Monocyte Count Is a Predictor of Novel Plaque Formation
A 7-Year Follow-up Study of 2610 Persons Without Carotid Plaque at Baseline
The Tromsø Study

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Background and Purpose—Activation of monocytes and differentiation into lipid-laden macrophages are fundamental events in generation of atherosclerotic lesions. There exist few data on monocyte activity and the risk for atherosclerosis. In this prospective population-based study, we examined whether monocyte count in blood is a predictor of future plaque formation in persons without pre-existing carotid atherosclerosis.

Methods—At baseline, we measured monocyte count, white cell count (WCC), fibrinogen, intima-media thickness (IMT), and traditional cardiovascular risk factors in 2610 men and women aged 25 to 82 years who on ultrasound had no plaque in their right carotid artery. After 7 years of follow-up, a new ultrasound screening was performed and the number of novel plaques was grouped as none, 1 plaque, and 2 or more plaques.

Results—In multivariate analysis, monocyte count, age, sex, total cholesterol, current smoking, systolic blood pressure, and IMT were independent predictors of novel plaque formation. No significant association was found between plaque formation and either WCC or fibrinogen. For 1 standard deviation (0.17×10⁶) increase in monocyte count, the risk of being in a higher plaque category increased by 18% (OR, 1.18; 95% CI, 1.08 to 1.29). In the highest monocyte quartile, the risk for having plaque compared with the lowest quartile was 1.85 (OR) (95% confidence interval, 1.41 to 2.43). Repeating the analysis without IMT did not change the monocyte estimate. Excluding subjects with cardiovascular disease and diabetes mellitus from analysis neither changed the monocyte estimate.

Conclusion—Monocyte count is an independent predictor of future plaque formation in subjects without pre-existing carotid atherosclerosis. (Stroke. 2005;36:715-719.)

Key Words: atherosclerosis ■ inflammation ■ intima-media thickness ■ risk factors ■ ultrasonography
and IMT at baseline (1994) in 2610 persons aged 25 to 82 years without plaque in their right carotid artery. After 7 years of follow-up, a new ultrasound screening was performed and the independent relationship between monocyte count and the number of novel plaques was assessed.

**Subjects and Methods**

The Tromsø Study is a single-center population-based prospective study with repeated health surveys of inhabitants in the city of Tromsø, Norway. The main focus is on cardiovascular diseases. All inhabitants aged 55 to 74 and 5% to 10% samples in the other 3-year birth cohorts older than 24 years of age were invited to an ultrasonographic examination of the right carotid artery in the fourth survey conducted in 1994 to 1995 (baseline). A total of 6889 attended and ultrasound examination was performed in 6727 persons.

For the fifth survey in 2000 to 2001, all subjects who attended the baseline study and were still alive and residing in the municipality of Tromsø were invited to a follow-up ultrasound screening. A total of 1869 persons were lost to follow-up screening, of which 532 persons had died between baseline and follow-up. Because of reduced ultrasound capacity, rescaning was not performed in 110 persons. The total number of subjects examined by ultrasound at baseline and follow-up was 4858. Of these, 2610 persons (53.7%) had no plaque at baseline, and this group comprises the study population. The Norwegian Data Inspectorate licensed all data. The Regional Committee for Research Ethics approved the study. Written informed consent was obtained from all participants.

**Cardiovascular Risk Factors**

At baseline, standardized measurements of height, weight, blood pressure, nonfasting lipids, monocytes, and fibrinogen were performed. Blood pressure was recorded with an automatic device (Dinamap Vital Signs Monitor, Tampa, Fla) by specially trained personnel. Serum total cholesterol and nonfasting triglycerides were analyzed by standard enzymatic methods (CHOD-PAP; Boehringer-Mannheim, Mannheim, Germany). Serum high-density lipoprotein cholesterol was measured after precipitation of low-density lipoprotein with manganese chloride. Fibrinogen was measured using the PT-Fibrinogen reagent (Instrumentation Laboratory). White blood cells and monocytes were measured with automated cell counters by standard techniques. The analyses were performed at the Department of Clinical Chemistry, University Hospital of Tromsø.

