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Improvement in Stroke Risk Prediction: Role of c-reactive protein (CRP) and Lipoprotein-Associated Phospholipase A2 (Lp-PLA$_2$) in the Women's Health Initiative

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Abstract

Background and Purpose—Classification of risk of ischemic stroke is important for medical care and public health reasons. Whether addition of biomarkers adds to predictive power of the Framingham Stroke Risk or other traditional risk factors has not been studied in older women.

Methods—The Hormones and Biomarkers Predicting Stroke (HaBPS) Study is a case-control study of blood biomarkers assayed in 972 ischemic stroke cases and 972 controls, nested in the Women's Health Initiative Observational Study of 93,676 postmenopausal women followed for an average of 8 years. We evaluated additive predictive value of two commercially available biomarkers: c-reactive protein (CRP) and Lipoprotein-Associated Phospholipase A2 (Lp-PLA$_2$) to determine if they added to risk prediction by the Framingham Stroke Risk Score (FSRS) or by traditional risk factors (TRF) which included lipids and other variables not included in the FSRS. As measures of additive predictive value, we used the c-statistic, Net Reclassification Improvement (NRI), category-less NRI, and Integrated Discrimination Improvement Index (IDI).

Results—Addition of CRP to Framingham risk models or additional traditional risk factors overall modestly improved prediction of ischemic stroke and resulted in overall NRI of 6.3%, (case NRI=3.9%, control NRI=2.4%) .In particular, hs-CRP was useful in prediction of cardioembolic strokes (NRI=12.0%; 95%CI: 4.3-19.6%) and in strokes occurring in less than 3 years (NRI=7.9%, 95%CI: 0.8-14.9%). Lp-PLA$_2$ was useful in risk prediction of large artery strokes (NRI=19.8%, 95%CI: 7.4 -32.1%) and in early strokes (NRI=5.8%, 95%CI: 0.4-11.2%).

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Conclusions—CRP and Lp-PLA2 can improve prediction of certain subtypes of ischemic stroke in older women, over the Framingham stroke risk model and traditional risk factors, and may help to guide surveillance and treatment of women at risk.

INTRODUCTION

Accurate stroke risk classification is useful for clinicians to apply to their patients, as well as for public health purposes. Most studies consider prediction and classification of overall cardiovascular risk (CVD) which includes both coronary heart disease and stroke. Few studies focus specifically on stroke, and few consider stroke risk in older women which is important because in women (but not in men) stroke accounts for a higher proportion of total CVD events than does coronary artery disease[1].

The Framingham risk score, described by Kannel, et al in 1976 [2], and its subsequent modifications in 1991 [3, 4] , have been widely used and validated as a general CVD risk profile. The Framingham risk prediction models specific for stroke (Framingham Stroke Risk Score, FSRS) used Cox proportional hazards regression models[3] to relate age, systolic blood pressure, diabetes mellitus, cigarette smoking, prior cardiovascular disease, atrial fibrillation, left ventricular hypertrophy by electrocardiogram, and the use of antihypertensive medication to the occurrence of stroke. The FSRS has limited predictive accuracy and as new biomarkers become available, it is of interest whether they improve risk prediction, or reclassify individuals to lower or higher risk groups better than do traditional risk factors or than the FSRS.

In this report we examine the additive predictive value of high-sensitivity C-reactive protein (hs-CRP) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2), when added to the Framingham Stroke Risk Score (FSRS) and traditional risk factors by looking at several indices of discrimination and of reclassification efficacy. These biomarkers are of special interest because high levels of these biomarkers have been independently associated with increased stroke risk[5, 6] and because they are commercially available for use by clinicians.

