

# Chronic Infections and the Risk of Carotid Atherosclerosis

## Prospective Results From a Large Population Study

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**Background**—Chronic infections have been implicated in the pathogenesis of atherosclerosis, yet from an epidemiological perspective, this concept remains controversial.

**Methods and Results**—The Bruneck Study is a prospective population-based survey on the pathogenesis of atherosclerosis. In 826 men and women 40 to 79 years old (1990 baseline), 5-year changes in carotid atherosclerosis were thoroughly assessed by high-resolution duplex scanning. The presence of chronic respiratory, urinary tract, dental, and other infections was ascertained by standard diagnostic criteria. Chronic infections amplified the risk of atherosclerosis development in the carotid arteries. The association was most pronounced in subjects free of carotid atherosclerosis at baseline (age-/sex-adjusted odds ratio [95% CI] for any chronic infection versus none, 4.08 [2.42 to 6.85];  $P < 0.0001$ ) and applied to all types of chronic (bacterial) infections. It remained independently significant after adjustment for classic vascular risk attributes and extended to low-risk individuals free of conventional risk factors. Among subjects with chronic infections, atherosclerosis risk was highest in those with a prominent inflammatory response. Markers of systemic inflammation, such as soluble adhesion molecules and circulating bacterial endotoxin, and levels of soluble human heat-shock protein 60 and antibodies to mycobacterial heat-shock protein 65 were elevated in subjects with chronic infections and predictive of an increased risk of atherosclerosis.

**Conclusions**—The present study provides solid evidence for a role of common chronic infections in human atherogenesis. Induction of systemic inflammation and autoimmunity may be potential pathophysiological links. (*Circulation*. 2001; 103:1064-1070.)

**Key Words:** atherosclerosis ■ inflammation ■ infection ■ carotid arteries ■ cardiovascular disease

Among the recent advances in understanding atherogenesis, the “infection hypothesis” is one of the most compelling, given the high prevalence of this putative risk factor in the general community and the potential availability of preventive interventions. Actually, this hypothesis is astonishingly old, with roots going back to the 19th century,<sup>1–3</sup> although the bulk of supportive evidence has accumulated in the past few decades.<sup>4–15</sup> Reasons for the remaining uncertainty and skepticism lie in the difficulties surrounding the ascertainment of atherogenesis and chronic infections in large clinically healthy populations. Previous prospective studies have focused on clinical end points rather than on the extent of and changes in underlying vessel pathological lesions, and most applied serological testing to define infection status. Accordingly, epidemiological evidence of an infectious risk factor in atherogenesis at this time is mainly indirect and restricted to certain pathogens.

The Bruneck Study<sup>16–18</sup> is appropriate for directly addressing the key hypothesis, given the thorough clinical characterization and diagnostic workup of study subjects, its focus on atherosclerosis, and the availability of extensive laboratory evaluations involving measurements of antibody titers of common bacterial and viral pathogens.<sup>13</sup>

## Methods

### Study Subjects

The Bruneck Study is a prospective population-based survey of the epidemiology and pathogenesis of atherosclerosis.<sup>16–18</sup> At the 1990 baseline evaluation (July to November), the study population was recruited as a sex- and age-stratified random sample of all inhabitants of Bruneck (Bolzano Province, Italy) 40 to 79 years old (125 women and 125 men in the fifth to eighth decades each). A total of 93.6% participated, with data assessment completed in 919 subjects. During 1990 and the reevaluation in 1995 (July to October), a subgroup of

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63 individuals died or moved away. In the remaining population, follow-up was 96.5% complete (n=826).<sup>16-18</sup>

### Clinical History and Examination

The average number of cigarettes smoked per day and the pack-years were noted for each smoker and ex-smoker. Regular alcohol consumption was quantified in terms of grams per day. Hypertension was defined as blood pressure (mean of 3 measurements)  $\geq 160/95$  mm Hg or the use of antihypertensive drugs. Diabetes mellitus was coded as present for subjects with fasting glucose levels  $\geq 140$  mg/dL and/or a 2-hour value (oral glucose tolerance test)  $\geq 200$  mg/dL. High, moderate, and low socioeconomic status were assumed if the subject had  $\geq 12$ , 11 to 9, or  $\leq 8$  years of education and/or the average monthly income of the person with the highest income in the household was  $\geq \$2000$ ,  $\$2000$  to  $\$1000$ , or  $\leq \$1000$ , respectively.

