Original Contribution

Eosinophil Cationic Protein, Carotid Plaque, and Incidence of Stroke

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Background and Purpose—ECP (eosinophil cationic protein) is a marker of eosinophil activity and degranulation, which has been linked to atherosclerosis and cardiovascular disease. We examined the relationship between ECP, carotid plaque, and incidence of stroke in a prospective population-based cohort.

Methods—The subjects participated in the Malmö Diet and Cancer Study between 1991 and 1994. A total of 4706 subjects with no history of stroke were included (40% men; mean age, 57.5 years). Carotid plaque was determined by B-mode ultrasound of the right carotid artery. Incidence of stroke was followed up during a mean period of 16.5 years in relation to plasma ECP levels.

Results—Subjects in the third tertile (versus first tertile) of ECP tended to have higher prevalence of carotid plaque (odds ratio: 1.18; 95% confidence interval: 1.003–1.39; P=0.044 after multivariate adjustments). A total of 258 subjects were diagnosed with ischemic stroke (IS) during follow-up. ECP was associated with increased incidence of IS after risk factor adjustment (hazard ratio, 1.57; 95% confidence interval: 1.13–2.18; for third versus first tertile; P=0.007). High ECP was associated with increased risk of IS in subjects with carotid plaque. The risk factor–adjusted hazard ratio for IS was 1.86 (95% confidence interval: 1.32–2.63) in subjects with carotid plaque and ECP in the top tertile, compared with those without plaque and ECP in the first or second tertiles.

Conclusions—High ECP is associated with increased incidence of IS. The association between ECP and IS was also present in the subgroup with carotid plaque. **(*Stroke*. 2017;48:00-00. DOI: 10.1161/STROKEAHA.117.018450.)**

Key Words: cohort studies ■ eosinophil cationic protein ■ epidemiology ■ risk factors ■ stroke

Many studies have showed increased risk of cardiovascular diseases in association with total leukocyte counts or neutrophil counts,[1,2] but only a few studies have reported about incidence of cardiovascular disease in relation to eosinophils, which normally accounts for 1% to 4% of the total leukocyte population.[3–5] ECP (eosinophil cationic protein) is produced and secreted by activated eosinophils[6] and is frequently used as a marker of eosinophil activity and degranulation. ECP has cytotoxic, fibrosis promoting and immune-regulatory functions, and raised ECP concentrations can be found in asthma and other atopic or inflammatory diseases.[6,7] ECP has cytotoxic properties, which are effective in the defense to invasive microorganisms. Small amounts of ECP are also found in neutrophil granulocytes and monocytes.[8]

Recent studies show that ECP could have a role in development of cardiovascular diseases. High ECP was associated with severity and extension of atherosclerosis in a study of patients with coronary artery disease.[9] High ECP concentrations have been associated with poor prognosis in patients undergoing percutaneous coronary interventions,[10] and it has been shown that eosinophilic granule proteins can promote a prothrombotic condition.[11,12] It was recently reported that increased levels of ECP is a risk factor for developing ischemic stroke,[13] independently of traditional stroke risk factors. However, this finding has not been replicated to date.

The purpose of the present study was to examine whether ECP is associated with incidence of stroke in a large study from the general population. We also assessed whether ECP is associated with atherosclerotic carotid plaque and whether ECP modified the risk of stroke in individuals with plaque.

Methods

Subjects

The Malmö Diet and Cancer Study is a population-based cohort study originally designed to investigate associations between dietary habits and health outcomes. All men and women who were born between 1926 and 1945 and were living in Malmö, Sweden, were invited to a screening examination between 1991 and 1996.[14] In all, 28 449 subjects (60% women) participated. Participation rate was 41%. A random sample of 6103 subjects was invited to a substudy of cardiovascular diseases.[15] The participants in the substudy were examined with ultrasound of the right carotid artery. In 5540 of the 6103 subjects, fasting blood samples were taken and stored at −80°C immediately after collection.

