The Natural Course of Atherosclerosis
Part I: Incidence and Progression

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Abstract—The natural course of early atherogenesis is not well established. The current prospective survey was designed to monitor 5-year changes in carotid atherosclerosis in a large, stratified random sample of the general population using high-resolution duplex ultrasound (Bruneck Study). Incidence rates of carotid atherosclerosis ranged from near zero to 184 per 1000 person-years. Most atherosclerotic lesions developed at sites with enhanced wall thickness. Incidence of atherosclerosis in premenopausal women was less than half of that observed in men of equal age. The sex difference disappeared within 5 years after menopause and may possibly be attributed to sex variations in body iron stores. Preexisting atherosclerotic lesions may experience 1 of 2 different types of disease progression. 1) The first main type of plaque growth causing nonstenotic or diffuse dilative atherosclerosis was characterized by slow and continuous plaque extension, which usually affected several lesions simultaneously and did not primarily focus on the carotid bifurcation. This step-by-step process relied on a cumulative exposure to well-known risk factors such as hyperlipidemia. Compensatory enlargement of the artery at the site of active atherosclerosis effectively preserved a (near) normal lumen. 2) The second main type of plaque growth was characterized by occasional prominent increases in lesion size. This process primarily occurred in the internal carotid artery and was mediated by procoagulant risk factors in a way that peak levels were relevant rather than cumulative exposure. As the main underlying pathomechanism, atherothrombosis may be hypothesized. Marked increases in plaque size and insufficient vascular remodeling acted synergistically in producing a significant compromise of the lumen. The current study provides novel insights into the natural course of early carotid atherosclerosis, thereby focusing on disease incidence and various types of spontaneous disease progression. Nonstenotic or diffuse dilating atherosclerosis and focal stenotic disease were found to constitute epidemiologically and etiologically distinct disease entities that develop and proceed independently of each other.


Key Words: carotid arteries • atherosclerosis • atherothrombosis • population study

Cardiovascular disease is the leading source of morbidity, disability, and mortality in Western industrialised countries, and atherosclerosis is unquestionably the main underlying pathology. Current knowledge on incidence and course of atherosclerosis mainly originates from patient-based angiographic and Doppler follow-up evaluations, which for methodological reasons are restricted to severe atherosclerosis (stenosis).1-4 In the late 1980s, advances in technique and resolution of duplex ultrasound scanning afforded the opportunity to noninvasively quantify and monitor atherosclerosis from its precursor lesions to occlusive disease. The carotid arteries are an appropriate target for such evaluations on account of accessibility, size, and limited wall movement. High coincidence of carotid atherosclerosis with vessel pathology in other vascular territories makes it an adequate window for systemic atherosclerosis.5-9 The Bruneck Study ranks among the few large ultrasound-based cohort studies10-16 aimed at assessing incidence and natural course of early atherogenesis in the general population.

Methods

Population Recruitment
The Bruneck Study is a prospective population-based survey on the epidemiology and etiology of carotid atherosclerosis.16 The baseline examination was performed between July 1990 and November 1990 in the semi-urban mountainous area of Bruneck (Bolzano Province, Italy). Financial and personnel resources allowed us to recruit a random sample of up to 1000 subjects. To best utilize population size in terms of accurate sex- and age-specific rates of atherosclerosis incidence and progression, equal contingents of men and women (n=125) in each the 5th to 8th decade were selected for inclusion.16 In the choice of the follow-up interval, we were mainly guided by the objective of ascertaining a sufficient number of incident carotid stenoses as the ultrasound endpoint with the lowest rate of occurrence. Extrapolation of cross-sectional data suggested that a follow-up period of 5 years suffices for this requirement. Accordingly, the first reevaluation was scheduled for July 1995 to October 1995. Participation and follow-up rates were high at 93.6% (91.9% with complete data assessment) and 96.5%, respectively. Study design, protocol, and characteristics of the survey area have been detailed previously.