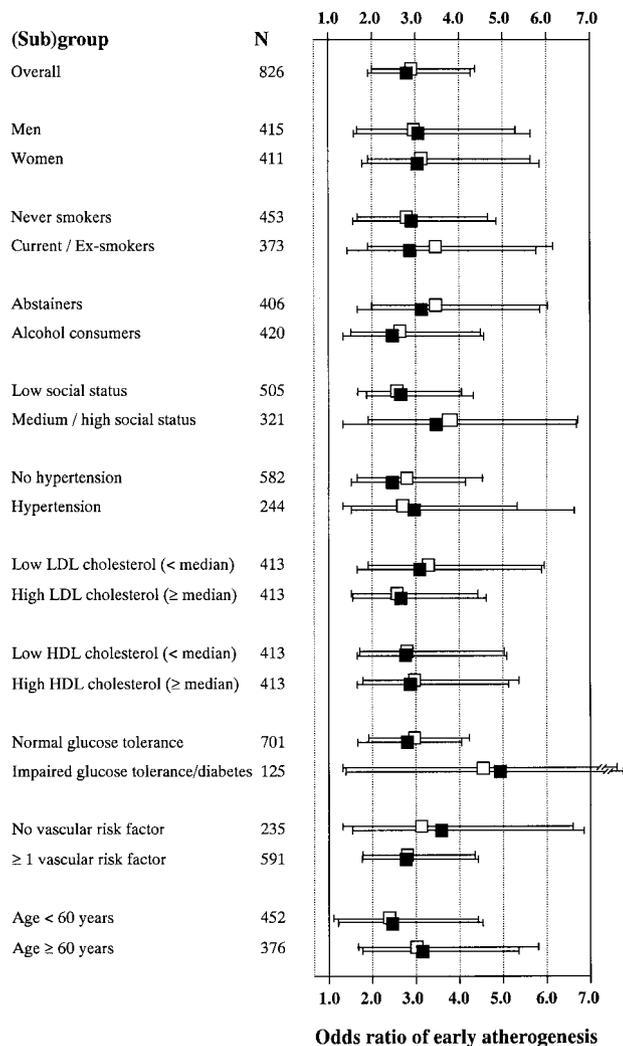
In an effort to identify subjects with chronic infections or conditions known to be associated with recurrent episodes of infectious exacerbation, such as chronic obstructive pulmonary disease, we began an extensive screening consisting of 2 consecutive phases.<sup>19,20</sup> The first step involved a detailed self-reported medical and medication history, thorough clinical examination, spirometry, extensive laboratory evaluations including urinary analysis, and a review of the Bruneck Hospital databases and other medical records. If the data were inconclusive, in a second step individuals were referred for further optional examinations. The diagnosis of common chronic infections was established according to standard diagnostic criteria by an expert committee including specialists from various medical fields. The diagnosis of chronic obstructive pulmonary disease required documentation of airway obstruction by spirometry (FEV<sub>1</sub>/FVC ratio  $< 0.70$ ) and the presence of typical symptoms, such as dyspnea, cough, expectoration, or wheeze. Bronchitis was defined as chronic when cough with expectoration lasted  $\geq 3$  months in  $> 2$  consecutive years. Urinary tract infections were regarded as recurrent in the case of  $\geq 3$  documented episodes. Periodontitis was defined by self-report. Chronic infections were considered in the present analysis when manifest at study entry (baseline 1990).

### Laboratory Methods

Blood samples were drawn after an overnight fast and 12 hours' abstinence from smoking. In subjects with acute infection, blood drawing was delayed for  $\geq 6$  weeks, ie, until  $\geq 4$  to 5 weeks after recovery from infectious illness. Markers of infection/inflammation were assayed as follows: C-reactive protein,  $\alpha_1$ -antitrypsin, and ceruloplasmin (nephelometry, Behring); soluble vascular-cell and intracellular adhesion molecules 1 and E-selectin (ELISA, R&D Systems and Bender); and endotoxin (limulus amoebocyte lysate test, Chromogenix<sup>19</sup>). ELISAs were used to determine antibody titers to mycobacterial heat-shock protein (HSP) 65,<sup>21</sup> anti-*C pneumoniae* IgA (seroCP-IgA, Savyon Diagnostics Ltd), anti-*H pylori* IgG (Medac), and anti-cytomegalovirus IgG antibodies (Medac<sup>13</sup>) and levels of soluble human HSP60. For the latter, monoclonal antibodies directed against the II-13 epitope (coated at microtiter plates) and the ML-30 epitope (labeled with biotin) were used. Standard curves were obtained by use of recombinant human HSP60 (StressGen Biotechnologies).<sup>22</sup> Other parameters were assayed by standard methods.<sup>18,20</sup>