METHODS

The study population in which CRP and Lp-PLA_2 were assayed came from the Women's Health Initiative Observational Study (WHI-OS) which is an ongoing prospective study of the major determinants of morbidity and mortality in 93,676 postmenopausal women ages 50-79 at baseline, who were enrolled from October 1993 through December 1998 in 40 clinical centers in the US, with methods and baseline characteristics described in detail elsewhere.[7,8]

In brief, WHI eligibility required that the women had no medical conditions associated with predicted survival of less than 3 years and gave written informed consent. The Hormones and Biomarkers Predicting Stroke (HaBPS) case-control study was nested in the WHI-OS after excluding 11,085 women who had a history of prior stroke or myocardial infarction (MI) or did not have sufficient blood samples for the biomarker assays, or after local adjudications for stroke were not confirmed centrally by trained neurologist adjudicators (N=627 of the 11,085 exclusions). Among the remaining 82,591 eligible WHI-OS

participants, the first 972 centrally adjudicated ischemic strokes were considered cases, and controls were selected in a time-forward manner, with one control for each case from the risk set at the time of the case's event. Matching was done on age at screening (+/− 2 years), race/ethnicity (White, Black, Hispanic, Asian, American Indian, Other/unspecified), date of study enrollment (+/−3 months), and follow-up time (control follow-up time ≥case follow-up time). Cases and controls were pulled from separate datasets, so cases could not be selected as controls. Mean follow-up in controls was 7.9 years, standard deviation (SD)=1.3 years and range from 1.9 to 10.5 years.

Data and Variables

At the WHI baseline visit, women completed questionnaires about medical history, lifestyle factors and personal habits, had a physical examination, and provided blood samples. Certified staff measured height and weight, and right arm blood pressure, using the average of two seated readings, after a 5 minute rest, and obtained at least 30 seconds apart. Blood pressure was measured before the blood draw or a minimum of 30 minutes after the blood draw. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Laboratory Measures

Fasting blood samples, collected at the WHI baseline visit, were labeled, centrifuged, and frozen on site in -70C freezers and later shipped to the central WHI specimen repository (McKesson BioServices, Rockville MD) for long-term storage. The case and control samples were extracted from the specimen archive and sent to Medical Research Laboratory International (MRL) for assay of hs-CRP, fasting plasma glucose as well as triglycerides, HDL-C and total cholesterol. From these measures, LDL-C was calculated for those women who had a triglyceride value less than 400. LDL-C values were set to missing for those women whose triglyceride value was >400 (n=35) or who were missing HDL, total cholesterol or triglyceride values (n=7). Samples were sent to the laboratory at diaDexus for assay of Lp-PLA_2. These laboratory tests were performed between September 2005 and March 2006, approximately 7 to 12 years since specimen collection (depending on when participants were enrolled).

Lp-PLA_2 mass was measured in plasma aliquots using an enzyme-linked immunoassay (PLAC™ test, diaDexus, Inc, South San Francisco, California). Samples were incubated in microtitre plate wells with immobilized monoclonal antibody (2C10) against Lp-PLA_2. The enzyme was identified by a second monoclonal anti-Lp-PLA2 antibody (4B4) labeled with horseradish peroxidase. The standard was recombinant Lp-PLA_2. The range of detection was 50 to 1000 ng/mL and the interassay coefficients of variation were 7.8% at 276ng/mL, 6.1% at 257ng/mL, and 13.5% at 105ng/mL. There was no cross-reactivity with other A2 phospholipases. All analyses were performed blinded to risk factors, biochemical, and clinical characteristics.

Stroke Ascertainment—Strokes were ascertained by thorough investigation of overnight hospitalizations identified through annual mail and/or telephone follow-up, and participant or third-party reports. For a potential stroke case, laboratory results, medical records and

available imaging study reports were obtained. Trained local physician adjudicators assigned a diagnosis according to standard criteria and all locally adjudicated strokes were sent for central adjudication by three highly trained neurologists. Only centrally confirmed ischemic strokes were used in this study and only stroke events that required hospitalization were considered as a potential outcome; transient ischemic attacks (TIA's,) or hemorrhagic strokes (determined on review of reports of brain imaging studies) were not included in the definition of stroke outcome. Ischemic stroke was defined as the rapid onset of a persistent neurologic deficit without evidence for other causes, attributed to an obstruction in the arterial circulation to the brain. The deficit must have lasted more than 24 hours unless death supervened, or there was a demonstrable lesion compatible with acute stroke on computed tomography (CT) or magnetic resonance imaging (MRI) scan. Additional details are provided elsewhere[9].

