Different patterns of interleukin-6 and von Willebrand factor antigen changes after coronary stenting in unstable versus stable angina

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Stenting;
Unstable angina.

Background. **Inflammation plays an important role in the pathogenesis of acute coronary syndromes. The purpose of our study was to evaluate the time course and the clinical relevance of inflammatory markers in patients with unstable angina undergoing successful coronary stent implantation.**

Methods. **Fifty-six patients (33 with unstable and 23 with stable angina) scheduled for single vessel coronary angioplasty followed by successful stent implantation were studied. Blood samples for measurements of interleukin-6 (IL-6) and von Willebrand factor antigen (vWf) were taken immediately before coronary angioplasty and 24 hours and 1 month after the procedure. Patients were clinically examined 1 month after the procedure.**

Results. **The mean levels of IL-6 before stenting were significantly higher in unstable than in stable angina patients (p = 0.002), whereas baseline values of vWf showed no difference between the two groups. In unstable angina, serum levels of IL-6 and of vWf did not change 24 hours after stent implantation, but significantly decreased 1 month after the procedure (p = 0.005 and p = 0.0015 respectively). In stable patients, serum levels of IL-6, but not of vWf, increased 24 hours after the procedure and returned to baseline levels 1 month after stent implantation (p = 0.046).**

Conclusions. **In unstable angina, successful treatment of the culprit lesion by coronary stenting results in a significant decrease in the serum levels of IL-6 and of vWf 1 month after the procedure, suggesting that, in this clinical condition, elevated levels of these parameters correlate with the instability of the atheromatous plaque and that their decrease after successful stent implantation is the result of plaque stabilization.**

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Introduction

Recent observations suggest a prominent role for inflammation in acute coronary syndromes, as activated T cells and monocytes promote plaque instability and rupture with superimposed thrombosis[1-6]. In unstable angina, an increase in the number of circulating activated lymphocytes, as well as of neutrophil and monocyte adhesion molecules has been described[7-9]. The cause of increased systemic markers of inflammation in this clinical condition is still debated. Ott et al.[10] demonstrated a functionally relevant interaction between activated neutrophils and platelets, suggesting a link between inflammation and thrombosis at the level of the active plaque. More recently however, Liuzzo et al.[11], studying the time course of C-reactive protein, of serum amyloid A protein and of interleukin-6 (IL-6) after coronary angiography and coronary angioplasty (PTCA), found that in unstable angina these markers increase as a result of the hyperresponsiveness of unstable patients to inflammatory stimuli rather than as a consequence of plaque rupture.

In the present study, we serially measured the serum levels of IL-6, an inflammatory cytokine[12,13], and the plasma concentrations of von Willebrand factor antigen (vWf), a marker of endothelial activation[14,15], in patients with unstable and stable angina undergoing PTCA followed by stent implantation.

The purpose of our investigation was to assess whether successful treatment of the unstable plaque leads to a reduction of these markers of inflammation 1 month after the procedure.

Methods

Study population. Fifty-six patients with either stable or unstable angina scheduled

for single vessel PTCA were studied. Patients with unstable angina were admitted to our Coronary Care Unit because of angina at rest associated with electrocardiographic changes suggestive of myocardial ischemia. At the time of admission all patients were in Braunwald class IIIB[16] and were treated with infusion of nitroglycerin and heparin. They were also treated with aspirin (all patients, at least 250 mg/day), beta-blockers (n = 18), diltiazem (n = 6), and oral or transdermal nitrates (n = 28). No patient had peak creatine kinase values exceeding twice the upper normal limit. All patients with stable effort angina showed significant (≥ 1 mm) ST-segment depression during the exercise test.

The exclusion criteria were prior PTCA, acute myocardial infarction, and intercurrent inflammatory conditions requiring the use of steroids or non-steroid drugs.

Patients gave informed consent and the protocol was approved by the Ethics Committee of the hospital.

