Prognostic value of iron, nutritional status indexes and acute phase protein in acute coronary syndromes

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Coronary artery disease; Ischemia; Nutrition.

Background. **The relation between the inflammatory status during unstable angina and nutritional parameters has not been well described. We sought to investigate the relation between the acute inflammatory status, as evaluated on the basis of high C-reactive protein (CRP) and serum amyloid A levels and laboratory indexes of iron and nutritional status in patients with unstable angina, and whether the variations in iron levels have a prognostic significance.**

Methods. **The study population consisted of 98 patients admitted to our facility with unstable (group 1: 64 consecutive patients, 52 males, 12 females, mean age 66 ± 10 years) or stable angina (group 2: 34 patients, 30 males, 4 females, mean age 65 ± 9 years). The hemoglobin levels, the erythrocyte mean cell volume, serum iron levels, the increase in transferrin levels, the decrease in the percent transferrin saturation, ferritin levels, the nutritional status, and the CRP and serum amyloid A levels were measured.**

Results. **On the basis of a CRP value > 1 mg/dl, 47 patients with unstable angina and 4 patients with stable angina were identified as having active inflammatory disease. The presence of inflammation was associated with significantly lower mean values of hemoglobin, erythrocyte mean cell volume, serum iron and transferrin levels, and percent transferrin saturation in comparison with patients without inflammation. A significant inverse correlation coefficient between a CRP level > 1 mg/dl and hemoglobin, transferrin levels and percent transferrin saturation was observed: the strongest correlation was with serum iron levels. The relative risk of total cardiac events was significantly greater in patients with low serum iron levels than in those with high serum iron levels.**

Conclusions. **Patients with acute inflammation present altered iron status indexes. Increased CRP levels and reduced serum iron levels are associated with a worse outcome in patients with unstable angina.**

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Introduction

Several studies have previously suggested the association of an acute inflammatory reaction with unstable angina. A raised plasma level of C-reactive protein (CRP), a prototypal acute-phase reactant, has been found in the majority of patients with unstable angina[1,2]. Raised levels of CRP at the time of hospital admission predict a poor outcome in patients with unstable angina[2]. On the other hand, it is generally accepted that serum iron levels are reduced during systemic inflammation[3,4]. Besides, low serum iron levels have been previously reported in patients with myocardial infarction[5]. There is no clear evidence about serum iron levels in patients with unstable or stable angina. The present study was undertaken with the aim of investigating the relation between the acute inflammatory status, as evaluated on the basis of high CRP and serum amyloid A levels and laboratory indexes of iron and nutritional status in patients with stable and unstable angina, and whether the variations in iron levels have a prognostic significance.

Methods

The study population consisted of 98 patients admitted to our Institution with unstable or stable angina. Group 1 consisted of 64 consecutive patients (52 males, 12 females, mean age 66 ± 10 years) and presented with unstable angina. The criteria for enrollment were the occurrence of chest pain lasting at least 20 min within the previous 6 hours, an ST segment shift in at least 2 precordial adjacent leads and no elevation in serum creatine kinase or lactate dehydrogenase concentrations at the time of admission.

All patients received aspirin, nitrates, calcium antagonists, and beta-blockers as

required and a continuous intravenous infusion of heparin, dose adjusted to maintain an activated partial thromboplastin time ratio between 2 and 3 times the upper value. Coronary angiography was performed within 6 to 9 days of admission in all patients.

Group 2 included 34 patients (30 males, 4 females, mean age 65 ± 9 years) with CCS class 2-3 chronic stable angina lasting > 1 year and that was angiographically confirmed. All patients were on beta-blockers, nitrates, aspirin, and calcium channel antagonists as required.

Criteria for exclusion were the presence of inflammatory or neoplastic diseases, anemia, renal or hepatic failure and cachexia. All causes of low iron were evaluated and excluded. A weekly dietary recall was completed at baseline in order to assess the habitual food intake. Data were converted to nutrient intake by using a computerized food composition program (Winfood). It was estimated that heme iron accounted for 40% of the total iron intake from meat, poultry and fish. All patients underwent Holter monitoring during hospitalization and follow-up.

The patient baseline values are given in table I.

All patients gave their informed consent to participate in the study and the protocol was approved by the Ethics Committee of the University of Modena and Reggio Emilia.

