Fibrin D-Dimer, Tissue-Type Plasminogen Activator, von Willebrand Factor, and Risk of Incident Stroke in Older Men

S. Goya Wannamethee, PhD; Peter H. Whincup, PhD; Lucy Lennon, MSc; Ann Rumley, PhD; Gordon D. Lowe, DSc

Background and Purposes—Abnormalities in blood coagulation and the fibrinolytic system have been associated with increased risk of stroke, but few prospective studies have studied the associations in older adults. We have examined the associations between fibrin D-dimer, tissue-type plasminogen activator, and von Willebrand factor (vWF) and risk of stroke in older men and examined their predictive roles separately in normotensive and hypertensive men.

Methods—Prospective study of 3358 men aged 60 to 79 years with no previous diagnosis of myocardial infarction or stroke and without atrial fibrillation followed-up for an average of 9 years, during which there were 187 incident stroke events.

Results—Increased levels of D-dimer and vWF were associated with significantly increased risk of major stroke events after adjustment for potential confounders, including systolic blood pressure (adjusted hazard ratios and 95% confidence interval per standard deviation increase in D-dimer and vWF were 1.24 [95% confidence interval, 1.08–1.44] and 1.25 [95% confidence interval, 1.09–1.45], respectively). No associations were seen with tPA after adjustment. The positive associations between D-dimer and vWF and incident stroke remained after additional adjustment for markers of inflammation (C-reactive protein, IL-6). D-dimer was associated with stroke in both normotensive and hypertensive men; vWF showed stronger associations in normotensive than in hypertensive men (test for interaction: P=0.52 for D-dimer; P<0.01 for vWF).

Conclusions—Fibrin D-dimer and vWF are associated with increased risk of stroke in older men. These associations were not explained by their associations with inflammation. D-dimer may be a useful marker to identify those at high risk for stroke among hypertensive men. (Stroke. 2012;43:1206-1211.)

Key Words: cerebrovascular disease ■ epidemiology ■ etiology ■ risk factors

Abnormalities in the coagulation and fibrinolytic system have been associated with increased risk of coronary heart disease in observational studies and in meta-analyses of these,1–3 but the roles of hypercoagulability and fibrinolysis in stroke have been less studied. Thrombus formation is a key mechanistic event in ischemic stroke and various markers of coagulation and fibrinolytic factors have been suggested as possible predictors of stroke.4 The von Willebrand factor (vWF) is synthesized primarily by vascular endothelial cells and promotes platelet adhesion and aggregation, leading to thrombus formation.5 Increased levels of vWF in stroke patients have been reported in several case-control studies,5–8 but prospective studies have been inconsistent, with some showing a positive relationship9,10 and others showing no relationship11–14 between vWF and stroke risk. Fibrin D-dimer is the primary degradation product of cross-linked fibrin and is a marker of activated coagulation and fibrinolytic function.2 Tissue-type plasminogen activator (tPA), produced and released mainly by endothelial cells, acts on plasminogen to form plasmin, and tPA antigen may be indicative of impaired fibrinolytic function.3 Circulating levels of D-dimer and tPA are increased in acute stroke16–18 and have been shown to be positively associated with incident stroke.13,14,17,18 The vWF, D-dimer, and tPA levels increase markedly with age,19 but few studies have examined the associations between coagulation markers, fibrinolytic function, and incident stroke in the elderly population, in whom incident stroke rates are particularly high. D-dimer and tPA are also implicated in the inflammatory response.1,2,3 However, the extent to which associations between D-dimer and tPA and stroke risk can be explained by associations with inflammation, which has been implicated in the pathogenesis of stroke,20 has not been well-studied. We therefore have examined the independent associations between fibrin D-dimer, tPA, and vWF levels...
and risk of incident stroke in a prospective cohort of older men followed-up for a mean period of 9 years. We also examined the prognostic role of these factors separately in hypertensive individuals, who are at particularly high risk for development of stroke.