Coronary angioplasty and stenting procedure. PTCA was performed with the use of conventional techniques and monorail balloons were used in all patients to predilate the lesions. A heparin bolus (10 000 IU) was injected at the beginning of the procedure. In no patient of this series was abciximab used during the procedure of stent implantation. However, all patients took clopidogrel (loading dose 300 mg) at least 12 hours before the procedure. Successful stent implantation was defined as complete passage across the target lesion with expansion of the stent. Stents were implanted using high inflation pressures (at least 12 atm) in order to achieve a better apposition of the stent struts to the vessel wall. The final balloon size was selected on the basis of the visual assessment of the angiographic reference diameter. High pressure inflation was repeated, if needed, to achieve a good angiographic result with a < 20% residual stenosis by visual estimate.

Study protocol and laboratory assays. Venous blood samples were taken immediately before PTCA and 24 hours after the procedure. Serum samples were stored at -80°. At the time of hospital discharge, all patients were prescribed a therapeutic regimen including clopidogrel (for 1 month), aspirin (100-325 mg/day), and statins (to be continued indefinitely). They were clinically examined 1 month after the procedure and a venous blood sample was also taken at that time. Serum levels of IL-6 were measured with a commercial assay kit (Quantikine human IL-6 R&D system, Minneapolis, MN, USA). The plasma concentrations of vWf were measured with an ELISA technique (Asserachrom von Willebrand factor, Boehringer Mannheim, Mannheim, Germany) with data expressed as a percent of that obtained in pooled normal plasma. In our assay laboratory, the coefficient of variation was < 10% for both the vWf and IL-6 assays. The reference values in 120 healthy control subjects aged 25 to 65 years (82 men and 38 women) measured at our laboratory were 60 to 128% for vWf and 0 to 2.5 pg/ml for IL-6.

Statistical analysis. Descriptive statistics are reported for all variables. Because of variance inhomogeneity between groups, baseline comparisons between stable and unstable angina have been performed using the Mann Whitney U test. Time changes have been assessed by means of a multiple regression model for repeat measurements. The within-patient correlation and inhomogeneity of variances have been accounted for by computing robust standard errors (Huber-White estimator). Stata 6.0 (StatCorp, College Station, TX, USA) was used for computation. The Bonferroni correction was used for all pairwise comparisons. A p value of < 0.05 was retained for statistical significance.

Results

Patients were divided into two groups for analysis. Group I included 33 patients with unstable angina: there were 25 men and 8 women with a mean age of 66 years (range 49-88 years). In Group II, there were 21 men and 2 women with stable angina and with a mean age of 58 years (range 50-76 years). The clinical and angiographic data are shown in table I.

Single stent implantation was achieved in all 33 patients of group I and in 21 patients of group II. Implanted stents were the NIR stents (Boston Scientific, Boston, MA, USA) in 12 patients and the Guidant Duet stent (Guidant, Advanced Cardiovascular Systems Inc., St. Paul, MN, USA) in 42 patients. Two patients of group II, in whom a stent could not be delivered at the target site, were not included in the final

Table I. Clinical and angiographic data of the study groups.

	Group I (n=33)	Group II (n=23)
Age (years)	66 (49-88)	58 (50-76)
Sex (M/F)	25/8	21/12
Prior MI	10 (30%)	8 (38%)
Prior CABG	2 (6%)	2 (9.5%)
Hypertension	20 (60%)	11 (53%)
Smoking	15 (45%)	11 (53%)
Diabetes	6 (18%)	3 (16%)
Cholesterol (mg%)	216 ± 48	212 ± 43
Triglycerides (mg%)	204 ± 107	196 ± 12
Treated lesions		
LAD	11	5
RCA	14	9
LCx	7	7
SVG	1	2
Angiographic thrombus	6	1

CABG = coronary artery bypass graft; LAD = left anterior descending coronary artery; MI = myocardial infarction; RCA = right coronary artery; LCx = left circumflex coronary artery; SVG = saphenous vein graft.