Study protocol. The hemoglobin (Hb) levels, the erythrocyte mean cell volume (MCV), serum iron levels, the increase in transferrin (TIBC) levels, the decrease in the percent transferrin saturation (%TS), ferritin levels, the nutritional status, and the CRP and serum amyloid A levels were measured. Blood specimens were drawn after an overnight fast. CRP was determined using the immunonephelometric method (APS 560, Beckman Coulter, Milan, Italy) (range 0.01-1.1 mg/dl), serum amyloid A using the BNII Nephelometer (Dade Behring, Newark, DE, USA) (range 0.4-1.2 mg/dl), and iron using the ferene method (Ironchem, Chemetro, Newark, DE, USA) (range 70-145 ng/dl). The TIBC was measured using the immunophelometric method (APS 360, Beckman) (range 259-429 mg/dl) and the ratio of serum iron to the TIBC was calculated (%TS). Ferritin was measured using the immunochemical method (ES300 BMI, New York, NY, USA) (range 15-400 ng/dl). Albumin was determined by means of colorimetry with bromonesol purple (range 3.4-5.0 g/dl). A single operator who was unaware of the patient's disease and treatment assayed all samples. With regard to CRP, a cut-off level > 1 mg/dl was arbitrarily chosen because the value is within the 99th percentile of normal distribution.

Follow-up. Follow-up consisted of visits, performed in an outpatients setting for 12 months. The diagnosis of myocardial infarction was based on the presence of two out of the three conventional criteria, i.e. typical chest pain, a diagnostic ECG or the presence of elevated serum levels of the biochemical markers of myocardial damage. During hospitalization, silent ischemia was recorded at continuous ECG monitoring and during follow-up at Holter recording.

Statistical analysis. Data are expressed as means ± SD. The Cox proportional-hazards model was used to examine the relation of serum iron to the risk of an event after dividing the sample into thirds. Confidence intervals were calculated at the 95% level. The χ^2 test was used to compare discrete variables. The unpaired test or variance analysis was used to compare clinical variables. A p value < 0.05 was considered statistically significant.

Table I. Clinical characteristics of group 1 (unstable angina) and group 2 (stable angina) patients.

Clinical parameters	Group 1 (n=64)	Group 2 (n=34)	p
Age (years)	66 ± 10	65 ± 9	NS
Sex (M/F)	52/12	30/4	
Hypercholesterolemia	18 (28%)	7 (20%)	NS
Smoking	42 (65%)	17 (50%)	NS
Previous myocardial infarction	2 (3%)	2 (5%)	NS
Body mass index	32 ± 3	33 ± 2	NS
Fe levels (admission, μg/dl)	104 ± 15	101 ± 14	NS
Fe levels (lowest value, μg/dl)	65.94 ± 29	103 ± 15	< 0.001
Hb (admission, g/dl)	13 ± 1.9	13.3 ± 1.7	NS
Red blood cells (millions)	4.7 ± 0.600	4.8 ± 0.650	NS
Reticolocytes	8 ± 2	7 ± 3	NS

Hb = hemoglobin; Fe = serum iron.

Results

On the basis of a CRP value > 1 mg/dl[6], 47 patients with unstable angina and 4 patients with stable angina were identified and evaluated. The mean CRP serum level in the group with unstable angina was 5.7 mg/dl (range 0.2-27 mg/dl); the mean value in the group with stable angina was 0.8 mg/dl (range 0.4-2.3 mg/dl). Other patients in the two groups with a CRP level ≤ 1 mg/dl were considered as being free from intercurrent inflammation. The mean values of the variables in the group with unstable angina and a CRP level > 1 mg/dl and in those patients with unstable angina and a CRP level ≤ 1 mg/dl are reported in table II. The presence of inflammation was associated with mean values of Hb, MCV, serum iron, TIBC and %TS which were significantly lower than those observed in patients without inflammation: the mean ferritin level was significantly higher in the group of patients with a CRP level > 1 mg/dl. The

Table II. Variables in group 1 and group 2 patients: comparison between patients with C-reactive protein (CRP) levels > 1 mg/dl and those with CRP levels < 1 mg/dl.

