INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 23, no. 4, 1245-1252 (2010)

CHLAMYDOPHILA PNEUMONIAE INFECTION IN PATIENTS UNDERGOING CAROTID ARTERY STENT

F. MANCINI, E. BOATTA[1], M.F. VESCIO, F. FANELLI[1], F.M. SALVATORI[1], R. PASSARIELLO[1], A. CASSONE and A. CIERVO

Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Parassitarie ed Immuno-mediate, Rome; [1]Università La Sapienza, Dipartimento di Scienze Radiologiche, Rome, Italy

Received April 20, 2010 - Accepted September 10, 2010

The first two authors contributed equally to this work

Although several reports have correlated *Chlamydophila pneumoniae* (CP) infection with carotid endarterectomy and coronary stent, no data have been reported on the potential relationship between this pathogen and carotid artery stenting (CAS). Hence, we evaluated 47 subjects, 27 symptomatic and 20 asymptomatic, before CAS intervention and during the follow up, for the presence of CP DNA and anti-CP antibodies, including chlamydial HSP60 (Cp-HSP60). Before stent placement, CP DNA was detected exclusively in symptomatic patients, all of whom were also positive for CP IgG and IgA and 85.7% of them also had CP-HSP60 antibodies. At the follow-up, all CP DNA positive and 11 out of the 13 symptomatic patients with Cp-HSP60 antibodies became negatives. In contrast, no change was observed for CP- IgA antibodies. Despite the small number of patients, the present study advocates an important role of CP infection in symptomatic patients with carotid artery disease. Our findings also suggest that stent placement and/or therapy might have a role in favouring resolution of inflammation, though not affecting persistence of CP infection.

Infections, with their inflammatory potential, may play an important role in the evolution of atherosclerosis and consequent cardiovascular events. In particular, *Chlamydophila pneumoniae* (CP) infections are associated with coronary artery disease and its progression (1-6).

CP is an obligate intracellular microbe showing an outstanding tropism for macrophages and circulating monocytes. Depending on host immunological conditions and individual genetic susceptibility, CP may enter into a reproductive cycle (acute infection) or a non-replicative, non-cultivable and antibiotic-refractory state (chronic infection and persistence) (7-8). Thus, an understanding of the genetic background of this persistence, which may continue ill-defined *in vivo*, is the key to efforts to eliminate the pathogen from chronic infection.

In particular, CP could also contribute to precipitating acute thrombotic complications of atheroma (2). Furthermore, activated CP-specific T cells within the infected human atheromas may enhance proteolytic and pro-apoptotic events, thus rendering the protective fibrous cap susceptible to rupture (4, 9).

Key words: Chlamydophila pneumoniae, Real-time PCR, stent, carotid, atheromatous plaques

Mailing address: Dr Alessandra Ciervo, PhD
Department of Infectious,
Parasitic and Immune-mediated Diseases
Istituto Superiore di Sanità,
Viale Regina Elena 299 00161-Rome, Italy
Tel: ++39 06 49903127 Fax: ++39 06 49387183
e-mail: alessandra.ciervo@iss.it

0394-6320 (2010)

In this context, several findings indicate that the chlamydial heat shock 60 protein (Cp-HSP60) and its molecular mimicry action, may represent a potent antigenic stimulus, capable of eliciting strong humoral and cell-mediated immune responses with immunopathological sequelae of chronic chlamydial infections (3, 10-11).

Interest has been focused on the possible pathophysiologic link between CP infection and either the manifestations of coronary atherosclerosis or adverse outcomes after coronary stent implantation (12). Furthermore, some studies suggest that CP infection participates in vascular trauma during stent implantation, which triggers the production of inflammatory processes and intima layer proliferation (12-13).

Until now, no data have been reported on the potential relationship between carotid artery stent (CAS) and CP infection. CAS procedure is considered a less invasive alternative to carotid endarterectomy, which represents the standard treatment for selected patients with carotid stenosis (CS) (14). CAS may be selectively indicated for high-risk patients such as those aged >80 years, with contra-lateral carotid occlusion or in patients with hostile necks who cannot undergo surgery (15).

