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An Oxidized Low-Density Lipoprotein Receptor Gene Variant Is Inversely Associated with the Severity of Coronary Artery Disease

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Summary

Background: A lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) is the major receptor of oxidized LDL in endothelial cells. The expression of LOX-1 was shown to be upregulated in atherosclerotic lesions. Recently, LOX-1 gene polymorphism (G501C) was reported to be associated with myocardial infarction (MI).

Hypothesis: Our study was undertaken to elucidate the association between this polymorphism and coronary artery disease (CAD).

Methods: We evaluated LOX-1 gene polymorphism using Invader assay in 586 patients undergoing coronary angiography.

Results: Study patients were categorized into three groups: normal/minimal stenosis (≤25%) (n = 128); mild stenosis (26–50%) (n = 39); and significant stenosis (>50%) (n = 419). Of the 419 patients with significant stenosis, 163 had single-vessel, 165 had double-vessel, and 91 had triple-vessel disease. Myocardial infarction was present in 171 patients. The frequency of LOX-1 gene variants (C/C or C/G) was lower in patients with significant than in those with normal/minimal stenosis (36 vs. 49%, $p < 0.01$). The frequency of LOX-1 gene variants did not differ between patients with and without MI (34 vs. 37%). However, a stepwise decrease in the frequency of such variants was found depending on the severity of CAD: 49% in normal/minimal stenosis, 41% in mild stenosis, 39% in single-vessel, 35% in double-vessel, and 32% in triple-vessel disease. Multivariate analysis demonstrated LOX-1 gene variants to be inversely associated with the presence of significant stenosis (odds ratio = 0.61; 95% confidence interval = 0.41–0.92).

Conclusions: The LOX-1 gene variants at 501 were found to be inversely associated with the severity of CAD. This polymorphism may be modifying the severity of CAD.

Key words: lectin-like oxidized low-density lipoprotein receptor-1, coronary artery disease, genetics

Introduction

A lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) is the major receptor of oxidized LDL in endothelial cells[1] that allows the uptake of oxidized LDL into endothelial cells;[2] it is also present in macrophages and smooth muscle cells.[3,4] The binding of oxidized LDL to LOX-1 decreases nitric oxide synthase, increases adhesion molecules, and induces apoptosis in endothelial cells.[5,6] The expression of LOX-1 is upregulated in atherosclerotic lesions, especially in early stage lesions.[7,8] These suggest that LOX-1 plays an important role in the development of atherosclerosis.

Tatsuguchi *et al.*[9] recently reported LOX-1 gene polymorphism (a G-to-C transition at position 501) to be associated with myocardial infarction (MI). They showed the percentage of patients who have C/C or C/G genotypes (LOX-1 gene variants) to be higher in those with MI than in healthy controls. However, no association between this polymorphism and coronary artery disease (CAD) has yet been elucidated. Using the Invader assay,[10,11] we investigated the association between LOX-1 gene polymorphism and CAD in 586 patients undergoing coronary angiography. To examine whether this polymorphism affects the metabolism of oxidized LDL, we also assessed serum malondialdehyde-modified LDL (MDA-LDL) levels, one of oxidized LDLs, using a recently developed sensitive enzyme-linked immunosorbent assay (ELISA).[12]

Methods

Study Patients

We evaluated LOX-1 gene polymorphism in 586 consecutive patients who underwent coronary angiography for suspected CAD at National Defense Medical College Hospital. Patients with a history of coronary artery bypass surgery were excluded. Of the 586 patients, 350 (60%) had hypertension

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(blood pressure ≥ 140/90 mmHg or on drugs), 244 (42%) had hyperlipidemia (total cholesterol level >240 mg/dl or on drugs), 172 (29%) had diabetes mellitus (fasting glucose level ≥ 126 mg/dl or on hypoglycemic drugs or insulin), and 380 (65%) were smokers (≥ 5 cigarettes/day). Our study was approved by the ethics committee of the hospital. After written informed consent was obtained, fasting blood samples were taken on the morning of the day of angiography. Serum total cholesterol, LDL cholesterol, and high-density lipoprotein (HDL) cholesterol levels were measured by standard laboratory methods. In the 177 samples randomly selected from our patients, serum MDA-LDL levels were measured by ELISA, as recently reported by Kotani *et al.*[12]

All angiograms were evaluated by Y.M., blinded to genotype data. Study patients were categorized into three groups: (1) normal/minimal stenosis, ≤25% stenosis (n = 128); (2) mild stenosis, 26–50% stenosis (n = 39); and (3) significant stenosis, >50% stenosis (n = 419), in any one major coronary artery. Myocardial infarction was confirmed by the documentation of coronary stenosis plus either elevations of cardiac enzymes or diagnostic changes on electrocardiograms.

Genotyping

We analyzed the LOX-1 gene polymorphism (a G-to-C base transition at 501) using Invader assay, which combines structure-specific cleavage enzymes and the universal fluorescent resonance energy transfer (FRET) system.[10] After genomic DNA was extracted from blood samples, the region (491 bp) containing the polymorphic site was amplified by polymerase chain reaction (PCR), as previously reported,[9] and PCR products were used for Invader assay according to our protocol.[11] The primary probes (probe 1 for C allele, acggacgcggagcttttcccagttaaatgagc, and probe 2 for G allele, cgcgccgagggttttcccagttaaatgagc) and the Invader probe (tggcatccaaagacaagcacttctcttggctt) were designed using the Invader Creator software package to obtain a theoretical annealing temperature of 63°C and 77°C, respectively. After putting the probes, PCR products, and $MgCl_2$ into the reaction wells of FRET detection plates, they were incubated at 63°C for 30 min. The fluorescent intensities of Fam dye (C allele) and Red dye (G allele) in each well were measured using the Cytoflour 4000 fluorescence plate reader for genotyping. The concordance rate of genotyping between Invader assay and PCR-restriction fragment length polymorphism analysis was 100% for 980 samples in our previous reports.[11,13]

Statistical Analysis

Differences between two groups were evaluated by unpaired *t*-test for continuous variables and by chi-square test for categorical variables. Differences among three or more groups were evaluated by analysis of variance (ANOVA) with Scheffe's test for continuous variables and by chi-square test for categorical variables. Forward stepwise multiple logistic regression analysis was used to identify any association between LOX-1 gene polymorphism and coronary stenosis. A p value of <0.05 was considered statistically significant. Results are presented as mean ± standard deviation (SD).

Results

Table I shows clinical characteristics of the three groups. Compared with patients with normal/minimal stenosis, those with significant stenosis were older, predominantly male, had higher rates of hypertension, hyperlipidemia, diabetes, and smoking, and also had lower HDL cholesterol levels. Regarding LOX-1 gene polymorphism, the percentages of patients who had C/C, C/G, and G/G genotypes were 6, 33, and 61%, respectively. The genotype distribution did not deviate from Hardy-Weinberg equilibrium. Unexpectedly, the percentage of patients who had either C/C or C/G genotypes (LOX-1 gene variants) was lower in patients with significant stenosis than in those with normal/minimal stenosis (36 vs. 49%, $p<0.01$) (Fig. 1). The frequency of C allele was also lower in patients with significant than in those with normal/minimal stenosis (21 vs. 28%, $p<0.025$). Multivariate analysis demonstrated LOX-1 gene variants to be inversely associated with the presence of significant stenosis independent of risk factors (Table

TABLE I Clinical characteristics of the three groups

	Normal/minimal stenosis (n = 128)	Mild stenosis (n = 39)	Significant stenosis (n = 419)	p Value
Age (years)	60 ± 10	65 ± 9	64 ± 9	<0.001
Gender (male) (%)	70 (55)	31 (79)	344 (82)	<0.001
Hypertension (%)	64 (50)	26 (67)	260 (62)	<0.05
Systolic blood pressure (mmHg)	129 ± 17	136 ± 14	134 ± 21	<0.05
Hyperlipidemia (%)	40 (31)	12 (31)	194 (46)	<0.005
Total cholesterol (mg/dl)	201 ± 36	206 ± 38	199 ± 36	NS
HDL cholesterol (mg/dl)	57 ± 16	53 ± 14	48 ± 14	<0.001
Diabetes (%)	23 (18)	6 (15)	143 (34)	<0.001
Smoking (%)	59 (46)	29 (74)	292 (70)	<0.001

Data are presented as mean ± standard deviation or the number (%) of patients.
Abbreviations: HDL = high-density lipoprotein, NS = not significant.

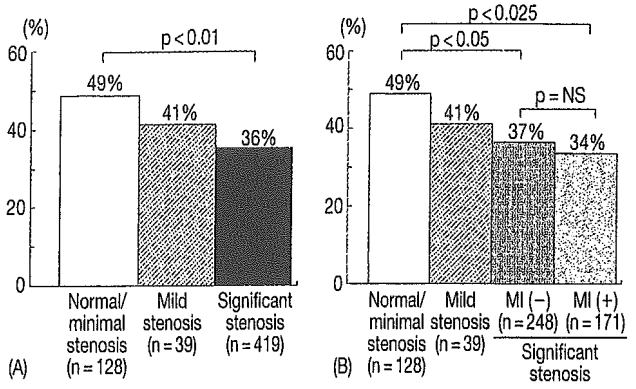

FIG. 1 Frequency of LOX-1 gene variants. The percentage of patients having LOX-1 gene variants was lower in patients with significant stenosis than in those with normal/minimal stenosis (A), but it did not differ between patients with and without myocardial infarction (MI) (B).