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The Malmö Diet and Cancer Study was approved by the Ethics Committee of Lund University (LU 51–90).

Exclusions

After exclusion of individuals with missing plasma samples (n=307) and missing information for clinical data (n=368), a total of 4865 samples were sent for analysis. After exclusion of samples because of technical reasons, information of ECP was available for 4739 subjects. Subjects with previous stroke at baseline were excluded (n=33). Thus, a total of 4706 subjects (2833 women and 1873 men) were available to study ECP in relation to incidence of stroke. Information on carotid plaque was available for 4533 of them. Complete information on all risk factors (ECP, age, sex, systolic blood pressure, low-density lipoprotein, diabetes mellitus, waist circumference, smoking status, blood pressure medication, white blood cells [WBC], and CRP [C-reactive protein]) was available for 4588 subjects and for 4420 subjects with information about carotid plaque.

Measurements

At baseline, all subjects were examined at the screening center. A self-administered questionnaire on smoking habits, medication, medical history, and lifestyle factors was administrated. Smoking habits were categorized as current smokers (including occasional smokers), former smokers, and never smokers.

Ultrasound imaging was used to investigate the presence of atherosclerotic plaque lesions in the right carotid artery of the subjects. The bifurcation area of the common carotid artery was scanned within a predefined window comprising 3 cm of the distal common carotid artery, the bifurcation, and 1 cm of the internal and external carotid arteries, respectively. Atherosclerotic plaque lesions were defined as a focal thickening of the intima–media layer of >1.2 mm.[16] The examination was performed by specialized and certified sonographers. Diabetes mellitus was defined as either self-reported physician diagnosis, current use of antidiabetic medication, or by fasting whole blood glucose of at least 6.1 mmol/L (corresponding to plasma glucose ≥7.0 mmol/L).

Height and weight were measured with light indoor clothing. Body mass index was calculated as weight in kilograms divided by squared height in meters (kg/m^2). Blood pressure was measured after a 10-minute rest in supine position. Low-density lipoprotein levels were calculated using the Friedewald formula.[15] High sensitive CRP was measured from frozen plasma samples using the Tina-quant CRP latex high-sensitivity assay. WBC were measured in fresh blood using a SYSMEX K1000 analyzer (TOA Medical Electronics, Kobe, Japan).

ECP was measured in plasma samples at the Science for Life Laboratory, Uppsala University, using the Olink Proseek Multiplex Cardiovascular panel.[17] The samples had been frozen in −80°C from the examination in 1991 to 1994 until analysis in 2015. ECP is expressed as normalized protein expression values as arbitrary units on a log2 scale. The within- and between run coefficients of variation were 6% and 18%, respectively. Lower and upper limits of quantification correspond to 488 and 62 500 pg/mL, respectively (www.olink.com).

Incidence of Stroke

The cohort was followed up from the baseline examination until the first stroke event, emigration from Sweden, death, or end of follow-up on December 31, 2010. The definition of stroke is rapidly developed clinical signs of local or global loss of cerebral function that lasted for >24 hours or led to death within 24 hours.[18] Thus, transient ischemic attacks were not included.[19] Computed tomography, MRI, or autopsy verification was required to subtype the stroke cases as ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage. One patient with an unspecified stroke event (ie, without computed tomography or magnetic resonance verification) was counted as ischemic stroke in this study. The stroke events were retrieved by linkage with the Stroke Register of Malmö,[20] and subjects who experienced stroke outside the city of Malmö were retrieved by linkage with the Swedish Hospital Discharge Register.[21] Ninety-three percent of all stroke cases were validated by review of hospital records.