Subjects and Methods

The British Regional Heart Study is a prospective study of cardiovascular disease involving 7735 men aged 40 to 59 years from 1 general practice in each of 24 British towns who were screened between 1978 and 1980.21 The population studied was socioeconomically representative of British men but consisted almost entirely of white Europeans (99%). In 1998 to 2000, all surviving men, now aged 60 to 79 years (mean age, 68.7 years), were invited for a 20-year follow-up examination. The men completed a questionnaire that included questions regarding their medical history and lifestyle behavior. They were requested to fast for a minimum of 6 hours, during which time they were instructed to drink only water and to attend for measurement at a prespecified time between 0800 and 1800 hours. All men were asked to provide a blood sample, collected using the Sarstedt Monovette system. The samples were frozen and stored at −20°C on the day of collection and transferred in batches for storage at −70°C until analysis, performed after no more than 1 freeze–thaw cycle. Twelve-lead electrocardiograms were recorded using a Siemens Sicard 460 instrument and were analyzed and coded in accordance with Minnesota coding definitions at the University of Glasgow ECG core laboratory based at Glasgow Royal Infirmary.22 Atrial fibrillation was defined according to Minnesota codes 8.3.1 and 8.3.3. The men were asked whether a doctor had ever told them that they had angina or myocardial infarction (heart attack, coronary thrombosis) or stroke, and they were asked to bring their medication to the examination session; 4252 men (77% of survivors) attended for examination and 4084 men had at least 1 of the 3 markers (vWF, tPA, or D-dimer) measured. We excluded men with a recall of a diagnosis of myocardial infarction or stroke (n = 613) and men with atrial fibrillation (n = 113) because D-dimer is known to be elevated in patients with atrial fibrillation who are at high risk for thromboembolic events,23 leaving 3358 men for analysis.

Cardiovascular Risk Factors

Anthropometric measurements including body weight, height, and waist circumference were performed. Details of measurements and classification methods for smoking status, physical activity, body mass index, waist circumference, social class, blood pressure, blood lipids, glucose, and forced expiratory volume in 1 second have been described.24–26 Men with a doctor’s diagnosis of diabetes or those with a fasting glucose of ≥7 mmol/L (World Health Organization

| Table 1. Baseline Characteristics (1998–2000) in 3358 Men Without Pre-Existing Myocardial Infarction or Stroke and Without Atrial Fibrillation According to Incident Stroke Status |
|---------------------------------|-----------------|-----------------|-----|
| No Stroke (N=3171)              | Development of Stroke (N=187) | P Value |
| Age (y) 68.2 (5.37)             | 71.0 (5.40)     | <0.0001        |
| BMI (kg/m²) 26.84 (3.64)        | 26.74 (3.44)    | 0.73           |
| WC (cm) 96.95 (10.24)           | 97.22 (9.83)    | 0.71           |
| Inactive (%) 32.3               | 37.4            | 0.15           |
| Current smokers (%) 13.0        | 13.9            | 0.73           |
| Manual workers (%) 53.4         | 56.7            | 0.38           |
| Moderate/heavy drinkers (%) 18.4| 20.4            | 0.44           |
| Using blood pressure lowering treatment (%) 19.5 | 26.2 | 0.03 |
| Angina (%) 8.4                  | 9.6             | 0.57           |
| Diabetes (%) 10.4               | 11.8            | 0.57           |
| ABO blood group O (%) 47.8      | 43.9            | 0.29           |
| ABO blood group A (%) 29.3      | 29.6            | 0.92           |
| SBP (mm Hg) 149.50 (23.65)      | 157.47 (25.24)  | <0.0001        |
| DBP (mm Hg) 85.39 (10.93)       | 87.35 (11.96)   | 0.01           |
| Cholesterol (mmol/L) 6.05 (1.05)| 6.11 (1.14)     | 0.50           |
| HDL-cholesterol (mmol/L) 1.33 (0.34) | 1.32 (0.36) | 0.65         |
| FEV1 (L) 2.64 (0.66)            | 2.46 (0.49)     | 0.0002         |
| Glucose* (mmol/L) 5.81 (5.25–6.08)| 5.92 (5.23–6.19)| 0.23         |
| CRP (mg/L)* 1.63 (0.78–3.21)   | 2.11 (1.05–3.90)| 0.002          |
| IL-6 (pg/mL)* 2.34 (1.51, 3.30)| 2.89 (1.73, 4.24)<0.0001 |
| Fibrinogen 3.23 (0.72)          | 3.37 (0.79)     | 0.01           |
| D-dimer (ng/mL)* 79.0 (47.0–77.48)| 103.5 (60.95, 160.77)<0.0001 |
| tPA (ng/mL) 10.78 (4.30)        | 11.39 (4.39)    | 0.05           |
| vWF (IU/dL) 136.54 (50.68)      | 150.04 (45.04)  | <0.0001        |