	CRP > 1 mg/dl	CRP ≤ 1 mg/dl	p
Group 1			
Hb (g/dl)	11.32 ± 1.51	13.41 ± 0.98	NS
MCV (fl)	87.92 ± 7.02	88.74 ± 6.42	NS
Fe (μg/dl)	53.42 ± 12.43	102.32 ± 15.44	< 0.01
SF (μg/dl)	398.4 ± 254.6	249.7 ± 213.52	< 0.001
TIBC	177.4 ± 62.4	252.9 ± 48.6	< 0.001
%TS	20.37 ± 16.82	40.04 ± 16.41	< 0.001
Nutritional status (g/dl)	3.39 ± 0.39	3.48 ± 0.37	NS
Group 2			
Hb (g/dl)	10.42 ± 1.05	13.78 ± 1.04	NS
MCV (fl)	89.54 ± 6.22	90.62 ± 7.12	NS
Fe (μg/dl)	49.23 ± 16.31	106.34 ± 21.42	< 0.001
SF (μg/dl)	361.92 ± 344.9	238.54 ± 208.99	< 0.001
TIBC	184.43 ± 64.81	255.04 ± 51.34	< 0.001
%TS	19.92 ± 14.80	38.91 ± 15.13	< 0.001
Nutritional status (g/dl)	3.41 ± 0.32	3.38 ± 0.28	NS

MCV = erythrocyte mean cell volume; SF = ferritin; TIBC = increased transferrin; %TS = decreased percent transferrin saturation. Other abbreviations as in table I.

MCV and nutritional status were not significantly different in the two groups. Table II also reports the mean values of the variables in patients with stable angina and a CRP level > 1 mg/dl and those of the patients with stable angina and a CRP level ≤ 1 mg/dl. The presence of inflammation was associated with significantly lower mean values of Hb, serum iron, TIBC, and %TS: the ferritin level was significantly higher in the group with a CRP level > 1 mg/dl. The MCV and nutritional status were not different in the two groups.

Table III reports the mean values of the variables in all the patients with a CRP level > 1 mg/dl and their comparison with those of patients with a CRP level ≤ 1 mg/dl independently of the type of angina. In this case too the presence of inflammation was associated with significantly lower mean values of Hb, serum iron, TIBC and %TS. The mean ferritin level was higher in patients with a CRP level > 1 mg/dl. MCV and nutritional status were not significantly different in the two groups.

Table IV reports the mean values of the variables in the group of patients with unstable angina and their comparison with those of patients with stable angina. The presence of unstable angina was associated with significantly lower mean values of Hb, serum iron, TIBC and %TS. The mean ferritin level was higher in patients with a CRP level > 1 mg/dl. MCV and nutritional status were not significantly different in the two groups.

The correlation coefficient between the CRP continuous levels and iron and the nutritional status variables are reported in table V. We also found a significant inverse correlation coefficient between CRP levels > 1 mg/dl and Hb levels, TIBC and the %TS: the strongest correlation was with serum iron.

Follow-up. The aim of follow-up was to investigate whether the variations in iron status have a long-term prognostic influence in patients with unstable angina. Serum iron levels were strongly correlated with CRP continuous levels. Follow-up lasted 12 months. After this period the patients were assessed. Patients with unstable angina were divided into two groups: the first group consisted of 52 patients (81%) with serum iron levels ≤ 70

Table III. Variables in all patients of both groups: difference between patients with C-reactive protein (CRP) levels > 1 mg/dl and patients with CRP levels < 1 mg/dl.

	CRP > 1 mg/dl	CRP ≤ 1 mg/dl	p
Hb (g/dl)	11.0 ± 1.68	13.37 ± 1.12	NS
MCV (fl)	88.62 ± 5.98	88.71 ± 6.99	NS
Fe (μg/dl)	51.28 ± 16.38	103.38 ± 16.79	< 0.001
SF (μg/dl)	389.53 ± 264.91	246.51 ± 210.71	< 0.001
TIBC	179.34 ± 63.71	254.91 ± 49.01	< 0.001
%TS	20.31 ± 15.72	40.0 ± 15.52	< 0.001
Nutritional status (g/dl)	3.40 ± 0.38	3.41 ± 0.46	NS

Abbreviations as in tables I and II.

Table IV. Comparison between the two groups of patients.