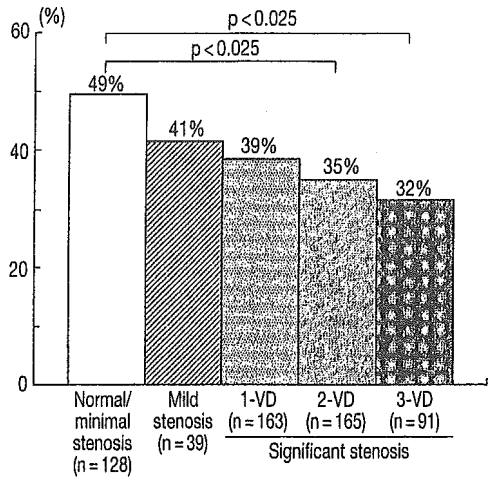

FIG. 2 Association between LOX-1 gene variants and the severity of coronary artery disease. A stepwise decrease in the frequency of LOX-1 gene variants was found depending on the number of >50% stenotic vessels. The lowest frequency of such variants was observed in patients with triple-vessel disease. 1-VD = single-vessel disease, 2-VD = double-vessel disease, 3-VD = triple-vessel disease.

II). The odds ratio for significant stenosis was 0.61 (95% confidence interval [CI] = 0.41–0.92) for LOX-1 gene variants.

Of the 419 patients with significant stenosis, 171 had MI. However, the frequency of LOX-1 gene variants did not differ between patients with and without MI (34 vs. 37%, p = NS) (Fig. 1). To elucidate the association between LOX-1 gene variants and the severity of CAD, 419 patients with significant stenosis were divided into three subgroups by the number of >50% stenotic vessels: 163 with single-vessel, 165 with double-vessel, and 91 with triple-vessel disease. As shown in Figure 2, a stepwise decrease in the percentage of patients with LOX-1 gene variants was found depending on the severity of CAD: 49% in normal/minimal stenosis, 41% in mild stenosis, 39% in single-vessel disease, 35% in double-vessel disease, and 32% in triple-vessel disease.

Table III shows serum MDA-LDL levels in 177 patients, of whom 70 had LOX-1 gene variants. Between patients with and without such variants, LDL cholesterol levels were similar. The MDA-LDL levels (87 ± 33 vs. 91 ± 31 mg/dl) and the ratio of MDA-LDL to LDL cholesterol (0.74 ± 0.24 vs. 0.78 ± 0.26) tended to be lower in patients with LOX-1 gene variants, but these differences did not reach statistical significance.

TABLE II Factors associated with significant stenosis

	Odds ratio	(95% CI)	p Value
Age (per 10 years increase)	1.71	(1.37–2.14)	<0.001
Gender (male)	2.64	(1.57–4.46)	<0.001
Hyperlipidemia	2.38	(1.55–3.65)	<0.001
HDL cholesterol (per 10 mg/dl increase)	0.70	(0.61–0.80)	<0.001
Diabetes	2.12	(1.31–3.44)	<0.005
LOX-1 gene variants	0.61	(0.41–0.92)	<0.02

The dependent variable was the presence of significant stenosis (>50% stenosis). The analysis included age, gender, hypertension, hyperlipidemia, HDL cholesterol, diabetes, smoking, and LOX-1 gene variants.

Abbreviations: LOX-1 = lectin-like oxidized low-density lipoprotein receptor-1, CI = confidence interval, HDL = high-density lipoprotein.

Discussion

We investigated LOX-1 gene polymorphism at 501 in 586 patients undergoing coronary angiography. We found the percentage of patients having LOX-1 gene variants to be low in patients with significant stenosis. The frequency of LOX-1 gene variants decreased as the severity of CAD increased, and the lowest frequency was observed in patients with triple-vessel disease. However, the frequency of the variants did not differ between patients with and without MI. The LOX-1 gene variants were found to be inversely associated with the severity of CAD.

In 2003, Tatsuguchi *et al.*[9] reported higher frequency of LOX-1 gene variants in 102 Japanese patients with MI than in 102 controls (38 vs. 18%). In contrast, our study showed the

TABLE III Serum malondialdehyde-modified low-density lipoprotein (MDA-LDL) levels in patients with and without lectin-like oxidized LDL-receptor-1 gene variants (C/C or C/G)

	C/C or C/G (n=70)	G/G (n=107)	p Value
Age (years)	63 ± 10	64 ± 9	NS
LDL cholesterol (mg/dl)	121 ± 30	121 ± 31	NS
MDA-LDL (mg/dl)	87 ± 33	91 ± 31	NS
MDA-LDL / LDL cholesterol	0.74 ± 0.24	0.78 ± 0.26	NS

Abbreviation: NS = not significant.

frequency of LOX-1 gene variants to be lower in patients with MI (34%) than in those with normal/minimal stenosis (49%). During the preparation of our manuscript, Mango *et al.*[14] reported the frequency of LOX-1 gene variants in 150 Italian patients with MI and 103 controls. They demonstrated the frequency of the variants to be lower in patients with MI than in controls (9 vs. 18%). Mango *et al.* recruited their controls from subjects with at least one risk factor, who were found to have normal coronary arteries on angiograms; their results are compatible with our results. Hence, the differences in the results of Tatsuguchi *et al.* and our studies may be due to differences in the methods of selecting the controls. Tatsuguchi *et al.* used age- and gender-matched apparently healthy subjects without hyperlipidemia as controls. They did not describe whether or not their controls had any stress test or angiography to rule out CAD. In contrast, we studied 586 consecutive patients undergoing angiography, who were divided into three groups by the severity of stenosis, namely, patients with normal/minimal stenosis, those with mild stenosis, and those with significant stenosis. All our study patients were suspected of having CAD, and most of them had some risk factors. However, some patients were found to have significant stenosis, but others did not. In our study, LOX-1 gene variants were a significant factor inversely associated with significant stenosis and were also inversely associated with the severity of CAD. Our results suggest that LOX-1 gene polymorphism may be modifying the severity of CAD in patients at high risk for CAD, such as those undergoing angiography.

The G-to-C transition at 501 of LOX-1 gene results in the Lys-to-Asn change at 167. The amino-acid residue 167 is located at the C-type lectin-like domain in the extracellular portion of LOX-1.[9] The lectin-like domain recognizes ligands, and these basic amino-acid residues are important for strengthening ligand binding.[15, 16] The Lys-to-Asn change causes reduced binding and internalization of oxidized LDL, suggesting that LOX-1 gene variants may exert a protective effect against atherogenesis.[14, 15] As LOX-1 is a receptor for oxidized LDL, this polymorphism may affect the metabolism of oxidized LDL. To examine its effect on oxidized LDL metabolism, we assessed serum MDA-LDL levels. However, MDA-LDL levels tended to be lower in patients with than in those without LOX-1 gene variants, but these differences did not reach statistical significance. Further study is needed to elucidate the functional effects of this polymorphism on LOX-1 activity and the mechanism by which it affects the severity of CAD.

Our study has some limitations. First, it is cross-sectional; such a study cannot establish causality. It shows some association and is hypothesis generating. To elucidate the association between LOX-1 gene polymorphism and CAD, further study in a prospective manner is needed. Second, we did not have healthy controls. We studied patients undergoing angiography, who are generally considered to be a highly selected population at high risk for CAD. This may have caused some selection bias and may have confounded the results. Third, our study was performed in the Japanese population. Our results may not be applicable to other ethnic populations.

Conclusion

The LOX-1 gene variants at 501 were found to be inversely associated with the severity of CAD, suggesting that this polymorphism may be modifying the severity of CAD.

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In vivo magnetic resonance evaluation of associations between aortic atherosclerosis and both risk factors and coronary artery disease in patients referred for coronary angiography

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Background Magnetic resonance imaging was recently reported to detect atherosclerotic plaques in thoracic and abdominal aortas.

Methods Using magnetic resonance imaging, we investigated associations of risk factors and plasma inflammatory markers with plaques in both thoracic and abdominal aortas in 102 patients undergoing coronary angiography. Associations between coronary artery disease (CAD) and aortic plaques were also evaluated.

Results Plaques in thoracic and abdominal aortas were detected in 61% and 90% of patients, respectively. Age and systolic blood pressure correlated with plaque extents in both the aortas. Serum LDL cholesterol level correlated with plaque extent in the thoracic aorta (r_s = 0.42). The degree of smoking correlated with plaque extent in the abdominal aorta (r_s = 0.43). In multivariate analysis, age and systolic blood pressure were associated with plaques in both the aortas. The LDL cholesterol and smoking were characteristically associated with plaques in the thoracic and abdominal aortas, respectively. Regarding inflammatory markers, fibrinogen and C-reactive protein levels correlated with total plaque extent in the aortas (r_s = 0.50 and r_s = 0.51). Compared with 24 patients without CAD, 78 with CAD more often had plaques in the thoracic (71% vs 29%) and abdominal (95% vs 75%) aortas. Although plaque extents in both the aortas correlated with the severity of CAD, only thoracic plaques were independently associated with CAD.