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1484
TABLE 1. Incidence and Progression of Carotid Atherosclerosis According to Age, Sex, and Menopausal Status (n=826)

<table>
<thead>
<tr>
<th>Age Range (y)</th>
<th>Incidence Rate</th>
<th>Years of Follow-Up</th>
<th>Progression Rate</th>
<th>Years of Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>40–44</td>
<td>32.8</td>
<td>158.5</td>
<td>5.3</td>
<td>150.0</td>
</tr>
<tr>
<td>45–49</td>
<td>51.1</td>
<td>270.0</td>
<td>13.4</td>
<td>239.0</td>
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<tr>
<td>50–54</td>
<td>45.7</td>
<td>245.0</td>
<td>15.7</td>
<td>102.1</td>
</tr>
<tr>
<td>55–59</td>
<td>47.1</td>
<td>148.5</td>
<td>13.3</td>
<td>15.0</td>
</tr>
<tr>
<td>60–64</td>
<td>93.2</td>
<td>124.5</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>65–69</td>
<td>118.6</td>
<td>72.5</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>70–74</td>
<td>183.8</td>
<td>55.5</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>75–79</td>
<td>170.5</td>
<td>30.5</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>80–84</td>
<td>160.0</td>
<td>5.0</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

Incidence and progression rates were calculated per 1000 person-years. Incidence rates were assessed in subjects free of carotid atherosclerosis at the 1990 baseline (n=500) and progression rates in those with preexisting atherosclerosis (n=326).

Scanning Protocol

Sonographic assessment of the extracranial carotid arteries was performed using a duplex ultrasound system (ATL8, Advanced Technology Laboratories) with 10-MHz scanning frequency in B-mode and 5-MHz scanning frequency in pulsed–Doppler mode. All subjects were examined in a supine position. The scanning protocol included imaging of the right and left common carotid arteries (CCA) and internal carotid arteries at the following locations: proximal CCA (15 to 30 mm proximal to the carotid bulb), distal CCA (<15 mm proximal to the carotid bulb), and proximal internal carotid artery (ICA) (carotid bulb, identified by loss of the parallel wall present in the CCA and the initial 10 mm of the vessel above the flow divider between external and internal carotid arteries). For each segment, the sonographer imaged the vessel in multiple longitudinal and transversal planes to identify the largest axial diameter of focal plaques and to adequately visualize the interface required to measure intima-media thickness (IMT). Pulsed Doppler was used to provide information on blood flow velocity and to identify the various arteries.

Atherosclerotic lesions were defined by 2 ultrasound criteria: (1) wall surface (protrusion into the lumen or roughness of the arterial boundary) and (2) wall texture (echogenicity). We did not use an IMT cut-off to discriminate plaques from wall thickening. The maximum axial diameter of each plaque was measured as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface. For the assessment of stenosis, Doppler criteria or, when no hemodynamic disturbances were detectable, the percentage of maximum diameter reduction in the B-mode images was applied.17 Peak systolic velocities exceeding 180 cm/s and 250 cm/s were considered indicative of stenosis >60% and 80%, respectively. Scanning was performed twice, namely in 1990 and 1995, by the same experienced sonographer, who was unaware of the subjects’ clinical and laboratory characteristics. For documentation purposes, short segments of real time ultrasonography and frozen longitudinal and transversal images were recorded for each vessel segment.

Reproducibility, Validity, and Data Quality

To assess the reproducibility of the ultrasound technique applied in the current evaluation, rescanning was performed in a representative subsample by the same sonographer (n=100). To avoid memory effects, we left a waiting period of 6 to 8 weeks between both assessments. In all, 800 vessel segments and 180 plaques served as the basis for computation of intra-observer variability. Main focus was on the quantification of plaque diameters. Relative measurement errors that describe the intra-observer error as a percentage of the pooled mean were low at 10% (CCA) and 15% (ICA), which enabled us to monitor changes in atherosclerosis in individuals over time. In an attempt to subdivide the overall error into various components we considered a variety of potential sources of bias: poor scanning quality, true changes in vascular status in the intervening period, administration error, attribution of lesions to neighboring segments, overestimation of “hidden” plaques, and the measurement error in its primary sense. Parallel rereading of the scans was performed to define the source of error responsible for deviations between both assessments. Poor scanning quality due to anatomical variations (high bifurcation, n=1), real progression of atherosclerosis (1 of 180 plaques), and oversizing of adequately-sized lesions in 1 of the scannings (1 of 180 plaques) was rare and contributed little to overall error. At <0.1%, administration and input error were kept low by multiple checks of the database. Variable attribution of plaques to neighboring segments requires attention. Pitfalls mainly occurred in...
The correct attribution of plaques to either the ICA or external carotid artery (4 of 118). In the CCA, lesions that approached or just crossed the limit between its distal and proximal section were occasionally counted twice (2 of 62). When this and all aforementioned sources of variability were eliminated, we arrived at the actual measurement error caused by physical limitations of the measuring device, lack of clear-cut interfaces, and the choice of distinct axes through lesions of complex shape. Rates were 8.5% (CCA) and 12.4% (ICA). To ensure that increases in plaque size during follow-up did not simply reflect measurement variability, only cases contributed, on average, 2.5 years of follow-up to the denominator of the incidence formula (for details see Reference 18). Cumulative A rates were converted into cumulative risks by means of the formula $P = 1 - \exp (- \Lambda)$, 18 Standardization of rates was performed according to the guidelines given by Breslow and Day. 18

