Multiple Biomarkers for the Prediction of Ischemic Stroke
The PRIME Study

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Objective—To simultaneously evaluate 14 biomarkers from distinct biological pathways for risk prediction of ischemic stroke, including biomarkers of hemostasis, inflammation, and endothelial activation as well as chemokines and adipocytokines.

Methods and Results—The Prospective Epidemiological Study on Myocardial Infarction (PRIME) is a cohort of 9771 healthy men 50 to 59 years of age who were followed up over 10 years. In a nested case–control study, 95 ischemic stroke cases were matched with 190 controls. After multivariable adjustment for traditional risk factors, fibrinogen (odds ratio [OR], 1.53; 95% confidence interval [CI], 1.03–2.28), E-selectin (OR, 1.76; 95% CI, 1.06–2.93), interferon-γ-inducible-protein-10 (OR, 1.72; 95% CI, 1.06–2.78), resistin (OR, 2.86; 95% CI, 1.30–6.27), and total adiponectin (OR, 1.82; 95% CI, 1.04–3.19) were significantly associated with ischemic stroke. Adding E-selectin and resistin to a traditional risk factor model significantly increased the area under the receiver-operating characteristic curve from 0.679 (95% CI, 0.612–0.745) to 0.785 and 0.788, respectively, and yielded a categorical net reclassification improvement of 29.9% (P=0.001) and 28.4% (P=0.002), respectively. Their simultaneous inclusion in the traditional risk factor model increased the area under the receiver-operating characteristic curve to 0.824 (95% CI, 0.770–0.877) and resulted in an net reclassification improvement of 41.4% (P<0.001). Results were confirmed when using continuous net reclassification improvement.

Conclusion—Among multiple biomarkers from distinct biological pathways, E-selectin and resistin provided incremental and additive value to traditional risk factors in predicting ischemic stroke. (***Arterioscler Thromb Vasc Biol*. 2013;33:659-666.**)

Key Words: biomarkers ■ epidemiology ■ ischemic stroke ■ risk prediction

Ischemic stroke is a leading cause of the global disease burden, making its prevention a major public heath challenge. Risk stratification on the basis of traditional risk factors enables identifying patients at particularly high stroke risk.[1] Beyond traditional risk factors, novel circulating biomarkers may improve risk stratification and, ultimately, clinical management of ambulatory patients.[2] Previous studies have mostly investigated single biomarkers and, more recently, some studies have evaluated several biomarkers from a given biological pathway. In the Prospective Epidemiological Study on Myocardial Infarction (PRIME), we have shown an incremental value of chemokines and adipocytokines in predicting ischemic stroke.[3,4] Biomarkers of hemostasis, inflammation, and endothelial activation represent further candidates for risk prediction, but evidence for their prospective association and prognostic relevance with regard to ischemic stroke is yet to be established. Moreover, simultaneous assessment of multiple biomarkers from distinct biological pathways, the so-called multimarker approach, has been hypothesized to enhance risk prediction in a clinically relevant manner.[5] Supporting evidence for this hypothesis has primarily been provided by studies investigating combined cardiovascular outcomes.[5–7] With regard to cerebrovascular disease, only 2 previous studies have used the approach, 1 for ischemic stroke among postmenopausal women and 1 for total stroke and transient ischemic attack in adult men and women.[8,9] Therefore, among healthy European middle-aged men from the PRIME study, our first objective was to newly quantify associations of 9 biomarkers of hemostasis (fibrinogen, plasminogen activator inhibitor-1 [PAI-1], von Willebrand factor antigen [vWF]), inflammation (high-sensitivity C-reactive protein [hs-CRP], interleukin-6 [IL-6]), and endothelial activation (E-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, CD-40 ligand [CD40L]) with the risk of ischemic stroke. Furthermore, associations between these biomarkers and ischemic stroke were examined, including 5 biomarkers that were previously identified to be associated with ischemic stroke in the PRIME

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study, namely 2 chemokines (regulated on activation normal T-cell expressed and secreted [RANTES], interferon-γ-inducible-protein-10 [IP-10]) and 3 adipocytokines (resistin, adipsin, total adiponectin).[3,4] This step aimed at identifying from an initial set of 14 biomarkers those most significantly associated with ischemic stroke. Our second objective was to put the results into a clinical perspective by studying the usefulness of the most significant biomarkers for individual risk prediction of ischemic stroke using metrics of discrimination and reclassification. With this step, we aimed to develop the most parsimonious model to facilitate future application in clinical settings.