A questionnaire on previous myocardial infarction and stroke, present angina pectoris and diabetes mellitus (yes/no), treated hypertension (never/previous/currently), and cigarette smoking (never/previous/currently, number of cigarettes per day) was enclosed in the letter of invitation. Coronary heart disease (CHD) was defined as prevalent angina pectoris or previous myocardial infarction.

**Ultrasonography and Measures of Atherosclerosis**

We used a duplex scanner (Acuson Xp10 128, ART-upgraded) equipped with a 7.5-MHz linear array transducer at baseline and at the follow-up examination. Duplex settings were fixed at start and not altered during the study. The sonographers were blinded to the baseline laboratory data and data from the questionnaires. The subjects were placed in a supine position, with the head turned slightly away from the probe. We used no fixed angle, but scanned the far and near walls of the right common carotid artery, bifurcation artery, and internal carotid artery (6 locations) for the presence of plaques as described previously. A plaque was defined as a localized protrusion of the vessel wall into the lumen (>50% compared with the adjacent intima-media layer). Locations with plaque were registered. Total numbers of plaques were calculated as the sum of plaques in each location, giving a maximum of 6 plaques and a minimum of zero. The actual distribution of novel plaques was as follows: none (n=1559), 1 plaque (n=667), 2 plaques (n=283), 3 plaques (n=83), 4 plaques (n=14), 5 plaques (n=2), and 6 plaques (n=2). Because of the low number in the upper plaque categories, we merged those with ≥2 plaques into 1 category to have fairly balanced and comparable groups. The interobserver and intraobserver agreement on plaque occurrence was substantial with κ-values (95% confidence interval [CI]) 0.67 (0.58 to 0.76) and 0.80 (0.70 to 0.91), respectively.

Automated measurements of baseline IMT were performed offline as described previously. The average IMT in the near and far walls of the common carotid artery and the far wall of the bifurcation were measured, and the mean of these averages was calculated and used in the analyses.

**Statistical Analyses**

In the regression models, plaque number at follow-up (none, 1 plaque, 2+ plaques) was defined as the dependent variable. Baseline characteristics were used as independent variables, first testing each independent variable separately, and then adding to the multivariate model those variables that were statistically significant in univariate analysis. Values of monocyte count and WCC were log-transformed before analyses, but because this had no impact on results, untransformed values were used. Diastolic blood pressure was not included in analyses because of the high intercorrelation with systolic blood pressure (Pearson r=0.77). Age- and sex-adjusted mean levels of risk factors in different plaque categories were calculated in a general linear model (the GLM procedure in the SAS statistical software), and linear trend across categories was tested by linear regression. Independent relationship between baseline risk factors and plaque numbers at follow-up was obtained from cumulative ordinal logistic regression, in which plaque number (1= no plaque, 2=1 plaque, and 3=2+ plaques) were treated as the dependent variable and risk factors were treated as explanatory variables. The model calculates the probability for being in a higher (ie, more plaques) category of the dependent variable. A score test confirmed that the proportional odds assumption was met. A backward selection procedure was performed to assess the predictive value of the explanatory variables. Interaction with sex was examined in a pooled analysis of women and men with plaque number as the dependent variable and the following independent variables: risk factor, sex, and risk factor*sex. Logistic regression was used to calculate the odds ratios (ORs) for having plaque at follow-up within quartiles of monocyte count using the lowest quartile as the reference. The SAS software package was used for all statistical analyses (SAS,V8, 2001). Two-sided P<0.05 was considered statistically significant.

**Results**

Table 1 presents baseline characteristics of the study population. The mean age for men was 59.0 years (range, 25 to 78), and for women it was 58.6 years (range, 25 to 82). Men had significantly higher monocyte count, WCC, and had lower total cholesterol, high-density lipoprotein cholesterol, and fibrinogen than women. There was no sex difference in the prevalence of current smoking, but women smoked fewer cigarettes per day than men. The correlation between monocyte count and WCC was 0.59 (P<0.0001) and between monocyte count and fibrinogen was 0.13 (P<0.0001) and were similar in men and women. The correlation between WCC and fibrinogen in men and women was 0.27 (P<0.0001) and 0.15 (P<0.0001), respectively. CHD was reported in 5.6% of the population, 1.4% reported stroke, and 1.8% reported diabetes mellitus.