Mean (SD) unless specified.
BMI indicates body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; FEV1, forced inspiratory volume in 1 second; HDL, high-density lipoprotein; IL, interleukin; IU, international units; SBP, systolic blood pressure; SD, standard deviation; tPA, tissue-type plasminogen activator; vWF, von Willebrand factor; WC, waist circumference.
*Geometric mean and interquartile range.
criteria) were considered to have prevalent diabetes. Because a high proportion of men had systolic blood pressure ≥140 mm Hg (63.6%) and the majority would be classified as having hypertension on the basis of systolic blood pressure of ≥160 mm Hg, as used in the Systolic Hypertension in the Elderly Program.27 Hypertension in this older cohort was defined as systolic blood pressure ≥160 or diastolic blood pressure ≥90 or using antihypertensive treatment.

Hemostatic and Inflammatory Biomarkers
After the 20-year examination, plasma levels of tPA antigen and D-dimer were measured with enzyme-linked immunosorbent assays (Biopool AB, Umea, Sweden), as was vWF antigen (DAKO, High Wycombe, UK). C-reactive protein (CRP) was assayed by ultrasensitive nephelometry (Dade Behring, Milton Keynes, UK). IL-6 was assayed using a high-sensitivity enzyme-linked immunosorbent assay (R & D Systems, Oxford, UK).

Follow-Up
All men have been followed up from initial examination (1978–1980) for cardiovascular morbidity and follow-up has been achieved for 99% of the cohort.28 In the present analyses, all-cause mortality and morbidity events are based on follow-up from rescreening in 1998 to 2000 at mean age 60 to 79 years to July 2008, a mean follow-up period of 9 years (range, 8–10 years). Information on death was collected through the established “tagging” procedures provided by the National Health Service registers. Fatal stroke episodes were those coded on the death certificate to International Classification of Diseases 430 to 438. Nonfatal stroke events were those that produced a neurological deficit that was present for >24 hours. Evidence regarding nonfatal stroke was obtained by ongoing reports from general practitioners, by biennial reviews of the patients’ practice records (including hospital and clinic correspondence) through to the end of the study period, and from repeated personal questionnaires to surviving subjects after initial examination. Supplementary information on CT/MRI scans was only available for 96 (51%) of the 187 stroke cases. Of these, 59 (61%) were ischemic stroke, 10 (10%) were hemorrhagic strokes, and 27 (29%) were indeterminate.

Statistical Methods
The distribution of D-dimer was skewed to the right and log transformation was used. Cox proportional hazards model was used to assess the multivariable adjusted hazards ratio (relative risk) in a comparison of tertiles of D-dimer, tPA, and vWF and for a 1 SD increase in these variables. Models were adjusted for potential confounding and mediating factors in a stepwise manner. In the adjustment, we included first age and then conventional risk factors that have been shown to be associated with stroke. To assess the contribution of inflammation, we additionally adjusted for inflammatory risk markers CRP and IL-6. In multivariable analyses, smoking (never, long-term ex-smokers [≥15 years], recent ex-smokers [<15 years], and current smokers), social class (manual versus nonmanual), physical activity (4 groups), alcohol intake (5 groups), diabetes (yes/no), and angina (yes/no) were fitted as categorical variables; body mass index, forced expiratory volume in 1 second, CRP, and IL-6 were fitted as continuous variables. Receiver-operating characteristic curves and areas under the curve (c-statistics) were used to assess the ability of D-dimer and vWF to predict stroke beyond routine clinical risk factors. We also conducted a supplementary analysis restricting cases to the 59 confirmed ischemic stroke cases.

Results
During the mean follow-up period of 9 years, there were 187 incident stroke cases (41 fatal and 146 nonfatal), a rate of 6.8 per 1000 person-years, in the 3358 men with no history of stroke or myocardial infarction and without atrial fibrillation. Table 1 shows baseline characteristics in the men who had development of stroke and in those who remained free of stroke. Men who had development stroke showed significantly higher rates of blood pressure treatment and higher mean levels of systolic and diastolic blood pressures, fibrinogen, CRP, vWF, D-dimer, and tPA, and lower levels of forced expiratory volume in 1 second.