Classification of etiologic subtypes of ischemic stroke was performed for 195 (of 258) ischemic stroke cases. The classification was performed by a senior neurologist according to Trial of Org 10172 in Acute Stroke Treatment.[22]

Statistics

Differences in baseline characteristics of the included subjects were assessed with the use of ANOVA (continuous variables) or Pearson χ^2 test (dichotomous variables). The results were presented as means±SD or percentages as appropriate. The subjects were split into tertiles, the highest concentration of ECP being the top tertile. Tertiles were chosen a priori to avoid too small number of stroke in each group. Cox' proportional hazards regression was used to compare incidence of all-cause stroke and ischemic stroke in relation to tertiles of ECP and per 1 SD (=0.80 U) increment of log-transformed ECP. Cox' regression was also used to compare incidence of all-cause stroke and ischemic stroke in groups defined by occurrence of carotid plaque (yes versus no) and high ECP (ie, third tertile versus tertiles 1–2). Hazard ratios (HR) were calculated in 3 separate models adjusted for (1) age and sex; (2) model 1 +systolic blood pressure, low-density lipoprotein, diabetes mellitus, waist circumference, smoking status, blood pressure medication; and (3) model 2 +CRP and WBC. Proportional hazards assumptions were tested using time-dependent covariate in the Cox' models, and no significant deviation was found. Logistic regression was used to compare occurrence of carotid plaques in relation to tertiles of ECP and to calculate odds ratios in the models previously described. Kaplan–Meier survival plots were used to illustrate incidence of stroke in relation to ECP levels in tertiles and in categories of carotid plaque and ECP levels.

Results

Baseline Characteristics

Baseline characteristics in individuals are presented in Table 1. Mean (±SD) ECP (in arbitrary units on a log scale) was 5.03±0.79 in men and 4.96±0.81 in women. Mean age was 57.6±6.08 years in men and 57.4±5.89 in women.

Most cardiovascular risk factors were more common with higher ECP concentrations (Table 1). However, the proportion of current smokers was not associated with concentrations of ECP.

ECP and Carotid Plaque

The relationship between occurrence of carotid plaque and tertiles of ECP is presented in Table 2. The proportion of plaque was 37.7% in the top tertile of ECP and 31.3% for those in tertile 1. The occurrence of plaque for those in the top tertile of ECP remained significant after adjustments for risk factors (odds ratio, 1.18; 95% confidence interval [CI], 1.003–1.39; *P* for trend=0.044). When ECP was modeled as a continuous variable (per 1 SD), ECP was significantly associated with plaque after adjustments for risk factors in model 2. However, the HR per 1 SD was 1.06 (95% CI, 0.996–1.14; *P*=0.065) after further adjustment for CRP and WBC (Table 2).

ECP and Incidence of Stroke

During a mean follow-up of 16.5 years, a total of 308 subjects were diagnosed with a first stroke event, of which 258 were ischemic stroke.

The cumulative incidence rates of stroke were higher for subjects with high plasma concentrations of ECP (Figure 1; Table 3). When comparing the highest tertile with the lowest,

Table 1. Relationships Between Tertiles of ECP and Different Cardiovascular Risk Factors

	Eosinophil Cationic Protein			
	T1	T2	T3	*P* Value (Trend)
n=4706	1568	1569	1569	
ECP, mean±SD (range), AU	4.16±0.42 (1.75–4.64)	4.94±0.18 (4.64–5.26)	5.87±0.50 (5.26–7.51)	
Men (%)	575 (37)	625 (40)	673 (43)	<0.001
Age, y	57.1±6.0	57.3±6.0	58.0±5.7	<0.001
Current smokers, n (%; n=4700)	340 (21.7)	357 (22.8)	313 (20.0)	0.244
DM, n (%)	77 (4.9)	137 (8.7)	142 (9.1)	<0.001
HbA1c (%)	4.8±0.58	4.9±0.75	4.9±0.75	<0.001
LDL, mmol/L (n=4700)	4.08±0.96	4.20±0.98	4.22±0.98	<0.001
SBP, mm Hg	138.7±18.3	141.9±19.2	142.2±18.9	<0.001
DBP, mm Hg	85.7±9.1	86.9±9.4	87.5±9.2	<0.001
BMI, kg/m^2	25.1±3.6	25.9±3.9	26.1±4.1	<0.001
Waist, cm	81.3±12.2	83.6±12.9	85.3±12.9	<0.001
CRP, mg/L (n=4603)	1.13	1.36	1.70	<0.001
WBC (10^9/L)	5.8±2.3	6.1±1.6	6.3±1.7	<0.001
BP drugs n (%)	215 (13.7)	237 (15.1)	298 (19.0)	<0.001
Asprin, n (%)	30 (1.9)	29 (1.8)	38 (2.4)	0.32
Statins, n (%)	26 (1.7)	21 (1.3)	23 (1.5)	0.66