Materials and Methods

Sampling Frame

PRIME is a prospective cohort study among male European Caucasians.[10] Briefly, the study was conducted in 4 collaborating centers of the World Health Organization MONICA Project (Multinational MONItoring trends and determinants in CArdiovascular disease) in France (Lille, Strasbourg, and Toulouse) and Northern Ireland (Belfast).

Baseline Assessment

A total of 10 602 men 50 to 59 years of age were examined between 1991 and 1994 using standardized methods from the World Health Organization MONICA Project. Height and weight were measured in individuals standing tall, wearing light cloths, and without shoes. Waist circumference was taken midway between the lower rib bow and the upper iliac crest. Blood pressure was measured after 5 minutes of rest using an automated device (Spengler, Asnières-sur-Seine, France). Diabetes mellitus was defined as self-reported diabetes or use of antidiabetic medication. Cigarette smoking was defined as the average number of cigarettes smoked over the last 5 years. Alcohol drinking was defined as the average alcohol intake in grams per day during the last week. Venous blood samples were drawn after a minimum fasting time of 12 hours. The following parameters were measured on fresh samples in the whole cohort: total and high-density lipoprotein cholesterol using an automated analyzer (Boehringer, Mannheim, Germany), fibrinogen by ELISA (Diagnostica Stago, Asnières-sur-Seine, France), and PAI-1 using an amidolytic method (SpectrolyseTM/Fibrin, Biopool, Umea, Sweden). The coefficients of variation for fibrinogen and PAI-1 were 4.3% and 7.0%, respectively. Plasma aliquots were stored at −80°C for subsequent laboratory analyses of further biomarkers.

Follow-up

Study participants were contacted annually over a period of 10 years and completed self-report questionnaires on clinical events. Among individuals with possible events, clinical information was obtained from medical records in hospital and general practice. All available data were gathered concerning hospital admissions, electrocardiograms, laboratory results, medical treatment, and interventions. Deceased patients' families and practitioners were contacted to clarify death circumstances, and death certificates were checked to complement clinical and post mortem information. Stroke events were adjudicated by an independent committee, and stroke was defined according to World Health Organization MONICA criteria as an acute focal cerebral function deficit, including acute hemiparesis or hemiplegia, hemianopia, diplopia, dysarthria or aphasia, ataxia, acute loss of balance or coordination or an acute global cerebral function deficit, including coma or 4 limbs and cranial nerve palsy, of a vascular origin, and persisting for >24 hours (except if symptoms were interrupted by a surgical intervention or death). Transient or permanent cerebral focal deficit caused by a blood disease, a cerebral tumor or metastasis, or secondary to a trauma were not considered by the medical committee. Clinical information, computerized tomodensitometry scans (compatible signs), angiographic and autopsy data were used to distinguish between ischemic and hemorrhagic stroke events.[3,4] After 10 years of follow-up, the stroke event status was available for 95.1% of the cohort. Details of an assessment of the quality of data have been published elsewhere.[11]

Nested Case–Control Study

Among 9771 men free of coronary heart disease and stroke at baseline, 98 first ischemic stroke cases were validated during 10 years of follow-up, but baseline plasma samples were available from 95 cases. A total of 190 controls, 2 for each stroke case, were randomly selected from the initial cohort. Controls were free of coronary heart disease and stroke at the time of event and matched on age (±3 years), study center, and date of recruitment (±3 days). Biomarker concentrations in plasma obtained at baseline examination were measured blind to the case–control status. Multiplex bioassays were conducted using measurement kits from the following manufacturers: Indicia Biotechnology (Oullins, France) for vWF using polyclonal antibodies from Diagnostica Stago (Asnières-sur-Seine, France); R&D Systems (Billerica, MA) for hs-CRP (LOB1707), CD40L (LUB201), IP-10 (LUB266), resistin (LOB1359), and adipsin (LOB1824); Bio-Rad (Hercules, CA) for RANTES (X500AIXMP4), intercellular adhesion molecule-1 (XF0000ZGAA), and vascular cell adhesion molecule-1 (171-A11123); Millipore (Billerica, MA) for E-selectin (HCVD1-67-AK); Linco Research Inc (Billerica, MA) for total adiponectin (human cardiovascular disease panel 1 multiplex immunoassay). Assays were performed according to the manufacturer specifications, plates were read on a Luminex 200 instrument system, and coefficients of variation ranged from 4.0% to 16.3%. IL-6 was measured using high-sensitivity ELISA (Bender MedSystems, Vienna, Austria; BMS213HS) with a coefficients of variation of 8.0%. Please refer to Table I in the online-only Data Supplement for biomarker assay specifications.

