ELSEVIER

Atherosclerosis 164 (2002) 355–359

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

C-reactive protein and multiple complex coronary artery plaques in patients with primary unstable angina

Michael N. Zairis *, Olga A. Papadaki, Stavros J. Manousakis, Maria A. Thoma, Demetrios J. Beldekos, Christopher D. Olympios, Cristina A. Festeridou, Spyros K. Argyrakis, Stefanos G. Foussas

Department of Cardiology, Tzanio Hospital, Piraeus, Greece

Received 30 January 2002; received in revised form 2 April 2002; accepted 11 April 2002

Abstract

The aim of this study was to investigate the possible association of plasma C-reactive protein (CRP) levels with the presence of angiographically multiple complex lesions (CLs) in patients with primary unstable angina (PUA). For the purpose of this study, 228 consecutive patients with PUA who underwent in-hospital catheterization were evaluated. Plasma CRP levels were measured upon patients' admission. Coronary plaques were classified as CL or non-CL according to Ambrose's criteria. There were 100 (43.9%) patients with no or one CL ($\leqslant 1$) and 128 (56.1%) patients with multiple CLs ($\geqslant 2$). Tertiles of plasma CRP levels upon admission were significantly associated with the number of CLs on angiographic studies. In particular there was a significant gradual increase in either the number of CLs, or the presence of apparently thrombus-containing CLs with increasing of CRP tertiles. By multivariate analysis CRP was independently associated with the presence of either multiple CLs (R.R. = 1.8, 95%CI = 1.5–2.2, $P < 0.001$), or angiographically apparent thrombus-containing CLs (R.R. = 1.4, 95%CI = 1.2–1.7, $P = 0.03$).

High plasma levels of CRP may reflect a multifocal activation of the coronary tree in patients with PUA. This finding suggests a generalized inflammatory reaction throughout the coronary tree in these patients. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: C-reactive protein; Primary unstable angina; Coronary angiography; Pathophysiology

1. Introduction

Primary unstable angina (PUA) results mainly from coronary artery thrombosis superimposed on sites of plaque fissure, rupture or superficial erosion [1]. The components of the disrupted plaque, the superimposed thrombosis, and intraplaque bleeding, all contribute to the delineation of the angiographically rough contour of the so called complex lesion (CL) [2–5].

The disruption of the fibrous cap occurs mainly at the rupture-prone shoulders of the coronary plaque, which are areas of accumulated inflammatory cells [6–9]. It has been postulated that activation of the plaque-infiltrating inflammatory cells may play an important role in plaques disruption resulting to the induction of acute coronary events.

Although the focus of inflammation of a single plaque is so small that systematic signs of inflammation might not be expected [10], however acute-phase reactants are increased in acute coronary events. In particular, plasma levels of C-reactive protein (CRP) are increased in a significant proportion of patients with unstable coronary artery disease and this increase is significantly associated with either short- or long-term ischemic complications [11].

Moreover, there is an increased body of evidence that supports a multifocal pattern of plaque disruption throughout the coronary artery tree during acute coronary events [12–15]. It is possible that in some patients, multiple coronary plaques become inflamed, vulnerable and probably ruptured during acute coronary syndromes. Accordingly, the hypothesis that more than one ruptured, thrombotic, and therefore angiogra-

* Corresponding author. Address: 273–277, Alkiviadou Street, Piraeus 18536, Greece. Tel.: +30-1-4284387; fax: +30-1-4533666
E-mail address: zairis@hellasnet.gr (M.N. Zairis).

0021-9150/02/$ - see front matter © 2002 Elsevier Science Ireland Ltd. All rights reserved.
PII: S0021-9150(02)00129-6

phically CLs may be found in patients with PUA and that the number of these CLs may be associated with plasma CRP levels, seems possible. The aim of this prospective study was to evaluate this hypothesis.

2. Patients and methods

2.1. Study patients

The study population constituted 271 consecutive eligible patients with PUA who were admitted at our institute. Eligible patients fulfilled the following criteria: (a) new rest angina during the last 12 h lasting $\geqslant 5$ but < 30 min; (b) diagnostic electrocardiographic ST-segment depression of at least 1, 2 mm after the J point in $\geqslant 2$ contiguous leads or T waves inversion > 1 mm in leads with predominant R waves. Exclusion criteria were: (a) elevated creatin kinase-MB ($\geqslant 17$ IU/l, upper normal value) or cardiac troponin T levels ($\geqslant 0.1$ μg/l) upon admission; (b) history of coronary artery bypass grafting; (c) significant valvular disease, uncontrolled hypertension, or congestive heart failure; (d) anemia (Hct $\leqslant 30\%$), fever or thyrotoxicosis; (e) left bundle branch block, left ventricular strain electrocardiographic pattern, or pacemaker rhythm; (f) sustained supraventricular or ventricular arrhythmia; (g) evolution to fatal or non-fatal myocardial infarction before in-hospital coronary angiography; (h) conditions known to affect plasma CRP levels (e.g. infection, malignancy, renal or hepatic insufficiency, inflammatory diseases); and (i) myocardial infarction, revascularization procedure or surgery in the last 6-months.