P values for trend across tertiles were calculated using ANOVA or Pearson χ^2. For continuous traits, mean±SD are given. Geometric means are presented for CRP. For categorical traits, % are given. AU indicates arbitrary units; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; DBP, diastolic BP; DM, diabetes mellitus; ECP, eosinophil cationic protein; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; SBP, systolic blood pressure; and WBC, white blood cells.

ECP was significantly associated with both total stroke and ischemic stroke. These associations remained significant after adjustments for cardiovascular risk factors, including WBC and CRP; HR for total stroke was 1.52 (95% CI, 1.13–2.05; *P*=0.006) and HR for ischemic stroke was 1.57 (95% CI, 1.13–2.18; *P*=0.007).

Information about etiologic classification of stroke was available for 195 ischemic stroke cases (of 258). However, the number in each category was small, and no significant differences were observed between tertiles of ECP after adjustments for age and sex. For large artery atherosclerosis (n=33), the HR was 1.81 (95% CI, 0.73–45) comparing the third versus first tertile. The corresponding HR were 1.34 (95% CI, 0.70–2.6) for cardioembolism (n=49), 1.44 (95% CI, 0.78–2.7) for small-artery occlusion (n=73), and 1.79 (95% CI, 0.77–4.2) for undetermined causes (n=39).

ECP, Carotid Plaque, and Incidence of Stroke

ECP was significantly associated with total stroke in subjects with plaque (HR per 1 SD, 1.26; 95% CI, 1.08–1.47; *P*=0.004; after adjustments in model 2) but not in subjects without carotid plaque (HR per 1 SD, 1.12; 95% CI, 0.94–1.33; *P*=0.21). HR for ischemic stroke was 1.33 per 1 SD of ECP (95% CI, 1.12–1.57; *P*=0.001) for those with carotid plaque and 1.06 (95% CI, 0.88–1.29; *P*=0.54) for those without carotid plaque. However, the interaction term for plaque and ECP did not reach statistical significance (*P*=0.12).

Table 2. Occurrence of Carotid Plaque in Relation to Tertiles of ECP

	T1	T2	T3	*P* Value (Trend)	Per 1 SD
n=4533	1533	1501	1499		
Plaque n (%)	480 (31.3)	492 (32.8)	565 (37.7)		
Model 1	1.00 (ref)	1.04 (0.89–1.22)	1.23 (1.05–1.43)	0.01	1.09 (1.03–1.17)
Model 2	1.00 (ref)	0.99 (0.84–1.16)	1.20 (1.02–1.40)	0.027	1.08 (1.01–1.15)
Model 3	1.00 (ref)	0.99 (0.84–1.17)	1.18 (1.003–1.39)	0.044	1.06 (0.996–1.14)

Values are odds ratios from a multiple logistic regression. Model 1: adjusted for age and sex (based on 4533 subjects, 1537 with plaque). Model 2: model 1 +systolic blood pressure, low-density lipoprotein, diabetes mellitus, waist circumference, smoking status, blood pressure medication (based on 4522 subjects, 1536 with plaque). Model 3: model 2 +c-reactive protein and white blood cell (based on 4420 subjects, 1493 with plaque). ECP indicates eosinophil cationic protein.