Despite post-CAS outcome having improved over the past 10 years, the incidence of neurological complications due to distal plaque debris embolization ranges from 3.2 to 10% even with the use of embolic protection devices EPDs (16-18). In this context, vascular chlamydial infection may modify the outcome of CAS, and the infected vessel wall with CP might trigger spontaneous thrombotic events (19).

The objectives of this study are to investigate in our CAS patients: 1) specific antibody titres against CP, including Cp-HSP60, before the intervention and during the follow-up; 2) the presence of the CP DNA in atheromas through EPDs and in the blood; 3) possible association of CP infection with cardiovascular risk factors and adverse events.

MATERIALS AND METHODS

Study population

The study group consisted of 47 CAS subjects, with or without symptoms, who had undergone successful carotid stent placement. The basic characteristics of the study patients which were noted were: age, sex, current smoking if not stopped during the last month, C-reactive protein (CRP) level, hypertension, hypercholesterolemia, diabetes mellitus, medication at the time of procedure, previous history of cardiovascular diseases, and degree of carotid artery disease as at least one lesion 50-80% diameter stenosis on carotid angiography. The severity of stenosis was quantified using the North American Symptomatic Carotid Endarterectomy Trial Criteria (20). In the case of CAS of more than one lesion, on the basis of plaque morphology, the most complex lesion was evaluated in the statistical analysis.

All patients received oral anti-platelet therapy with ticlopidine 250 mg or clopidogrel 75 mg at least 48 hours prior to CAS and continued for at least 1 year following the procedure combined with statins and β-blockers.

All data were collected prospectively and entered into a central database. Clinical follow-up information was obtained by contacting all patients at 12 months and performing Ultrasond-Color-Doppler in order to evaluate stent patency. Source documents of potential events were obtained.

Study end points were the composite occurrence, during hospitalization and at the follow-up, of major cardiac (death, nonfatal myocardial infarction, repeated revascularization) or cerebrovascular (stroke, transient ischemic attacks, revascularization syndrome) events and restenosis.

All studies were performed after patient written informed consent and approved by the Ethics Committee of our Institution, conformed to the principles outlined in the Declaration of Helsinki.

Procedures

Patients were scheduled for carotid angiography and the intervention was carried out on the basis of their clinical stabilization or clinical indications. Under local anaesthesia femoral or brachial access was gained and a bolus of unfractionated heparin (70 U/kg) was given intravenously. Blood pressure and ECG were monitored throughout the procedure. Angiography of the extracranial and intracranial carotid system was carried out routinely in all standard projections. Stenosis predilatation (with balloon diameter ranging from 2.5 to 3 mm) was undertaken in case of severe stenosis when a distal protection device could not be advanced through the lesion. Distal protection devices, EPI filter wire (Boston Scientific) or Angioguard filters (Boston Scientific), were used in 22 (47%) and 25 (53%) patients, respectively. Appropriate self-expandable carotid stent, Wallstent (Boston Scientific) or Precise (Cordis Corporation), was selected on the basis of carotid anatomy and plaque morphology, and implanted. The stent difference consists

in the body structure of the 2 medical devices. Essentially, the skeleton of the Precise stent provides moderate column strength with open cells, while the Wallstent column shape is conceived with close cells.

Once the distal protection device was deployed above the lesion, carotid stent was dilated with a balloon catheter (diameter ranging from 5.5 mm to 6mm). Before dilation, 1 mg of athropine was intravenously administered in order to avoid the effects related to the carotid sinus reflex.

