Published by Elsevier Science Inc.

ISSN 0735-1097/03/$30.00
doi:10.1016/S0735-1097(02)02899-1

Combined Role of the Lewis Antigenic System, *Chlamydia Pneumoniae*, and C-Reactive Protein in Unstable Angina

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OBJECTIVES The goal of this study was to assess the prognostic role of the Lewis antigenic system, *Chlamydia pneumoniae* (CP) seropositivity (CP+), and C-reactive protein (CRP) levels in unstable angina (UA).

BACKGROUND The role of CP infection in acute coronary syndromes is contradictory. The Lewis antigenic system, a genetically determined blood group system associated with infections and several disorders, including ischemic heart disease, might influence the susceptibility to CP infection, inflammatory response, and risk of cardiac ischemic events.

METHODS The CRP levels, Lewis antigens, and CP+ were measured in 54 patients with Braunwald's class IIIB UA. All patients were followed up for one year, and the occurrence of new coronary events (coronary death, myocardial infarction, and recurrence of instability) were recorded.

RESULTS Twenty-five coronary events occurred during follow-up. At univariate analysis CRP >3 mg/l (CRP+) ($p = 0.0056$), Lewis antigen b (Leb+) ($p = 0.028$), and the combination of Leb+ and CP+ ($p = 0.006$) and of CRP+ and Leb+ ($p = 0.003$) were associated with new coronary events, while CP+ alone was not. At multivariate analysis, CRP+ ($p = 0.008$) and combined Leb+CP+ ($p = 0.03$) were independent predictors of worse outcome. The event rate was 64% in CRP+ patients, 67% in Leb+CP+ patients, and 86% in CRP+Leb+CP+ patients. Combined Leb+CP+, but not Leb+ and CP+ alone, was related to CRP levels ($p = 0.03$). Among CP+ patients, CRP levels were higher in Leb+ than Leb- ($p = 0.028$).

CONCLUSIONS Our data demonstrate that in UA the Lewis antigenic system plays an important role, probably determining individual susceptibility to the detrimental effects of CP infection and by determining an enhanced inflammatory response. (J Am Coll Cardiol 2003;41:546–50)
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In the majority of patients with unstable angina (UA), systemic signs of inflammation are detectable (1,2). Such an inflammatory component has a short- and long-term prognostic value in patients with UA and in normal subjects (3–6). However, the cause of such inflammatory response is unclear. Recently, the possibility that some infectious agents may contribute to atherogenesis has been raised. In particular, *Chlamydia pneumoniae* (CP) has been associated with ischemic heart disease (IHD) (7,8). However, data in literature are contradictory, and the evidence for such association is not conclusive (9,10), possibly because of an uneven sensitivity to this pathogen. The high prevalence of CP infection in the adult population suggests the possibility that the individual host response may be crucial and that CP may have a role only in patients having a specific genetic susceptibility.

In this setting a possible role may be played by the Lewis antigenic system. This genetically determined blood group system is composed of two antigens, Lewis a (Lea) and Lewis b (Leb), whose expression is determined by two fucosyltrasferase genes located on chromosome 19. According to an individual's genotype and, therefore, to the expression of either one or of both putative genes, three phenotypes (Lea+, Leb+, Lea-b-) are observed in the Caucasian population (11). A rare Lewis phenotype, Lea+b+, has also been described in some Asian ethnic groups, but it is generally not observed in our population (12). The Lewis antigens are localized on cell surfaces and in organic liquids and are known to be associated with a variety of infectious diseases, including pulmonary infections and *Helicobacter pylori* (HP) infection (13,14); it is hypothesized that these antigens may act through an adhesive mechanism (15). Furthermore, the Lewis antigenic system has been associated with several clinical disorders associated with IHD such as elevated levels of factor VIII and von Willebrand factor, metabolic syndrome X, insulin resistance, and dyslipidemia (16–18). The Lewis antigenic system has also been recognized as a genetic marker of IHD (19,20), and polymorphisms of a fucosyltrasferase gene involved in Lewis antigen expression have been suggested to be associated

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Manuscript received February 6, 2002; revised manuscript received November 12, 2002, accepted November 19, 2002.

