Markers of Inflammation and Infection Influence the Outcome of Patients With Baseline Asymptomatic Carotid Lesions
A 5-Year Follow-Up Study

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Background and Purpose—It is still in debate whether the evaluation of markers of infection and inflammation may be of importance for cerebrovascular and cardiovascular prevention, and we aimed to investigate this field in a prospective 5-year clinical follow-up study in patients with early stages of atherosclerosis.

Methods—We studied 668 subjects divided in 3 groups according to the results of carotid ultrasound examination: (1) normal subjects, if intima-media thickness (IMT) was <0.9 mm; (2) with IMT, if IMT was between 0.9 and 1.5 mm; and (3) with asymptomatic carotid plaque, if IMT was >1.5 mm. Traditional cardiovascular risk factors were investigated, and laboratory analysis included measurement of plasma lipids, fibrinogen, C-reactive protein, IgG antibodies for helicobacter pylori (HP), cytotoxic HP, cytomegalovirus, and chlamydia pneumoniae.

Results—Cerebrovascular or cardiovascular events were registered in 18% of patients during the follow-up, and at multivariate analysis we found that the high levels of fibrinogen (\(P<0.0001\)) and C-reactive protein (\(P=0.014\)), the seropositivity to cytotoxic HP (\(P=0.001\)) and chlamydia pneumoniae (\(P=0.026\)), the presence of IMT or asymptomatic carotid plaque (\(P<0.0001\)), and the total burden of infections (\(P<0.0001\)) were the variables predictive of the clinical events.

Conclusions—Beyond traditional cardiovascular risk factors, markers of inflammation and infections seem to significantly influence the occurrence of cerebrovascular and cardiovascular events in patients with baseline asymptomatic carotid lesions. (Stroke. 2006;37:000-000.)

Key Words: atherosclerosis ■ carotid arteries ■ infection ■ inflammation

Atherosclerosis is a high-cost disease, and its complications represent the first cause of death in most of the industrialized countries; thus, the early recognition of any condition potentially leading to coronary artery disease has a great clinical relevance. However, the absence of the “traditional” risk factors does not totally protect from the disease, and new “emerging” risk factors have been identified, including inflammation and infectious agents. Regarding markers of inflammation, observational and epidemiological data have suggested the potential predictive role of fibrinogen and C-reactive protein (CRP) in coronary artery disease (CAD). In addition, the evaluation of markers of infection and inflammation may also be of importance for an effective prevention of cerebrovascular risk.

However, the role of markers of infection and inflammation on atherosclerosis development and progression is still on debate, and less data are available on the role of such markers in the early stages of atherosclerosis, such as in asymptomatic patients with carotid intimal-media thickness (IMT). Therefore, the aim of our study was to evaluate the influence of traditional cardiovascular risk factors (eg, older age, male gender, obesity, hypertension, diabetes, smoking habit, family history of CAD, and dyslipidemia) and of markers of inflammation (eg, elevated levels of plasma fibrinogen or CRP) and infection [eg, seropositivity for helicobacter pylori (HP), cytotoxic (CTX) HP, cytomegalovirus (CMV), and chlamydia pneumoniae (CMP)] on cerebrovascular and cardiovascular events in a 5-year follow-up of patients with baseline asymptomatic carotid lesions.

Methods

Patients
We studied 668 subjects, 326 males and 342 females, selected from a total number of \(\approx 1100\) patients, all referred to our Unit of Cardiovascular Prevention for a clinical evaluation. The project design included a medical examination, biochemical analyses, and the ecocolordoppler of carotid arteries. The adopted procedures were...
approved by the Ethic Council of our institution. All of the subjects gave their informed consent to participate to the study.

Subjects were excluded from the study if they had a past history of atrial fibrillation, peripheral artery disease, coronary revascularization, angina pectoris, myocardial infarction, carotid surgery, or cerebrovascular event. None of the subjects included in the study had clinical evidence of connective tissue disease, liver dysfunction, or renal failure or received treatment with antibiotics or steroids shortly before commencing the study. Among the main cardiovascular risk factors, the presence of family history of CAD (in a first-degree relative <55 years of age), hypertension (systolic or diastolic blood pressure >140 and 90 mm Hg, respectively, or pharmacological therapy with antihypertensive drugs), diabetes (fasting glucose plasma concentrations >126 mg/dL or pharmacological therapy with antidiabetic drugs or insulin), and smoking habits were considered.