At baseline, 1144 men and 1466 women had no plaque (Table 2). After 7 years, plaques had developed in 513 (44.8%) men and 538 (36.7%) women (P<0.0001 for sex difference).

In Table 3, age- and sex-adjusted levels of risk factors are tabulated according to plaque number at follow-up. Except
for fibrinogen that was a significant predictor in men and of borderline significance in women (P/H11005 = 0.06), the trends were similar among sexes and are therefore presented as pooled analyses of men and women. There were no significant interactions with sex.

In a cumulative ordinal logistic regression model including both sexes, monocyte count was an independent predictor of plaque together with IMT, age, sex, total cholesterol, current smoking, and systolic blood pressure (Table 4). For 1 standard deviation increase in monocyte count (0.17/10^9/L), the risk of being in a higher plaque category increased by 18%. Fibrinogen and WCC were not associated with carotid plaque in this model. Men had ≈30% higher risk for plaque compared with women (OR, 1.33; 95% CI, 1.11 to 1.59). In Table 5, the plaque prevalence at follow-up is stratified in quartiles of monocyte count at baseline. The risk for having plaque was significantly increased in the highest quartile compared with the lowest quartile (OR, 1.85; 95% CI, 1.41 to 2.43).

When we excluded from analysis subjects who reported CHD (n = 262), diabetes mellitus (n = 107), or stroke (n = 91)...

### TABLE 1. Baseline Characteristics of the Study Population (N=2610): the Tromsø Study

| Social Demographic Variables | No (n=1559) | % | Sex, %, females | 2610 | Age, y | 56.7 (10.5) | Sex, %, females | 56.2 | BMI, kg/m² | 25.9 (3.7) | Serum lipids, mmol/L | 6.54 (1.22) | HDL cholesterol | 1.55 (0.42) | Triglycerides | 1.54 (0.90) | Smoking | Current smoker, % | 27.4 | No. of cigarettes/day | 12.0 (6.1) | Past smoker, % | 33.1 | Blood pressure, mm Hg | 134.9 (18.7) | Diastolic BP | 78.3 (10.9) | Inflammatory markers | Fibrinogen, mmol/L | 3.23 (0.80) | Monocyte count, 10^9/L | 0.57 (0.17) | White cell count, 10^9/L | 6.83 (1.86) | Use of drugs, % | Antihypertensive medication | 8.4 | Cholesterol-lowering medication | 1.3 | Self reported disease, % | History of CHD | 5.6 | History of stroke | 1.4 | History of diabetes | 1.8 | Intima-media thickness, mm | 0.772 (0.129) |
|-----------------------------|-------------|---|-----------------|------|--------|-------------|-----------------|-------|-------------|-------------|---------------------|-------------|-------------------|-------------|-------------|-------------|---------|---------------------|--------|---------------------|-------------|-------------------|-------------|---------------------|-------------|-------------------|-------------|

Values are unadjusted means (SD) or percentages.
BMI indicates body mass index; BP, blood pressure; CHD, coronary heart disease; HDL, high-density lipoprotein.

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### TABLE 2. Incidence of Novel Plaques: the Tromsø Study

<table>
<thead>
<tr>
<th>No Plaque</th>
<th>631 (54.0)</th>
<th>928 (64.2)</th>
<th>&lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 new plaque</td>
<td>323 (28.8)</td>
<td>344 (23.0)</td>
<td>0.0008</td>
</tr>
<tr>
<td>2+ new plaques</td>
<td>190 (17.2)</td>
<td>194 (12.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Sum</td>
<td>1144 (100.0)</td>
<td>1466 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

Percentages are age-adjusted.