Statistical Analysis

Baseline characteristics of ischemic stroke cases and controls were compared by univariate conditional logistic regression analysis. In the control data set, correlations between biomarkers were examined by partial Spearman ρ adjusted for matching variables. Biomarker values were log-transformed for further analyses. Odds ratios (ORs) of ischemic stroke per SD increase in biomarker levels among controls were estimated using conditional logistic regression. First, each biomarker was examined separately in univariate analysis and in multivariable analysis adjusted for traditional risk factors (systolic blood pressure, antihypertensive treatment, cigarette smoking, alcohol drinking, total cholesterol, high-density lipoprotein cholesterol, waist circumference, and diabetes mellitus). Second, biomarkers independently associated with ischemic stroke in separate models were mutually analyzed in a single multivariable model. This model was fitted using stepwise backward selection from traditional risk factors and, subsequently, including eligible biomarker variables. Calibration of the model was assessed by the Hosmer–Lemeshow test. Interactions were explored by including cross-product terms and evaluated using the Wald-test. Metrics of risk discrimination and reclassification were assessed using unconditional logistic regression analysis adjusted for matching variables. Hereby, the matched case–control design was taken into account. The area under the receiver-operating characteristic curve (AUC) of a traditional risk factor model and of additional models, including biomarkers, were compared with the method described by DeLong et al.[12] Categorical and continuous net reclassification improvement (NRI) was quantified at the inclusion of biomarkers in the traditional risk factor model after adjustment for the incidence rate in the original cohort and the case–control ratio.[13,14] In the absence of accepted thresholds, risk cutoffs for categorical NRI estimation were arbitrarily based on tertiles of risk as predicted by the more parsimonious model. Frequencies of available measurements across biomarker variables among cases and controls ranged from 90.5% to 97.9% and from 95.8% to 97.4%, respectively. Multiple imputation

using Markov chain Monte Carlo methods[15] was performed to fill incomplete biomarker variables with values within observed ranges. We separately conducted all analyses on 10 complete data sets and averaged statistical measures across runs. All tests were 2-tailed and an α level of 0.05 was chosen to indicate statistical significance. The statistical analysis was carried out using Statistical Analysis Software version 9.2 (SAS Institute, Cary, NC).

Results

Baseline Characteristics

Ischemic stroke cases showed higher levels of systolic blood pressure and cigarette smoking and more frequently were diabetic than controls (Table II in the online-only Data Supplement). Higher plasma concentrations of fibrinogen, PAI-1, vWF, CD40L, E-selectin, RANTES, resistin, adipsin, and total adiponectin were observed among cases when compared with controls (Table 1).

Correlations

Correlations between biomarkers were moderate with coefficients ranging from –0.185 to 0.464 (Table 2), minimizing the issue of collinearity. Most biomarkers of endothelial activation significantly correlated with chemokines and adipocytokines, but less frequently showed significant correlations with biomarkers of hemostasis and inflammation.

Separate Associations

The Figure depicts ORs of ischemic stroke separately for each biomarker and per SD increase in plasma levels (see also Table III in the online-only Data Supplement). In univariate analyses, higher concentrations of fibrinogen, PAI-1, vWF, hs-CRP, CD40L, and E-selectin were significantly associated with ischemic stroke. In sensitivity analysis, consistent univariate associations were observed for embolic (n=28) and atherothrombotic (n=51) stroke events (Table IV in the online-only Data Supplement). Associations remained statistically significant for fibrinogen (OR, 1.34; 95% confidence interval [CI], 1.01–1.80), vWF (OR, 1.49; 95% CI, 1.08–2.04), and E-selectin (OR, 2.83; 95% CI, 1.87–4.29) after adjustment for traditional risk factors.