DNA purification from EPDs and PBMC

Each Embolic Protection Device specimen was washed twice in PBS and the material was concentrated by centrifugation at 22,000 x g for 30' at 4°C. PBMC was extracted from whole heparinised blood before the stent placement and at the follow-up. Samples were diluted 1: 1 with PBS and were layered onto Ficoll-Paque PLUS (Amersham Biosciences AB, Uppsala, Sweden) and centrifuged for 30 min at 400 x g. The buffy-coat layer was then collected and washed with an equal volume of PBS twice for 10 min at 100 g. PBMC cells were suspended in 0.3 ml of PBS and frozen at -70°C. DNAs from balloons, EPDs and PBMC samples were extracted by Nucleospin Tissue kit (Macherey–Nagel GmbH, Düren, Germany), using the standard protocol for bacterial samples, according to the manufacturer's instructions.

Assessment of CP real-time PCR

PCR reaction mixture was performed using a final volume of 20 µl into glass capillary tubes (Roche Diagnostics GmbH, Germany). The quantitative real-time PCR was performed as previously described (5). Briefly, the PCR mixture contained 2 µl of DNA template (100 ng of total DNA from human samples, or CP DNA ranging from 10 to 10^5 copies). The quantitative analysis was made with LigthCycler quantification software using the cycle threshold of the standard CP DNA (10^5 to 10 genomic copies).

Serological analyses

Anti-Cp IgG and IgA antibody levels, before the surgical procedure and at the follow-up, were measured by a commercially available microimmunofluorescence assay (Labsystems Oy, Helsinki, Finland) according to the manufacturer's instructions. IgG or IgA titers ≥1:32 were regarded as positive. Anti-Cp-HSP60 IgG levels were measured by an in-house ELISA, as previously described (3). All sera were diluted 1:50, and seropositivity was defined as an ELISA reading of ≥ 0.4, corresponding to twice the maximum value of antigen-only-coated wells. CRP was measured by a high-sensitivity method on latex-nephelometry (Dade-Behring, City, Country).

Statistical analysis

Variables were reported as percentages. Comparison between symptomatic and asymptomatic groups was made by chi squared test. Two-tailed significance level was 0.05. All analyses were performed using STATA 10.

RESULTS

Baseline characteristics

We evaluated 47 consecutive patients (20 asymptomatic and 27 symptomatic), for endovascular intervention for CAS. The patients' clinical profiles are summarized in Table I. No statistically significant differences between the two patient categories were found for age, gender, hypercholesterolemia, hypertension, CRP levels, diabetes, family history of cardiovascular diseases, complex lesions and therapy administration. On the other hand, smoking was significantly more common in the symptomatic group. The stenosis grade of 50-70% was significantly more frequent in symptomatic patients, while the stenosis grade > 70% was predominantly found in the asymptomatic group. CAS implant was technically successful in all patients.

Evaluation of CP infection, cardiovascular risk factors and clinical status at the stent placement

Antibodies against CP (IgG and IgA) and against Cp-HSP60 (IgG) were sought in all subjects at the stent placement. As shown in Table II, the symptomatic subjects had overall higher frequencies of antibodies, with a statistically significant difference in the anti-chlamydial IgA and anti-HSP60 IgG. In particular, all CP IgA positive subjects (46.8%) were also positive for IgG ≥ 64, but there was a statistically significant difference for symptomatic patients. Table II also shows that CP DNA was detected exclusively in some symptomatic patients, namely in 7 atherosclerotic lesions by the EPDs recovering (from 350 to 1600 DNA copy numbers), and of these 7 patients, 4 were also PCR positive in PBMC samples (from 500 to 800 DNA copy numbers). Worthy of note, all PCR positive subjects were IgG and IgA positive and 6/7 (85.7%) had antibodies against Cp-HSP60. Overall, the above findings demonstrate that symptomatic patients were more likely to have CP-DNA and serological (CP-IgA, Cp-IgG and Cp-HSP60) markers of CP infection than the asymptomatic subjects.