### Results

Tables 1 and 2 depict age-, sex-, and site-specific incidence rates of atherosclerosis. Premenopausal women showed excellent protection against atherosclerosis, which was gradually lost within a 5-year period after menopause along with prominent iron accumulation (Figure 1a). Thereafter, incidence rates were virtually identical in men and women of equal age (Tables 1 and 2), which results in a convergence of disease manifestation between sexes with advancing age (Tables 1 and 2). Manifestation of >1 atherosclerotic lesion during the 5-year follow-up period was as frequent as the occurrence of a single plaque (percentage of subjects with 1, 2, 3, and ≥4 incident plaques: 57%, 27%, 12%, and 4%). Figure 2 describes disease activity as a function of preexisting atherosclerosis.

For comparison purposes, rates were adjusted to the age/sex structure of the standard European population, 18 thereby obtaining 1 universal estimate of atherosclerosis incidence in the middle-aged and elderly (aged 40 to 84 years), which amounted to 69.9 per 1000 person-years.
Next, we focused on spontaneous progression of atherosclerotic lesions. Plots of plaque growth against the lesion size ultimately achieved identified 2 distinct types of growth kinetics. Atherosclerosis that did not cause lumen obstruction.40% usually grew slowly and evoked (over)compensatory local dilation of vessel segments (Figure 3). In contrast, stenosis.40% usually originated from occasional marked increases in plaque size and insufficient or even lacking vascular remodelling (Figure 3). Both types of lesion progression started from plaques of similar diameters (Figure 3; 1.3 versus 1.6 mm), with the distribution of changes in plaque size showing only minor overlap (mean [95%CI]: 1.1 mm [0.1 to 2.2 mm] versus 2.4 mm [1.3 to 3.6 mm]; P=0.0001). Progression rates of nonstenotic atherosclerosis showed age- and sex-trends similar to those observed for incident atherosclerosis (Tables 1 and 2) and an amplification of disease activity with an increasing number of preexisting plaques (Figure 2). In contrast, incidence rates of stenosis in middle-aged and elderly subjects emerged as independent of sex and age (Table 2). In most instances, nonstenotic atherosclerosis manifested in several plaques simultaneously, whereas incident stenosis usually occurred only once in the 5-year follow-up period (Figure 4). Further differences in the epidemiology of both processes are opposed in Table 3.

The risk profile of nonstenotic atherosclerosis consisted of traditional risk factors (high LDL and low HDL cholesterol, hypertension, and smoking) supplemented by less well-established risk conditions such as prominent body iron stores,20,21 severe alcohol consumption, or chronic infections (unpublished data from the Bruneck Study). Stenotic atherosclerosis emerged as a domain of a procoagulant state involving high fibrinogen and Lp(a), low antithrombin III and APC ratio (factor V mutation), and clinical conditions known to shift hemostasis toward coagulation. As to cigarette smoking, the risk of nonstenotic atherosclerosis was best described by measures of cumulative exposure (pack-years) (OR [95%CI], 1.26 [1.06 to 1.50] for a 1-standard deviation unit change) and did not normalize within a 5- to 10-year period after cessation (1.24 [1.03 to 1.49]). In contrast, peak levels of exposure (number of cigarettes currently smoked) were superior to cumulative measures in predicting the risk of stenotic atherosclerosis (2.57 [1.74 to 3.80]). After cessation of smoking, the risk of focal stenotic disease normalized (OR for ex-smokers 1 to 5 years after cessation, 1.29 [0.33 to 5.04]).