Combined Associations

Among traditional risk factors, systolic blood pressure and cigarette smoking were independent predictors of ischemic stroke after stepwise backward selection in a model that additionally included matching variables (hereafter referred to as the traditional risk factor model). When significant biomarkers identified in separate models were mutually analyzed in this traditional risk factor model, fibrinogen (OR, 1.53; 95% CI, 1.03–2.28), E-selectin (OR, 1.76; 95% CI, 1.06–2.93), IP-10 (OR, 1.72; 95% CI, 1.06–2.78), resistin (OR, 2.86; 95% CI, 1.30–6.27), and total adiponectin (OR, 1.82; 95% CI, 1.04–3.19) were the sole biomarkers significantly related to ischemic stroke (Table 3; Hosmer–Lemeshow test P=0.53). No interaction was observed between these biomarkers and either systolic blood pressure or cigarette smoking.

Discrimination

As shown in Table 4, the traditional risk factor model yielded an AUC of 0.679 (95% CI, 0.612–0.745). When separately

Table 1. Plasma Levels of Biomarkers Among Ischemic Stroke Cases and Matched Controls

Biomarker Group	Cases (n=95)	Controls (n=190)	*P* Value*
Hemostasis			
Fibrinogen, mg/dL	324.8 (281.8–380.6)	308.7 (267.4–352.7)	0.027
PAI-1, ng/mL	15.1 (8.8–22.0)	11.4 (6.9–16.9)	0.008
vWF, UI/mL	1.4 (0.9–2.0)	1.1 (0.8–1.5)	0.012
Inflammation			
IL-6, pg/dL	40.5 (1.5–88.2)	27.6 (0.1–59.5)	0.221
hs-CRP, mg/dL	2.9 (0.1–4.8)	2.1 (1.1–4.0)	0.270
Endothelial activation			
ICAM-1, ng/mL	299.4 (238.8–367.5)	268.5 (236.5–330.9)	0.068
VCAM-1, ng/mL	438.9 (360.3–530.4)	422.8 (370.5–525.3)	0.279
CD40L, ng/mL	5.4 (1.9–9.0)	3.7 (1.5–7.0)	0.014
E-selectin, ng/mL	34.5 (22.3–55.8)	19.0 (12.5–27.8)	<0.001
Chemokines			
RANTES, ng/mL	62.5 (43.4–96.2)	52.1 (31.6–73.4)	0.003
IP-10, pg/mL	10.7 (7.0–19.1)	8.5 (5.2–15.4)	0.157
Adipocytokines			
Resistin, ng/mL	6.9 (5.0–9.5)	4.8 (1.5–7.0)	<0.001
Adipsin, ng/mL	4.1 (3.2–4.9)	2.9 (2.3–3.9)	<0.001
Total adiponectin, mg/dL	17.9 (11.4–22.0)	11.0 (7.3–16.3)	<0.001

Variables are presented as median (25th-75th percentile). CD40L indicates CD-40 ligand; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; IP-10, interferon-γ-inducible-protein-10; PAI-1, plasminogen activator inhibitor-1; RANTES, regulated on activation normal T-cell expressed and secreted; VCAM-1, vascular cell adhesion molecule-1; and vWF, von Willebrand factor antigen.

*Probability values from univariate conditional logistic regression analysis.

Table 2. Spearman Partial Correlation Coefficients Between Plasma Biomarkers in the Control Data Set (n=190)

Biomarker Group	Hemostasis			Inflammation		Endothelial Activation				Chemokines	
	Fibrinogen	PAI-1	vWF	IL-6	hs-CRP	ICAM-1	VCAM-1	CD40L	E-selectin	RANTES	IP-10
Adipocytokines											
Total adiponectin	–0.082	0.263	0.117	0.144	0.101	0.291‡	0.428‡	0.105	0.234†	0.098	0.127
Adipsin	–0.125	–0.009	0.073	–0.078	0.030	–0.028	0.076	0.120	0.283‡	0.099	–0.031
Resistin	–0.118	–0.012	0.149	0.125	–0.021	0.187*	0.097	0.277†	0.172*	0.187*	–0.185*
Chemokines											
IP-10	–0.059	0.127	0.018	0.078	0.132	0.184*	0.274‡	–0.163*	0.070		
RANTES	0.171*	0.001	0.134	–0.035	0.130	0.268‡	0.245†	0.464‡	0.013		
Endothelial activation											
E-selectin	–0.033	0.277‡	–0.048	0.042	0.144						
CD40L	0.059	0.020	0.076	0.183	0.136						
VCAM-1	–0.014	0.047	0.166*	0.028	0.131						
ICAM-1	0.115	0.102	0.165*	0.137	0.252‡						
Inflammation											
hs-CRP	0.397‡	0.083	0.084								
IL-6	0.090	–0.019	0.054								