Conclusions The thoracic and abdominal aortas may have different susceptibilities to risk factors. However, plasma inflammatory markers appear to reflect total extent of aortic atherosclerosis. Although aortic plaques are common in patients with CAD, only thoracic plaques are an independent factor for CAD. (Am Heart J 2004;148:137–43.)

Atherosclerotic disease is a major cause of death. The identification of contributing factors to atherosclerosis leads to a better understanding of its pathogenesis and is also fundamental for its prevention. Several autopsy studies have shown some association between conventional risk factors and atherosclerosis in both the thoracic and abdominal aortas.[1-4] However, such autopsy studies may be subject to selection bias. Risk factors were evaluated during life, but atherosclerosis was evaluated by postmortem examination. Recently, we[5] and another group[6] reported that magnetic resonance imaging (MRI) allows us to make a noninvasive evaluation of atherosclerosis in both the thoracic and abdominal aortas. Using MRI, we therefore investigated the association of risk factors and plasma inflammatory markers with atherosclerotic plaques in both the thoracic and abdominal aortas in 102 patients referred for coronary angiography. We also evaluated the association between coronary artery disease (CAD) and plaques in both aortas.

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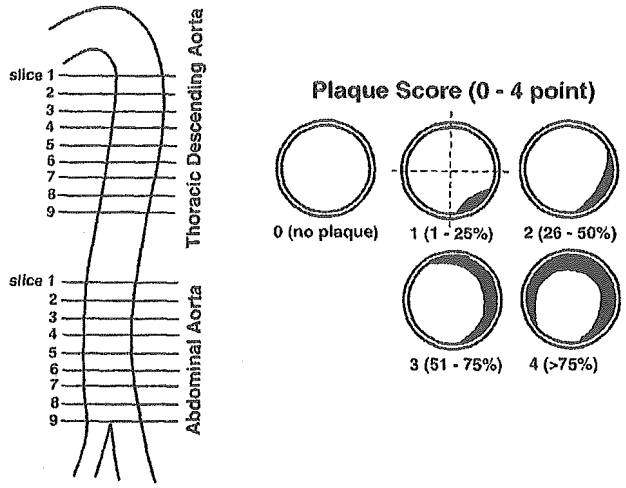
Methods

Patients characteristics and correlation factors

From February 2000 to March 2003, 226 patients were admitted to the National Defense Medical College Hospital to

Figure 1



MRI slices of aorta and plaque scores. In each patient, 9 slices of the thoracic aorta and 9 slices of the abdominal aorta were obtained at 12-mm intervals, which each covered about 10-cm portions of the thoracic aorta below the arch and of the abdominal aorta above the bifurcation. In each slice, plaque extent was scored 0 to 4 point by the percent of the luminal surface involved by a plaque.

undergo elective coronary angiography for suspected CAD. Any patients with acute coronary syndrome or those who had a history of cardiovascular surgery were excluded. Of the 226 patients, 102 (75 men; mean age, 64 ± 8 years; range, 45 to 78 years) gave informed consent to have MRI, and MRI of the aorta was performed at the Iruma Heart Hospital within 2 weeks of angiography. Of the 102 patients, 53 (52%) had hypertension (blood pressures ≥140/90 mm Hg or taking drugs), of whom 40 were taking antihypertensive drugs, and 50 (49%) had hyperlipidemia (total cholesterol level >240 mg/dL or taking drugs), of whom 37 were taking lipid-lowering drugs. Diabetes mellitus (fasting plasma glucose level ≥126 mg/dL or taking hypoglycemic drugs or insulin) was present in 25 (25%) patients, and 64 (63%) patients were smokers (≥10 packs per day times years smoked). Blood pressures were measured in the sitting position on the day of admission, and the pulse pressure, which is the difference between the systolic and diastolic blood pressures, was calculated. Blood samples were taken in a fasting state on the morning of the day when angiography was performed. Serum lipid levels were measured by standard laboratory methods. Plasma fibrinogen and high-sensitivity C-reactive protein (CRP) levels were measured by the thrombin time method (Dade Behring, Liederbach, Germany) and by nephelometry (Dade Behring), respectively. Since lipid-lowering drugs affect lipid and CRP levels,[7] correlations between such levels and aortic plaques were evaluated in only 60 patients who had no lipid-lowering drugs.

On coronary angiograms, CAD (>50% luminar diameter stenosis) was found in 78 (76%) patients, of whom 30 had 1-vessel disease, 32 had 2-vessel disease, and 16 had 3-vessel disease. Of the 78 patients with CAD, 63 (81%), 37 (47%), and 42 (54%) had >50% stenosis in the left anterior descending artery, circumflex artery, and right coronary artery, respectively. However, 24 patients had a history of percutaneous coronary intervention more than 6 months ago.

MRI of aorta

MRI was performed on the Signa 1.5-T CVI scanner (GE Medical Systems, Mount Prospect, Ill), using a commercially available phased-array body coil. Since the T_2-weighted image is the most useful image for plaque assessment with a high contrast-to-noise ratio,[8,9] the transverse T_2-weighted images of the thoracic descending and abdominal aortas were obtained with an ECG-gated, breath-hold, double-inversion-recovery fast spin-echo sequence, as we previously reported.[5] The imaging parameters were TR = 2 R-R intervals, TE = 60 ms, 20-cm FOV, 4-mm slice thickness, 8-mm interslice gap, 256 × 256 acquisition matrix, and 32 echo-train. In each patient, 9 slices of the thoracic aorta and 9 slices of the abdominal aorta were obtained at 12-mm intervals, which each covered about 10-cm portions of the thoracic aorta below the arch and of the abdominal aorta above the bifurcation of the common iliac artery (Figure 1).

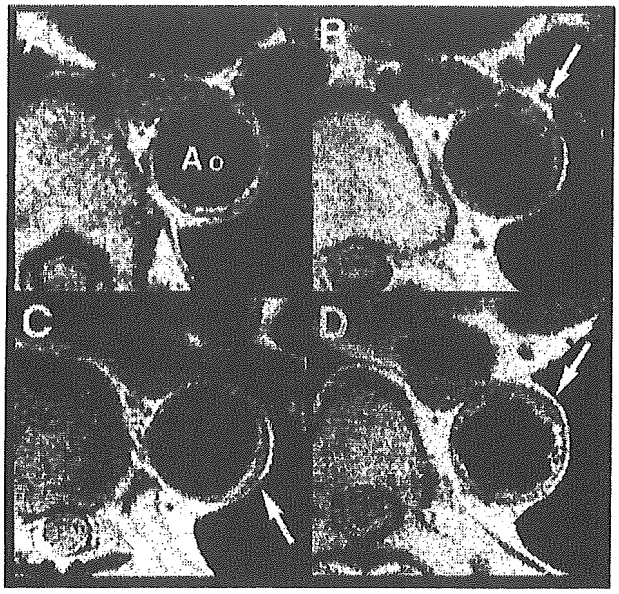
Atherosclerotic plaque analysis

For each patient, we assessed the presence and extent of plaque in 9 slices of the thoracic descending aorta and 9 slices of the abdominal aorta. The plaque extent in each slice was scored from 0 to 4 points by the percentage of the luminal surface involved by a plaque: 0 (no plaque), 1 (1% to 25%), 2 (26% to 50%), 3 (51% to 75%), and 4 points (>75%) (Figures 1 and 2). The plaque extents in the thoracic and abdominal aortas were evaluated as the number of slices with plaques (plaque slice number) and the sum of the scores of the 9 slices (plaque extent score). The plaque extents were all evaluated by 2 cardiologists, and any discrepancy was resolved by consensus. The intra-observer and interobserver agreement for the assessment of plaque extents were evaluated in 20 patients (360 slices), and they were 98% (κ value = 0.96) and 92% (κ value = 0.86) of slices, respectively.

Statistical analysis

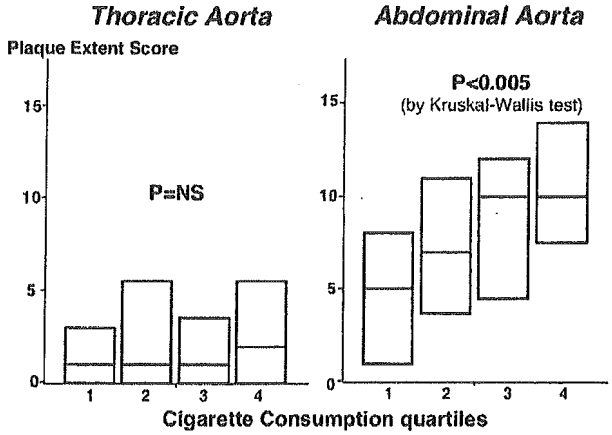
Any differences between 2 groups were evaluated by the unpaired t test for parametric variables, by the Mann-Whitney U test for nonparametric variables, and by the χ^2 test for categoric variables. Any differences among 3 or more groups were evaluated by analysis of variance (ANOVA) with Scheffé test for parametric variables, by the Kruskal-Wallis rank test for nonparametric variables, and by the χ^2 test for categoric variables. Since the distributions of the measured plaque extents were highly skewed, correlations of plaque extents with risk factors and inflammatory markers were evaluated by the Spearman rank correlation test. Forward stepwise multiple logistic regression analysis was also used to elucidate associations of aortic plaques with risk factors and CAD. A P value of <.05 was considered to be statistically significant. Results are presented as the mean value ± SD or the median value.

