimates were checked to en-

sure that other variables were not affected by our dropping the least significant variable. This resulted in the retention of only those factors that were significant at *P*  $<0.05$  in the final model. SAS software version 9.4 was used for all statistical analyses.

## Results

The study sample consisted of 175 subjects who had chronic arterial hypertension. Among the cohort, baseline sociodemographic characteristics suggested a vulnerable population. Overall, 117 patients were Latino (67%), 117 were female (67%), and 92 had not completed high school (53%) (Table I). Nearly half of the patients had established diabetes mellitus, and the median body mass index in the study population was 32 kg/m<sup>2</sup>.

Among the 175 subjects, 137 (78%) had at least 2 measurements performed. At baseline, increasing age and male sex were significantly associated with a higher CIMT (both *P*  $<0.001$ ). Evaluation of a total of 345 longitudinal CIMT measurements yielded a significant decrease over the study period, an average of 0.0058 mm/yr (*P*  $<0.001$ ) (Fig. 2). There was also a corresponding decrease in systolic blood pressure (*P*  $<0.001$ ) and total cholesterol (*P* = 0.02) in the cohort. Only arterial PWV (*P* = 0.002) and total cholesterol level (*P* = 0.03) were significantly associated with CIMT upon use of a time-varying covariate analysis. Higher PWV over time was significantly associated with higher CIMT over time (estimate = 0.00116 mm/100-U increase in PWV; SE = 0.00035). No other serum inflammatory marker (Lp-PLA<sub>2</sub> or hs-CRP) or vascular marker (AIx or brachial artery flow-mediated dilation) was significantly correlated with longitudinal changes in CIMT.

During a median follow-up period of 80 months, there were 17 deaths from any cause. Systolic blood pressure, arterial PWV, and CIMT were significantly associated with all-cause death (Table II). A trend toward an association between global (Framingham) risk score and death was also observed (*P* = 0.05).

## Discussion

In this prospective, observational cohort study, temporal changes in arterial PWV correlated with changes in CIMT and total cholesterol level, suggesting a relationship between vascular stiffness and anatomic atherosclerosis among patients with hypertension. No other clinical variables, including systolic blood pressure, age, circulating biomarkers, AIx, brachial artery flow-mediated dilation, or central aortic pressure, correlated with CIMT over time. This finding is consistent with our previously reported observation that arterial PWV exhibited the strongest linear relationship with CIMT in a baseline cross-sectional analysis,<sup>8</sup> and it corroborates the results of the larger Rotterdam cross-sectional study,

which similarly revealed a positive association between arterial PWV and CIMT among 3,000 participants.<sup>18</sup> To our knowledge, ours is the first study to prospectively show that arterial stiffness is associated with changes in preclinical atherosclerosis over time. The evaluation of novel CVD risk markers in our population of lower socioeconomic status might be particularly relevant from a health-policy standpoint, because economic depriva-

tion, beyond ethnicity, is a significant, independent predictor of CVD events.<sup>19</sup>

Arterial PWV has been clearly shown to improve the prediction of CVD events beyond standard modifiable risk factors<sup>6</sup>; and in our study, it was a more robust predictor than was the global CVD risk score using the Framingham formula. However, a causal pathobiological relationship between increased arterial PWV and clinical atherosclerosis remains less certain at this time.<sup>20</sup> Our finding that arterial stiffness has the strongest correlation with preclinical atherosclerosis suggests that further studies are warranted to investigate the temporal causality of this relationship. We are not aware of prospective, randomized interventional trials that have targeted a reduction in PWV and revealed reduced CVD events. Nonetheless, novel pharmacologic therapies also have the potential to affect vascular remodeling and ameliorate arterial stiffness and preclinical atherosclerosis. A molecular intervention that targets elastic fiber cross-linking is under investigation.<sup>21</sup>

Increased arterial PWV and CIMT were both associated with higher mortality rates in our population over nearly 7 years of follow-up. Although the global CVD (Framingham) risk score trended with mortality rate in the current study, the strength of the association was more apparent with PWV and CIMT. This affirms the paradigm that novel risk markers might have greater prognostic usefulness than do traditional risk factors in the medical safety net, although no larger-scale interventional trial has validated the public-health benefits of widespread population screening. In addition, our results are consistent with previous, larger studies in which these measures were predictive of actual CVD events.<sup>22</sup>

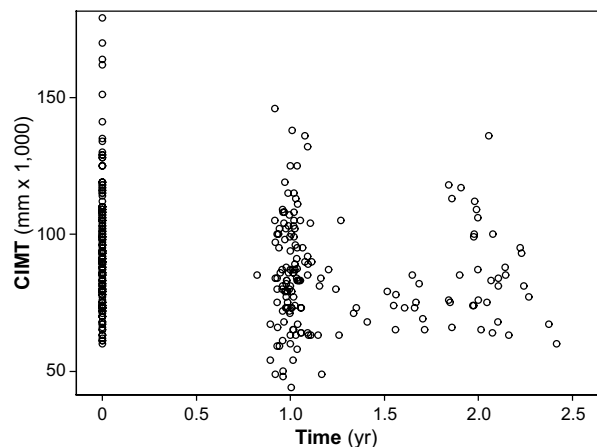
Our findings of the relationship between preclinical atherosclerosis and arterial stiffness and their positive association with higher all-cause mortality rates suggests