Spearman partial correlation coefficients adjusted for matching variables. CD40L indicates CD-40 ligand; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; IP-10, interferon-γ-inducible-protein-10; PAI-1, plasminogen activator inhibitor-1; RANTES, regulated on activation normal T-cell expressed and secreted; VCAM-1, vascular cell adhesion molecule-1; and vWF, von Willebrand factor antigen.

*P value<0.05.

†P value<0.01.

‡P value<0.001.

included in this model, E-selectin and resistin, but not fibrinogen and IP-10, significantly increased the AUC to 0.785 (P=0.004) and 0.788 (P=0.008), respectively. A borderline significant increase in the AUC to 0.744 (P=0.072) was observed at the inclusion of total adiponectin. The AUC increased to 0.824 (95% CI, 0.770–0.877) at the simultaneous inclusion of E-selectin and resistin in the traditional risk factor model. There was some indication that the traditional risk factor model, including both E-selectin and resistin, yielded better discrimination than the traditional risk factor model with either E-selectin (P=0.124) or resistin (P=0.069). By contrast, no incremental predictive information was provided by the inclusion of total adiponectin in the traditional risk factor model with either E-selectin (P=0.709) or resistin (P=0.279) or both E-selectin and resistin (P=0.686).

Reclassification

As shown in Table 5, the separate inclusion of total adiponectin, resistin, and E-selectin, but not of fibrinogen and IP-10, in the traditional risk factor model consistently yielded significant continuous and categorical NRI. The highest NRI was observed at the simultaneous addition of E-selectin and resistin to the traditional risk factor model. The traditional risk factor model with both E-selectin and resistin provided a significant NRI when compared with traditional risk factor models with either E-selectin or resistin (Table V in the online-only Data Supplement). By contrast and as with the AUC, adding total adiponectin to the traditional risk factor model with resistin and E-selectin alone or in combination resulted in nonsignificant NRI.

Discussion

Among healthy middle-aged men from the PRIME study, the simultaneous assessment of 9 biomarkers of hemostasis, inflammation, and endothelial activation revealed significant associations of fibrinogen, vWF, and E-selectin with ischemic stroke over 10 years of follow-up after adjustment for traditional risk factors. When concurrently evaluated with chemokines and adipocytokines, fibrinogen, E-selectin, IP-10, resistin, and total adiponectin remained significantly associated with ischemic stroke. Finally, only 2 of these biomarkers, namely E-selectin and resistin, provided incremental and additive value over traditional risk factors for risk prediction of ischemic stroke.

Associations

The first contribution of the present study consists in quantifying associations of several biomarkers of hemostasis, inflammation, and endothelial activation with ischemic stroke risk. Among investigated biomarkers of hemostasis, fibrinogen and vWF, but not PAI-1, were significantly associated with ischemic stroke in separate multivariable models. Our finding of increased stroke risk with elevated fibrinogen concentration is consistent with results of an individual participant meta-analysis, including 31 prospective studies.[16] The adverse relationship between higher vWF levels and ischemic stroke as observed in our study is in line with results of the Atherosclerosis Risk in Communities Study[17] and recent findings of the Rotterdam Study.[18] Similarly to our study, the Caerphilly Study, a cohort of British middle-aged men, found a significant association of PAI-1 with ischemic