**Discussion**

**Initiation of Atherosclerosis**

Precursor lesions of atherosclerosis (intima-media thickening) may occur as early as adolescence, but the frequency of definite atherosclerotic lesions remains low until age 40 in men and onset of menopause in women (prevalence <1.0% each). Protection against atherosclerosis in women was gradually lost within a 5-year period after menopause (Figure 1a). Afterward, incidence rates were virtually identical to those observed in men of equal age. In other words, atherogenesis in females usually started at higher ages than in men but proceeded at a similar pace once a postmenopausal period of 5 years had elapsed. Plots of cumulative risk rates in men and women best visualized the convergence in disease manifestation with advancing age (Figure 1b).
As detailed previously, differences in prevalence and incidence of carotid atherosclerosis evident between premenopausal women and men disappeared when sex variations in body iron stores were taken into account. Iron accumulation in (post)menopause corresponded excellently with the breakdown of female protection against atherogenesis (Figure 1b). Growing epidemiological and experimental evidence suggests a crucial role of prominent iron stores in lipid-induced atherogenesis even though a general consensus in this field has not yet been reached.

As expected, the proximal ICA was the site of predilection for manifestation of atherosclerosis. In 1 out of 2 subjects with incident atherosclerosis, plaques occurred simultaneously in 2 or more carotid segments. The risk of incident atherosclerosis amplified with size and number of preexisting lesions (Figure 2), which may well reflect a perpetuation of adverse risk profiles but could, speculatively, also point to some kind of auto-catalytic propagation of atherosclerosis. A previous investigation in this cohort yielded evidence of an (auto)-immune component in human atherogenesis and identified heat-shock protein 65 as a potential target antigen.

Occasionally, plaques evolved as focal lesions within a normal vasculature. In most instances, however, atherosclerosis developed at sites with an IMT beyond the 50th percentile (ICA, 88.8%; CCA, 97.8%). Thus, our survey supported the view that wall thickening commonly precedes definite atherosclerosis, possibly in the sense of a precursor lesion of one and the same disease process.

Progression of Atherosclerosis

From a pathoanatomical perspective, 2 distinct types of atherosclerosis progression may be distinguished: 1) In small and medium-sized lesions slow and continuous plaque growth predominates, which is mediated by a variety of complex biological step-by-step phenomena such as lipid-induced atherogenesis or smooth muscle cell proliferation. 2) This type of plaque growth may be occasionally accelerated by plaque fissuring, thrombosis and fibrous organization of mural thrombi. The latter mechanism, further on referred to as plaque thrombosis, gains increasing weight with advancing lesion size and may even be the key event in the development of stenotic lesions. A comprehensive discussion of the dualism of conventional atherosclerosis and atherothrombosis in human vessel pathology is given by Fuster et al and Badimon et al. The current prospective survey provides strong in vivo support for this concept and suggests that a shift in the relevance of both pathomechanisms occurs when a plaque causes >40% diameter reduction (Figure 3 and Table 3).

Nonstenotic atherosclerosis expanded slowly (Figure 3). Such processes usually paralleled in several atherosclerotic lesions independently of plaque location (Figure 4), ie, they were a continuous and ubiquitous process. In analogy to the initiation of atherosclerosis, the risk of disease progression amplified with advancing age and number of atherosclerotic lesions (Figure 2). Once more than 3 plaques preexisted in a single subject, further disease extension was an almost obligatory phenomenon (78%), which reinforces the possibility of an auto-catalytic component in this type of atherogenesis. Compensatory enlargement of the vessel at the site of active atherosclerosis effectively preserved a normal lumen or was even over-compensatory in the early course of disease (see Part II: Vascular Remodeling). Diffuse dilative athero-
sclerosis may be assumed as a final stage of this type of disease progression.