Abbreviations and Acronyms

ACS = acute coronary syndromes
CABG = coronary artery bypass grafting
CI = confidence interval
CP = *Chlamydia pneumoniae*
CRP = C-reactive protein
HP = *Helicobacter pylori*
IgG = immunoglobulin G
IHD = ischemic heart disease
Lea = Lewis a antigen
Leb = Lewis b antigen
OR = odds ratio
UA = unstable angina

with atherosclerotic disease and inflammatory response (21). In particular, because the Leb antigen has been associated with infectious disease, such as HP infection (14), which has also been associated with IHD, and with pulmonary infections (13), we studied the relation of this antigen with C-reactive protein (CRP) levels, CP seropositivity, and prognosis in UA.

METHODS

Population. We studied 54 consecutive patients with Braunwald's class IIIB UA (42 men; mean age, 61 ± 9 years) admitted to our coronary care unit. Inclusion criteria on admission were angina at rest, with >2 ischemic episodes or 1 episode lasting >20 min in the last 24 h with diagnostic ST-segment shift and negative creatine kinase-MB and troponin T, in order to avoid any confounding effect of myocardial damage on the inflammatory response. Patients were excluded if they had any known condition associated with inflammatory response and conditions likely to be associated with reduced survival, including ejection fraction <40% and age over 75 years (3). All patients were followed up for one year, during which the occurrence of new coronary events defined as coronary death, myocardial infarction, and recurrence of instability, with documented electrocardiogram changes, requiring readmission to hospital, were recorded.

The study was approved by the Ethics Committee of the Catholic University, and all patients gave their informed consent.

Blood and laboratory assays. All patients were bled at hospital admission. The CRP plasma levels were measured using a high-sensitivity nephelometric system (BNII, Dade-Behring, Marburg, Germany). The CP antibody titers were measured by a microimmunofluorescence assay (MRL Diagnostics MIF kit, Santa Barbara, California). Samples were diluted from 1:8 to 1:64; CP seropositivity was defined as the presence of specific immunoglobulin (Ig)G antibodies ≥1:16. Lewis antigens (Lea, Leb) were determined by a direct hemoagglutination method (Seraclone anti-Lea, anti-Leb, Biotest AG, Dreieich, Germany).

Statistical analysis. Because CRP values were not normally distributed, data are presented as median and range. Nonparametric tests were used for comparisons of CRP levels between groups (Mann-Whitney *U* test). Chi-square analysis was used for noncontinuous variables using Yates correction. Univariate and multivariate logistic regression analysis, after log transformation of CRP values, were carried out to assess the determinants of outcome, and multiple regression analysis was used to assess the parameters associated with CRP levels. As confounders, we analyzed CRP >3 mg/l (CRP+), positivity towards CP and Leb, combination of these three confounders (Leb+CP+, Leb+CRP+, CP+CRP+, Leb+CP+CRP+), gender, age, hypertension (defined as systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg, or the need for antihypertensive medication), presence of diabetes mellitus (defined as fasting glucose levels ≥140 mg/dl, including both insulin-dependent and noninsulin-dependent diabetes mellitus), hypercholesterolemia (defined as total cholesterol >200 mg/dl or the need for lipid-lowering therapy), family history of IHD (defined as history of IHD in siblings, parents, or first cousins ≤65 years of age), severity of coronary disease (according to number of diseased vessels with a diameter stenosis ≥70% assessed by quantitative coronary angiography), and smoking habits (current smokers or former smokers within the previous month). Only parameters with a trend toward significance ($p < 0.1$) were included in the multiple regression analysis; however, as inclusion of CRP, Leb+, CRP+Leb+, CP+Leb+, and CRP+Leb+CP+ led to colinearity, only CRP+ and the association of Leb+CP+ were included for multivariate analysis as they did not lead to colinearity. All other associations (CRP+CP+, CRP+Leb+, CRP+Leb+CP+), in fact, contained at least one of the other confounders, implicating that they were not independent.