Figure 2



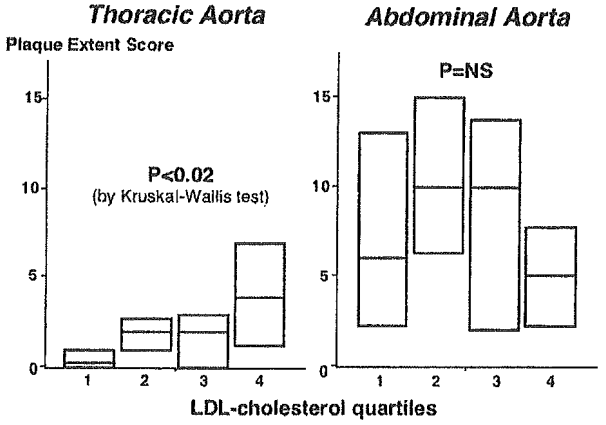
T2-weighted images of the thoracic aorta. **A,** No plaque; **B,** plaque of score = 1 point; **C,** 2 points; and **D,** 4 points. *Arrows* indicate plaques.

Figure 3



Plaque extents in thoracic and abdominal aortas based on quartiles of cigarette consumption. Quartile ranges for cigarette consumption (packs per day times years smoked) were 0, 5 to 34, 35 to 45, and 50 to 150 pack-years. Median plaque extent score in the abdominal aorta increased with higher quartiles of cigarette consumption. *Central line* represents median value; *boxes* span from 25th to 75th percentiles.

Figure 4

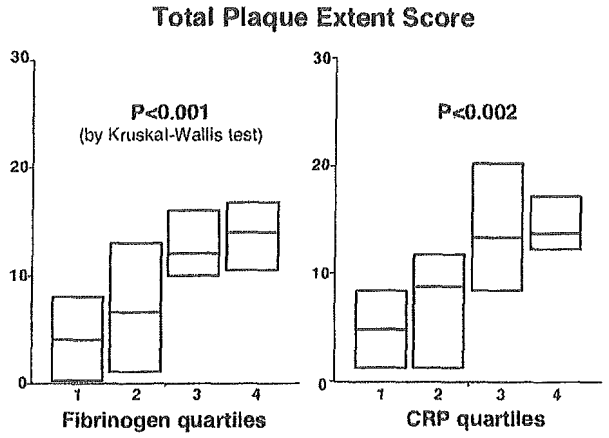


Plaque extents in thoracic and abdominal aortas based on the quartiles of serum LDL-C level. Quartile ranges for LDL-C level were 74 to 114, 115 to 133, 135 to 150, and 151 to 249 mg/dL. Median plaque extent score in the thoracic aorta increased with higher quartiles of LDL-C level. *Central line* represents median value; *boxes* span from 25th to 75th percentiles.

Results

Plaques in the thoracic descending aorta and the abdominal aorta were detected by MRI in 62 (61%) and 92 (90%) patients, respectively. Age correlated with the plaque slice numbers and plaque extent scores in both the thoracic (r_s = 0.34 and r_s = 0.35 by the Spearman rank correlation test) and abdominal (r_s = 0.26 and r_s = 0.26) aortas ($P < .01$). Systolic blood pressure also correlated with the plaque slice numbers and extent scores in the thoracic (r_s = 0.43 and r_s = 0.43) and abdominal (r_s = 0.30 and r_s = 0.30) aortas ($P < .005$). Notably, the degree of smoking (pack-years) correlated with the plaque slice number and extent score in the abdominal aorta (r_s = 0.36 and r_s = 0.43, $P < .001$), but it did not correlate with those in the thoracic aorta. Moreover, as shown in Figure 3, the median plaque extent score in the abdominal aorta was found to increase with higher quartiles of cigarette consumption. In contrast, the serum total cholesterol level correlated with the plaque slice number and extent score in the thoracic aorta (r_s = 0.41 and r_s = 0.42, $P < .002$) but not in the abdominal aorta. The LDL cholesterol (LDL-C) level also correlated with the plaque slice number and extent score only in the thoracic aorta (r_s = 0.39 and r_s = 0.42, $P < .005$). As shown in Figure 4, the median plaque extent score in the thoracic aorta was found to increase with higher quartiles of the LDL-C level. The HDL-C level correlated inversely with the plaque slice numbers and extent scores in the thoracic (r_s = −0.23 and r_s = −0.22) and abdominal (r_s = −0.38 and r_s = −0.39) aortas ($P < .05$).

Figure 5



Total aortic plaque extent based on quartiles of plasma fibrinogen and CRP levels. Quartile ranges for fibrinogen level were 180 to 231, 235 to 268, 275 to 320, and 321 to 469 mg/dL. Quartile ranges for CRP level were 0 to 0.02, 0.03 to 0.06, 0.07 to 0.15, and 0.18 to 0.61 mg/dL. Median total plaque extent score increased with higher quartiles of fibrinogen and CRP levels. *Central line* represents median value; *boxes* span from 25th to 75th percentiles.

Plaques were more prevalent in the abdominal aorta (45% of slices) than in the thoracic aorta (21% of slices) ($P < .001$). However, of the 92 patients with plaques, 13 (14%) had more plaques in the thoracic aorta than in the abdominal aorta and had a higher LDL-C level (164 ± 35 vs 123 ± 26 mg/dL $P < .001$) compared with 79 patients with more plaques in the abdominal aorta than in the thoracic aorta. Of the 13 patients with more plaques in the thoracic aorta, 10 (77%) had an LDL-C level of >150 mg/dL, whereas only 10 of the 79 patients (13%) with more plaques in the abdominal aorta had such a level ($P < .001$).

Clinical variables (age, sex, blood pressures, LDL-C and HDL-C levels, smoking, and diabetes) were entered into a multivariate logistic regression model to test their independent associations with the aortic plaques. The multivariate analysis in the 102 patients revealed that age and systolic blood pressure were independent factors associated with plaques in both the thoracic and abdominal aortas. However, the LDL-C level and smoking were found to be characteristically associated with plaques in the thoracic aorta and the abdominal aorta, respectively. The HDL-C level was also a factor associated with only plaques in the thoracic aorta.

Regarding plasma inflammatory markers, the fibrinogen level correlated with the plaque slice numbers and plaque extent scores in the thoracic ($r_s = 0.50$ and $r_s = 0.47$) and abdominal ($r_s = 0.39$ and $r_s = 0.42$) aortas ($P < .005$). The CRP level also correlated with the plaque slice numbers and extent scores in the thoracic ($r_s = 0.42$ and $r_s = 0.39$) and abdominal ($r_s = 0.46$ and $r_s = 0.46$) aortas ($P < .005$). However, the total plaque slice number (total number of slices with plaques in both the thoracic and abdominal aortas) and the total plaque extent score (the sum of the scores of both the aortas) correlated better with the fibrinogen ($r_s = 0.52$ and $r_s = 0.50$, $P < .001$) and CRP ($r_s = 0.57$ and $r_s = 0.51$, $P < .001$) levels. As shown in Figure 5, the median total plaque extent score was found to increase with higher quartiles of the fibrinogen and CRP levels.

Table I. Clinical characteristics in patients with and without CAD

	CAD(+) (n = 78)	CAD(−) (n = 24)	P
Age (y)	65 ± 8	63 ± 10	NS
Sex (male)	61 (78)	15 (63)	NS
Hypertension	42 (54)	11 (46)	NS
Systolic BP (mm Hg)	135 ± 21	128 ± 17	NS
Pulse BP (mm Hg)	60 ± 14	51 ± 11	<.005
Hyperlipidemia	41 (53)	9 (38)	NS
Total cholesterol (mg/dL)	207 ± 36	206 ± 29	NS
LDL-C (mg/dL)	129 ± 32	126 ± 29	NS
HDL-C (mg/dL)	47 ± 11	59 ± 13	<.001
Diabetes mellitus	21 (27)	4 (17)	NS
Smoking	53 (68)	11 (46)	NS
MRI of aorta			
Plaques in thoracic aorta	55 (71)	7 (29)	<.001
Plaques in abdominal aorta	74 (95)	18 (75)	<.025

Data are presented as the mean value ± SD or the number (%) of patients.
BP, Blood pressure.

Of the 102 patients, 78 had CAD. Compared with the 24 patients without CAD, the 78 with CAD more often had plaques in the thoracic (71% vs 29%) and abdominal (95% vs 75%) aortas ($P < .025$) (Table I). To clarify the associations between the aortic plaques and the severity of CAD, the 102 patients were divided into 4 groups by the number of >50% stenotic coronary vessels. As shown in Table II, stepwise increases in the prevalence and extents of plaques in both the thoracic and abdominal aortas were found depending on the number of stenotic coronary vessels. However, a multivariate analysis revealed that the thoracic aortic plaques were a significant factor associated with CAD (odds ratio = 4.1; 95% CI, 1.4 to 11.9) independent of risk factors, but the abdominal aortic plaques were not.