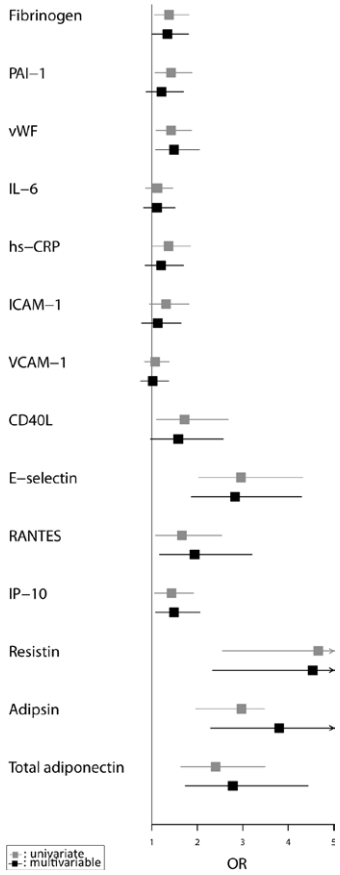

Figure. Univariate and multivariable odds ratios (ORs) of ischemic stroke presented separately for each plasma biomarker. ORs from conditional logistic regression analysis per SD increase in log-transformed biomarker levels. Univariate analysis accounts for matching variables only. Multivariable analysis is adjusted for systolic blood pressure, antihypertensive treatment, cigarette smoking, alcohol drinking, total chsolesterol, high-density lipoprotein cholesterol, waist circumference, and diabetes mellitus. CD40L indicates CD-40 ligand; hs-CRP, high-sensitivity C-reactive protein; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; IP-10, interferon-γ-inducible-protein-10; PAI-1, plasminogen activator inhibitor-1; RANTES, regulated on activation normal T-cell expressed and secreted; VCAM-1, vascular cell adhesion molecule-1; and vWF, von Willebrand factor antigen.

stroke that disappeared when adjusting for traditional risk factors.[19]

With regard to investigated biomarkers of inflammation, neither hs-CRP nor IL-6 showed a significant multivariable association with ischemic stroke. The former association was attenuated to nonsignificance when adjusted for traditional risk factors. Of note, the magnitude of the multivariable association of hs-CRP with ischemic stroke in our study was similar to that from a recent meta-analysis of individual participant data (OR, 1.20 versus hazard ratio, 1.27 per SD increment in the log-transformed variable).[20] The lack of a significant association between IL-6 and ischemic stroke in the present study is consistent with results of the above mentioned Caerphilly Study,[21] but contrasts with findings in older populations.[8,22,23] IL-6 concentration varies with time and, therefore, regression dilution bias may have contributed to the lack of a significant association with ischemic stroke in the present study.[24]

Table 3. Mutually Adjusted Odds Ratios of Ischemic Stroke for Plasma Biomarkers

Biomarker	Odds Ratio	95% CI	*P* Value
Fibrinogen	1.53	1.03–2.28	0.036
vWF	0.84	0.55–1.28	0.422
E-selectin	1.76	1.06–2.93	0.030
RANTES	1.77	0.89–3.54	0.105
IP-10	1.72	1.06–2.78	0.027
Resistin	2.86	1.30–6.27	0.009
Adipsin	1.68	0.88–3.21	0.119
Total adiponectin	1.82	1.04–3.19	0.036

Odds ratios (OR) from conditional logistic regression analysis per SD increase in log-transformed biomarker levels adjusted for all covariates in the table plus systolic blood pressure (OR, 1.02; 95% CI, 1.00–1.05) and cigarette smoking (OR, 1.05; 95% CI, 1.01–1.10). Model was fitted using stepwise backward selection from traditional risk factors and, subsequently, including eligible biomarker variables. CI indicates confidence interval; IP-10, interferon-γ-inducible-protein-10; RANTES, regulated on activation normal T-cell expressed and secreted; and vWF, von Willebrand factor antigen.

Among investigated biomarkers of endothelial activation, only E-selectin, but not intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and CD40L, was associated with ischemic stroke independently from traditional risk factors. We are not aware of any previous study that investigated the association of intercellular adhesion molecule-1 and CD40L with future ischemic stroke in the general population. The Hormones and Biomarkers Predicting Stroke (HaBPs) Study among postmenopausal women has recently reported nonsignificant associations for vascular cell adhesion molecule-1 and E-selectin with regard to ischemic stroke.[8] However, an adverse association between E-selectin concentration and macrovascular events, including stroke, has recently been reported among type 2 diabetic patients, with a hazard ratio per 1-U increase in log E-selectin of 2.3 over 10 years of follow-up.[25] Furthermore, a case–control study, including 200 stroke patients and 205 community controls, observed a 3.4-fold and 7.9-fold increased likelihood of ischemic stroke among individuals in the third and fourth quartiles of the E-selectin distribution.[26]

The concurrent examination of significant biomarkers in a single multivariable model allowed us to obtain a more parsimonious set of biomarkers of potential importance for ischemic stroke risk prediction. Accordingly, only fibrinogen, E-selectin, IP-10, resistin, and total adiponectin were identified as the most relevant biomarkers for ischemic stroke. The observed increase of ischemic stroke risk with higher total adiponectin contrasts with results of previous investigations.[27,28] However, elevated total adiponectin has already been associated with future coronary heart disease[29] and cardiovascular mortality.[30] Furthermore, high plasma adiponectin levels have been related to the presence of atrial fibrillation,[31] a major risk factor for ischemic stroke of cardioembolic origin.