The Figure shows the Kaplan-Meier estimates of the cumulative incidence of stroke by tertiles of D-dimer, vWF,
and tPA. Risk of stroke increased significantly with increasing levels of D-dimer and vWF, even after adjustment for age, smoking, physical activity, social class, systolic blood pressure, prevalent diabetes, angina, use of antihypertensive treatment, and forced expiratory volume in 1 second (Table 2). No significant association was seen with tPA after adjustment. Adjustment for ABO blood group made no difference to the findings. Further adjustment for CRP attenuated the associations between vWF and D-dimer with incident stroke, but the trends remained significant. Adjusting for IL-6 instead of CRP yielded results similar to that seen with adjustment for CRP. The adjusted hazards ratio (HR) and 95% CI for a 1 SD increase in log D-dimer was 1.20 (95% CI, 1.02–1.39; P = 0.02) and the corresponding HR for vWF was 1.20 (95% CI, 1.04–1.42; P = 0.009).

The addition of D-dimer to a multivariate model using routinely measured clinical variables and biomarkers to predict stroke (including age, smoking, alcohol intake, body mass index, systolic blood pressure, use of antihypertensive treatment, prevalent diabetes, and lung function) increased the C-statistic from 0.675 (95% CI, 0.637–0.712) to 0.688 (95% CI, 0.651–0.726; P = 0.006). The further addition of vWF increased the C-statistic to 0.693 (95% CI, 0.655–0.729; P = 0.003) when vWF was added to the routine model.

The vWF and D-dimer were significantly associated with both fatal and nonfatal strokes. The adjusted HR (95% CI) for fatal and nonfatal stroke were 1.36 (95% CI, 1.00–1.82; P = 0.04) and 1.23 (95% CI, 1.04–1.44; P = 0.01) for a 1 SD increase in log D-dimer. The corresponding adjusted HR for vWF were 1.51 (95% CI, 1.15–1.98; P = 0.007) and 1.19 (95% CI, 1.01–1.40; P = 0.04), respectively. The tPA showed no appreciable association with either fatal or nonfatal strokes (adjusted HR, 1.24; 95% CI, 0.92–1.68; P = 0.14 and adjusted HR, 1.04; 95% CI, 0.88–1.24; P = 0.62, respectively).

We also conducted an additional analysis restricting incident cases to confirmed ischemic strokes (n = 59 cases). D-dimer and vWF were both significantly associated with ischemic stroke after adjustment for factors in model 1. The adjusted HR (95% CI) for a 1 SD increase in log D-dimer was 1.55 (95% CI, 1.24–1.94; P = 0.0002) and the corresponding HR for vWF was 1.31 (95% CI, 1.01–1.73; P = 0.03).
Normotensive men and hypertensive men had similar age-adjusted mean levels of vWF (137.2 versus 137.1 IU/dL; \( P=0.95 \)) and D-dimer (geometric mean, 79.0 versus 80.6 ng/mL; \( P=0.48 \)). In analyses performed separately in normotensive and hypertensive men (Table 3), D-dimer predicted stroke in both normotensive men and in hypertensive men (test for interaction, \( P=0.52 \)). By contrast, vWF showed stronger associations in normotensive men than in hypertensive men (test for interaction, \( P=0.01 \)).

### Discussion

In this large population study of British men aged 60 to 79 years, we have confirmed the findings of some previous prospective studies\(^9,10,13,14\) that circulating levels of hemostatic markers are associated with incident stroke. We have shown that fibrin D-dimer and vWF, but not tPA antigen, are independently associated with incident stroke in older men and their measurements modestly improved prediction of stroke as assessed by improvement in the C statistics. Our findings extend those of other studies of coagulation and fibrinolysis and risk of stroke by observing that these associations are independent from markers of inflammation (CRP and IL-6), an observation not previously reported, and by examining their prognostic role in hypertensive men who are at particularly high risk for development of stroke.

The vWF was shown to be predictive of stroke in the Rotterdam and Atherosclerosis Risk in Communities (ARIC) studies\(^9,10\), but others have reported no independent association between vWF and stroke\(^11,14\); however, many of these studies were based on smaller participant numbers. Our results, generated in a more homogenous population of predominantly white men, provide additional support for an independent association between vWF and stroke that is not explained by inflammation. This finding was similar using CRP (an acute phase protein associated with stroke risk\(^20\)) and IL-6 (a proinflammatory cytokine that stimulates release of vWF from vascular endothelium\(^20\)). This association with stroke independent of inflammation might reflect release of vWF as a marker of endothelial disturbance or a prothrombotic tendency because vWF promotes platelet adhesion and aggregation.