Table I. *Characteristics and cardiovascular risk factors of patients at baseline.*

	Asymptomatic n=20	Symptomatic n=27	*P*
Age, years (mean ± SD)	73±7	73±10	
Male sex, n (%)	12 (60)	17 (63)	
Smoke, n (%)	6 (30)	16 (59)	*
Hypercholesterolemia, n (%)	7 (35)	10 (37)	
Hypertension, n (%)	14 (70)	19 (70)	
CRP > 3 mg/L n (%)	9 (45)	17 (63)	
Diabetes, n (%)	4 (20)	10 (37)	
Family History of Cardiovascular risks, n (%)	9 (45)	12 (44)	
ACS, n (%)	3 (25)	9 (33)	
CAD, n (%)	6 (30)	3 (11)	
Stenosis			**
grade 50-70%, n (%)	8 (40)	21 (78)	
grade >70%, n (%)	12 (60)	6 (22)	
Complex lesions, n (%)	8 (40)	14 (52)	
Therapy on admission			
Aspirin, n (%)	7 (35)	8 (30)	
Statin, n (%)	10 (50)	13 (48)	
Beta-Blockers, n (%)	10 (50)	14 (52)	
Ace-inhibitors, n (%)	8 (40)	10 (37)	

** P < 0.05; **P< 0.001*

Table II. *Relation of CP seropositivity to cardiovascular risk factors, PCR positivity and clinical status at stent placement.*

	IgG			IgA			Cp-HSP60		
	Asymptomatic	Symptomatic	*P*	Asymptomatic	Symptomatic	*P*	Asymptomatic	Symptomatic	*P*
Patients, n (%)	13 (65)	22 (81)		6 (27)	16 (73)	*	3 (19)	13 (81)	*
Smoke, n (%)	4 (31)	16 (73)	*	1 (17)	10 (62)	*	0 (0)	8 (61)	*
Hypercholesterolemia, n(%)	5 (38)	8 (36)		2 (33)	7 (44)		2 (67)	6 (46)	
Hypertension, n (%)	9 (69)	16 (64)		4 (67)	10 (62)		2 (67)	9 (69)	
CRP > 3mg/L, n (%)	6 (46)	16 (73)		3 (50)	13 (81)	*	1 (33)	12 (92)	**
Diabetes, n (%)	3 (23)	7 (32)		2 (33)	7 (44)		2 (67)	5 (38)	
History of Cardiovascular Diseases, n (%)	5 (38)	9 (41)		3 (50)	6 (37)		3 (100)	5 (38)	
Stenosis									
grade 50-70%, n (%)	5 (38)	17 (77)		1 (17)	13 (81)	*	1 (33)	9 (69)	
grade >70%, n (%)	8 (61)	5 (23)		5 (83)	3 (19)	*	2 (67)	4 (31)	
Complex lesions, n (%)	7 (54)	12 (54)		2 (33)	8 (50)		0 (0)	7 (54)	*
CP PCR EPDS, n (%)	0 (0)	7 (100)	**	0 (0)	7 (100)	**	0 (0)	6 (86)	**
CP PCR PBMC, n (%)	0 (0)	4 (100)	**	0 (0)	4 (100)	**	0 (0)	4 (100)	**

** P < 0.05; **P< 0.001*

We also assessed antibody differences according to the presence or absence of specific risk factors for cardiovascular disease. As shown in Table II, among smokers, IgG, IgA and Cp-HSP60 antibodies were more likely to be found in symptomatic than in non-symptomatic subjects (73% *vs* 31%). In

Table III A. *Relation of CP infection (serology and PCR) to clinical status of patients at stent placement.*

	IgG	IgA	Cp-HSP60	PCR EPDs	PCR PBMC	Total positive	Negative
Asymptomatic, n (%)	13 (65)	6 (27)	3 (19)	0 (0)	0 (0)	14 (38)	6 (60)
Symptomatic, n (%)	22 (81)	16 (73)	13 (81)	7 (100)	4 (100)	23 (62)	4 (40)
P		*	*	**	**		
Total	35	22	16	7	4	37	10