Statistical Analyses—The objective of the statistical analyses was to determine if the two commercially available assays, hs-CRP and Lp-PLA_2, add predictive value over and above the FSRS or traditional risk factors and if they reclassify individuals into a different risk category. To evaluate the utility of these assays for risk stratification we first calculated unconditional logistic regression to obtain odds ratio for stroke risk using the variables from the FSRS for women[3]. The FSRS variable included: age, systolic blood pressure, being on antihypertensive medications, history of diabetes, smoking, atrial fibrillation, left ventricular hypertrophy (LVH) and prevalent CVD. In Framingham CVD includes history of MI, angina pectoris, coronary insufficiency, intermittent claudication, or congestive heart failure (CHF), but in our dataset those with history of MI were excluded. We also assume no LVH for all women since the LVH variable was not available in our dataset and since the prevalence of LVH is low. We then calculated unconditional logistic regression estimates for stroke risk adding each of the biomarkers in their continuous form (log-transformed hs-CRP or Lp-PLA_2) one at a time, and also together, to the model with the variables used in the FSRS equation.

In order to classify participants into absolute risk categories from the above models, we had to adapt methods used in prospective studies to our case-control study[10, 11]. To calculate predicted probabilities of stroke with and without the biomarker, we added the term: $log\left(\frac{p}{1-p}\cdot\frac{n_{controls}}{n_{cases}}\right)$ to the intercept of the unconditional logistic regression models; p was estimated as the incidence of stroke in the parent study (the WHI-OS) which was 0.0029 annually, times the average follow-up of 8 years. Since this was generally a low to intermediate risk population (by virtue of the fact that we excluded all those with a previous stroke or MI), the risk categories we chose were: < 2%, 2% to <5%, 5% to <8%, ≥8%. These categories roughly correspond to low, intermediate and high risk levels used in decisions to initiate treatment to prevent stroke in persons with atrial fibrillation [12].

Similarly, we added each biomarker one at a time and both together to models with traditional risk factor variables generally available to clinicians: age, race, current smoking, systolic blood pressure, self-reported current blood pressure medication use, self reported history of diabetes, self-reported history of atrial fibrillation, self reported history of vascular disease (angina, revascularization, peripheral vascular disease or congestive heart failure),

body mass index, alcohol use (none, <7, 7 or more drinks per week), self-reported depression, current hormone therapy use, and low-density lipoprotein, triglycerides. We included triglycerides since in the HaBPS study it was found that high triglyceride levels increase risk (unpublished data) and triglyceride levels are also usually readily available to clinicians.

We assessed various indices of additive predictability of two biomarkers, as proposed by Pencina.[10]

1. Discrimination as measured by the c-statistic reflecting the area under the curve (AUC) from a Receiver Operating Characteristic (ROC) curve (which is a plot of sensitivity on Y axis vs. 1-specificty on X axis). It is the probability that a randomly selected person with the event will have a higher predicted risk than a randomly selected person without an event. Higher c-statistic values indicate better discrimination.