RESULTS

Demographic and clinical characteristics of patients according to the presence or absence of the Leb antigen are summarized in Table 1. As only four patients were Leb-CP- (one of whom developed a cardiovascular event), this group was not included for subgroup analysis. During one-year follow-up, 25 new coronary events were observed. In the overall population, CRP median levels were 4.5 mg/l (0.5 to 188 mg/l); 21/33 (64%) patients with CRP >3 mg/l versus 4/21 (19%) patients with CRP <3 mg/l had new coronary events ($p = 0.0035$). Median CRP levels were 7.3 mg/l (0.7 to 188 mg/l) in patients with new coronary events and 2.9 mg/l (0.5 to 32.9 mg/l) in those without events ($p = 0.015$); 37/54 (68.5%) patients were Leb+, and 34/54 (63%) patients were CP+ at hospital admission. The Leb antigen expression, but not CP seropositivity, was associated with new coronary events after one year ($p = 0.048$) (Table 2). No difference was observed in CP IgG titers between

Table 1. Demographic and Clinical Characteristics of the Study Population

	Leb+	Leb−
Number of patients (%)	37/54 (68.5)	17/54 (31.5)
Age, yrs, mean ± SD	61 ± 10	62 ± 8
Gender (M/F)	27/10	15/2
Risk factors		
Family history of IHD (%)	19 (51)	5 (29)
Hypercholesterolemia (%)	16 (43)	7 (41)
Diabetes (%)	9 (24)	3 (18)
Hypertension (%)	21 (57)	9 (53)
Smoking (%)	20 (54)	8 (47)
Medications		
Nitrates (%)	23 (62)	11 (65)
Beta-blockers (%)	23 (62)	9 (53)
Calcium blockers (%)	21 (57)	10 (59)
Aspirin (%)	33 (89)	16 (94)
Other antiplatelet agents (%)	4 (11)	1 (6)
Lipid-lowering agents (%)	17 (46)	6 (35)
ACE inhibitors (%)	7 (19)	3 (17)
CRP > 3 mg/l (%)	25 (67.5)	10 (59)
IgG-CP (≥1:16) (%)	21 (57)	13 (76)

All p were NS.
ACE = angiotensin-converting enzyme; CP = *Chlamydia pneumoniae*; CRP = C-reactive protein; IHD = ischemic heart disease; Leb = Lewis antigen b.

patients with and without events. Combined positivity for CRP >3 mg/l and Leb+ increased the event rate to 72%.

New coronary events were significantly associated with the combined positivity toward Leb and CP, as they were observed in 67% of Leb+CP+ patients, versus 44% Leb+CP- and 23% Leb-CP+ patients (p = 0.043). The highest incidence of new events was observed in patients with combined positivity for CRP >3 mg/l, Leb, and CP (86%) (Table 3).

In a logistic regression analysis, CRP >3 mg/l (p = 0.0056), Leb antigen expression (p = 0.028), but not CP seropositivity per se (p = 0.48), were associated with outcome. Only CRP >3 mg/l (p = 0.008; odds ratio [OR], 5.74; confidence interval [CI], 1.5 to 21.7) and Leb+CP+ (p = 0.03; OR, 4.1; CI, 1.4 to 14.9) were independently associated with outcome (Table 4). In a multiple regression analysis, CRP levels were not related to CP seropositivity, Leb antigen expression, or any of the other confounders analyzed, but to the combination of CP seropositivity and Leb antigen expression (p = 0.03). Among CP+ patients,

Table 2. Event Rate in the Study Population

	Total Events	UA	MI	D
CRP > 3 mg/l	21/33 (64%)	19/33	2/33	0
CRP < 3 mg/l	4/21 (19%)	4/21	0	0
P	0.0035	0.0121	NS	NS
Leb+	21/37 (56.8%)	19/37	2/37	0
Leb−	4/17 (23.5%)	4/17	0	0
p Value	0.048	NS	NS	NS
CP+	17/34 (50%)	15/34	2/34	0
CP−	8/20 (40%)	8/20	0	0
p Value	NS	NS	NS	NS

D = coronary death; MI = myocardial infarction; UA = recurrence of instability requiring readmission to hospital. Other abbreviations as in Table 1.