### Scanning Protocol and Definition of Ultrasound End Points

The ultrasound protocol involves the scanning of the internal (bulbous and distal segments) and common carotid arteries (proximal and distal segments) of either side with a 10-MHz imaging probe and a 5-MHz Doppler probe.<sup>16,17</sup> Atherosclerotic lesions were defined according to 2 ultrasound criteria: (1) wall surface (protrusion or roughness of the arterial boundary) and (2) wall texture (echogenicity). The maximum axial diameter of plaques was assessed in each of the 8 vessel segments, and an atherosclerosis score was calculated by addition of all diameters. The intima-media thickness was measured at the far wall of the common carotid arteries (intraobserver coefficient of variation, 7.9% [n=100]).<sup>16,17</sup> Scanning was per-

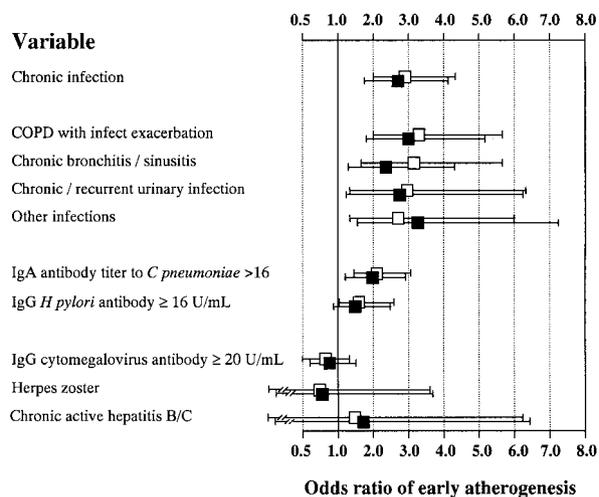


**Figure 1.** ORs (95% CIs) of new carotid plaques calculated for chronic infection status according to subject subgroups. ORs were adjusted for age, sex, and baseline atherosclerosis (□) + LDL and HDL cholesterol, lipoprotein (a), hypertension, smoking (pack-years), alcohol consumption, ferritin, hypothyroidism, and microalbuminuria (■).

formed twice in 1990 and 1995 by the same experienced sonographer, who was unaware of the subjects' clinical and laboratory characteristics. The development of new carotid plaques (early atherogenesis) and occurrence/progression of vessel stenosis  $> 40\%$  (advanced atherogenesis) was assessed in all subjects.<sup>16-18</sup> Reproducibility of the ultrasound categories was "nearly perfect" ( $\kappa$  coefficients  $> 0.8$  as derived from 2 independent measurements performed by the same sonographer in a reproducibility sample, n=100<sup>16,17</sup>).

### Statistical Analysis

Differences in the means of vascular risk attributes and markers of inflammation in subjects with and without chronic infections were analyzed with Student's *t* test ( $\chi^2$  test for proportions). The association of chronic infection status with the development of carotid plaques and carotid stenosis was examined by logistic regression analysis.<sup>23</sup> Multivariate equations were adjusted for a fixed set of covariates<sup>18</sup> or fitted by a forward stepwise selection procedure. Analyses performed in the entire population sample (Figures 1 through 4) included the baseline atherosclerosis score as a covariate, because there is evidence that preexisting vessel disease promotes atherogenesis beyond the injurious action of the vascular risk profile.



**Figure 2.** ORs (95% CIs) of new carotid plaques calculated for various types of chronic infections. Adjustment, see Figure 1.

This procedure had virtually no effect on the risk estimates. Population attributive fractions were adjusted for other risk variables.<sup>24</sup> Logistic regression models were supplemented and confirmed by linear regression analysis by use of 5-year changes in the atherosclerosis score or the intima-media thickness as continuous outcome variables.