Discussion

Transesophageal echocardiography (TEE) is often used to evaluate atherosclerosis in the thoracic aorta.

Some studies used TEE to evaluate any associations between risk factors and atherosclerosis in the thoracic aorta.[10,11] TEE provides high-resolution images of the thoracic aorta. However, TEE is not a noninvasive tool and can assess only a small portion of the abdominal aorta. Computed tomography (CT) may be useful for the detection of protruding plaques in both the thoracic and abdominal aortas.[12] Using CT, Takasu et al[13] investigated the association between risk factors and plaques in both the aortas. However, CT requires the exposure to ionizing radiation and usually needs the injection of a contrast agent for vascular imaging.

Recently, MRI became a useful tool for noninvasively detecting plaques in both the thoracic and abdominal aortas.[5,6] Regarding this MRI method, we[14-16] and another group[17] showed good correlations for the aortic plaque extent between the in vivo MRI findings and the histopathologic findings in rabbits and between the ex vivo MRI findings and the histopathologic findings in a porcine model. In human beings, we reported that MRI evaluations of the thoracic aorta closely correlated with the TEE findings regarding the plaque extent.[5] Using this MRI method, the current study showed the associations of risk factors, plasma inflammatory markers, and CAD with plaques in the thoracic and abdominal aortas. Plaques in both the thoracic and abdominal aortas were associated with age and systolic blood pressure, but plaques in the thoracic aorta and the abdominal aorta were found to be characteristically associated with hyperlipidemia and smoking, respectively. Plasma inflammatory markers appear to reflect the total extent of aortic plaques.

In the current study, age and systolic blood pressure were associated with plaques in both the thoracic and abdominal aortas. These results were compatible with those in autopsy studies.[3,4,18] Such associations were reported in vivo in the thoracic aorta with the use of TEE [11,19,20] and in the abdominal aorta with the use of conventional ultrasonography.[21,22] Takasu et al[13] also reported age and systolic blood pressure to be factors associated with plaques in both the aortas by using CT.

Regarding hyperlipidemia, Tribouilloy et al[23] reported an association of the serum LDL-C level with thoracic aortic plaques using TEE. In contrast, Giral et al[21] showed no association between the LDL-C level and abdominal aortic plaques by ultrasonography. Takasu et al[13] reported the total cholesterol level to be weakly related to plaques in both the thoracic and abdominal aortas by using CT. However, they did not measure the LDL-C level and assessed only the 2- to 5-cm portion of the middle thoracic aorta and the infrarenal portion of the abdominal aorta. Our study showed the LDL-C level to correlate with the plaque extent in the thoracic aorta but not in the abdominal aorta. In a multivariate analysis, the LDL-C level was found to be an associating factor for only thoracic aortic plaques. The HDL-C level was also a factor for thoracic aortic plaques.

Table II. Associations between the severity of CAD and the extents of plaques in the thoracic and abdominal aortas

	CAD(−) (n = 24)	1VD (n = 30)	2VD (n = 32)	3VD (n = 16)	P
Age (y)	63 ± 10	63 ± 9	65 ± 8	68 ± 7	NS
Sex (male)	15 (63)	23 (77)	26 (81)	12 (75)	NS
Thoracic aorta					
Plaque(+)	7 (29)	19 (63)	22 (69)	14 (88)	<.005
Plaque slice number	0.0	1.0	1.0	2.0	<.05
Plaque extent score	0.0	1.0	1.5	2.5	<.05
Abdominal aorta					
Plaque(+)	18 (75)	28 (93)	30 (94)	16 (100)	<.05
Plaque slice number	2.0	5.0	4.0	5.0	<.01
Plaque extent score	3.5	8.0	8.0	9.0	<.005

Data are presented as the mean value ± SD or the number (%) of patients. Plaque slice number and plaque extent score are presented as the median value. *1VD*, 1-Vessel disease; *2VD*, 2-vessel disease; *3VD*, 3-vessel disease.

Plaques were more prevalent in the abdominal aorta than in the thoracic aorta, as previously shown in autopsy studies.[1,18] It was interesting to note that patients with more plaques in the thoracic aorta than in the abdominal aorta characteristically had a much higher LDL-C level compared with patients with more plaques in the abdominal aorta than in the thoracic aorta. An autopsy study also reported that patients with type II hyperlipidemia had more severe plaques in the thoracic aorta than in the abdominal aorta.[18] The thoracic aorta may therefore tend to have a high susceptibility to plaque formation associated with hyperlipidemia.

An autopsy study[3] reported that smoking was associated with plaques in both the aortas, but other autopsy studies[2,4] showed that smoking was more strongly associated with plaques in the abdominal aorta than in the thoracic aorta. Giral et al[21] reported in vivo studies that smoking was associated with abdominal aortic plaques by ultrasonography, whereas Tribouilloy et al[11] reported an association between smoking and thoracic aortic plaques by TEE. Takasu et al[13] reported no association between the degree of smoking (cigarettes per day) and plaques in the thoracic or abdominal aortas by CT. However, we demonstrated the degree of smoking (packs per day times years smoked) to correlate with the plaque extent in the abdominal aorta but not in the thoracic aorta and to be a significant factor for only abdominal aortic plaques. In contrast to hyperlipidemia, the abdominal aorta may have a high susceptibility to plaque formation associated with smoking.

Plaques in the thoracic aorta and the abdominal aorta were characteristically associated with hyperlip-

idemia and smoking, respectively. The thoracic and abdominal aortas are thus suggested to have different susceptibilities to risk factors. The mechanism of different susceptibilities has not yet been clarified, but some differences in their structures have been reported. The abdominal aorta tapers geometrically and has higher blood pressures than the thoracic aorta.[24] The abdominal aorta is also stiffer, with less elastin and more collagen.[24] Moreover, vasa vasorum is common in the thoracic aorta but rare in the abdominal aorta, thus suggesting that the oxygen and nourishment of the abdominal aorta comes mainly by diffusion from the aortic lumen.[25] These may be the reasons why the abdominal aorta has more plaques than the thoracic aorta while also being more susceptible to plaque formation associated with smoking. An autopsy study reported that fatty streaks were more common in the thoracic aorta, especially in areas with high shearing strain on the aortic wall, than in the abdominal aorta.[26] Areas just distal to the ostia of the intercostal arteries were commonly spared by fatty streaks. The thoracic aorta was also shown to have more atheromatous lesions with more cholesterol accumulation than the abdominal aorta in rabbits fed by a cholesterol diet.[27] Plaque formation associated with hyperlipidemia may thus be associated with high shearing strain in the thoracic aorta.

Inflammation has also been suggested to be involved in the pathogenesis of atherosclerosis.[28] Tribouilloy et al[29] reported that the extent of thoracic aortic plaques detected by TEE correlated with the plasma fibrinogen level. Levenson et al[22] also evaluated the prevalence of plaques at 3 arterial sites (carotid, femoral arteries, and abdominal aorta) by ultrasonography and showed the extent of atherosclerosis (1, 2, or 3 sites) to be related to the fibrinogen level. Wang et al[30] reported the degree of carotid atherosclerosis assessed by ultrasonography to be associated with the CRP level. They also reported the extent of subclinical coronary calcification detected by electron-beam CT to correlate with the CRP level.[31] In our study, the plasma fibrinogen and CRP levels correlated with plaque extents in the thoracic and abdominal aortas. Of note was that the fibrinogen and CRP levels correlated better with the total plaque extent in the thoracic and abdominal aortas. These findings suggest that plasma inflammatory markers appear to reflect the total extent of aortic atherosclerosis rather than the extent of atherosclerosis in either the thoracic or abdominal aortas.

Some studies reported an association between CAD and thoracic aortic plaques through the use of TEE.[19,32,33] The number of stenotic coronary vessels was also shown to be associated with thoracic aortic plaques.[32] Regarding abdominal aortic plaques, an autopsy study reported that plaques in the abdominal aorta were more severe in patients with cardiac catastrophe than in those without it.[34] Using CT, Takasu et al[13] reported plaques in both the thoracic and abdominal aortas to be associated with CAD. However, they also documented that plaques in the thoracic aorta were more closely associated with CAD than those in the abdominal aorta. Our study showed that there were stepwise increases in the prevalence and extents of plaques in both the thoracic and abdominal aortas as the severity of CAD increased. However, the thoracic aortic plaques were found to be an independent factor associated with CAD, but the abdominal aortic plaques were not.

Study limitations

First, our study was performed in a relatively small number of Japanese patients referred for coronary angiography. Because of the highly selected study population and Japanese ethnicity, our results may not be applicable to the general population and other ethnic groups. Moreover, our patients were predominantly male (75%). To elucidate sex differences, further study in a larger population is needed. Second, of the 102 patients, 40 (39%) and 37 (36%) were taking antihypertensive and lipid-lowering drugs, respectively. Such treatment may have caused some bias for our results.

Conclusions

Plaques in the thoracic aorta and abdominal aorta were found to be characteristically associated with hyperlipidemia and smoking, respectively. Our in vivo MRI study suggests that even the thoracic and abdominal aortas may have different susceptibilities to atherosclerotic risk factors. However, plasma inflammatory markers appear to reflect the total extent of aortic atherosclerosis. Although plaque extents in both the aortas correlated with the severity of CAD, only thoracic aortic plaques were an independent factor for CAD.