In contrast, stenosis >40% usually developed focally at sites of high hemodynamic stress (ICA) based on occasional marked increases in plaque size (mean, 2.4 mm). Subjects with preexisting stenotic disease were at a clearly elevated risk of developing a further stenosis in a different segment of the carotid arteries (23 of 55 [41.8%]). On the other hand, such events appeared to be so rare that manifestation of more than 1 stenosis during the 5-year follow-up period was definitely the exception and not the rule (Figure 4). Compensatory enlargement of the vessel as typical for nonstenotic atherosclerosis did not occur at all or only insufficiently, which acted synergistically with the rapid growth pattern in producing significant lumen compromise (Figure 3). Actually, 95% of stenosis producing significant lumen compromise (Figure 3). Actu-

TABLE 3. Distinct Epidemiology and Etiology of Nonstenotic and Stenotic Carotid Atherosclerosis in the Bruneck Study Cohort (1990 to 1995) (n=826)

<table>
<thead>
<tr>
<th>Site of predilection</th>
<th>Nonstenotic Atherosclerosis “Diffuse Dilative Atherosclerosis”</th>
<th>Stenotic Atherosclerosis “Focal Stenotic Disease”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque growth</td>
<td>Slow, continuous and diffuse growth</td>
<td>Focal rapid plaque extension</td>
</tr>
<tr>
<td>Vascular remodeling</td>
<td>Prominent or even over-compensatory</td>
<td>Insufficient or even lacking</td>
</tr>
<tr>
<td>Risk profile</td>
<td>Hyperlipidemia (cofactor body iron stores), hypertension, smoking</td>
<td>Procoagulant state</td>
</tr>
<tr>
<td>Association with risk factors</td>
<td>Cumulative</td>
<td>Peak levels</td>
</tr>
<tr>
<td>Suspected pathological sequela</td>
<td>Conventional atherogenesis (step-by-step mechanisms)</td>
<td>Plaque thrombosis</td>
</tr>
</tbody>
</table>

Both types of atherosclerosis develop and proceed independently of each other. Of subjects with >1 atherosclerotic lesion at the 1990 baseline (n=219) 131 showed nonstenotic plaque growth and 88 did not. In both groups, the percentage of subjects with incident stenosis >40% was strikingly similar at 38.6% and 37.4% (χ²=0.03, P=0.85; coefficient Φ=0.01, P=0.91).

In our survey, long-lasting regression was quite rare (5.2% of all lesions, n=45) and confined to lesions with signs of plaque hemorrhage (19 of 45 [42%]), healing of ulcerated atherosclerosis (2 of 45 [4%]), and disappearance of small plaques (18 of 45 [40%]).

Regression

A variety of angiographic and ultrasound follow-up evaluations outlined the possibility of spontaneous or drug-induced regression of increased IMT and atherosclerotic lesions.28–31 In our survey, long-lasting regression was quite rare (5.2% of all lesions, n=45) and confined to lesions with signs of plaque hemorrhage (19 of 45 [42%]), healing of ulcerated atherosclerosis (2 of 45 [4%]), and disappearance of small plaques (18 of 45 [40%]).

Limitations and Merits of the Study Design

1) Accuracy of descriptive epidemiologic data mainly depends on the choice of an appropriate study population and the sample size. The current cohort was a random sample of the general population with a near complete follow-up (96.5%) and an observation period of more than 33 000 segment-years (4000 person-years). 2) The progression model developed and applied in the current study permitted us to monitor and characterize changes in vascular status in individuals (person-based approach), which is a clear advantage over the more commonly used approach of assessing changes in IMT over time. 3) Ultrasound evaluations were performed twice, namely in 1990 and 1995. Systematic interim scanning was not available. Thus, we cannot construct precise growth curves of atherosclerotic lesions. In particular, it remains to be elucidated whether slow plaque growth stands for a continuous or stepwise process or for successive plaque propagation and repair (regression) with progression overweight.
Conclusions
The current study may well be the first to provide population-based incidence rates of carotid atherosclerosis and detailed insights into the course of early disease progression. These findings contribute to a better understanding of the nature of atherogenesis and possibly assist in clinical decision making. Atherogenesis was found to be a heterogeneous disease that subserves epidemiologically and etiologically distinct disease entities. Fighting the same risk factors in all individuals, as is common clinical practice, ignores the actual complexity of the disease.

Appendix
The Bruneck Study Group
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References
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