analysis. In all the other patients the procedure was successfully performed and no complications occurred during hospitalization. In no patient did the serum levels of total creatine kinase and of its MB isoform rise above twice the upper normal limit. Treated lesions were the left anterior descending artery in 11 patients of group I and 5 patients of group II, the right coronary artery in 14 patients of group I and 9 patients of group II, the circumflex artery in 7 patients of group I and 7 patients of group II and a vein graft in 1 patient of group I and 2 patients of group II. All patients were discharged on ticlopidine, which was continued for 1 month, aspirin and statins, which were continued indefinitely.

Inflammatory response to coronary stenting (Table II). Before stenting, the mean serum levels of IL-6 were significantly higher in unstable than in stable angina patients (7.1 ± 7.4 vs 2.9 ± 3.5 pg/ml, p = 0.002), whereas no significant difference was found between baseline values of vWf between the two groups of patients (group I 120 ± 38%; group II 104 ± 40%, p = 0.21).

Table II. Mean concentrations of interleukin-6 (IL-6) and von Willebrand factor antigen (vWf) in the two groups of patients at baseline and 24 hours and 1 month after stenting.

	Baseline	24 hours	1 month
IL-6 (pg/ml)			
Group I	7.1 ± 7.4 *	7.5 ± 6.5**	2.9 ± 3.4
Group II	2.9 ± 3.5***	6.1 ± 6.3	3.2 ± 3.7
vWf (%)			
Group I	120 ± 38§	127 ± 27§§	107 ± 12
Group II	104 ± 40	107 ± 31	108 ± 20

* = p = 0.006 baseline vs 1 month; ** = p = 0.003 24 hours vs 1 month; *** = p = 0.02 baseline vs 24 hours; § = p = 0.03 baseline vs 1 month; §§ = p = 0.004 24 hours vs 1 month.

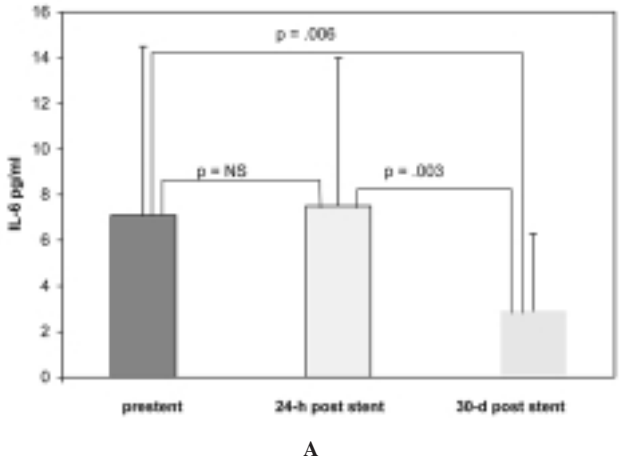
In group I, the serum levels of IL-6 varied from 7.1 ± 7.4 pg/ml before stent implantation, to 7.5 ± 6.5 pg/ml 24 hours after the procedure, and to 2.9 ± 3.4 pg/ml at 30 days (p = 0.005) (Fig. 1A). In group II, the serum levels of IL-6 changed from 2.9 ± 3.5 pg/ml at baseline, to 6.1 ± 6.3 pg/ml 24 hours after stent implantation, and to 3.2 ± 3.7 pg/ml at 30 days (p = 0.04) (Fig. 1B).

In group I, the serum levels of vWf varied from the baseline value of 120 ± 38% to 127 ± 27% 24 hours after coronary stenting, to 107 ± 12% at 1 month (p = 0.0015) (Fig. 2A). On the contrary, no significant variations were found in group II patients (baseline 104 ± 40%; 24 hours post-stent 107 ± 31%; 30 days 108 ± 20%, p = 0.91) (Fig. 2B).