Of the 271 eligible patients, 13 with normal coronary angiograms, 18 who experienced in-hospital fatal or non-fatal myocardial infarction before angiographic evaluation and 12 without in-hospital coronary angiography were excluded. Thus, 228 patients with in-hospital coronary angiography comprised the final study cohort. The local Ethics Committee approved the study protocol and informed consent was obtained from all participants.

2.2. Collection of blood samples and biochemical assays

Upon admission, venous blood samples were obtained before the intravenous administration of drugs. Coded plasma samples were stored at -80 °C for CRP analysis at the end of the study. CRP was measured utilizing a quantitative nephelometric method (The Binding Site, Birmingham, UK). The lower limit of detection was 0.12 mg/l. For values below the limit of detection, the lower limit value was used for statistical analysis. Plasma cardiac troponin T levels were measured upon admission by an enzyme based immunoassay method (Boehringer Mannheim, Germany). The lower limit of detection was 0.01 μg/l, and the diagnostic threshold level was 0.1 μg/l.

2.3. Treatment

All patients received aspirin, intravenous unfractioned heparin, intravenous nitroglycerin, and an oral β-blocker upon admission in the absence of contraindications, or intolerance. Chewed aspirin was given in a dose of 160–325 mg upon admission, and was continued indefinitely. Intravenous infusion of unfractioned heparin or nitroglycerin was continued for at least 24 h in uncomplicated cases. Thereafter patients received a low molecular weight heparin subcutaneously as well as oral or topical nitrates. Further, antiischemic therapy was titrated to patients' blood pressure and heart rate at the discretion of the attending physician.

2.4. Cardiac catheterizations and analysis of the angiographic characteristics

All catheterizations were performed by the femoral approach using the Seldinger technique. The same non-ionic contrast agent was used in all patients. At least five and two views for the left and right coronary system were included, respectively. All obstructive lesions were visualized in two orthogonal views. Two independent and experienced angiographers, who were blinded to the study, performed qualitative analysis of the coronary atheromatic lesions ($\geqslant 50\%$ diameter stenosis) in the culprit and non-culprit arteries. Two atheromatic lesions, located in the same coronary artery, were considered as distinct if there was at least 3 cm apparently normal lumen between them. Atheromatic plaques were qualitatively classified as simple or complex according to the morphologic criteria introduced by Ambrose et al. and reconfirmed by other investigators [2–5]. Flow in the culprit arteries was graded according to the TIMI criteria.

2.5. Culprit artery, culprit lesion, and CL determination

Culprit artery was identified, taking into account the coronary anatomy, the localization of electrocardiographic changes and the segmental wall motion abnormalities of the left ventricle. Culprit lesion was determined by correlating the presence of a CL and the aforementioned criteria. An atheromatic lesion was considering as complex if angiographic characteristics indicative of irregular borders, ulcerations, or the presence of intraluminal thrombus were presented [2–5].

Irregular borders were characterized by ulceration of the plaque, or saw-toothed contour suggesting a friable surface, and intimal flaps. Intimal flap was characterized by a mobile and radiolucent extension of plaque surface into the arterial lumen. Plaque ulceration was defined as

a small and discrete crater with hazy contour beyond the vessel lumen. Intracoronary thrombus was defined as a filling defect at the area of an atheromatic plaque, visible in multiple projections, with at least three edges surrounded by contrast agent [2–5]. Total or subtotal occluded arteries (TIMI flow grade 0 or 1) were considered as CL as well. Restenotic lesions following previous percutaneous coronary angioplasty were not included.