2. The Integrated Discrimination Improvement (IDI) measures the separation between people who develop the outcome and those who do not, by comparing the average predicted risks for people who develop the outcome and those who do not [7]. Smaller p-values for IDI indicate better discrimination. IDI is the difference in the mean predicted probability of being a case and being a control in the model with the new biomarker minus the difference in the mean predicted probability of being a case and being a control in the model without the new biomarker:

$$\mathrm{IDI}=(\mathrm{p(new\ model,\ cases)}-\mathrm{p(new\ model,\ controls)})-(\mathrm{p(old\ model,\ cases)}-\mathrm{p(old\ model,\ controls)}),\ \text{where p is the mean predicted probability.}$$

3. Net Reclassification Improvement Index (NRI) which measures whether the new model with the biomarker included, sufficiently changes a person's risk to move them into a different risk category and thus potentially affect treatment decisions. It distinguishes between individuals correctly and incorrectly reclassified and quantifies the correct movement in categories (upward for events and downward for non-events). The NRI is calculated as :

$$NRI=[\hat{p}\,(up|D=1)-\hat{p}\,(down|D=1)]+[\hat{p}\,(down|D=0)-\hat{p}\,(up|D=0)]$$

4. Category-less NRI. As noted by Pencina [10, 11], one drawback of the reclassification-based measure is its dependence on the choice of absolute risk categories. This limitation can best addressed by using a category-less NRI or an integrated discrimination index (IDI). The category-less NRI is the percent of all subjects whose risk estimates are changed in the correct direction (increased risk in model with biomarker compared to model without biomarker for cases and decreased risk for controls) minus the percent changed in the incorrect direction (decreased risk in model with biomarker compared to model without biomarker for cases and increased risk for controls). It is calculated as:

$$\mathrm{P}(\text{risk with biomarker}>\text{old model risk}|\text{case})+\mathrm{P}(\text{risk with biomarker}<\text{old model risk}|\text{control})]-\mathrm{P}(\text{risk with biomarker}>\text{old model risk}|\text{control})-\mathrm{P}(\text{risk with biomarker}<\text{old model risk}|\text{case}).$$

We also conducted analyses stratified by stroke subtype according to the TOAST (The Trial of Org 10172 Acute Stroke Trial)[13] criteria, by hormone use, by LDL-c levels and by time of stroke after baseline.

RESULTS

There were 902 centrally adjudicated stroke cases and 909 controls who met our criteria of no prior stroke or MI, and adequate blood samples for Lp-PLA_2 and hs-CRP assays. Due to missing covariate information models using FSRS variables were limited to 1,751 (868 cases and 883 controls). Since the TRF models had more covariates, these models were limited further to 1,625 participants with non-missing data (794 cases and 831 controls). Stroke cases compared to controls were more likely at baseline to be current smokers, to have cardiovascular co-morbidities, diabetes, higher BMI, systolic blood pressure, triglycerides, hs-CRP levels and Lp-PLA_2 levels (Table 1, p-values are for unmatched data).

Table 2 summarizes the C-statistic and IDI for all models. It should be noted that the FSRS alone has a rather low c-statistic of 0.644 indicating it is not a very good discriminator. Adding the two biomarkers singly or jointly does not affect the c-statistic appreciably. The IDI was highly significant for hs-CRP, (p<.001), for Lp-PLA2 (p=.001), and for both biomarkers together (p<.001.).

Table 3 illustrates an example calculation of the NRI based on the FSRS for 868 cases and 883 controls. There are 8 cases with calculated FSRS corresponding to a less than 2% risk of stroke over 8 years (first row of the table). After adding log of hs-CRP to the Framingham model, there are 19 cases at a less than 2% risk. The numbers on the diagonal indicate that these individuals did not change their risk category with the addition of log hs-CRP to the model. Cases above the diagonal (N=112 out of the 868 strokes or 12.9%), correctly moved up at least one risk category and cases below the diagonal (78 out of 868 = 9.0%) incorrectly moved down in risk. Thus the net improvement for cases was 12.9%-9.0% = 3.9%. Among controls, adding the biomarkers resulted in 116 controls moving down in risk correctly (13.1%) and 95 controls incorrectly moving up in risk (10.8%) resulting in a net difference of 2.3%. Thus the Net Reclassification Improvement is 3.9% + 2.3 % = 6.3% (after rounding).