### TABLE 3. Risk Factor Level at Baseline According to Number of Plaques at Follow-up: the Tromsø Study

| No Plaque | 56.7 (10.5) | Sex, %, females | 56.2 | BMI, kg/m² | 25.9 (3.7) | Serum lipids, mmol/L | 6.54 (1.22) | HDL cholesterol | 1.55 (0.42) | Triglycerides | 1.54 (0.90) | Smoking | Current smoker, % | 27.4 | No. of cigarettes/day | 12.0 (6.1) | Past smoker, % | 33.1 | Blood pressure, mm Hg | 134.9 (18.7) | Diastolic BP | 78.3 (10.9) | Inflammatory markers | Fibrinogen, mmol/L | 3.23 (0.80) | Monocyte count, 10^9/L | 0.57 (0.17) | White cell count, 10^9/L | 6.83 (1.86) | Use of drugs, % | Antihypertensive medication | 8.4 | Cholesterol-lowering medication | 1.3 | Self reported disease, % | History of CHD | 5.6 | History of stroke | 1.4 | History of diabetes | 1.8 | Intima-media thickness, mm | 0.772 (0.129) |
|-----------|------------|-----------------|--------|-------------|-------------|---------------------|-------------|-------------------|-------------|-------------------|-------------|---------------------|-------------|-------------------|-------------|---------------------|-------------|-------------------|-------------|---------------------|-------------|-------------------|-------------|---------------------|-------------|-------------------|-------------|

Values are unadjusted means (SD) or percentages.
BMI indicates body mass index; BP, blood pressure; CHD, coronary heart disease; HDL, high-density lipoprotein.

### TABLE 4. Predictors of Novel Plaque Formation: the Tromsø Study

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>14.1</th>
<th>1.18 (1.08–1.29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.2</td>
<td>1.61 (1.42–1.81)</td>
</tr>
<tr>
<td>Male</td>
<td>9.2</td>
<td>1.33 (1.11–1.59)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>25.2</td>
<td>1.26 (1.15–1.38)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>28.4</td>
<td>1.73 (1.41–2.11)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>23.2</td>
<td>1.25 (1.14–1.37)</td>
</tr>
<tr>
<td>Intima-media thickness, mm</td>
<td>46.8</td>
<td>1.43 (1.29–1.58)</td>
</tr>
</tbody>
</table>

R^2 | 0.19 |

The multivariate adjusted odds ratio predicts the probability of being in a higher category (more plaques) for 1 SD increase in the independent continuous variable or for being a male or current smoker.
TABLE 5. OR for Having Plaque in Quartiles of Monocyte Count: the Tromsø Study

<table>
<thead>
<tr>
<th>Monocyte Count x 10^3/L</th>
<th>% With Plaque*</th>
<th>OR†</th>
<th>OR‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First quartile &lt;0.41</td>
<td>34.9</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Second quartile 0.41–0.59</td>
<td>38.1</td>
<td>1.16</td>
<td>1.17 (0.91–1.51)</td>
</tr>
<tr>
<td>Third quartile 0.60–0.69</td>
<td>37.8</td>
<td>1.15</td>
<td>1.12 (0.85–1.46)</td>
</tr>
<tr>
<td>Fourth quartile ≥0.70</td>
<td>49.1</td>
<td>1.96</td>
<td>1.85 (1.41–2.43)</td>
</tr>
<tr>
<td>P for trend</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Percentages are age- and sex-adjusted.
†OR adjusted for age and sex.
‡OR adjusted for age, sex, total cholesterol, current smoking, systolic blood pressure, and IMT.

Discussion
The main and novel finding in this study was that monocyte count is an independent predictor of future plaque formation. In addition, we found that IMT, age, sex, total cholesterol, current smoking, and systolic blood pressure were independent predictors of later plaque development. As far as we know, this is the first study aimed at defining risk factors for plaque formation in previously plaque-free arteries.

The fact that the monocyte count was higher in persons who 7 years later had plaque(s) develop supports the concept that inflammation plays an etiological role in early atherogenesis and does not only appear as an epiphenomenon. Inflammatory mechanisms may be important through all stages of atherogenesis, from its initiation of fatty streaks to its progression to plaque rupture. Modified low-density lipoprotein cholesterol acts chemotactically for monocytes and may upregulate the expression of genes for macrophage colony-stimulating factor. This stimulates recruitment of monocytes from the peripheral blood to the intima of the artery wall and replication of monocyte-derived macrophages.