Table III B. *Relation of CP infection (serology and PCR) to clinical status of patients at the end of follow-up.*

	IgG	IgA	Cp-HSP60	PCR PBMC	Total positive	Negative
Asymptomatic, n (%)	11 (37)	6 (27)	2 (50)	0 (0)	13 (39)	7 (50)
Symptomatic, n (%)	19 (63)	16 (73)	2 (50)	0 (0)	20 (61)	7 (50)
P		*				
Total	30	22	4	0	33	14

*$P < 0.05$; **$P < 0.001$*

symptomatic patients, CRP was related to IgA and Cp-HSP60 antibodies, and hypertension with IgA and Cp-HSP60 antibodies. Moreover, Cp-HSP60 antibodies were also correlated to lesion complexity and stenosis grade >70%.

We also evaluated possible correlations between clinical status, adverse events, cardiovascular risk factors, stent types and CP infection. No correlation was detected (data not shown).

Serologic and PCR findings at the follow-up

Clinical follow-up was obtained from all patients. During this period the patients received ticlopidine 250 mg/day or clopidogrel 75 mg/day, statins and β-blockers, with no differences among the study groups (data not shown). At 1 year of follow-up, no stent re-stenosis (assessed by Eco-color-Doppler analysis) or deaths were reported. Only 2 of the 13 symptomatic patients positive for Cp-HSP60 antibodies remained positive, and the only 4 patients with PBMC CP DNA positive at stent placement converted to negativity. In contrast, no change was observed for CP IgA antibodies and no differences were noticed in the asymptomatic group before the surgical treatment and at the follow-up (Table III, A and B).

DISCUSSION

Atherosclerosis is considered to be a chronic inflammatory disease with multifactorial aetiology (21-23). Infections are the main causes of inflammation and they seem to confer an increased risk for development of atherosclerosis, even in subjects without conventional cardiovascular risk factors (24). In particular, chronic CP infection could be a possible initiator or facilitator of chronic inflammation in atherosclerosis. It may trigger spontaneous thrombotic events, increase the risk of thrombotic or adverse events and modify the outcome of CAS intervention (1, 5-6, 25-27).

The first objective of our study was to evaluate

antibody levels against CP in our patient groups at stent placement. In symptomatic patients we found a potential relationship between IgA seropositivity with smoking, CRP and stenosis grade of 50-70%. We also observed the correlation between anti-CP-HSP60 antibodies with smoking, CRP protein and lesion complexity. Additionally, IgG, IgA and Cp-HSP60 seropositivity perfectly matched all patients with CP DNA infected plaques, and in 4 of them (50%) the bacterial DNA was also detected in PBMC samples. Overall, CP DNA detection and serologic markers, such as anti CP-HSP60, were closely correlated with clinical symptoms, suggesting a role of CP in determining/amplifying atherosclerosis symptomatology.

The above conclusion appears to be strengthened by the follow-up observations of a drastic decrease of Cp-HSP60 antibodies in 85% symptomatic patients, and conversion to negativity of the previously PBMC CP DNA positive patients. Intriguingly, CP IgA antibodies remain unchanged in the same patients, and no significant association or differences were observed in asymptomatic patients before the stent procedure and during the follow-up.

These results would imply that the state of chronic CP infection and the level of Cp-HSP60, both significantly associated to CRP, may contribute to the systemic inflammation. Chlamydial HSP60 cross reaction with other microbial and human HSP60s could mediate cytotoxicity on stressed endothelial cells and pro-atherogenic effects (3-4, 9, 11). The chronic infection and the overexpression of Cp-HSP60 may contribute to vascular injury by causing a breakdown of self-tolerance and subsequent immune attack against exposed HSP60s.