Table 4 displays number and percent of cases and controls who moved in the correct and incorrect direction of risk and the associated NRI. Adding log hs-CRP to the FSRS results in a Net Reclassification Improvement of 6.3% (p=<0.01), with 12.9% of the cases and 13.1% of the controls being correctly reclassified. The corresponding figures for adding Lp-PLA_2 were 4.3% for cases and 3.1% for controls being correctly reclassified. Similar relationships pertained to the traditional risk factors.

The category-less NRI with hs-CRP was 18.9% (95%CI: 9.5, 28.3) and 15.2% (95% CI: 5.5, 24.9) in the FSRS and TRF models respectively. NRI with Lp-PLA_2 was 8.4% (95%CI: -0.9, 17.8) and 9.8% (95%CI: 0.1, 19.5) for FSRS and TRF respectively (data not shown). It should be noted that under the null hypothesis the NRI is expected to be 0%.

We also did analyses stratified by hormone use, LDL-C levels, stroke subtype and time of stroke after baseline. There were no differences in reclassification when we stratified by current vs non-current hormone use at baseline, or LDL level (i.e. LDL<130 vs those with LDL>= 130) [data not shown]. However, after stratifying by stroke subtype, (Table 5) we found that hs-CRP aids in reclassification of cardioembolic strokes with an NRI of 12.0% (95%CI: 4.3-19.6%), while Lp-PLA_2 aids in reclassification of large artery strokes with an NRI of 19.8% (95%CI: 7.4 -32.1%) . The category-less NRI, (which measures the percent of people moving in risk in the right direction without regard to the magnitude of the change in risk), is 26.6% for hs-CRP for cardioembolic strokes and 30.3% for Lp-PLA_2 for large artery strokes. Both hs-CRP and Lp-PLA_2 showed a significantly increased NRI for strokes occurring less than 3 years after baseline than for later strokes (Table 5). The NRI for the early strokes was 7.9% (95%CI: 0.8-14.9%) for hs-CRP and 5.8% (95%CI: 0.4-11.2%). for Lp-PLA_2.

DISCUSSION

We have found in a case-control study of 868 ischemic strokes and 883 controls in postmenopausal women, that the addition of hs-CRP to a model with Framingham Stroke Risk variables, overall modestly improved risk prediction and significantly increased the c statistic, the IDI and the NRI and substantially improved risk prediction for cardioemoblic strokes and for strokes occurring less than 3 years after baseline. While the addition of Lp-PLA_2 to Framingham variables or traditional risk factors overall did not result in a higher net reclassification improvement among all women, it did significantly and substantially improve risk prediction for large artery strokes and early strokes compared to those occurring after 3 years .

A potential limitation of our study is its case-control rather than prospective design. However, we used statistical corrections to adapt methods used in prospective studies to our case-control study, as suggested by Pencina [11]. In addition, generalizability may be limited because our sample is predominantly white (86%) and excludes women with any prior history of stroke or myocardial infarction. The strengths of our study include large numbers of ischemic strokes (N= 868), rigorous ascertainment and adjudication of stroke cases by trained neurologists, high quality of phenotypic data in WHI, availability of the two biomarkers which can be commercially measured and thus may be useful to clinicians, and the different indices we used in exploring the additive usefulness of biomarkers in risk prediction.