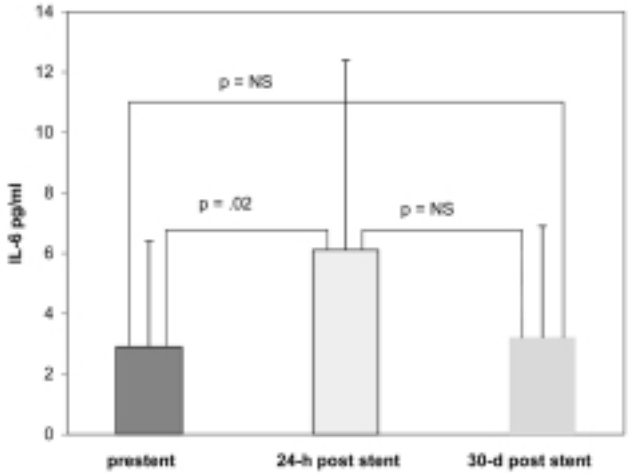
Discussion

Our findings show that baseline concentrations of IL-6 are increased in unstable angina as compared to stable angina patients, confirming previous similar observations[12]. The cause of such an increase however is still unknown, because the relation between coronary plaque disruption and the systemically detectable inflammatory response has been questioned[11]. In our study a different response to coronary stenting was observed in patients with unstable angina as compared to those with stable angina. Serum levels of IL-6 remained unchanged in unstable angina patients 24 hours after stenting, whereas they significantly increased in stable angina patients. One month after the procedure, IL-6 values significantly decreased in both groups of patients and reached similar levels.

Inflammatory response to coronary angioplasty and stenting. It is known that PTCA induces an inflammatory reaction which may have potential implications in



A



B

Figure 1. *Mean concentrations of interleukin-6 (IL-6) in 33 patients with unstable angina* (A) and *in 21 patients with stable angina* (B).

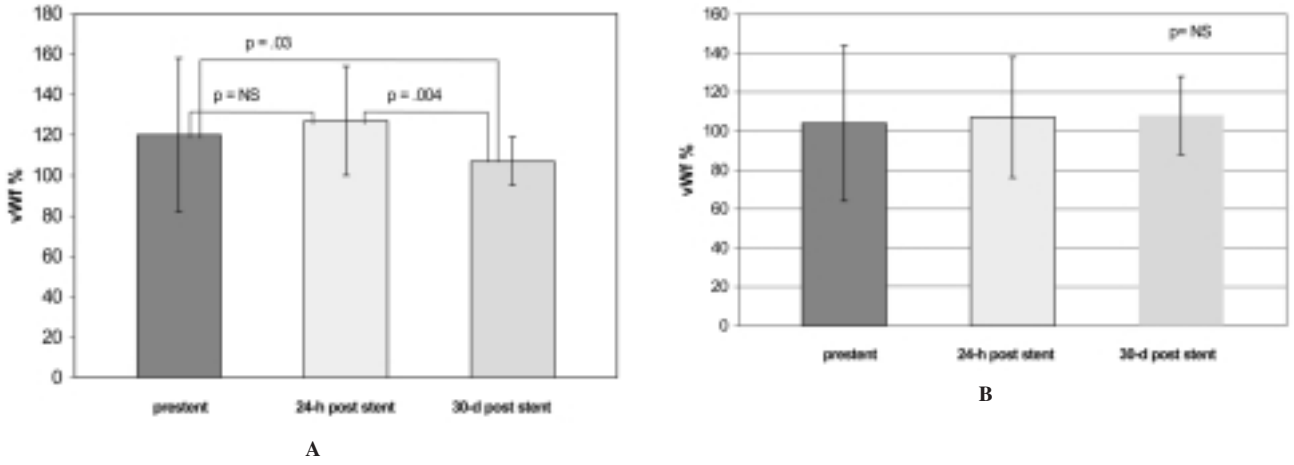


Figure 2. *Mean concentrations of von Willebrand factor antigen (vWf) in patients with unstable angina* (A) *and in patients with stable angina* (B).