Sitting blood pressure was measured twice on the right arm with a random-zero sphygmomanometer. The average of 2 measurements obtained on 1 occasion, separated by a count of the pulse rate, was used in the present analysis. Height and weight were recorded, and body mass index was expressed as kg/m². Participants were categorized as having obesity if body mass index was ≥30 kg/m².

Echocardiography was not included in the study protocol, and this may lead to a lack of valuable information, such as the exclusion of vascular events because of cardiac embolism (eg, patent foramen ovale).

Biochemistry
A blood sample was drawn in the morning, before the medical examination, after a 12- to 14-hour overnight fast. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol were quantified by standard enzymatic-colorimetric methods; low-density lipoprotein cholesterol was calculated by the Friedewald formula.

Fibrinogen determination was rapidly performed according to the coagulative method of Von Claus; using this method, high levels are considered to be those >350 mg/dL. High-sensitive CRP was determined by the nephelometric method (Beckman Instrument APS), and high levels are considered those >3 mg/L.

IgG-specific antibodies for CMP, CMV, and HP were measured by ELISA. IgG titers ≥1.1 U/mL to CMP were considered positive, and anti-CMV IgG titers ≥11 U/mL were also considered positive. IgG-specific antibodies to HP were considered elevated when the anti-HP IgG concentration was >30% and the anti-CTX-HP IgG titers were >7.5 U/mL. The total burden of infections was considered as the concomitant seropositivity to the 4 antibodies.

Eccodoppler Examination of Carotid Arteries
B-mode real-time ultrasound was used to evaluate the arterial wall thickness in the carotid arteries using a machine Toshiba 270 SS with a probe of 7.5 to 10.0 MHz. The power output, focus, depth of measurement, and gain were standardized by using the preset program incorporated within the software package of the ultrasound equipment. The IMT was defined as the distance between the echogenic line representing the intima blood interface and the outer-echogenic line representing the adventitia junction. After freezing the image, the measurement was made with electronic calipers.

Patients were examined in the supine position, and each carotid wall and segment was examined to identify the thickest intimal-medial site. Three segments were identified and measured in anterior and posterior planes on each side: the distal 1 cm of the common carotid proximal to the bifurcation, the bifurcation itself, and the proximal 1 cm of the internal carotid artery. At each of these sites, we have determined the IMT, automatically measured, and detected any possible plaque. We primarily used the maximum carotid IMT value, which was determined as the mean of the maximum IMT of near- and far-wall measurements of both the left and right side arteries for each of the 3 arterial segments. If data on 1 of the walls or 1 of the sides were missing, maximum thickness of the available wall and side was used. The percentage of missing data in ICA was ~35%.

Ultrasound examination was performed by 1 investigator (E.C.), in blind and with no possibility of reproducing the IMT measurement. Carotid ultrasonography was carried out by 1 sonographer to limit the risk of a large interobserver variability. However, for methodological correctness, the intraobserver agreement for sono- graphic measurement was calculated with a 4.1% to 5.0% coefficient of variation for repeated scans.

According to the most recent guidelines of the joint European Society of Hypertension/European Society of Cardiology, we considered “normal” patients to be those with IMT <0.9 mm, “with IMT” to be those with IMT between 0.9 and 1.5 mm, and “with asymptomatic carotid plaque” to be those with IMT >1.5 mm.

Follow-Up
We performed a 5-year follow-up study in all of the patients to evaluate cerebrovascular and cardiovascular morbidity and mortality and to assess if baseline clinical and laboratory variables were predictive of these clinical events. Regarding the use of cardiovascular medications, during the follow-up patients received treatment with β-blockers (35%), angiotensin-converting enzyme inhibitors (42%), calcium entry blockers (36%), diuretics (20%), statins (70%), and antiplatelet drugs (76%). The protocol included that none of the patients had to receive antibacterial or antiinflammation therapy during the follow-up. However, we cannot exclude that patients took such therapies in 5 years; this fact, however, did not lead to dropout patients from follow-up, because we were observing the investigated population during their normal life and activity. In addition, we cannot exclude that the large use of statin therapy during the follow-up did influence the inflammation parameters.