The OR for monocyte count did not change when persons with CHD, stroke, and/or diabetes were excluded from the model, which is a further indication of a primary effect of monocytes in atherogenesis rather than being a response to clinical disease. Our data correspond well with results from previous population-based studies reporting elevated levels of circulating adhesion molecules, neopterin, and tumor necrosis factor receptor in subjects with carotid atherosclerosis. In a recent community-based cross-sectional study from Australia of 1111 men and women aged 27 to 77 years, the monocyte count was an independent risk marker of carotid plaque. Our study population is larger, covers the same age span, has a balanced sex distribution and similar baseline prevalence of cardiovascular diseases, and the study design is prospective.

Although there was a highly significant linear trend for plaque presence across strata of monocyte count, the plaque prevalence was increased mainly in the upper quartile. This may indicate a threshold effect of monocytes more than a true linear relationship.

Diffuse thickening of the intima-media layer is generally considered to be an early marker of generalized atherosclerosis because such thickening has been associated with an unfavorable risk profile, the presence of atherosclerosis elsewhere in the arterial system, and an increased risk of CHD and stroke. The mechanisms that determine diffuse thickening of the intima-media layer versus local plaque formation are not fully understood. This study showed no significant association between monocyte count and either baseline IMT or follow-up IMT in persons without plaque. In the Australian study, no association between monocyte count and IMT was found for the whole population, but an association was found in persons older than 53 years. In our study, there was no age–monocyte interaction and the monocyte effect seems to be entirely linked to later plaque formation. Therefore, it may be hypothesized that differences in monocyte activity is a determining factor for plaque formation but not for diffuse thickening of the intima-media layer.

In this study, fibrinogen was not an independent predictor of early plaque formation. Previous studies have found cross-sectional associations between carotid atherosclerosis and plasma fibrinogen. In these studies, the populations were older or had prevalent cardiovascular disease, indicating a more advanced atherosclerosis compared with our study. Fibrinogen has effects on vasoconstriction and platelet aggregation and reflect ongoing tissue damage associated with atherothrombosis. Thrombosis superimposed on a preexisting atherosclerotic plaque seems to be a major factor in plaque growth, suggesting that fibrinogen is more important in plaque growth than in plaque formation. Data relating WCC to carotid atherosclerosis are weak. We found no independent association between WCC and carotid plaque. Monocytes and lymphocytes, but not neutrophils, are the main cellular components of the circulation in generating atherosclerotic lesions. Monocyte count may therefore be a better and more specific marker than WCC of the inflammatory activity in atherosclerosis.

The large number, the high participation rate, and the prospective design of the study strengthen our findings. Monocyte count was measured only once and therefore intrapersonal variation cannot be assessed. Those lost to follow-up were older, smoked more, and had higher systolic blood pressure, fibrinogen, monocyte count, and more CHD, stroke, and diabetes compared with the study population. This healthy participant bias may weaken the true relationship between risk factors at baseline and plaque at follow-up. Another potential limitation to this study is that only one carotid and femoral artery was studied. Inclusion of the left carotid and femoral arteries might have given a better description of the individual plaque burden.
In conclusion, this 7-year follow-up study shows that monocyte count is an independent predictor of novel plaque formation in persons with no plaque at baseline. The monocyte effect does not seem to be a response to clinical cardiovascular disease. The effect of monocytes seems to be linked to plaque formation and not to diffuse thickening of the intima-media layer. In a healthy population, the monocyte count may be used to predict the risk for future plaque formation.

Acknowledgments
This study was supported by grants from the Norwegian Research Council, and was conducted in collaboration with the Norwegian Institute of Public Health, Oslo, Norway.

References
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Stroke. 2005;36:715-719; originally published online March 3, 2005;
doi: 10.1161/01.STR.0000158909.07634.83

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