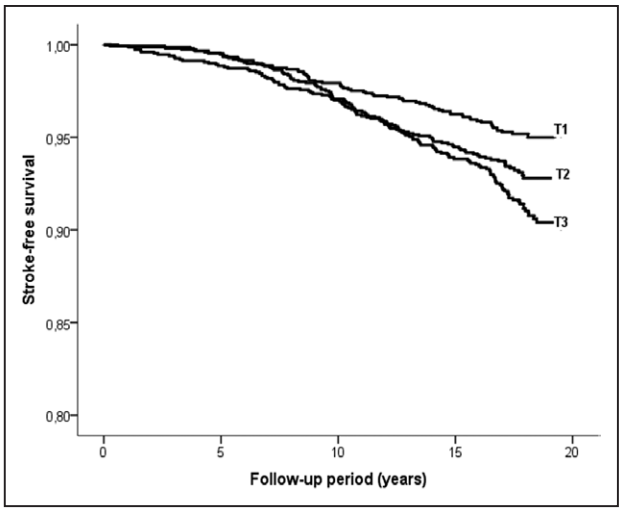

Figure 1. Incidence of stroke in relation to tertiles of eosinophil cationic protein.

Subjects with carotid plaque and levels of ECP in the third tertile had increased risk for stroke compared with those without carotid plaque and levels of ECP in the first or second tertile (Table 4; Figure 2). For total stroke, the HR was 1.77 (95% CI, 1.28–2.44; *P*=0.001) after adjustments for cardiovascular risk factors, including WBC and CRP. For ischemic stroke, the corresponding HR was 1.86 (95% CI, 1.32–2.63; *P*<0.001; Table 4). There was a significant difference in incidence of ischemic stroke between individuals with carotid plaque and high ECP and those with carotid plaque and ECP in tertile 1 or 2 (Table 4).

Discussion

ECP is an inflammatory protein that is secreted mainly by eosinophil granulocytes and a biomarker of eosinophil activation. It has been reported that ECP is associated with degree of coronary atherosclerosis,[9] and ECP was recently associated with ischemic stroke in a study of 70-year-old men and women.[13] The present study shows that ECP is associated with occurrence of carotid plaque and with incidence of ischemic stroke in a study from the general population. Individuals with carotid plaque and high ECP levels had significantly higher risk of ischemic stroke than those with carotid plaque and low or moderate ECP levels. The results persisted after adjustments for major cardiovascular risk factors.

Even though many studies have reported increased risk of stroke in relation to high leukocyte levels, few have studied the role of eosinophils. Recently, a study reported increased occurrence of complex aortic arch plaque in stroke patients with high eosinophils.[23] Studies of patients with coronary syndromes have reported higher ECP in those with high degree of coronary atherosclerosis.[9] ECP was not significantly associated with carotid plaque in a study of 70-year-old men and women.[24] The effect size of the relationship with carotid plaque was moderate in our study, and association was only borderline significant (*P*=0.044) after risk factor adjustments. However, the results are in accordance with findings of atherosclerosis in patients with stroke and coronary disease.[9,23]

It is unclear whether ECP could have a causal role in atherogenesis and development of stroke. It has been reported that eosinophils can be activated and degranulated by adhesion molecules linked to endothelial cells,[25] and the eosinophils seem to be involved in the pathogenesis of various forms of vascular inflammation.[26] ECP stimulates fibroblast migration and fibrosis,[26,27] which hypothetically could be of importance for atherosclerosis.[9] ECP also interacts with several other proteins, such as complement factors and coagulation proteins.[28,29] Activated eosinophils have prothrombotic functions,[9,11] and ECP has been shown to shorten coagulation time