## Results

Of the 826 study subjects, 268 met the diagnostic criteria of chronic infections (Table 1). Susceptibility to infectious illness increased with advancing age, low social status, and risk behaviors such as cigarette smoking and heavy drinking. Serum levels of various markers of inflammation and infection were substantially enhanced in the chronic infection condition (Table 2). Furthermore, subjects with chronic infections showed multiple unfavorable changes in vascular risk factors, most of which were quantitatively low (Table 2).

During a mean of 5 years' follow-up, new carotid plaques emerged in 332 of 826 subjects (41%, population-adjusted rate 33%), with the risk of atherosclerosis development being

**TABLE 1. Chronic Infections in a Population 40 to 79 Years Old (N=826)**

Respiratory infections, n	
COPD with recurrent infectious exacerbation	141
Chronic bronchitis	86
Chronic upper respiratory tract infections	3
Urinary tract infections, n	
Recurrent lower urinary tract infections	32
Pyelonephritis	2
Other infections, n	
Chronic pancreatitis	4
Diverticulitis	3
Periodontitis	19
Recurrent bacterial skin infections	3
Diabetic foot ulcers	11

COPD indicates chronic obstructive pulmonary disease. A total of 26 subjects suffered from 2 types of chronic infection.

markedly elevated among individuals with chronic infection (adjusted OR for any chronic infection versus none, 2.78,  $P<0.001$ ; Figure 1). In the subgroup of subjects free of carotid atherosclerosis at baseline ( $n=500$ ), a total of 125 (25%, population-adjusted rate 20%) showed a manifestation of first carotid plaques. Again, those with chronic infections faced a several-fold risk of atherosclerosis regardless of whether the analysis was adjusted for age and sex only (OR [95% CI], 4.08 [2.42 to 6.85];  $P<0.0001$ ) or fitted with a forward-stepwise logistic regression procedure (adjusted OR, 4.10; Table 3). Exclusion of subjects with manifest cardiovascular diseases had little effect on the risk estimates obtained (adjusted OR, 4.05). In the subgroup of subjects with preexisting carotid atherosclerosis, chronic infection status was of predictive significance for the development of further lesions (disease extension) as well (adjusted OR, 2.31;  $P<0.001$ ).

To account for the potentially confounding effect of cigarette smoking, which increases susceptibility to infections and per se represents a vascular risk factor, the analyses were repeated in lifetime nonsmokers. This and the following analyses were performed in the entire population sample to ensure adequate sample sizes. Results were not substantially attenuated (Figure 1). In line with this, separate analyses limited to abstainers or various age and other subgroups yielded results similar to those derived from the original computation (Figure 1), as did analyses performed after exclusion of subjects receiving aspirin and/or those with manifest cardiovascular disease and/or the few subjects reporting repeated or long-term therapy with antibiotics ( $n=21$ ).

The strong association with the development of new atherosclerotic lesions applied to respiratory, urinary, and other types of chronic infectious illness, including infections with *C pneumoniae* (Figure 2). In contrast, seropositivity to the cytomegalovirus, manifest infections with the herpes zoster virus, and chronic active hepatitis B/C appeared to be unrelated to this early stage in atherogenesis.

Chronic infection status conferred a markedly increased risk of atherosclerosis development, even in the absence of other vascular risk factors (Figure 1). Among subjects with chronic infections, there was a clear tendency for atherosclerosis risk to increase when C-reactive protein levels exceeded 1 mg/L (60th percentile) (Figure 3).

In subjects with preexisting carotid atherosclerosis ( $n=326$ ), chronic infections conferred an increased risk for the manifestation/progression of carotid stenosis  $>40\%$  (OR [95% CI] 1.63 [1.03 to 2.58];  $P<0.05$ ), which disappeared after adjustment for other risk variables, some of which were increased in the chronic infection condition (eg, fibrinogen). Finally, analyses that applied continuous measures of atherosclerosis extension replicated the results derived from our categorical progression model. In linear regression analyses adjusted for age, sex, and other vascular risk factors, chronic infection status ranked among the variables most strongly associated with atherosclerosis progression (regression coefficients [95% CI] for change in atherosclerosis score, 0.85 [0.47 to 1.23] and intima-media thickness, 0.05 [0.02 to 0.08];  $P<0.01$  each).