An intriguing observation of our study is that, at the follow-up, the state of chronic infection, as inferred by the persistent CP IgA antibodies level, did not match with the evident lack of CP seropositivity and the decrease of anti-HSP60 response. In this context, the role of drug treatments and supportive measures should be taken into account. Our patients were continually treated with statins, β-blokers and anti-platelet drugs. At least for statins, there are some clinical and experimental observations, strongly suggesting that these drugs could contribute to lowering inflammation (28-29).

The risk factor represented by persistent CP infection adds to other risk factors, such as anti-HSP60 auto-antibodies and elevated CRP concentration, and support the hypothesis proposed by Wick et al. (30) that chronic CP infection increases the expression of host HSP60, and may progressively result in autoimmunity with systemic inflammation and elevated CRP.

In interpreting our data, caution should be exercised due the study size and the particularly low number of symptomatic subjects.

Nevertheless, the present study advocates a prominent role of CP infection in symptomatic patients with carotid artery diseases and suggests the occurrence of the pathogen in systemic inflammation and possibly autoimmunity. Our findings encourage future investigations in CAS patients and CP infectious/inflammatory diseases in large patient series.

ACKNOWLEDGEMENTS

We thank Maria Losardo for the technical help. The work was supported by funds from the Italian Ministry of Research and University, Rome- Italy, FIRB grant: *"Costruzione di un laboratorio nazionale per le resistenze agli antimicrobici"*.

REFERENCES

1. Kuo CC, Shor A, Campbell LA, Fukushi H, Patton DL, Grayston JT. Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries. J Infect Dis 1993; 167:841-9.
2. Libby P, Egan D, Skarlatos S. Roles of infectious agents in atherosclerosis and restenosis: an assessment of the evidence and need for future research. Circulation 1997; 96:4095-103.
3. Ciervo A, Visca P, Petrucca A, Biasucci LM, Maseri A, Cassone A. Antibodies to 60-kDa heat shock protein and outer membrane protein 2 of Chlamydia pneumoniae in patients with coronary heart disease. Clin Diagn Lab Immunol 2000 9:66-74.
4. Benagiano M, Azzurri A, Ciervo A, et al. T helper type-1 lymphocytes drive inflammation in human atherosclerotic lesions. PNAS 2003; 100:6658-63.
5. Ciervo A, Petrucca A, Cassone A. Identification and quantification of Chlamydia pneumoniae in human

atheromatous plaques by LightCycler real time PCR. Mol Cell Probes 2003; 17:107-11.