Table 3. Incidence of Stroke and Hazard Ratios in Relation to Tertiles of ECP

	T1	T2	T3	*P* Value (Trend)	HR per 1 SD
n=4706	1568	1569	1569		
Total stroke					
Incidence, n (n/1000 p-y*)	71 (2.70)	103 (3.99)	134 (5.20)		
Model 1	1.00 (ref)	1.43 (1.06–1.93)	1.73 (1.30–2.31)	<0.001	1.26 (1.12–1.40)
Model 2	1.00 (ref)	1.33 (0.98–1.80)	1.55 (1.16–2.08)	0.003	1.21 (1.08–1.35)
Model 3	1.00 (ref)	1.31 (0.96–1.78)	1.52 (1.13–2.05)	0.006	1.19 (1.06–1.34)
Ischemic stroke					
Incidence, n (n/1000 p-y*)	58 (2.21)	84 (3.25)	116 (4.50)		
TOAST class (n=195) (LAA/CE/SAO/OTH/UND)	7/15/17/0/8	12/12/30/0/15	14/22/26/1/16		
Model 1	1.00 (ref)	1.42 (1.02–1.98)	1.82 (1.33–2.50)	<0.001	1.27 (1.12–1.43)
Model 2	1.00 (ref)	1.32 (0.94–1.84)	1.62 (1.17–2.22)	0.003	1.21 (1.07–1.37)
Model 3	1.00 (ref)	1.29 (0.92–1.82)	1.57 (1.13–2.18)	0.007	1.19 (1.04–1.35)

Model 1: adjusted for age and sex (based on 4706 subjects [308 stroke, 258 ischemic stroke]). Model 2: model 1 +systolic blood pressure, low-density lipoprotein, diabetes mellitus, waist circumference, smoking status, blood pressure medication (based on 4693 subjects [305 stroke, 255 ischemic stroke]). Model 3: model 2 + log c-reactive protein and white blood cell (based on 4588 subjects [297 stroke, 247 ischemic stroke]). CE indicates cardioembolism; ECP, eosinophil cationic protein; HR, hazard ratio; LAA, large artery atherosclerosis; OTH, other causes; SAO, small artery occlusion; TOAST, Trial of ORG 10172 in Acute Stroke Treatment; and UND, undetermined causes.

*Person-years.

Table 4. Incidence of Stroke by Occurrence of Carotid Plaque and High ECP (ie, Top Tertile)

	Low ECP, No Plaque	High ECP, No Plaque	Low ECP, Plaque	High ECP, Plaque
n=4533	2062	934	972	565
Total stroke				
Incidence, n (n/1000 p-y*)	86 (2.47)	53 (3.37)	81 (5.19)	69 (7.67)
Model 1	1.00 (ref)	1.41† (1.03–1.93)	1.57† (1.16–2.12)	2.19 (1.59–3.00)
Model 2	1.00 (ref)	1.30 (0.94–1.80)	1.39 (1.02–1.88)	1.81 (1.32–2.49)
Model 3	1.00 (ref)	1.24† (0.89–1.72)	1.32 (0.97–1.80)	1.77 (1.28–2.44)
Ischemic stroke				
Incidence, n (n/1000 p-y*)	74 (2.12)	44 (2.79)	64 (4.10)	63 (7.00)
TOAST class (n=184; LAA/CE/SAO/UND)	7/17/22/11	2/6/11/7	12/10/23/10	11/14/12/9
Model 1	1.00 (ref)	1.36 (0.96–1.93)	1.45 (1.04–2.03)	2.33 (1.66–3.26)
Model 2	1.00 (ref)	1.24† (0.87–1.77)	1.29† (0.92–1.81)	1.93 (1.37–2.71)
Model 3	1.00 (ref)	1.16† (0.80–1.66)	1.21† (0.86–1.71)	1.86 (1.32–2.63)

Model 1: adjusted for age and sex (based on 4533 subjects, 289 stroke, 245 ischemic stroke). Model 2: model 1 +systolic blood pressure, low-density lipoprotein, diabetes mellitus, waist circumference, smoking status, blood pressure medication (based on 4521 subjects, 287 stroke, 243 ischemic stroke). Model 3: model 2 +white blood cell and log c-reactive protein (based on 4420 subjects, 279 stroke, 235 ischemic stroke). CE indicates cardioembolism; ECP, eosinophil cationic protein; LAA, large artery atherosclerosis; SAO, small-artery occlusion; TOAST, Trial of ORG 10172 in Acute Stroke Treatment; and UND, undetermined causes.