In patients with atherosclerotic risk factors, the detection of atherosclerosis is important to prevent its development and progression. Because patients have various risk factors and because different vascular beds may have different susceptibilities to risk factors, it appears to be preferable to evaluate atherosclerosis in multiple vascular beds than in just one bed. The use of MRI may be helpful for evaluating the degree of atherosclerosis in multiple vascular beds in the same examination session, thereby determining the degree of the systemic atherosclerotic involvement more accurately.

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Association of *Mycoplasma pneumoniae* infection with coronary artery disease and its interaction with chlamydial infection

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Abstract

Mycoplasma pneumoniae (MP) seropositivity was reported to be associated with coronary events. MP organisms were detected with *Chlamydia pneumoniae* (CP) in coronary plaques. We investigated MP and CP seropositivity in 549 patients undergoing coronary angiography. Coronary artery disease (CAD) was found in 396 patients, of whom 154 had myocardial infarction (MI). MP seropositivity was more prevalent in patients with CAD than without CAD (14% versus 6%, $P < 0.01$). The highest prevalence was found in patients with MI. In contrast, the prevalence of CP seropositivity was similar in patients with and without CAD (62% versus 59%). To clarify interaction with CP infection, 549 patients were divided into two groups with and without CP seropositivity. Among patients with CP seropositivity, MP seropositivity was more prevalent in patients with CAD than without CAD (17% versus 5%, $P < 0.01$), whereas among patients without CP seropositivity, MP seropositivity did not differ between patients with and without CAD (9% versus 6%). In multivariate analysis, MP seropositivity was associated with CAD only in patients with CP seropositivity (odds ratio = 5.1, 95% CI = 1.8–14.9). Thus, MP seropositivity was associated with CAD. However, this association was confined to patients with CP seropositivity. Coinfection by MP and CP may be an important cofactor for CAD.

Keywords: *Mycoplasma*; *Chlamydia*; Infection; Coronary artery disease

1. Introduction

Inflammation has been recognized to be involved in the pathogenesis of atherosclerosis [1]. Infectious agents may play a role in the development of coronary artery disease (CAD). *Chlamydia pneumoniae* (CP), one of the human common respiratory pathogens, has often been reported to be associated with CAD in seroepidemiological studies [2,3]. CP organisms were detected within atheroma [4–6]. However, the potential contribution of CP infection to CAD remains unclear. Two prospective studies [7,8] failed to show any association between CP infection and CAD.

Recently, one prospective study [9] has shown that CP seropositivity was associated with myocardial infarction (MI) or coronary death. They also reported *Mycoplasma pneumoniae* (MP) seropositivity to be associated with such events [9]. MP is another common respiratory pathogen. MP and CP show similar epidemiological behaviors and antibiotic susceptibility [10], but one characteristic of MP is that it requires cholesterol for its survival. Interestingly, MP organisms were recently reported to be detected in the lipid cores of coronary plaques, together with CP organisms [11,12]. Our study analyzed the association between MP seropositivity and the presence of CAD, and also a possible relationship between MP seropositivity and CP seropositivity or biochemical lipid data.

2. Methods

2.1. Study patients

We investigated the prevalence of MP and CP seropositivity in 549 consecutive patients (mean age 63 ± 9 years,

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range 35 to 84 years), who underwent coronary angiography for suspected CAD at National Defense Medical College Hospital from 1999 to 2002. These results were compared with clinical and angiographic data. Of the 549 patients, 274 (50%) had hypertension (blood pressures ≥160/95 mmHg or on drugs), 230 (42%) had hyperlipidemia (total cholesterol level ≥240 mg/dl or on drugs), 124 (23%) had diabetes mellitus (fasting glucose level ≥126 mg/dl or on insulin or hypoglycemic drugs), and 259 (47%) were smokers (≥4 cigarettes/day). Our study was approved by the ethics committee of our hospital. After admission, written informed consent was obtained, and blood samples were taken in a fasting state in the morning on the day of angiography. All the coronary angiograms were evaluated by Y. Momiyama, blinded to the serology data. CAD was defined as at least one coronary artery having >50% luminar diameter stenosis on angiograms. MI was confirmed by the documentation of coronary artery stenosis plus either elevations of cardiac enzymes or diagnostic changes on electrocardiograms. Any patient who had a history of coronary artery bypass grafting was excluded.

2.2. MP and CP serology

Serum MP-specific antibody titer was measured using a complement fixation test by SRL Co., Japan. This assay used MP glycolipid hapten as an antigen, and the measured titer is considered to mainly reflect IgG antibody. The titer of ≥1/4 was considered to be seropositive according to the manufacturer's instruction. Serum CP-specific IgG titer was measured using an enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (HITAZYME CP TM, Hitachi Chemical, Japan) by R. Ohmori. This assay used the CP outer membrane complex as a CP-specific antigen and showed the high agreement rate (90%) for CP IgG seropositivity with the micro-immunofluorescence test [13]. The cut-off index of ≥1.10 was considered to be seropositive.

2.3. Statistics

Any differences among the groups of patients were evaluated by unpaired *t*-test and analysis of variance with Scheffe's test for continuous variables and by chi-square test for categorical variables. Stepwise multiple logistic regression analysis was used to elucidate the association between MP seropositivity and CAD. A *P*-value of <0.05 was considered to be statistically significant. Results are presented as the mean value ± S.D.

3. Results

Of the 549 patients, 396 (72%) were found to have CAD, of whom 154 had MI. The diagnosis of acute or old MI was given to 101 or 53 patients, respectively. Compared to 153 patients without CAD, 396 with CAD were older, predominantly male, and had higher rates of hypertension, hyperlipidemia, diabetes, and smoking (Table 1). MP antibody titer of ≥1/4 tended to be more prevalent in patients with CAD than in those without CAD (29% versus 21%, P = NS). Of note was that MP antibody titers of ≥1/8 and ≥1/16 were more

Table 1
Clinical characteristics and the prevalence of MP and CP seropositivity in patients with and without CAD

	CAD (−)(n = 153)	CAD (−) vs. (+)	CAD (+)			
			n = 396	MI (−) (n = 242)	MI (−) vs. (+)	MI (+) (n = 154)
Age (years)	61 ± 10	<0.001	64 ± 9	65 ± 8	<0.02	63 ± 10
Gender (male)	101 (66%)	<0.001	323 (82%)	186 (77%)	<0.005	137 (89%)
Hypertension	57 (37%)	<0.001	217 (55%)	145 (60%)	<0.025	72 (47%)
Systolic BP (mmHg)	131 ± 17	NS	135 ± 21	138 ± 20	<0.001	130 ± 22
Hyperlipidemia	51 (33%)	<0.025	179 (45%)	126 (52%)	<0.001	53 (34%)
TC (mg/dl)	204 ± 37	NS	202 ± 36	206 ± 35	<0.005	195 ± 35
HDL-C (mg/dl)	57 ± 16	<0.001	48 ± 14	50 ± 15	<0.05	47 ± 12
Diabetes	20 (13%)	<0.005	104 (26%)	72 (30%)	NS	32 (21%)
Smoking	58 (38%)	<0.01	201 (51%)	115 (48%)	NS	86 (56%)
MP seropositivity						
≥1/4	32 (21%)	NS	115 (29%)	54 (22%)	<0.001	61 (40%)
≥1/8	9 (6%)	<0.01	57 (14%)	28 (12%)	NS	29 (19%)
≥1/16	2 (1%)	<0.05	24 (6%)	11 (5%)	NS	13 (8%)
CP seropositivity						
≥1.10	91 (59%)	NS	246 (62%)	152 (63%)	NS	94 (61%)

Data are presented as the mean value ± S.D. or the number (%) of patients. BP, blood pressure; TC, total cholesterol; HDL-C, high density lipoprotein-cholesterol.