6. Ciervo A, Mancini F, Sale P, Russo A, Cassone A. Real-time polymerase chain reaction and laser capture microdissection: an efficient combination tool for Chlamydophila pneumoniae DNA quantification and localization of infection in atherosclerotic lesions. Int J Immunopathol Pharmacol 2008; 21:421-8.
7. O'Connor CM, Dunne MW, Pfeffer MA, Muhlestein JB, Yao, Gupta LS. Azithromycin for the secondary prevention of coronary heart disease events: the WIZARD study: a randomized controlled trial. JAMA 2003; 290:1459-66.
8. Hogan RJ, Mathews SA, Mukhopadhyay S, Summersgill JT, Timms P. Chlamydial persistence: beyond the biphasic paradigm. Infect Immun 2004; 72:1843-55.
9. Benagiano M, D'Elios MM, Amedei A, et al. Human 60-kDa heat shock protein is a target autoantigen of T cells derived from atherosclerotic plaques. J Immunol 2005; 174:6509-17.
10. Kol A, Bourcier T, Lichtman AH, Libby P. Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. J Clin Invest 1999; 103:571-7.
11. Mayr M, Metzler B, Kiechl S, Willeit J, Schett G, Xu Q, Wick G. Endothelial cytotoxicity mediated by serum antibodies to heat shock proteins of Escherichia coli and Chlamydia pneumoniae: immune reactions to heat shock proteins as a possible link between infection and atherosclerosis. Circulation 1999; 99: 1560-6.
12. Neumann F, Kastrati A, Miethke T, et al. Treatment of Chlamydia pneumoniae infection with roxithromycin and effect on neointima proliferation after coronary stent placement (ISAR-3): a randomised, double-blind, placebo-controlled trial. Lancet 2001; 357: 2085-9.
13. Feldman LJ, Aguirre L, Ziol M, Bridou JP, Nevo N, Michel JP, Steg PG. Interleukin-10 inhibits intimal hyperplasia after angioplasty or stent implantation in hypercholesterolemic rabbits. Circulation 2000; 101: 908-16.
14. Fayad P. Endarterectomy and stenting for asymptomatic carotid stenosis a race at breakneck speed. Stroke 2007; 38:707-14.
15. Mozes G, Sullivan TM, Torres-Russotto DR, et al. Carotid endarterectomy in SAPPHIRE-eligible high-risk patients: implications for selecting patients for carotid angioplasty and stenting. J Vasc Surg 2004; 39:958-66.
16. McCabe DJ, Pereira AC, Clifton A, Bland JM, Brown MM, CAVATAS Investigators. Restenosis after carotid angioplasty, stenting or endarterectomy in the carotid and vertebral artery transluminal angioplasty study (CAVATAS). Stroke 2005; 36: 281-6.
17. Wholey MH, Al-Mubarak N, Wholey MH. Updated review of the global carotid artery stent registry. Catheter Cardiovasc Interv 2003; 60:259-66.
18. Fanelli F, Bezzi M, Boatta E, Passariello R. Techniques in cerebral protection. Eur J Radiol 2006; 60:26-36.
19. Dechend R, Maass M, Gieffers J, Dietz R, Scheidereit C, Leutz A, Gulba DC. Chlamydia pneumoniae infection of vascular smooth muscle and endothelial cells activates NF-kappaB and induces tissue factor and PAI-1 expression: a potential link to accelerated arteriosclerosis. Circulation 1999; 100:1369-73.
20. North American Symptomatic Carotid Endarterectomy Trial Collaborators. The beneficial effects of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 1991; 325:445-53.
21. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005; 352: 1685-95.
22. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, Ellis SG, Lincoff AM, Topol EJ. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 2003; 290:898-904.
23. Gupta R. Recent trends in coronary heart disease epidemiology in India. Indian Heart J 2008; 60:4-18.
24. Kiechl S, Egger G, Mayr M, et al. Chronic infections and the risk of carotid atherosclerosis: prospective results from a large population study. Circulation 2001; 103:1064-70.
25. Spagnoli LG, Pucci S, Bonanno E, Cassone A, Sesti F, Ciervo A, Mauriello A. Persistent Chlamydia pneumoniae infection of cardiomyocytes is

correlated with fatal myocardial infarction. Am J Pathol 2007; 170:33-42.

26. Kern JM, Maass V, Maass M. Chlamydia pneumoniae-induced pathological signaling in the vasculature. FEMS Immunol Med Microbiol 2009; 55:131-9.
27. Dechend R, Maass M, Gieffers J, Dietz R, Scheidereit C, Leutz A, Gulba DC. Chlamydia pneumoniae infection of vascular smooth muscle and endothelial cells activates NF-kappaB and induces tissue factor and PAI-1 expression: a potential link to accelerated arteriosclerosis. Circulation 1999; 100:1369-73.
28. Liuba P, Pesonen E, Forslid A, Paakkari I, Kornerup-Hansen A, Kovanen P, Pentikäinen M, Persson K, Østergård G. Protective effects of simvastatin on coronary artery function in swine with acute infection. Atherosclerosis 2006; 186:331-6.
29. Schmeck B, Beermann W, N'Guessan PD, et al. Simvastatin reduces Chlamydophila pneumoniae-mediated histone modifications and gene expression in cultured human endothelial cells. Circ Res 2008; 102:888-95.
30. Wick G, Perschinka H, Xu Q. Autoimmunity and atherosclerosis. Am Heart J 1999; 138:444-9.