Clinical events were registered in 18% of patients and included transient ischemic attack (TIA), minor and major stroke, effort or unstable angina, acute myocardial infarction (AMI), peripheral arterial disease (PAD), and cerebrovascular and cardiovascular death. TIA was defined as a brain deficit caused by vascular disease that clear completely in <24 hours. Minor and major stroke were distinguished by the modified Rankin Scale. Effort angina was defined by the presence of chest pain on walking that was relieved within 10 minutes after stopping or by ST segment of ECG down-sloping in a standard 12-lead electrocardiogram during chest pain or by positive stress testing. AMI was defined by a prolonged episode of chest pain with electrocardiogram and/or specific myocardial enzymes changes, involving hospitalization. PAD was defined by the self-reported presence of pain in the lower extremities while walking that was relieved within 10 minutes after standing still, with an ankle/brachial pressure index <0.90 and a positive treadmill test. The causes of death were retrieved directly from the families and were confirmed in all of the cases by the general practitioners on the basis of their own records (which may include hospital records).

Statistical Analysis
Statistical analyses was performed using the Statview Program (Abacus Concepts Inc). All of the differences in the investigated parameters among the study groups were assessed by ANOVA. A subsequent ANCOVA was performed to correct the dependent key variables (eg, each marker of inflammation and infection and the total burden of infections) for significant confounders (eg, the different traditional cardiovascular risk factors: age, male gender, obesity, hypertension, diabetes, smoking habit, family history of CAD, and dyslipidemia). Independent associations of the studied variables with the clinical events registered in the follow-up were assessed by logistic regression analysis.

Results
The progression in the IMT score (from normal to IMT to asymptomatic carotid plaque, Table 1) was in relation with most of the traditional cardiovascular risk factors (eg, male gender, older age, hypertension, diabetes, and dyslipidemia) and with all markers of inflammation and infection. In a
subsequent ANCOVA analysis (data not shown), to correct the dependent key variables (eg, fibrinogen, CRP, seropositivity to each agent, and total burden of infections) for possible significant confounders (eg, all the different traditional cardiovascular risk factors: age, male gender, obesity, hypertension, diabetes, smoking habit, family history of CAD, and dyslipidemia), only the seropositivity to each agent, and total burden of infections) were the only variables predictive of total burden of infection or baseline carotid lesions.

As shown in Table 2, the progression in the IMT score was in relation with ischemic stroke, AMI, PAD, and total events as registered in the 5-year follow-up. Regarding the subsets of ischemic stroke (data not shown), we found that the total incidence of minor stroke was 64% and major stroke was 36%. No significant relations were found for minor or major stroke with progression in the IMT score (data not shown). At univariate analysis (Table 3), patients with clinical events had significantly older age, higher levels of fibrinogen and high sensitivity (hs)-CRP, seropositivity to CTX-HP and CMP, and the presence of baseline carotid lesions, or total burden of infections were the only variables predictive of baseline carotid lesions.

In a subsequent ANCOVA analysis (data not shown), to correct the dependent key variables (eg, fibrinogen, CRP, seropositivity to each agent, and total burden of infections) for possible significant confounders (eg, all the different traditional cardiovascular risk factors: age, male gender, obesity, hypertension, diabetes, smoking habit, family history of CAD, and dyslipidemia), only the seropositivity to each agent, and total burden of infections) were the only variables predictive of total burden of infection or baseline carotid lesions.

We also assessed by logistic regression analysis whether a specific marker may predict a specific vascular event (data not shown), and we found the following significant associations: fibrinogen with TIA (P=0.0007), ischemic stroke (P=0.0003), effort or unstable angina (P=0.037), and AMI