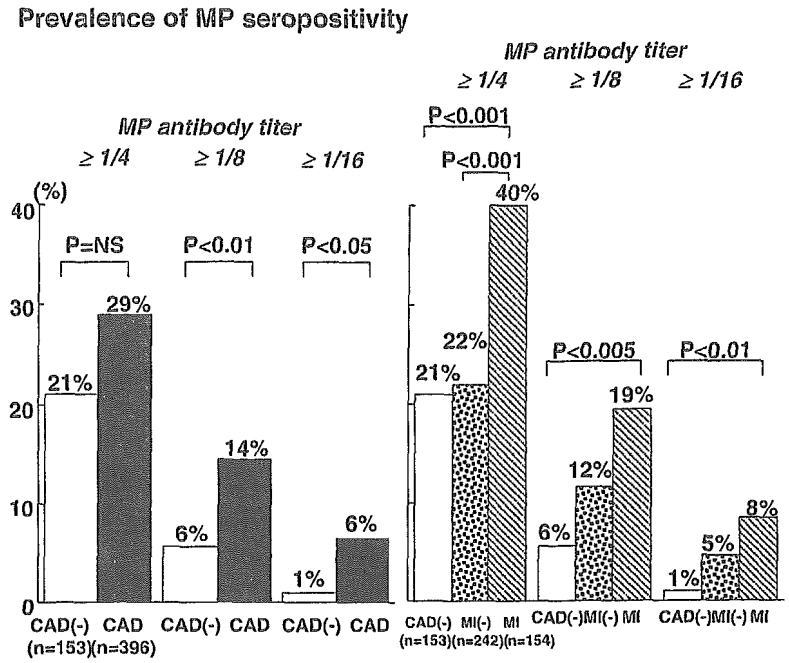

Fig. 1. Prevalence of MP seropositivity in patients with and without CAD. MP antibody titers of ≥1/16 and ≥1/8 were more prevalent in patients with CAD than in those without CAD. Among the patients without CAD, CAD patients without MI, and those with MI, MP seropositivity was the most prevalent in patients with MI.

prevalent in patients with CAD than without CAD (14% and 6% versus 6% and 1%, $P < 0.05$) (Fig. 1, left). Among the three groups of patients without CAD, CAD patients without MI, and those with MI, MP seropositivity was most prevalent in patients with MI (Fig. 1, right). The prevalence of MP antibody titers of ≥1/4, ≥1/8, and ≥1/16 was 40%, 19%, and 8% in patients with MI, 22%, 12%, and 5% in CAD patients without MI, and 21%, 6%, and 1% in patients without CAD, respectively. Compared to patients without CAD, those with MI more often had MP seropositivity ($P < 0.01$). Regarding CP seropositivity, its prevalence did not significantly differ between patients with and without CAD (62% versus 59%) (Table 1). In multivariate analysis, MP seropositivity (titer ≥1/8) was associated with the presence of CAD (odds ratio = 2.7, 95%CI = 1.2–5.9, $P < 0.02$) independent of conventional risk factors, but CP seropositivity was not (Table 2). For MP antibody titer of ≥1/16, the odds ratio was 4.6 (95%CI = 1.1–20.7, $P < 0.05$) for the presence of CAD.

The percentage of patients with combined MP seropositivity (antibody titer ≥1/8) and CP seropositivity was higher in patients with CAD than in those without CAD (11% versus 3%, $P < 0.01$). Moreover, this percentage was higher in CAD patients with MI and those without MI than in patients without CAD (14% and 9% versus 3%, $P < 0.01$). In contrast, the percentage of patients with combined MP seropositivity and hyperlipidemia did not differ between patients with and without CAD (5% versus 3%, P = NS). Moreover, this percentage was similar in the three groups of CAD patients with MI, those without MI, and patients without CAD (5%, 5%, and 3%; P = NS). To clarify the interaction between MP and CP infection, the 549 patients were divided into two groups: 337 with CP seropositivity and 212 without it. Among the patients with CP seropositivity, MP seropositivity (≥1/8) was more prevalent in patients with CAD than in those without CAD (17% versus 5%, $P < 0.01$) (Fig. 2, left). Moreover, it was much more prevalent in patients with MI than in those without CAD (22% versus 5%, $P < 0.005$) (Fig. 2, right). In contrast, among the patients without CP seropositivity, the prevalence of MP seropositivity did not differ between patients with and without CAD (9% versus 6%, P = NS). Moreover, no significant difference in MP seropositivity was

Table 2
Factors associated with the presence of CAD (multiple logistic regression analysis in 549 patients)

	Odds ratio	(95% CI)	P-value
Age (years)	1.06	1.04–1.09	<0.001
Gender (male)	1.85	1.11–3.09	<0.02
Hypertension	1.90	1.23–2.93	<0.005
Hyperlipidemia	2.07	1.33–3.21	<0.002
HDL-cholesterol (mg/dl)	0.96	0.95–0.98	<0.001
Diabetes	2.03	1.16–3.57	<0.02
Smoking	1.65	1.04–2.61	<0.05
MP seropositivity (≥1/8)	2.68	1.20–5.94	<0.02
CP seropositivity	0.79	0.51–1.23	NS

The dependent variable was the presence of CAD. The analysis included age, gender, hypertension, hyperlipidemia, HDL-cholesterol, diabetes, smoking, MP seropositivity (titer ≥1/8), and CP IgG seropositivity.

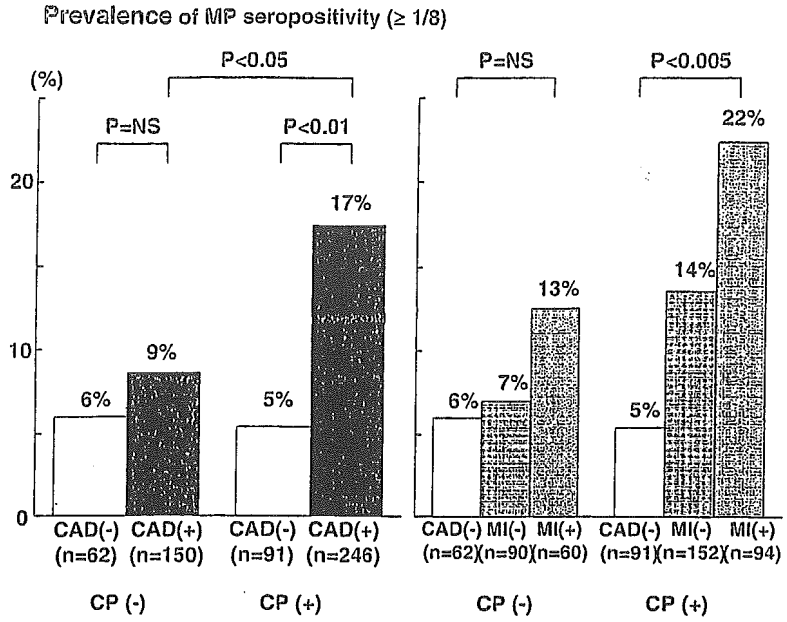

Fig. 2. Prevalence of MP seropositivity in patients with and without CP seropositivity. Among the patients with CP seropositivity, MP seropositivity wa more prevalent in patients with CAD than without CAD. In contrast, among the patients without CP seropositivity, the prevalence of MP seropositivit did not differ between patients with and without CAD. Compared to CAD patients without CP seropositivity, those with CP seropositivity more ofte had MP seropositivity.

found among the three groups of CAD patients with MI, those without MI, and patients without CAD (13%, 7%, and 6%; P = NS). Compared to CAD patients without CP seropositivity, CAD patients with CP seropositivity were found to more often have MP seropositivity (17% versus 9%, $P < 0.05$) (Fig. 2, left). In multivariate analysis, MP seropositivity (titer ≥1/8) was found to be associated with the presence of CAD only in patients with CP seropositivity (odds ratio = 5.1, 95%CI = 1.8–14.9) (Table 3). The association between MP titer of ≥1/16 and CAD in patients with CP seropositivity did not reach statistical significance.

Table 3
Factors associated with the presence of CAD (multiple logistic regression analysis in 337 patients with CP seropositivity and 212 without CP seropositivity)

	Odds ratio	(95% CI)	P-value
CP seropositivity (+) (n = 337)			
Age (years)	1.05	1.02–1.08	<0.001
Hyperlipidemia	2.38	1.32–4.29	<0.005
HDL-cholesterol (mg/dl)	0.94	0.92–0.96	<0.001
Diabetes	2.75	1.34–5.63	<0.01
MP seropositivity (≥1/8)	5.15	1.77–14.93	<0.005
CP seropositivity (−) (n = 212)			
Age (years)	1.09	1.05–1.14	<0.001
Gender (male)	3.37	1.63–6.70	<0.002
HDL-cholesterol (mg/dl)	0.97	0.95–0.99	<0.02

The dependent variable was the presence of CAD. The analysis included age, gender, hypertension, hyperlipidemia, HDL-cholesterol, diabetes, smoking, and MP seropositivity (titer ≥1/8).

4. Discussion

The present study investigated MP and CP seropositivity in 549 patients undergoing coronary angiography. The prevalence of CP IgG seropositivity did not differ between patients with and without CAD. MP antibody titer was measured using the complement fixation test, and the titer of ≥1/4 was considered to be seropositive. The prevalence of MP titer of ≥1/4 did not differ between patients with and without CAD, but the titers of ≥1/8 and ≥1/16 were significantly more prevalent in patients with CAD, especially in those with MI. However, MP seropositivity (titer ≥1/8) was found to be associated with CAD only in patients with CP seropositivity. Our results suggest that the coinfection by MP and CP appears to be an important cofactor for CAD and that the combination of CP IgG seropositivity and MP seropositivity (titer ≥1/8) may be a risk marker for CAD.

CP was often reported to be associated with CAD in seroepidemiological studies [2,3]. The association between CP and CAD was strengthened by the detection of the organism within atheroma using direct immunofluorescence and PCR [4,5]. A viable organism was also isolated from atheroma [6]. CP infection accelerates atherosclerosis in a rabbit model [14]. However, the contribution of CP infection to CAD remains controversial. Two prospective studies [7,8] and one recent case-control study [15] failed to show any association between CP seropositivity and CAD. We also found no significant difference in the prevalence of CP seropositivity between patients with and without CAD.