3. Statistical analysis

Continuous variables were expressed as mean ± SD for normally distributed and as median with range for non-normally distributed CRP values. Dichotomous variables were presented as percentages. Patients were classified into three groups according to the tertiles of CRP values upon admission. Comparisons of continuous variables among CRP groups were made using ANOVA test, or Kruskal-Wallis test, as appropriate. Bonferroni in ANOVA, *t*-test, or Mann–Whitney U test were used, as appropriate, for pairwise comparisons between CRP groups. Associations between two categorical variables were tested by Chi-square test, or Fisher's exact test, as appropriate. Correlations were evaluated by Spearman's r. Inter-observer agreement in the classification of the CLs was tested by Cohen's kappa method. Univariate and multivariate logistic regression analyses were constructed for the determination of univariate and multivariate predictors of either the presence of multiple CLs or the detection of angiographically apparent thrombus-containing CLs. All possible predictors (presented in Table 1) as well as electrocardiographic pattern (ST depression vs. T inversion) upon admission were evaluated and all variables with $P < 0.1$ were included into multivariate models. All tests were two-tailed and $P < 0.05$ was considered as the level of statistical significance. Statistical analysis was performed with SPSS statistical software (release 10.0, SPSS, Chicago, IL).

4. Results

The mean age of the 228 study patients was 62 ± 9 years (range: 35 through 82 years). There were 168 (73.7%) males. Median CRP value upon admission was 2.35 mg/l (range: 0.12 through 29.9 mg/l). The mean time interval from patients' symptoms initiation through admission (pre-hospital delay) was 6.3 ± 3.4 h. There was no significant relationship between pre-hospital delay and CRP values upon patients' admission ($r = 0.09$, $P = 0.24$). Differences among tertiles of CRP regarding to the baseline characteristics are presented in Table 1.

There were 770 simple or CLs on the angiographic studies. Inter-observer agreement in the classification of CLs was 'very good' (Cohen's kappa = 0.97, 95%CI = 0.93–1.0). There were 337 CLs in patent arteries and 12 patients had one (8 patients) or two (4 patients) arteries with TIMI flow grade 0 or 1. Thus, there were 353 CLs and 417 non-CLs. There were 20 patients with no CL, 80 with a single CL, and 128 with multiple CLs. Concerning the patients with multiple CLs, 112 had two, and 16 had three CLs. There were no significant differences in the number of the diseased arteries or the number of the total coronary plaques among the groups defined by CRP tertiles. However, there was a significant gradual increase in the number of angiographically apparent CLs with increasing of CRP tertiles (Table 2). Forty-two patients (18.4%) had angiographically apparent throm-

Table 1
Comparison of baseline data among tertiles of CRP

	First ($n = 78$)	Second ($n = 71$)	Third ($n = 79$)	P
CRP median (range) mg/l	0.95 (0.12–1.0)	2.3 (1.1–4.3)	7.0 (4.5–29.9)	
Age (years), mean ± SD	62.4 ± 9.9	61.3 ± 8.8	62.4 ± 9.5	0.69
Male gender, %	75.6	85.9	60.8	0.002
BMI (kg/m^2), mean ± SD	26.9 ± 3.9	27.1 ± 3.0	26.9 ± 3.1	0.91
Hypertension, %	62.8	55.7	59.1	0.66
Current smoking, %	57.7	50.7	45.6	0.32
Diabetes mellitus, %	15.4	23.9	38.0	0.005
Family history of CAD, %	56.4	56.3	46.8	0.39
Hypercholesterolemia[a], %	55.1	63.4	63.3	0.49
History of stable angina, %	7.7	12.7	11.4	0.58
History of MI[b], %	9.0	11.3	24.1	0.02
History of PCI[b], %	10.3	16.9	24.1	0.07
Time from onset of pain through admission in hours, mean ± SD	6.0 ± 3.6	6.2 ± 3.3	6.6 ± 3.4	0.56

CRP, C-reactive protein; BMI, body mass index; CAD, coronary artery disease; MI, myocardial infarction; PCI, percutaneous coronary intervention; SD, standard deviation.

[a] Previously treated elevated cholesterol levels or total plasma cholesterol values > 200 mg/dl upon admission.

[b] No in the last 6 months.

Table 2
Comparison of electrocardiographic and angiographic data among tertiles of CRP

	First ($n=78$)	Second ($n=71$)	Third ($n=79$)	P
ST depression[a],%	76.9	74.6	74.7	0.93
Time interval between admission through in-hospital CA in days, mean ± SD	3.7 ± 1.3	3.6 ± 1.2	3.8 ± 1.2	0.59
Left main or 3-vessels CAD, %	35.9	35.2	41.8	0.65
Total coronary lesions per patient, mean ± SD	3.3 ± 1.1	3.4 ± 1.1	3.4 ± 1.1	0.48
Number of CLs per patient, mean ± SD	1.1 ± 0.8	1.5 ± 0.7	2.0 ± 0.7	< 0.001
Patients with no CL, %	20.5	4.2	1.3	< 0.001
Patients with a single CL, %	48.7	49.3	8.9	< 0.001
Patients with two CLs, %	28.2	40.8	77.2	< 0.001
Patients with three CLs, %	2.6	5.6	12.7	0.04
Patients with multiple CLs (⩾ 2), %	30.8	46.3	89.9	< 0.001
Patients with thrombus-containing CLs, %	10.3	15.5	29.1	0.02

CRP, C-reactive protein; CAD, coronary artery disease; CL, complex lesion; SD, standard deviation.
[a] On admission electrocardiogram.

bus-containing CLs in open arteries (with 2 or 3 TIMI flow) (Table 2).