The strongest associations with stroke in the present study were observed for D-dimer. Prospective studies on the association between D-dimer and risk of stroke in persons with sinus rhythm are limited. Our findings are consistent with results of 2 previous prospective studies showing D-dimer to predict incident stroke in the general population.\(^13,14\) No significant association was seen in the Three-City French cohort study,\(^11\) but the number of stroke cases was relatively small. The associations between D-dimer and stroke persisted even after adjustment for CRP or IL-6, suggesting that they are not attributable to persisting inflammatory reactions as measured by these inflammatory markers. By contrast, we found no association between tPA and risk of stroke that is consistent with other prospective studies.\(^13,14\)

Hypertensive patients have been shown to have elevated levels of D-dimer and vWF in some, but not all, studies.\(^30\) D-dimer or vWF was not elevated in hypertensive men in this general older population, but D-dimer was a significant predictor of stroke in hypertensive men, in contrast to vWF, which showed weak associations in hypertensive men. On the basis of these findings, D-dimer rather than vWF is more likely to help in the assessment of stroke risk, particularly in patients with hypertension. Further large studies in hypertensive individuals are needed to formally evaluate whether inclusion of D-dimer assessment as a part of screening would be clinically useful to reduce stroke risk in this population at high risk (eg, by selective use of aspirin).
Our study has some limitations. It was based on an older and predominantly white male population, so the results cannot be generalized directly to women, younger men, or other ethnic groups. The findings were based on a single measurement at the baseline re-examination rather than on repeated assessments, which may lead to underestimation of the true strength of associations. However, we have published estimated regression dilution ratios for D-dimer and vWF over periods of up to 4 years. Taking account of regression dilution would have only modest effects on the adjusted hazards ratios per SD increase, which would increase from 1.24 to 1.33 for D-dimer and from 1.25 to 1.36 for vWF. Although we did not have information on type of stroke in all men, 85% of stroke cases in Great Britain are attributable to ischemia, and nonfatal strokes are likely to be predominantly ischemic. Moreover, additional analysis of confirmed ischemic stroke cases in this study confirmed a relationship between vWF and D-dimer with ischemic stroke. The number of confirmed hemorrhagic stroke in this study was too small to make any inferences about the relationships with hemorrhagic stroke.

In conclusion, we have shown that vWF and fibrin D-dimer are independent predictors of stroke in older men, which is likely to reflect an association with ischemic stroke, and these associations were independent of inflammation. Whether lowering levels of D-dimer and vWF in susceptible older population groups by lifestyle measure or pharmacological means is beneficial remains to be elucidated.

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Disclosures
None.

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Abstract

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Abstract

High-risk men incident stroke in older men

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Background and objectives: Blood coagulation and fibrinolytic system abnormalities are associated with a higher risk of stroke among elderly. However, there are few prospective studies looking at these relationships. In the current study, we examined the relationship between fibrin D-dimer, tissue-type plasminogen activator, von Willebrand factor, and risk of incident stroke in older men.

Methods: We conducted a prospective study of 3358 men aged 60-79 years without prior diagnosis of stroke or myocardial infarction and no atrial fibrillation. During the average 9-year follow-up period, 187 strokes occurred.

Results: After adjustment for confounding factors, the risk of serious stroke events and D-dimer and von Willebrand factor levels increased significantly [for D-dimer and von Willebrand factor, adjusted hazard ratios (95% CI) were 1.24 (1.08 - 1.44) and 1.25 (1.09 - 1.45), respectively]. Adjustment for t-PA did not alter the results. D-dimer and von Willebrand factor were associated with stroke rate in both men with normal blood pressure and hypertension. The association was stronger for hypertension than for normal blood pressure (interaction test p < 0.05 for D-dimer and p < 0.01 for von Willebrand factor).

Conclusions: Fibrin D-dimer and von Willebrand factor are associated with an increased risk of stroke in older men. These associations were not explained by inflammation. D-dimer is a useful marker for identifying the risk of stroke in hypertensive men.

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