Table 3. Event Rate for Combined Factors

	Total Events	UA	MI	D
Leb+CP+	14/21 (67%)	12/21	2/21	0
Non-Leb+CP+	11/33 (33%)	11/33	0	0
p Value	0.048	NS	NS	NS
Leb+CRP > 3 mg/l	18/25 (72%)	16/25	2/25	0
Non-Leb+CRP > 3 mg/l	7/29 (24%)	7/29	0	0
p Value	0.028	NS	NS	NS
CP+CRP > 3 mg/l	14/18 (78%)	12/18	2/18	0
Non-CP+CRP > 3 mg/l	11/36 (30%)	11/36	0	0
p Value	0.007	NS	NS	NS
Leb+CP+CRP > 3 mg/l	12/14 (86%)	10/14	2/14	0
Non-Leb+CP+CRP > 3 mg/l	13/40 (32%)	13/40	0	0
p Value	0.004	0.015	NS	NS

Chi-square analysis with Yates correction.
Abbreviations as in Tables 1 and 2.

CRP levels were higher in Leb+ (median, 6.5 mg/l; range, 0.85 to 188 mg/l) than Leb- (median, 2.88 mg/l; range, 0.5 to 14.9 mg/l) patients (p = 0.028). The CP seropositivity was not associated with any Lewis antigen.

In the overall study population, 53/54 patients performed coronary angiography. Coronary revascularization was performed in 49/54 patients (20 percutaneous transluminal coronary angioplasty and 29 coronary artery bypass grafting [CABG] interventions). Percutaneous transluminal coronary angioplasty was performed in 16 patients and CABG in 19 patients in the Leb+ group. Percutaneous transluminal coronary angioplasty was performed in 4 patients and CABG in 10 patients in the Leb- group (p = 0.27).

DISCUSSION

Our study demonstrates that the Leb antigen is associated with new coronary events in a carefully selected homoge-

Table 4. Univariate and Multivariate Logistic Analysis of Outcome Determinants

	p Value	OR (95% CI)
Univariate logistic analysis		
CRP > 3 mg/l	0.0056	5.67 (1.6–19.9)
Leb+	0.028	4.26 (1.13–16)
CP+	0.48	1.5 (0.47–4.7)
CP+Leb+	0.006	5.5 (1.5–19)
CP+CRP+	0.001	9.02 (2.2–36)
CRP+Leb+	0.003	6.25 (1.8–21.5)
CP+CRP+Leb+	0.0002	8.67 (2–38.6)
Severity of coronary disease	0.82	1.2 (0.56–2.06)
Family history of IHD	0.88	0.92 (0.29–2.86)
Diabetes	0.79	1.2 (0.29–4.9)
Hypercholesterolemia	0.67	1.26 (0.40–3.9)
Smoking habits	0.33	1.75 (0.54–5.6)
Age	0.83	0.99 (0.93–1.05)
Gender	0.10	0.3 (0.06–1.3)
Hypertension	0.54	1.4 (0.46–4.4)
Revascularization	0.89	0.9 (0.7–1.3)
Multivariate logistic analysis		
CRP > 3 mg/l	0.008	5.74 (1.5–21.7)
CP+Leb+	0.03	4.1 (1.4–14.9)

CI = confidence interval; OR = odds ratio. Other abbreviations as in Table 1.

neous group of patients with severe UA Braunwald's class IIIB and that such a relation is strengthened when associated with raised levels of CRP and CP seropositivity. Conversely, CP seropositivity is associated with new coronary events only when combined with Leb and CRP > 3 mg/l. Patients CP+, Leb+ having CRP > 3 mg/l have the greatest risk. Therefore, our data suggest that the inflammatory response is a major determinant of the association between infections and acute coronary syndromes (ACS).