**TABLE 2. Baseline Characteristics of Subjects With and Without Chronic Infection\***

Characteristic	Chronic Infection		P	P†
	No (N=558)	Yes (N=268)		
Demographic variables				
Male sex, n (%)	264 (47)	151 (56)	0.015	...
Age, y	54±10	65±10	<0.001	...
Social status, n (%)				
Low	302 (64)	203 (76)	<0.001	...
Medium	141 (25)	37 (14)		
High	115 (21)	28 (10)		
Risk factors				
LDL cholesterol, mg/dL	138±36	142±43	0.191	0.956
HDL cholesterol, mg/dL	58±15	56±13	0.042	0.013
Lipoprotein (a), mg/dL	16±18	16±18	0.851	0.963
Hypertension, n (%)	132 (24)	112 (42)	<0.001	0.023
Ferritin, ng/mL	137±15	172±19	0.008	0.597
Smoking, n (%)	122 (22)	77 (29)	0.031	<0.001
Alcohol consumption, n (%)				
Abstainers	289 (52)	117 (44)	<0.001	0.005
1–50 g/d	174 (31)	70 (26)		
51–99 g/d	67 (12)	49 (18)		
≥100 g/d	28 (5)	32 (12)		
Glucose tolerance, n (%)				
Normal glucose tolerance	495 (89)	206 (76)	<0.001	0.195
Impaired glucose tolerance	36 (6)	31 (12)		
Diabetes mellitus (type II)	27 (5)	31 (12)		
Hypothyroidism, n (%)	32 (6)	27 (10)	0.023	0.067
Microalbuminuria, mg/L	21±62	68±308	0.001	0.045
Markers of inflammation/infection				
C-reactive protein, mg/L	1.7±3.7	5.4±11.2	<0.001	<0.001
α <sub>1</sub> -antitrypsin, mg/dL	192±33	214±40	<0.001	<0.001
Ceruloplasmin, mg/dL	26±5	29±6	<0.001	<0.001
White blood cell count, ×10 <sup>3</sup> /μL	6.2±1.6	6.8±1.8	<0.001	<0.001
Neutrophil count, ×10 <sup>3</sup> /μL	3.4±1.1	3.9±1.3	<0.001	<0.001
Fibrinogen, mg/dL	252±56	277±59	<0.001	0.168
sICAM-1, ng/mL‡	318±86	350±102	<0.001	<0.001
sVCAM-1, ng/mL‡	641±157	710±193	0.078	0.028
E-selectin, ng/mL‡	52±21	56±22	0.064	0.025
Endotoxin, pg/mL‡	22±29	34±51	0.005	0.015
Soluble HSP60, ng/mL§	230±706	392±1281	0.015	0.045
HSP65 antibody, titer‡	273±435	343±406	0.024	0.027
IgA <i>C pneumonia</i> antibody, titer§	51±45	60±47	0.007	0.064
IgG <i>H pylori</i> antibody, U/mL§	64±50	65±52	0.864	0.814
IgG cytomegalovirus antibody, U/mL§	15±15	17±16	0.112	0.291

\*Plus-minus values are mean±SD. sICAM-1 and sVCAM-1 denote soluble intracellular and vascular-cell adhesion molecule 1, respectively.

†P values after adjustment for age, sex, and social status.

‡Data available in subgroups: sICAM-1 (n=750), E-selectin (n=750), HSP65 antibodies (n=750), endotoxin (n=466), and sVCAM-1 (n=160).

§Data assessed as part of the 1995 evaluation.

**TABLE 3. Association of Chronic Infections and Vascular Risk Attributes With Incident Carotid Atherosclerosis in Subjects Free of Atherosclerosis at Baseline (N=500)**

Variable	-2 Log LR	Step of Entry	Odds ratio (95% CI)	P
Chronic infection	25.1	1	4.10 (2.37–7.10)	<0.0001
Ferritin	8.4	2	1.45 (1.13–1.87)	0.005
Hypertension	6.4	3	2.01 (1.19–3.42)	0.011
LDL cholesterol	11.5	4	1.51 (1.18–1.93)	<0.001
Age	6.6	5	1.39 (1.08–1.78)	0.010
Alcohol consumption	9.8	6		0.019
Abstainers			1.00	
1–50 g/d			0.55 (0.32–0.99)	0.042
51–99 g/d			1.48 (0.44–5.42)	0.525
≥100 g/d			2.91 (1.38–6.13)	0.006
Hypothyroidism	3.6	7	2.31 (1.00–5.45)	0.050
Microalbuminuria	3.7	8	1.25 (0.99–1.60)	0.075
HDL cholesterol	3.3	9	0.81 (0.64–1.02)	0.081