the phenomenon of restenosis[17-20]. We showed that neutrophil activation occurs during PTCA leading to the release of proteolytic enzymes and to the generation of oxygen free radicals which may aggravate the endothelial damage caused by the balloon-related trauma[17]. It has been demonstrated that such an inflammatory process is more pronounced in patients who later develop restenosis[18] and that late lumen loss is related to the production of IL-1β by stimulated monocytes[19]. Moreover, complexes formed by leukocytes and thrombin-activated platelets are more frequently seen after the procedure in patients who experience clinical events during follow-up[20]. Recently Liuzzo et al.[11] found that the trauma of PTCA is followed by an increase in IL-6 levels 24 hours after the procedure only in those unstable angina patients with raised levels of inflammatory markers before the procedure, whereas no change in IL-6 levels was observed after the procedure in stable angina patients or in unstable angina patients without increased baseline values of this cytokine before PTCA. On the basis of these findings, the authors concluded that plaque rupture *per se* is not the main cause of the acute-phase protein increase in unstable angina and that increased baseline levels of acute-phase proteins are a marker of the hyperresponsiveness of the inflammatory system to even small stimuli. In our study, the reduction in the serum levels of the inflammatory markers IL-6 and vWf 1 month after the stenting procedure paralleled the remission of clinical instability. Treatment of the active plaque with coronary angioplasty and stent implantation may have resulted in a decrease in the intensity of inflammatory outburst. In view of the lack of a control group in our study, it may be objected that the same phenomenon would have occurred if the patients had been treated differently. However, Biasucci et al.[21] found persistently elevated C-reactive protein values 3 months after discharge in 81% of patients with severe unstable angina and increased levels of the acute-phase protein on admission. In that series no patient was treated with coronary stenting. A decrease in the serum levels of the inflammatory markers 4 weeks after coronary angioplasty, partially in patients treated with abciximab, was also shown by Lincoff et al.[22] in a selected population of the EPIC study. It is also possible that in our patients the use of an aggressive medical treatment (including statins and clopidogrel) has significantly contributed to the decrease in the serum levels of IL-6 and of vWf 1 month after the procedure. Further studies comparing the effects of different therapeutic regimens on inflammatory markers in unstable angina are needed to solve this issue.

Information concerning the effects of coronary stenting on inflammatory markers is limited. Gaspardone et al.[23] studied 81 patients with stable effort angina and single vessel disease who underwent coronary stent implantation. Venous blood samples, obtained on admission and after the procedure, were analyzed for the concentrations of C-reactive protein, an acute-phase protein produced by hepatocytes stimulated by IL-1 and IL-6. A significant increase in the serum levels of C-reactive protein was observed 48 hours following stent implantation in such patients. These data are consistent with our findings showing an elevation in the levels of IL-6 24 hours after the procedure in stable angina patients. On the contrary, we did not observe any increase in the levels of inflammatory markers in the unstable angina group. We hypothesize that two opposing factors played a role in this phenomenon. On one hand, as observed in stable angina patients, the inflammatory stimulus represented by stent implantation probably increased the serum levels of IL-6. On the other hand, dilation of the culprit lesion resulting in a larger lumen diameter, improved flow dynamics and less residual thrombus would have caused a reduction in the intensity of the inflammatory outburst related to the unstable plaque. As a result, no changes in the serum levels of the inflammatory markers were observed in the group of patients with unstable angina.

In conclusion, our data indicate that successful treatment of the culprit lesion by coronary stenting in unstable angina results in a significant decrease in the serum levels of IL-6 and of vWf in patients with unstable angina 1 month after the procedure, suggesting that in this clinical condition elevated levels of these parameters correlate with the instability of the atheromatous plaque, and that their decrease after successful stent implantation is the result of plaque stabilization.

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