**TABLE I.** Baseline Clinical Characteristics of the 175 Patients

Variable	Value
Age (yr)	61.7 ± 9.5
Female	117 (67)
Ethnicity	
Hispanic	117 (67)
Non-Hispanic white	58 (33)
Educational level	
No high school	54 (31)
Some high school	38 (22)
High school graduate	35 (20)
Beyond high school	48 (27)
Employment status	
Employed	33 (19)
Unemployed	82 (47)
Disabled	60 (34)
Body mass index (kg/m <sup>2</sup> )	32
Blood pressure (mmHg)	139/80
Total cholesterol (mg/dL)	182
Triglycerides (mg/dL)	152
HDL cholesterol (mg/dL)	49
LDL cholesterol (mg/dL)	95
Diabetes mellitus	86 (49)
Current smoker	38 (22)
Statin prescription filled in year before enrollment	94 (54)
Antihypertensive prescription filled in year before enrollment	142 (81)
Framingham risk score (%)	10
Lp-PLA <sub>2</sub> mass (ng/mL)	170
Lp-PLA <sub>2</sub> activity (nmol/min/mL)	134
Central aortic pressure (mmHg)	55
Augmentation index (%)	86
Pulse-wave velocity (cm/s)	1,658
Brachial artery flow-mediated dilation (%)	9.8
Carotid intima-media thickness (mm)	0.091
High-sensitivity C-reactive protein (mg/L)	3.1

HDL = high-density-lipoprotein, LDL = low-density-lipoprotein; Lp-PLA<sub>2</sub> = lipoprotein-associated phospholipase A<sub>2</sub>

Data are presented as mean ± SD, median, or number and percentage.



**Fig. 2** Scatter plot shows carotid intima-media thickness (CIMT) values in individual patients.

that measures of arterial stiffness play a role in CVD prediction and risk stratification within the growing U.S. Latino population. Evaluating sociodemographically disadvantaged subjects serves to broaden the ap-

plicability of research findings to those individuals who are underrepresented in large-scale clinical trials.<sup>23</sup>

### Limitations of the Study

Our study has several important limitations. First, this was a single-center experience with a limited sample size. Not all the subjects had CIMT measurements performed at each time point, and the follow-up period for measuring preclinical markers was only 2 years, with data collection occurring at baseline, 1 year, and 2 years. In contrast, clinical and death outcomes were evaluated over a median period of nearly 7 years. In addition, we observed regression in CIMT over time. It is known that CIMT generally progresses gradually as human beings age,<sup>24</sup> but regression upon the use of statin medication has also been observed in interventional studies<sup>25</sup>—and our penetration of statin use was 94 (54%) at baseline. We also noted decreased systolic blood pressure and total cholesterol levels during 2 years of follow-up. Therefore, regression was most likely attributable to increased medication compliance within the structured confines of a clinical cohort study and might not apply to general, unselected population studies. Another methodologic limitation is the relatively small number of deaths, which restricts our ability to discern predictive variables in the context of a multivariable model. One final limitation is the use of ankle-brachial rather than carotid-femoral PWV to evaluate arterial stiffness. Although the latter is considered the gold standard, results of previous studies suggest reasonable correlation between the 2 methods,<sup>26</sup> and the former is much more easily performed in care-delivery settings.

### Conclusion

The results of this observational study suggest that arterial stiffness, as measured by PWV, is temporally associated with longitudinal changes in preclinical carotid artery atherosclerosis. Increases in both of these vascular markers were positively associated with overall mortality rates in this Latino, female-predominant cohort. Novel inflammatory biomarkers had no such association with preclinical atherosclerosis, nor were they associated with all-cause death. Given ongoing concerns about the limitations of using traditional risk factors in evaluating racial and ethnic minorities, female patients, and socioeconomically disadvantaged populations, arterial PWV might be a promising, noninvasive risk-stratification tool. Further evaluation of arterial stiffness in epidemiologic and interventional studies seems to be warranted.

### Acknowledgments

We thank Martiniano Soto for performing ultrasonographic examinations and Inez Smith for providing study support.

**TABLE II.** Clinical Characteristics Associated with All-Cause Death in the 175 Subjects

Variable	Survived (n=158)	Died (n=17)	P Value
Age (yr)	60.9 ± 9.6	67.7 ± 7.4	0.11
Female	107 (68)	8 (47)	0.08
Latino	107 (68)	8 (47)	—
Non-Latino (white)	51 (32)	9 (53)	0.09
Body mass index (kg/m <sup>2</sup> )	32.5	32	0.31
Blood pressure (mmHg)			
Systolic	138	149	0.02
Diastolic	80	85	0.18
Total cholesterol (mg/dL)	178	188	0.53
Triglycerides (mg/dL)	154	146	0.47
HDL cholesterol (mg/dL)	49	54	0.23
LDL cholesterol (mg/dL)	95	88	0.63
Diabetes mellitus	77 (49)	7 (41)	0.52
Current smoker	32 (20)	6 (35)	0.21 F
Statin medication*	87 (55)	8 (47)	0.53
Antihypertensive medication*	130 (82)	11 (65)	0.1 F
Framingham risk score (%)	10	13	0.05
Lp-PLA <sub>2</sub> mass (ng/mL)	164	186	0.09
Lp-PLA <sub>2</sub> activity (nmol/min/mL)	132	135	0.42
Central aortic pressure (mmHg)	54	61	0.06
Augmentation index (%)	86	88	0.4
Pulse-wave velocity (cm/s)	1,644	1,913	0.005
Brachial artery flow-mediated dilation (%)	10.3	6.9	0.07
Carotid intima-media thickness (mm)	0.9	1.17	0.001
High-sensitivity C-reactive protein (mg/L)	3	3.7	0.23

HDL = high-density-lipoprotein; LDL = low-density-lipoprotein; Lp-PLA<sub>2</sub> = lipoprotein-associated phospholipase A<sub>2</sub>

\*Prescription filled in year before study enrollment

Unless otherwise stated, data are presented as mean ± SD, median, or number and percentage. The *t* test was used for age; the Wilcoxon rank-sum test was used for all other continuous variables. F = Fisher's exact test was used.

*P* < 0.05 was considered statistically significant.

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