Multivariate analysis showed that plasma CRP levels upon admission were positively related with a significantly increased risk of the presence of either multiple CLs (⩾ 2 CLs, Table 3) or thrombus-containing CLs (in arteries with 2 or 3 TIMI flow) (Table 4) on angiographic studies.

5. Discussion

The primary finding of this study is that in a significant proportion of patients with PUA a multifocal activation of the coronary artery tree is present and the extent of this activation is positively related to plasma CRP levels. The results of the present study suggest a generalized active inflammatory process throughout the coronary tree during acute coronary syndromes. This generalized inflammatory process may induce both a multifocal destabilization of multiple coronary plaques and elevation of the plasma CRP concentrations as well.

Multifocal activation of the coronary artery tree during acute coronary syndromes has been previously documented by pathologic [12,13] or angiographic studies [14,15]. In particular, Falk [12] as well as Roberts and Buja [13] have found, by postmortem pathologic examination, the presence of multiple thrombi in discrete ruptured coronary atherosclerotic plaques in patients who died because of acute myocardial infarction. Guazzi et al. [14] as well as Golstein et al. [15] have shown the presence of multiple angiographically CLs, in a significant proportion of patients with acute myocardial infarction. However, in these angiographic studies CRP levels were not measured. Moreover, a positive correlation between plasma CRP levels and complex angiographic morphology of the culprit lesion in patients with unstable angina has been described by Katritsis et al. [16] and by Moucarbel et al. [17]. The results of the present study showing a strong association between plasma CRP levels and angiographically apparent multiple CLs may confirm and expand the findings of the aforementioned reports.

The underlying mechanism of this multifocal activation during acute coronary syndromes is not well-known but may include an extensive inflammatory response throughout the coronary tree induced by several extrinsic or intrinsic factors. It is possible that in some patients, virus [18,19], bacteria [20] or oxidized low-density lipoprotein molecules [10] to induce a generalized stimulation of atherosclerotic plaques-associated inflammatory cells (e.g. macrophages, T lymphocytes).

Table 3
Univariate and multivariate predictors of multiple CLs (⩾ 2)

	Univariate logistic regression		Multivariate logistic regression	
	R.R. (95%CI)	P	R.R. (95%CI)	P
Diabetes mellitus	3.8 (1.9–7.4)	< 0.001	2.3 (1.1–5.3)	0.04
History of MI[a]	1.8 (0.9–3.8)	0.1		
ST-depression[b]	2.3 (1.2–4.2)	0.01	3.7 (1.5–8.6)	0.003
CRP, mg/l	1.7 (1.5–2.1)	< 0.001	1.8 (1.5–2.2)	< 0.001

R.R., relative risk; MI, myocardial infarction; CRP, C-reactive protein.
[a] No in the last 6 months.
[b] On electrocardiogram upon admission.

Table 4
Univariate and multivariate predictors of thrombus-containing CLs

	Univariate logistic regression		Multivariate logistic regression	
	R.R. (95%CI)	P	R.R. (95%CI)	P
Diabetes mellitus	3.0 (1.5–6.1)	0.002	2.5 (1.2–5.2)	0.01
CRP, mg/l	1.5 (1.2–1.8)	0.006	1.4 (1.2–1.7)	0.03

CL, complex lesion; R.R., relative risk; CRP, C-reactive protein.

This inflammatory cell activation may result in an enhanced secretion of metaloproteinases, or cytokines, which may disrupt the matrix components [1,10] in several atherosclerotic plaques (multiple CLs) and trigger the liver for an enhanced production of CRP.

5.1. Limitations of the study

Coronary angiography, which provides only the silhouette of the vessel lumen, is less sensitive than angioscopy and intravascular ultrasound for the detection of CLs. However, coronary angiography is highly specific for the detection of CLs. The identification of a CL by coronary angiography does not always indicate an acutely ruptured plaque. Some CLs may remain stable for a long period [21]. Accordingly, the presence of multiple CLs in patients with high plasma CRP levels observed in this study may only mean that these patients had disruption of the coronary plaques in the past. More studies are needed to elucidate these issues.