Since a large proportion of all strokes occur in people with no standard risk factors for stroke, a biomarker which adds to prediction of risk may be very useful. The Jupiter trial[14] indicated that people with normal or even low levels of LDL but who had elevated hs-CRP benefitted from statin therapy by having lower rates of cardiovascular events, with risk of

stroke reduced by 48% among those on rosuvastatin compared to placebo. Our finding that hs-CRP improves the risk prediction of ischemic stroke in older women, particularly cardioembolic strokes and early strokes, is consistent with the implications of this study and suggests that this biomarker may be useful in assessing stroke risk more accurately than Framingham risk factors or traditional risk factors alone. Lp-PLA_2 was useful in improving stroke risk prediction for large artery strokes and early strokes. The role of Lp-PLA_2 in prediction of large artery strokes is consistent with a report by Koledgie[15] which shows a relationship of Lp-PLA_2 to plaque progression in coronary arteries and a report by Serruys[16] which demonstrates that a specific Lp-PLA_2 inhibitor stabilizes the plaque necrotic core which is a key determinant of plaque vulnerability. The reclassification improvement with both of these biomarkers for early strokes compared to later occurring strokes implies that they are more acute predictors.

In summary, we found that hs-CRP may be useful to improve stroke risk prediction overall in older women, and particularly for cardioembolic strokes, while Lp-PLA_2 has additive predictive value above the Framingham Stroke Risk Score for large artery strokes. Both biomarkers are useful for prediction of more acute strokes than for those occurring later.

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Table 1

Baseline Characteristics by Case-Control Status (n=1751)

	Controls (n=883)		Cases (n=868)		
	N	**%**	**N**	**%**	**p-value**
White race	760	86.1	750	86.4	0.84
Current Smoking	31	3.5	70	8.1	<0.001
Alcohol Consumption					0.37
Non-Drinker	375	42.5	394	45.4	
1 to <7 drinks per week	402	45.5	366	42.2	
7 or more drinks per week	106	12	107	12.3	
Current Hormone Use	339	38.4	342	39.4	0.67
History of Vascular Disease	74	8.4	111	12.8	<0.01
History of Angina	50	5.7	70	8.1	0.048
History of Revascularization	11	1.3	33	3.8	<0.01
History of Congestive Heart Failure	6	0.7	16	1.8	0.03
History of Peripheral Arterial Disease	27	3.1	26	3.0	0.94
History of Diabetes	46	5.2	102	11.8	<0.001
History of Atrial Fibrillation	49	5.6	84	9.7	0.001
Current Blood Pressure Medication Use	254	29	347	40.9	<0.001
Depression	72	8.4	86	10.3	0.17
	mean	SD	mean	SD	p-value
Age (years)	68.8	6.4	68.7	6.4	0.89
Systolic Blood Pressure (mmHg)	130.0	18.0	136.9	19.1	<0.001
Body Mass Index (kg/m^2)	27.0	5.3	27.8	5.9	<0.01
Triglycerides (mg/dl)	161.4	81.5	179.1	90.5	<0.001
Low Density Lipoprotein (mg/dl)	139.1	36.5	141.9	37.3	0.10
hs-C-Reactive Protein (log transformed)	0.9	1.1	1.2	1.1	<0.001
Lp-PLA_2 (ng/mL)	296.7	87.5	307.4	97.1	0.02

Table 2

C-Statistics and Integrated Discrimination Index (IDI) for hs- CRP and Lp-PLA_2

	C-Statistic	p-value*	IDI	p-value
Framingham Stroke Risk (FSRS) Variables				
All Women (n=1751)				
868 cases; 883 controls				
FSRS	0.6436			
FSRS+ln hs-CRP	0.6534	0.08	0.0028	<0.001
FSRS+Lp-Pla_2	0.6442	0.78	0.0010	0.001
FSRS+ln hs-CRP&Lp-Pla_2	0.6548	0.06	0.0039	<0.001
Traditional Risk Factor (TRF) Variables				
All Women (n=1625)				
794 cases; 831 controls				
TRF	0.6492			
TRF+ln hs-CRP	0.6563	0.15	0.0024	<0.001
TRF+Lp-Pla_2	0.6498	0.73	0.0007	<0.01
TRF+ln hs-CRP&Lp-Pla_2	0.6572	0.12	0.0031	<0.001

* p-value for C-Statistic is comparing the added predictive ability of models with biomarker(s) to the model with only the FSRS variable or TRF variables