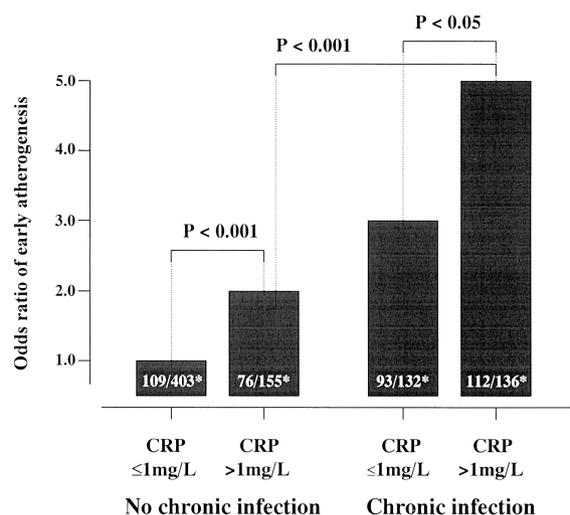
LR denotes likelihood ratio. ORs were derived from multiple stepwise logistic regression analysis. Variables were selected for inclusion from among those listed in Table 2 (except for those measured in 1995 or available in subgroups only). ORs were calculated for a 1-SD unit increase in given variables (except for categorical variables).

Figure 4 depicts various markers of infection, inflammation, and immunity. Baseline levels of these variables were significantly enhanced in subjects who developed atherosclerotic lesions during follow-up.

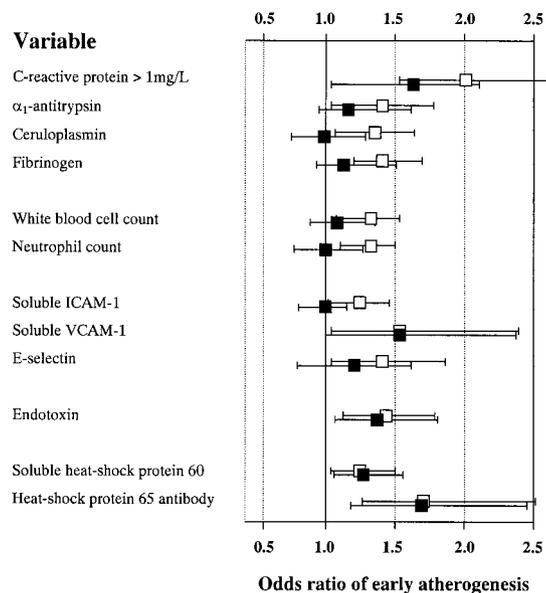
### Discussion

Experimental work in the late 1970s and 1980s has prompted a revival of interest in the old hypothesis of an infectious risk factor for atherosclerosis.<sup>1,4–7</sup> Epidemiological confirmation of this concept has not yet been furnished, although (mainly) indirect supportive evidence recently became available.<sup>7,8,10–15,19,21,22,25,26</sup> High concen-

trations of C-reactive protein were observed to predict the risk of future cardiovascular disease and may arise in part from the chronic inflammatory state evident in persistent infections.<sup>25,26</sup> In most but not all seroepidemiological evaluations, subjects with a prominent immune response to *C pneumoniae* or (virulent strains of) *H pylori*, as indicative of chronic infection status, faced an excess risk of coronary heart disease, stroke, and vascular death.<sup>7,13–15,27–29</sup> Finally, chronic bronchitis, as defined by self-reported clinical symptoms, and periodontal disease have



**Figure 3.** ORs of new carotid plaques according to chronic infection status and level of C-reactive protein. ORs were adjusted for age, sex, and baseline atherosclerosis. \*Numbers of subjects with incident atherosclerosis per number of patients at risk.