Role of the Lewis antigenic system. The mechanisms by which the Lewis antigens may be involved in IHD and their interaction with CP and inflammation are speculative. First, the Lewis genes are located on chromosome 19, nearby the loci of the low-density lipoprotein receptor, the glycogen synthetase, and the insulin receptor, suggesting a possible interaction between these genes and development of IHD (19). Second, the Lewis antigens are also soluble in plasma (11), where they may interact with circulating CP antigens and eventually favor plaque localization. According to such hypothesis, plaque localization of CP antigens and the development of IHD may be secondary to the release of CP antigens into plasma from an extracardiac localization (22–24). Third, a polymorphism of the Lewis genes (11) determines a quantitative difference in antigen expression, which might explain a different susceptibility towards CP infection and/or a different inflammatory response. Therefore, the previous evidence of the Lewis antigenic system as a genetic marker of IHD (19,20) may have both a biological and molecular explanation. In fact, the abovementioned pathogenic mechanisms, although speculative, are based on previous studies (11,21–24) concerning this antigenic system although not directly applied to IHD. The genetic background of this blood group system and the presence of polymorphisms regulating antigen expression, along with the modulating properties towards several microorganisms, suggest that the Lewis antigens may play a role in the multifactorial (endogenous and environmental components) pathophysiology of IHD.

Previous studies. Previous studies, mainly retrospective, have suggested an association between CP infection and IHD (8,25–27); this hypothesis has also generated a number of prospective studies on the role of specific antibiotic treatment in IHD patients (28–31). However, more recent prospective data have failed to confirm such an association (9,10,32,33), and a meta-analysis published by Danesh et al. (33) excluded the existence of any strong association between CP IgG titers and incident coronary artery disease. Conversely, studies assessing the association of CP or other infective agents with markers of inflammation have suggested a strong association between infections and risk of IHD in the presence of elevated inflammatory markers (34,35). Such an observation, and our finding that CRP levels are associated with the combined positivity toward CP and Leb, suggests that the role of infectious agents in atherogenesis and its complications may be, in part, related to the amount of inflammatory response to infections, which, in turn, may depend on a host's genetic background (36,37).

Role of the host response. Seropositivity towards CP infection is quite frequent in the adult population, but very few of these individuals develop ACS. This suggests that CP infection alone is not able to determine the development of ACS, but other factors are required. In particular, in our study we analyzed a small, but well characterized, group of patients with severe UA. In UA, the inflammatory process plays an important pathogenic role, and inflammatory markers are related to prognosis. Our findings confirm CRP as a major prognostic determinant but also demonstrate that patients with combined CP seropositivity and Leb antigen expression are characterized by a greater inflammatory response and a greater risk of future coronary events. Our findings suggest an important role of the host response in the association between infections and ACS and contribute to explaining the controversial results on the role of infectious agents in the pathogenesis of ACS and on the beneficial effects of antibiotics in patients with ACS.

Although this study included a small number of patients (which might, in part, explain the lack of correlation of the traditional risk factors with prognosis) and lack of measurement of specific CP antigens (38), it suggests, in line with recent observations (35), that the association between CP and UA may be mediated by inflammation and by the host's genetic background.

Conclusions. Our study demonstrates that patients with UA carrying the Leb antigen have a worse outcome at 12 months; among these, those who are seropositive to CP and have elevated CRP levels have the highest risk of new coronary events. Thus, the Lewis antigenic system might have a pathogenic role in the susceptibility to infectious agents in UA and influence patients' outcome. The CP infection may not produce adverse coronary events in nonresponsive patients. Further studies are needed to confirm the role of the Lewis antigens in the development of ACS and to clarify their interaction with infectious agents and inflammation.

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