Usefulness for Risk Prediction

The second contribution of our study rests on investigating the usefulness of biomarkers of hemostasis, inflammation, and endothelial activation for risk prediction of ischemic stroke in the context of chemokines and adipocytokines recently put

Table 4. Area Under the Receiver-Operating Characteristic Curve (AUC) of Multivariable Models Predicting Ischemic Stroke Risk

Model	AUC	95% CI	*P* Value*
Traditional risk factor model	0.679	0.612–0.745	…
+ Fibrinogen	0.688	0.621–0.755	0.825
+ IP-10	0.697	0.631–0.760	0.650
+ Total adiponectin	0.744	0.687–0.802	0.072
+ E-selectin	0.785	0.729–0.842	0.004
+ Resistin	0.788	0.733–0.843	0.008
+ Resistin + Total adiponectin	0.809	0.756–0.861	0.002
+ E-selectin + Total adiponectin	0.795	0.741–0.849	0.002
+ E-selectin + Resistin	0.824	0.770–0.877	<0.001
+ E-selectin + Resistin + Total adiponectin	0.830	0.779–0.882	<0.001

AUC from unconditional logistic regression analysis adjusted for matching variables. Traditional risk factor model includes systolic blood pressure and cigarette smoking after stepwise backward selection from these risk factors and antihypertensive treatment, alcohol drinking, total cholesterol, high-density lipoprotein cholesterol, waist circumference, and diabetes mellitus. AUC indicates area under the receiver-operating characteristic curve; CI, confidence interval; and IP-10, interferon-γ-inducible-protein-10.

*Probability values for the comparison of AUCs with the traditional risk factor model using the DeLong test.[12]

forward in the PRIME study. Although significantly associated with ischemic stroke in the final model, fibrinogen did not provide incremental value over traditional risk factors in predicting stroke risk. This is in line with results of the EUROSTROKE Project, a case–control study of males nested in 3 population cohorts,[32] and recent findings of the Strong Heart Study, a community-based cohort of American Indians.[33] The present analysis confirms the usefulness of resistin for risk prediction of ischemic stroke in the context of a larger set of investigated biomarkers.[4,28] Furthermore, our study newly identified E-selectin as a biomarker providing incremental predictive value over traditional risk factors and additive predictive value to resistin. E-selectin is a glycoprotein expressed on endothelial cells in response to their activation by cytokines and mediates leukocyte adhesion to the endothelium, an early step in atherogenesis.[34,35] Circulating E-selectin corresponds to its cleaved extracellular domain, of which higher levels have been associated with carotid atherosclerosis and coronary heart disease.[36] Resistin is an adipose-derived peptide hormone that, in addition to its local activity, exerts effects outside the adipose tissue, including expression and secretion of cytokines and activation of endothelial cells.[37,38] Involvement of E-selectin and resistin in convergent pathways of atherothrombosis may explain the additive value of these biomarkers in the prediction of ischemic stroke.

The results of our multimarker approach should be interpreted in the context of 2 recently published analyses from the HaBPs Study and the Framingham Offspring Study. In the former, a nested case–control study among postmenopausal women 50 to 79 years of age, none of 13 investigated biomarkers individually improved risk discrimination of ischemic stroke.[8] Differences in population characteristics between the HaBPs Study (older age, women) and the study at hand (middle age, men) may explain the discrepant findings. By contrast, the Framingham Offspring Study identified plasma B-type natriuretic peptide and urinary albumin/creatinine ratio from a set of 8 biomarkers to significantly improve risk prediction.[9] However, the different biomarker panel (besides hs-CRP and PAI-1) and end point (total stroke and transient ischemic attack) of that study limit the comparison of these results with our findings.