**Figure 4.** ORs (95% CIs) of new carotid plaques for various markers of infection, inflammation, and immunity. ORs were calculated for a 1-SD unit increase in given variables (except for C-reactive protein). Adjustment, see Figure 1 (+ chronic infection status in the multivariate equation).

been suggested to constitute risk factors for cardiovascular disease.<sup>10–12</sup>

In the Bruneck Study, common chronic infections, as defined by standard diagnostic procedures, predicted a markedly increased risk of atherogenesis in the carotid arteries. All major results were consistently obtained from 2 ultrasound progression models of atherosclerosis. The one commonly used in the literature uses continuous outcome variables, such as atherosclerosis scores or intima-media thickness, and analyzes changes in these variables over time. The second, person-based approach, developed and validated in the Bruneck Study, permits differentiation of 2 distinct stages of atherogenesis, namely the development of new (early) plaques and the occurrence/progression of vessel stenosis, which was shown to rely primarily on atherothrombotic pathomechanisms.<sup>16–18</sup> As expected, chronic infections were preferentially related to the former stage. In subjects without atherosclerosis at baseline, ≈40% of newly developed atherosclerosis was attributable to chronic infection (population attributable risk), making it a leading risk predictor for this initiating stage of vessel pathology (Table 3). Chronic infections conferred an increased risk of atherosclerosis development even in low-risk subjects free of conventional vascular risk factors.

Subjects with respiratory, urinary, and other types of infections all faced an increased risk of atherosclerosis (Figure 2), with the spectrum of underlying pathogens being highly heterogeneous. *C pneumoniae*, which attracted preferential attention in past research, ranked among these pathogens.<sup>13</sup> In accordance with our results, atherosclerosis can be induced in animal models by a variety of microorganisms, such as chlamydia, streptococci, and salmonella.<sup>1,3,9,30</sup> As to infection with the cytomegalovirus (assessed by serological criteria)<sup>13</sup> and other viral agents, our study did not furnish evidence of a role in atherogenesis, which, however, may be a peculiarity of our study population and does not rule out pathogenetic relevance in special subgroups, such as subjects receiving immune-suppressive therapy or undergoing coronary angioplasty.<sup>4–8,31</sup>

Among subjects with chronic infections, risk of atherosclerosis tended to be higher in those with a prominent inflammatory response (Figure 3), which may indicate high virulence of the underlying pathogen or atherogenic host-pathogen interactions.<sup>13,14,27,31</sup> Induction of systemic inflammation has been proposed to be of pathogenetic relevance in the association of infection and atherosclerosis and may rely in part on the endothelial toxicity of bacterial endotoxin and the action of proinflammatory cytokines.<sup>7,19</sup> Recently, we demonstrated that in smokers and subjects with chronic infections, high concentrations of circulating endotoxin predict a substantially increased risk of atherosclerosis (Figure 4).<sup>19</sup> The present evaluation extends this finding to various other markers of inflammation, all of which predicted the development of atherosclerosis and were substantially enhanced in subjects with chronic infections (Table 2, Figure 4).

Another well-founded proatherogenic property of infectious illness may be the induction of autoimmunity.<sup>32</sup> All bacteria expose immunogenic HSP60 on their surface, which shows a high structure homology to its human counterpart.

Human HSP60 expression is increased in atherosclerotic lesions, notably in areas subject to hemodynamic stress.<sup>32</sup> Serum antibodies to mycobacterial HSP65 correlate with the prevalence and incidence of carotid atherosclerosis,<sup>21</sup> cross-react with human HSP60, and mediate cytotoxicity on stressed endothelial cells.<sup>33</sup> Chronic infections may contribute to vascular injury by causing a breakdown of self-tolerance and subsequent immune attack against human HSP exposed on endothelium stressed by atherogenic risk factors.

In the interpretation of our results, it must be considered that no consensus exists on the types of illness to be subsumed under the term “chronic infections.” In the present analysis, this potential source of bias was at least in part overcome by an a priori definition of the variable. Infection status was defined by standard diagnostic guidelines, which closely resembles diagnostic practice in clinical routine and thus warrants high accuracy. As a further potential limitation, the study population is purely white. Results are not necessarily representative of other ethnic populations, which may differ in the genetic background of immune defense as well as target cell (endothelial cell) antigen expression.

In conclusion, the present study advocates a prominent role of common (bacterial) infections in atherogenesis and suggests 2 important pathophysiological clues: the induction of systemic inflammation and autoimmunity. Our findings may assist in identifying subjects at high risk of atherosclerosis and offer a preliminary basis for future anti-infectious and anti-inflammatory prevention trials.

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