Framingham Stroke Risk (FSRS) Variables include: age, white vs non-white race, systolic blood pressure, current smoker, self reported history of atrial fibrillation, self reported history of diabetes, self reported history of vascular disease (angina, revascularization, peripheral arterial disease or congestive heart failure) and hypertension*SBP interaction

Traditional Risk Factor (TRF) Variables include: age, white vs. non-white race, current smoking, systolic blood pressure, use of antihypertensives, body mass index, history of atrial fibrillation, history of CHD, alcohol consumption (none, <7, 7 or more drinks per week), self reported depression, low-density lipoprotein, triglycerides and current hormone therapy use

IDI=Integrated Discrimination Index; lnCRP=log transformed hs-C-Reactive Protein; Lp-Pla_2= lipoprotein-associated phospholipase A_2

Table 3

Example calculation of NRI: addition of hs-CRP to a model with FSRS variables

ALL
WOMEN

Reclassification among cases and controls

FSRS + ln hs-CRP

Stroke cases

Risk category	<2.0%	2.0%-5.0%	5.0%-8.0%	>=8.0%	total	P(up|case)	112	12.9%
<2.0%	5	3	0	0	8		868	
2.0%-5.0%	14	323	66	0	403			
5.0%-8.0%	0	43	167	43	253	P(down|case)	78	9.0%
>=8.0%	0	0	21	183	204		868	
total	19	369	254	226	868			

Controls

Risk category	<2.0%	2.0%-5.0%	5.0%-8.0%	>=8.0%	total	P(up|control)	95	10.8%
<2.0%	17	8	0	0	25		883	
2.0%-5.0%	62	467	60	0	589			
5.0%-8.0%	0	42	114	27	183	P(down |control)	116	13.1%
>=8.0%	0	1	11	74	86		883	
total	79	518	185	101	883			

diagonal cells indicate no change in risk prediction by adding biomarker

NRI=(P(up|case)-P(down|case))-((Pup|control)- P(down|control))

NRI= 6.3% p<0.01

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Table 4

Net Reclassification Improvement Parameters for hs- CRP and Lp-PLA$_2$

	NRI (%)	p-value	#(%) cases moving up in risk: *correctly reclassified*	#(%) cases moving down in risk: incorrectly reclassified	case NRI (%)	#(%) controls moving down in risk: correctly classified	#(%) controls moving up in risk: incorrectly reclassified	control NRI (%)
Framingham Stroke Risk (FSRS) Variables								
868 cases; 883 controls								
FSRS								
FSRS+ln hs-CRP	6.3 (1.8, 10.8)	<0.01	112 (12.9)	78 (9.0)	3.9	116 (13.1)	95 (10.8)	2.4
FSRS+Lp-Pla$_2$	0.1 (−2.4, 2.7)	0.93	37 (4.3	33 (3.8)	0.5	27 (3.1)	30 (3.4)	−0.3
FSRS+ln hs-CRP&Lp-Pla$_2$	3.7 (−0.8, 8.2)	0.11	112 (12.9)	87 (10.0)	2.9	106 (12.0)	99 (11.2)	0.8
Traditional Risk Factor (TRF) Variables								
794 cases; 831 controls								
TRF								
TRF+ln hs-CRP	7.6 (3.3, 12.0)	<0.01	93 (11.7)	61 (7.7)	4	102(12.3)	72 (8.7)	3.6
TRF+Lp-Pla$_2$	−0.3 (−2.7, 2.2)	0.83	23 (2.9)	27 (3.4)	−0.5	28 (3.4)	26 (3.1)	0.2
TRF+ln hs-CRP&Lp-Pla$_2$	7.2 (2.7, 11.8)	<0.01	93 (12.8)	69 (8.7)	3	115(13.8)	80 (9.6)	4.2