Perspectives and Limitations

A number of questions remain to be addressed before advocating the measurement of E-selectin and resistin in clinical practice. First, our results need to be validated in further prospective studies. Second, the benefit of systematically measuring these biomarkers for guiding clinical decision-making should be examined. As a first step, post hoc analyses of randomized controlled trials may help investigating this issue. Third, future interventions that might reduce plasma levels of

Table 5. Net Reclassification Improvement Using Multivariable Models Predicting Ischemic Stroke Risk

Model	Categorical NRI			Continuous NRI		
	%	95% CI	*P* Value*	%	95% CI	*P* Value*
Traditional risk factor model	…	…	…	…	…	…
+ Fibrinogen	6.6	–2.9 to 16.2	0.265	12.0	–8.1 to 32.1	0.340
+ IP-10	7.5	–3.9 to 18.9	0.280	38.7	18.9 to 58.5	0.002
+ Total adiponectin	17.1	3.8 to 30.3	0.040	56.5	37.5 to 75.5	<0.001
+ Resistin	28.4	14.2 to 42.5	0.002	74.4	56.5 to 92.4	<0.001
+ E-selectin	29.9	16.5 to 43.4	0.001	74.7	56.2 to 93.3	<0.001
+ Resistin + Total adiponectin	31.9	18.0 to 45.9	<0.001	78.8	60.8 to 96.6	<0.001
+ E-selectin + Total adiponectin	34.8	20.9 to 48.7	<0.001	80.6	62.9 to 98.4	<0.001
+ E-selectin + Resistin	41.4	27.5 to 55.4	<0.001	96.1	79.0 to 113.2	<0.001
+ E-selectin + Resistin + Total adiponectin	38.1	23.9 to 52.3	<0.001	90.5	73.3 to 107.8	<0.001

NRI from unconditional logistic regression analysis adjusted for matching variables. Categorical NRI based on tertiles of risk as predicted by the traditional risk factor model. Traditional risk factor model includes systolic blood pressure and cigarette smoking after stepwise backward selection from these risk factors and antihypertensive treatment, alcohol drinking, total cholesterol, high-density lipoprotein cholesterol, waist circumference, and diabetes mellitus. CI indicates confidence interval; IP-10, interferon-γ-inducible-protein-10; and NRI, Net Reclassification Improvement.

*Probability values for the null-hypothesis of NRI=0 as opposed to the traditional risk factor model using an asymptotic test proposed by Pencina et al.[13]

these biomarkers and ischemic stroke risk should be identified. So far, nonspecific agents have been suggested to lower resistin concentrations in human subpopulations and in experimental studies.[39–41] Fourth, from a mechanistic point of view, circulating endothelial microparticles are discussed as more specific markers of endothelial activation than soluble factors, such as E-selectin.[42] However, the clinical usefulness of such markers for stroke risk prediction remains to be examined in prospective investigations. Finally, the predictive performance of E-selectin and resistin should be further explored in the context of biomarkers not included in the present study and in the upcoming era of omics.

Among limitations of our study was the relatively modest number of ischemic stroke events that makes study implications tentative and precluded us from conducting in-depth subgroup analyses by phenotype. However, univariate biomarker associations were similar for embolic and atherothrombotic stroke. We cannot exclude underreporting of stroke events by study participants in the annual health questionnaire; however, case adjudication using extensive medical record review as in the PRIME study has been shown to be highly reliable.[43,44] Biomarkers were measured only once so that possible changes in their concentration over time could not be taken into account. We acknowledge the possibility of residual confounding by baseline or history of atrial fibrillation and diabetes mellitus because of unavailable and self-report information, respectively. Reverse causality by carotid artery stenosis that may contribute to elevated plasma biomarkers cannot be excluded as well. The restriction to Caucasian men of narrow age range (50–59 years) reduces the role of age in cardiovascular risk prediction. Consequently, the predictive value of biomarkers may be manifested more strongly than otherwise. Thus, our results are not directly transferable to other populations and should be confirmed in cohorts with broader age range, but also among women and other ethnic groups. Results from reclassification analysis should be interpreted with caution, because the choice of cut points was arbitrary for categorical NRI; on the contrary, continuous NRI is a category-free but very sensitive measure of reclassification.

Conclusion

In conclusion, the simultaneous evaluation of multiple biomarkers from distinct biological pathways, including hemostasis, inflammation, and endothelial activation with chemokines and adipocytokines, revealed that E-selectin and resistin provided incremental and additive value to traditional risk factors in risk prediction of ischemic stroke.

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Disclosures

None.

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