*Per 1000 person-years.

†$P<0.05$ vs group with high ECP, plaque.

in normal plasma in a dose-dependent manner, by interacting with factor 12.[28] However, the relationships between ECP and coagulation are complex, and ECP has also been reported to interact with the coagulation cascade system in a way that promotes fibrinolytic activity[12] and to inhibit platelet aggregation,[30] which may prevent the development of thrombosis.

Yet another possibility is that ECP reflects unspecific activation of the adaptive immune system. The eosinophil has many regulatory functions and is regarded as a key orchestrator of allergic responses.[31] It has been shown that human eosinophils interact with and activate other hematopoietic cells, such as macrophages and lymphocytes.[31] The risk of stroke was mainly seen in subjects with carotid plaque in this study. Because inflammation and high macrophage density in carotid plaque are associated with plaque vulnerability,[32] it could be speculated that raised ECP and eosinophil activation is associated with plaque rupture through effects on inflammatory cells in the plaque tissue.

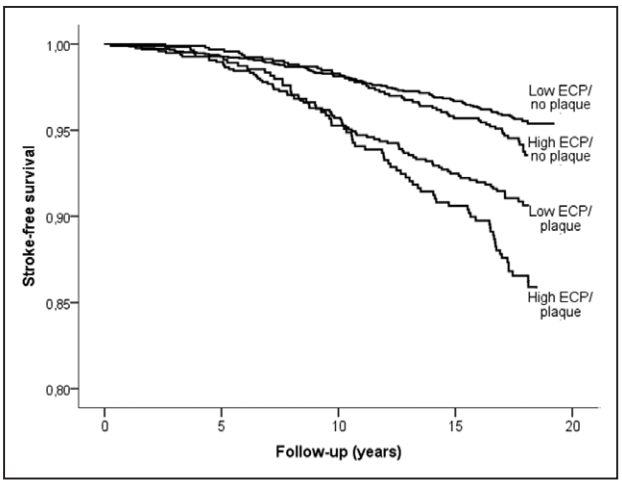

Figure 2. Incidence of stroke in relation to categories of ECP (eosinophil cationic protein; tertile 3 vs tertiles 1–2) and carotid plaque (yes vs no).

Strength and Limitations

The large population-based cohort, a long follow-up time, and a relatively large number of stroke cases are major strengths of this report. Information about risk factors was collected at baseline before the incident events. The end points were retrieved from nationwide and local registers, and the diagnosis was validated by review of hospital records in >90% of the cases.

There are also several limitations. The Malmö Diet and Cancer cohort is from a homogeneous white population of middle-aged men and women, and it is unknown whether the results can be generalized to other ethnic groups. ECP and risk factors were assessed at 1 point in time, and we do not know to what extent individual risk factors and preventive medication varied over the years. For example, statins were not commonly prescribed in the beginning of 1990s, and statin prescriptions have increased substantially in the past years. Elevated ECP levels are not specific for stroke but are also seen in conditions, such as asthma[33] and other eosinophilic diseases.[34] However, changes in ECP levels over time would probably dilute the associations with stroke and bias the results toward null.

The assessment of carotid plaque was performed by well-trained and certified sonographers,[35] and the reproducibility in the Malmö Diet and Cancer Study has been shown to be good.[36] The ultrasound measurement was only provided

for the right carotid artery, which is another limitation. However, plaque prevalence is comparable with previous studies, measuring both carotid arteries.[37] The occurrence of carotid plaque depends on the definition, and it is unknown what effect different definitions may have had on the relationships with ECP. Moreover, stroke is an etiologically heterogeneous disease, and the number of specific etiologic subtypes was small. More detailed information about plaque subtypes would also be of interest to investigate further in future studies.

In conclusion, high ECP is associated with increased incidence of stroke, in particular ischemic stroke. The association between ECP and ischemic stroke was also present in the subgroup with carotid plaque.

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Disclosures

None.

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