Framingham Stroke Risk (FSRS) Variables include: age, white vs. non-white race, systolic blood pressure, current smoker, self reported history of atrial fibrillation, self reported history of diabetes, self reported history of vascular disease (angina, revascularization, peripheral arterial disease or congestive heart failure) and hypertension*SBP interaction

Traditional Risk Factor (TRF) Variables include: age, white vs. non-white race, current smoking, systolic blood pressure, use of antihypertensives, body mass index, history of atrial fibrillation, history of CHD, alcohol consumption (none, <7, 7 or more drinks per week), self reported depression, low-density lipoprotein, triglycerides and current hormone therapy use

NRI= Net Reclassification Improvement Index; ln hs-CRP=log transformed C-Reactive Protein; Lp-Pla2= lipoprotein-associated phospholipase A$_2$

Table 5

Net Reclassification Improvement Parameters for hs- CRP and Lp-PLA$_2$ by Time of Stroke and Subtype of Stroke

	C-Statistic	IDI	p-value	NRI% (95%CI)	p-value	# cases	# controls	# cases up	# cases down	case NRI (%)	# controls down	# controls up	control NRI (%)
BY TIME FROM BASELINE													
Framingham Stroke Risk (FSR) Variables													
< 3 years													
FRS	0.6836												
FRS + ln CRP	0.6968	0.005	<0.01	7.9 (0.8, 14.9)	0.03	262	883	42	27	5.7	126	107	2.2
FRS + Lp-Pla2	0.6861	0.0035	<0.01	5.8 (0.4, 11.2)	0.04	262	883	26	15	4.2	70	56	1.6
>=3 years													
FRS	0.6323												
FRS + ln CRP	0.6409	0.0024	<0.01	3.7 (−0.8, 8.2)	0.11	606	883	71	46	4.1	80	84	−0.5
FRS + Lp-Pla2	0.633	0.0006	0.01	−1.0 (−3.5, 1.5)	0.42	606	883	18	16	0.3	22	34	−1.4
IDI	C-Statistic	IDI	p-value	NRI% (95%CI)	p-value	# cases	# controls	# cases up	# cases down	case NRI (%)	# controls down	# controls up	control NRI (%)
BY STROKE SUBTYPE													
Framingham Stroke Risk (FSR) Variables													
Small Vessel Occlusion													
FRS	0.6857												
FRS + ln CRP	0.6903	0.0038	<0.01	5.3 (−1.1, 11.7)	0.11	232	883	27	20	3	88	68	2.3
FRS + Lp-Pla2	0.6854	0.0002	0.09	2.1 (0.2, 4.0)	0.03	232	883	4	0	1.7	9	6	0.3
Cardioembolism													
FRS	0.6792												
FRS + ln CRP	0.6937	0.0061	<0.01	12.0 (4.3, 19.6)	<0.01	185	883	29	14	8.1	135	101	3.9
FRS + Lp-Pla2	0.6776	0.0018	0.03	1.2 (−1.9, 4.3)	0.46	185	883	5	2	1.6	16	20	−0.5
Large Artery													
FRS	0.6858												
FRS + ln CRP	0.6902	0.0086	<0.01	0.1 (−11.0, 11.0)	0.98	80	883	8	10	−2.5	120	97	2.6
FRS + Lp-Pla2	0.7042	0.0112	<0.01	19.8 (7.4, 32.1)	<0.01	80	883	18	7	13.8	153	100	6

	C-Statistic	IDI	p-value	NRI% (95%CI)	p-value	# cases	# controls	# cases up	# cases down	case NRI (%)	# controls down	# controls up	control NRI (%)
Undetermined/Non-specific													
FRS	0.616												
FRS + ln CRP	0.6272	0.002	<0.01	2.4 (−2.6, 7.4)	0.34	370	883	38	24	3.8	73	85	−1.4
FRS + Lp-Pla2	0.6165	0.0012	0.02	−2.3 (−5.7, 1.0)	0.18	370	883	11	15	−1.1	36	47	−1.2