One prospective study [9] recently showed that CP seropositivity was associated with coronary events. This

study also reported the association between MP IgG seropositivity and coronary events. Regarding MP seropositivity, Horne et al. [16] reported that MP IgA seropositivity was more prevalent in patients with CAD than in controls. The same group [17] also showed MP seropositivity to be associated with further coronary events in patients with CAD. Moreover, cerebral infarction and vasculitis associated with MP infection were reported in some cases [18,19]. MP organisms were also reported to be detected in 3% of aortic atherosclerotic specimens but in none of non-atherosclerotic specimens obtained from patients undergoing cardiac surgery [20,21]. MP can oxidize the host cell membrane, thereby inducing apoptosis and increase the release of cytokines by inflammatory cells [12,22]. In a rabbit model, MP infection induced periaortitis but not atherosclerotic lesions without a cholesterol diet [23]. Notably, Higuchi et al. [11,12] have recently reported that MP organisms were often detected in coronary plaques, mainly in the lipid cores of ruptured plaques, together with CP organisms in patients who died of MI. They suggested that the coinfection by MP and CP might increase the virulence of these organisms and thus be an important cofactor for plaque formation and instability. In our study, the prevalence of MP seropositivity was high in patients with CAD, especially in those with MI. Although our patients with CAD were older than those without CAD, and MP antibody titer measured by ELISA was suggested to increase with age [24], MP seropositivity was a significant factor associated with CAD, independent of age. However, MP seropositivity was found to be associated with CAD only in patients with CP seropositivity. Our results also suggest that the coinfection by MP and CP would be an important cofactor for the development of CAD.

Recently, Zhu et al. [25] investigated antibodies against five infectious pathogens (CP, cytomegalovirus (CMV), herpes simplex type 1 and 2, and hepatitis A virus) in 233 patients undergoing coronary angiography, and showed that the number of pathogens to which an individual has been exposed, namely, the infectious burden, was associated with CAD. They also reported the infectious burden to be associated with further coronary events in patients with CAD [26]. Reunanen et al. [9] tested antibodies against five pathogens (CP, MP, CMV, enterovirus, and adenovirus), and showed the infectious burden to be associated with coronary events in healthy men. However, two other prospective studies [27,28] failed to show any association between the infectious burden (CP, *Helicobactor pylori*, CMV, and herpes simplex virus) and cardiovascular events in healthy people. Conflicting results from these prospective studies may be due to the differences in the infectious pathogens tested, and some coinfections, such as that by CP and MP, may play a more important role in CAD than those by other infectious pathogens.

MP organisms characteristically require cholesterol for their survival and tend to be detected mainly in the lipid cores of coronary plaques in patients with MI [11,12]. We hypothesized that the combination of MP infection and hyperlipidemia may be an important cofactor for CAD. However, in contrast to the combination of MP and CP seropositivity, the percentage of patients with combined MP seropositivity and hyperlipidemia did not differ between patients with and without CAD. In our study, we could not find any interaction between MP infection and hyperlipidemia for CAD.

Our study was not without limitations. First, we measured MP antibody titer using a complement fixation test, because any ELISA kit to measure MP IgG or IgA titers is not commercially available in Japan. Second, a recent case-control study [29] suggested CP IgA titer to be more strongly related to CAD than IgG. However, prospective studies [28,30] showed that neither CP IgA nor IgG titers were strongly predictive of CAD. Because MP antibody titer that we measured mainly reflects IgG titer and because CP IgG titer is more commonly used in seroepidemiological studies than IgA titer, we measured CP IgG titer. Third, our study is cross-sectional. Such a study cannot establish causality. It shows some association and is hypothesis-generating. Finally, we had no healthy controls. We studied 549 consecutive patients undergoing angiography, who were divided into two groups with and without CAD. Some patients without CAD had mild, but not significant, coronary stenosis. These may have caused some selection bias and have confounded the results.

In conclusion, MP seropositivity was found to be associated with CAD. However, this association was confined to patients with CP seropositivity, thus suggesting that the coinfection by MP and CP may be an important cofactor for the development of CAD.

Acknowledgements

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Total Cavopulmonary Connection: Open Anastomosis of an Extracardiac Conduit With Vacuum-Assisted Venous Drainage

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Insertion of a tube conduit for total cavopulmonary connection is sometimes technically demanding due to the crumpled stump of the inferior vena cava caused by a tourniquet of the inferior vena cava near the division line. Herein we describe an alternative in which the anastomosis is completed during removal of the tourniquet with the application of vacuum-assisted venous drainage. This new technique may alleviate, if not completely eliminate, a concern associated with total cavopulmonary connection with extracardiac conduit in small patients.

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Total cavopulmonary connection using an extracardiac conduit technique has become one of the most commonly used modifications of Fontan-type operations [1, 2]. However, the insertion of a tube conduit is sometimes technically demanding due to the crumpled stump of the inferior vena cava (IVC) caused by a tourniquet of the IVC near the division line. We present a new technique using an open anastomosis of the IVC and a tube conduit with the application of vacuum-assisted venous drainage.

Technique

After general anesthesia is administered and the patient is prepared and draped, a midline sternotomy is performed. For patients with a previous bi-directional cavopulmonary shunt the pericardial adhesions are dissected only around the IVC and the neighboring right branch pulmonary artery. Cardiopulmonary bypass is established after cannulation of the ascending aorta, superior vena cava, and IVC. Straight and pliable venous cannula (Thin-Flex Single Stage Venous Drainage Cannula [Edwards Lifesciences LLC, Irvine, CA]) are used. The tip of the IVC venous cannula is positioned as usual, 2 cm below the diaphragm level. A tourniquet is applied to the IVC at the diaphragm level. The IVC is divided at the cavo-atrial junction, and the atrial stump is primarily closed by sutures. A slightly oversized polytetrafluoroethylene tube graft is selected and trimmed. Venous drainage is augmented with a vacuum-assisted negative pressure of between 40 and 60 mm Hg. An air bubble sensor is interposed in the venous drainage tube to recognize excessive air drawing, which could potentially lead to air blockage of the tube. The tourniquet for the IVC is released in order to achieve an open anastomosis of the graft with the IVC. Essentially no venous blood is spilled from the IVC stump. The cardiotomy sucker tip is placed in the IVC lumen to further facilitate the bloodless anastomosis technique that is needed (Fig 1). The other end of the conduit is anastomosed to a transverse incision in the inferior aspect of the right branch pulmonary arterial wall. Again, vacuum-assisted venous drainage is potent enough to eliminate the need for vascular clamping of the pulmonary artery. Finally, cardiopulmonary bypass is terminated.

Comment

Extracardiac conduit total cavopulmonary connection has been increasingly accepted as the procedure of choice for modified Fontan operations, because the hemodynamic properties in the reconstructed systemic venous route are excellent, and the suture load on the atrial wall is minimal. These characteristics promise better long-term morbidity and mortality. As an increasing number of patients undergo total cavopulmonary connection at a younger age, a small-sized tube graft is inevitably implanted, although an over-sized tube graft is desirable for such growing patients. The conventional technique with a tourniquet of the IVC near the division line may result in a heavily crumpled IVC stump. This makes the end-to-end anastomosis technique of the IVC stump and the tube graft highly demanding, especially in cases with a significant size mismatch between the two. The open technique allows a full expansion of the IVC wall and the placement of the sucker in the IVC lumen, thus assuring the surgeon more precise suture performance in such a difficult situation.

The open technique with vacuum-assisted venous drainage has been used in adult patients undergoing cardiac transplantation with bi-caval anastomosis [3], in

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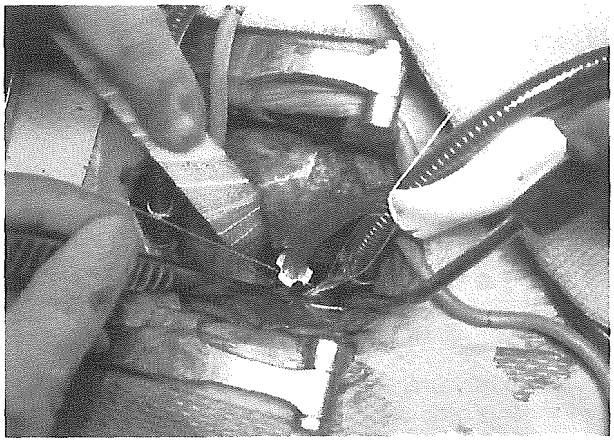

Fig 1. Surgeon's view of an open anastomosis with a tube graft and the inferior vena cava stump. Note a full expansion of the inferior vena cava wall and the placement of the sucker in the inferior vena cava lumen, thus assuring the surgeon's more precise suture performance.

which the IVC drainage was through the femoral vein. In our case, a direct IVC cannulation rather than peripheral venous drainage was used because of the small sizes that were involved. Our experience shows that direct IVC cannulation does not necessarily exclude an open IVC technique. Potential drawbacks of this technique include air blockage of the circuit tube and failure to suck some of the hepatic venous blood. Each surgical team applying this technique should individualize the position and type of the IVC cannula to achieve optimal venous drainage.

In summary, open IVC anastomosis with vacuum-assisted venous drainage through direct access is a feasible, safe, and useful procedure even in pediatric patients. This new technique may alleviate, if not completely eliminate, a concern associated with extracardiac conduit total cavopulmonary connection in small patients.

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