5.2. Conclusions

In conclusion, high plasma levels of CRP may be associated with the number of activated, unstable, ruptured and consequently complex atherosclerotic lesions in patients with PUA. The underlying mechanism of this multifocal activation is not well-known but may include an extensive inflammatory response throughout the coronary tree induced by several extrinsic or intrinsic factors.

References

[1] Shah PK. New insights into the pathogenesis and prevention of acute coronary syndromes. Am J Cardiol 1997;79:17–23.

[2] Gotsman M, Rosenheck S, Nassar H, et al. Angiographic findings in the coronary arteries after thrombolysis in acute myocardial infarction. Am J Cardiol 1992;70:715–23.

[3] Davies SW, Marchant B, Lyons JP, Timmis AD, Rothman MT, Layton CA, et al. Coronary lesion morphology in acute myocardial infarction: demonstration of early remodeling after streptokinase treatment. J Am Coll Cardiol 1990;16:1079–86.

[4] Ambrose JA, Winters SL, Arora RR, et al. Coronary angiographic morphology in myocardial infarction: a link between the pathogenesis of unstable angina and myocardial infarction. J Am Coll Cardiol 1985;6:1233–8.

[5] Levin DC, Gardiner GA. Complex and simple coronary artery stenoses: a new way to interpret coronary angiograms based on morphologic features of lesions. Radiology 1987;164:675–80.

[6] Davies MJ, Richardson PD, Woolf N, Katz DR, Mann J. Risk of thrombosis in human atherosclerotic plaques: role of extracellular lipid, macrophage, and smooth must cells content. Br Heart J 1993;69:377–81.

[7] Kragel AH, Reddy SG, Wittes JT, Roberts WC. Morphometric analysis of the composition of coronary arterial plaques in isolated unstable angina pectoris with pain at rest. Am J Cardiol 1990;66:562–7.

[8] Lacraz S, Isler P, Vey E, Welgus HG, Dayer JM. Direct contact between T-lymphocytes and monocytes is a major pathway for reduction of metalloproteinase expression. J Biol Chem 1994;269:22027–33.

[9] Kaartimen M, Pentilla A, Kovanen PT. Accumulation of activated mast cells in the shoulder region of human coronary atheroma: the predilection site of atheromatous rupture. Circulation 1994;90:1669–78.

[10] Lee RT, Liby P. The unstable atheroma. Arterioscler Thtomb Vasc Biol 1997;17:1859–67.

[11] Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000;321:199–204.

[12] Falk E. Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis: characteristics of coronary atherosclerotic plaques underlying fatal occlusive thrombi. Br Heart J 1983;50:127–34.

[13] Roberts WC, Buja LM. The frequency and significance of coronary arterial thrombi and other observations in fatal acute myocardial infarction: a study of 107 necropsy patients. Am J Med 1972;52:425–43.

[14] Guazzi MD, Bussotti M, Grancini L, Cesare ND, Guazzi M, Pera IL, et al. Evidence of multifocal activity of coronary disease in patients with acute myocardial infarction. Circulation 1997;96:1145–51.

[15] Golstein AJ, Demetriou D, Grines LC, Pica M, Shoukfeh M, O'Neill WW. Multiple complex coronary plaques in patients with acute myocardial infarction. N Engl J Med 2000;343:915–22.

[16] Katritsis D, Korovesis S, Giazitzoglou E, et al. C-reactive protein concentration and angiographic characteristics of coronary lesions. Clin Chem 2001;47:882–6.

[17] Moucarbel GV, Arnaout MS, Alam SE. C-reactive protein is a marker for a complex culprit lesion anatomy in unstable angina. Clin Cardiol 2001;24:506–10.

[18] Tillet HE, Smith JW, Gooch CD. Excess deaths attributed to influenza in England and Wales: age at death and certified cause. Int J Epidemiol 1983;12:344–52.

[19] Melnick JL, Adam E, DeBakey ME. Possible role of cytomegalovirus in atherogenesis. JAMA 1990;263:2204–7.

[20] Patel P, Mendall MA, Carrigton D, et al. Association of *Helicobacter pylori* and *Chlamydia pneumonia* infection with coronary heart disease and cardiovascular risk factors. BMJ 1995;311:711–4.

[21] Haft JI, al-Zarka AM. The origin and fate of complex coronary lesions. Am Heart J 1991;121:1050–61.