<table>
<thead>
<tr>
<th>Event</th>
<th>Normal (n=212)</th>
<th>IMT (n=162)</th>
<th>ACP (n=294)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIA</td>
<td>2</td>
<td>2.5</td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>0.5</td>
<td>2.5</td>
<td>3</td>
<td>0.05</td>
</tr>
<tr>
<td>Effort or unstable angina</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>AMI</td>
<td>3</td>
<td>5.5</td>
<td>11</td>
<td>0.0005</td>
</tr>
<tr>
<td>PAD</td>
<td>1.5</td>
<td>4</td>
<td>7</td>
<td>0.005</td>
</tr>
<tr>
<td>Cardiovascular or cerebrovascular death</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>Total events</td>
<td>8.5</td>
<td>18</td>
<td>24</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**TABLE 2. Clinical Events (%) Registered During the Follow-up in Correlation With the Ultrasonographic Findings**
The detection of asymptomatic carotid lesions is made by a safe and noninvasive technique, such as the ecocolordoppler duplex scanning. A number of clinical studies have confirmed the association between the traditional cardiovascular risk factors and, consistent with that, we found in our patients that the progression in the IMT score was correlated with male gender, older age, hypertension, diabetes, and dyslipidemia.

Several studies have demonstrated the association between IMT and the occurrence of cardiovascular and cerebrovascular events. In agreement with previous reports, we found increased progression in the IMT score was correlated with male gender, cardiovascular disease, and we recently found increased levels of such parameters in patients with asymptomatic carotid lesions.

Because it is known that cardiovascular medications may influence event risk, we performed ANCOVA analysis (data not shown) for each individual group of treatment (included in the 5-year follow-up). Preliminarily, we found that the progression in the IMT score was in correlation with hs-CRP and fibrinogen levels, as well as with the seropositivity to all of the investigated infectious agents. A few studies have already investigated this topic in this category of patients, but with contrasting results.

In the present study, we examined a group of patients with baseline asymptomatic carotid lesions in order to evaluate the influence of both markers of inflammation and infection, beyond traditional cardiovascular risk factors, on their cardiovascular and cerebrovascular outcome in a 5-year follow-up. Notably, we found an increased percentage of PAD, but this may be not surprising, because such disease is a common manifestation of systemic atherosclerosis and increases significantly with age.

Several studies have suggested that the evaluation of markers of infection and inflammation may be of importance for an effective prevention of cardiovascular and cerebrovascular events. However, even if the role of such markers in CAD development and progression has been extensively studied, very few studies addressed their significance in early stages of atherosclerosis. Enhanced levels of CRP, interleukin 6, and soluble CD40 ligand are associated with high risk of cardiovascular disease, and we recently found increased levels of such parameters in patients with asymptomatic carotid lesions.
result at both univariate and multivariable analyses; this may probably be explained by the fact that no significant differences were found in hypertension, diabetes, and dyslipidemia (at both univariate and multivariable analyses) in patients with or without the clinical events, and, therefore, it is likely that such treatments were well balanced between the 2 groups. To our knowledge, such influence of markers of infection and inflammation on the occurrence of future cardiovascular and cerebrovascular events has not been evaluated previously in patients with early stages of atherosclerosis, as in asymptomatic patients with carotid IMT.

Conclusions

In conclusion, our study confirmed the existence of an association between increased carotid IMT and the presence of traditional cardiovascular risk factors or new, emerging factors, including markers of infection and inflammation. We also confirmed the existence of a strong association between increased carotid IMT and subsequent cardiovascular and cerebrovascular events.

Nevertheless, the present study suggested a possible role of markers of infection and inflammation, beyond traditional cardiovascular risk factors, in influencing the cardiovascular and cerebrovascular outcome in patients with early stages of atherosclerosis, such as in asymptomatic patients with carotid IMT. As shown in the present study, the distinct markers of infection and inflammation probably play a different role in this category of patients, and additional studies are needed to clarify this point in the future, ensuring consistency in groups with different age, gender, or ethnicity.

Beyond the use of such markers in the prediction of early and late stages of atherosclerosis and, subsequently, on their association with cerebrovascular and cardiovascular events, the clinical and therapeutic implications of these results remain to be evaluated. Although antibiotic treatment of infections in CAD patients had no impact on mortality in large prospective trials, promising data are coming from large prospective trials, promising data are coming from large prospective trials, promising data are coming from infections in CAD patients had no impact on mortality in late stages of atherosclerosis and, subsequently, on their association with cerebrovascular and cardiovascular events.

Markers of infection and inflammation probably play a different role in this category of patients, and additional studies are needed to investigate the possibility of allowing this category of high-risk patients to undergo such therapeutic approaches of primary prevention.

References


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