	Group 1	Group 2	p
Hb (g/dl)	10.7 ± 1.54	14.7 ± 2.12	< 0.05
MCV (fl)	87.62 ± 5.98	88.71 ± 6.99	NS
Fe (μg/dl)	52.16 ± 17.42	104.27 ± 18.80	< 0.001
SF (μg/dl)	391.6 ± 251.84	245.6 ± 211.76	< 0.001
TIBC	180.42 ± 62.57	255.34 ± 45.83	< 0.001
%TS	20.51 ± 14.29	41.1 ± 16.34	< 0.001
Nutritional status (g/dl)	3.44 ± 0.68	3.40 ± 0.36	NS

Abbreviations as in tables I and II.

Table V. Correlation coefficients between C-reactive protein, iron serum levels and nutritional status.

	r	p
Hb (g/dl)	-0.31	< 0.05
MCV (fl)	-0.06	0.610
Fe (μg/dl)	-0.78	< 0.001
SF (μg/dl)	0.39	< 0.001
TIBC	-0.38	< 0.001
%TS	-0.14	0.038
Nutritional status (g/dl)	-0.03	0.71

Abbreviations as in tables I and II.

μg/dl and the second group included 12 patients with serum iron levels > 70 μg/dl.

Among the patients of the first group 22 ischemic episodes were observed, 9 of which were clinically silent and were recorded at Holter monitoring. Seven patients needed hospitalization during the follow-up period: 5 patients had acute myocardial infarction, 1 patient underwent urgent angioplasty, and 1 coronary artery bypass grafting. In the second group, 5 episodes of ischemia were observed, one of which was silent; no acute myocardial infarction was reported in this group and no patient required hospitalization or needed urgent revascularization (Table VI). The relative risk of total cardiac events was significantly greater in patients of the first group than in patients of the second group (2.0 vs 1.0, 95% confidence interval 1.1-2.8 vs 0.7-3.1, $p < 0.001$).

The prognostic significance of the CRP levels was evaluated in patients with unstable angina. Patients were classified into two groups identified as group A (CRP > 1 mg/dl) and group B (CRP < 1 mg/dl)[6]. Group A consisted of 47 patients and group B of 17 patients. Among the patients of group A, 19 ischemic episodes were observed, 7 of which were clinically silent; 5 patients had acute myocardial infarction, and 1 patient died on day 10. One patient underwent urgent angioplasty and 1 coronary artery bypass grafting. In group B we observed 5 episodes of ischemia, one of which was silent; no acute myocardial infarction occurred in this group. The trends of cardiac events were not significantly different in patients with low serum iron levels and in those with high CRP values. Analysis of patients with unstable angina who developed events during the follow-up revealed that the mean serum iron levels were lower than in patients who did not experience cardiac events (63 ± 20 vs 105 ± 36 μg/dl, $p < 0.01$). Conversely the mean CRP value was higher in patients with cardiac events (19 ± 8 vs 2.4 ± 10 mg/dl, $p < 0.01$).

Discussion

This study indicated that in patients with stable or unstable angina and high CRP and/or serum amyloid A levels, an alteration in iron status expressing a disorder in iron metabolism occurs. Serum iron levels are a marker of inflammation. The iron alteration is signifi-

Table VI. Cardiovascular events during follow-up in patients with unstable angina according to the lowest value of serum iron levels.

Cardiac events	Serum iron levels ≤ 70 ng/dl	Serum iron levels > 70 ng/dl
Acute MI within 30 days	5	0
Acute MI after the first month	2	0
Silent ischemic episodes during hospitalization	9	3
Silent ischemic episodes during follow-up	4	2
Urgent angioplasty within 30 days	1	1
Urgent bypass grafting within 30 days	1	0
Total events	22	6

MI = myocardial infarction.

cantly more frequent in patients with unstable angina than in those with stable angina; this picture is the result of inhibition of iron release from macrophages so that tissue levels of iron increase and circulating levels decrease. This is very common in the presence of inflammation[7,8]. Elevated CRP and/or serum amyloid A levels indicate almost certain interference by the inflammatory disease.

Hb levels were inversely correlated with CRP values, indicating that inflammation had negative effects on hematopoiesis; as shown by the absence of any correlation with CRP levels, inflammation did not influence the MCV. The study concerned patients with acute inflammatory disease which had developed some time previously, too short a period for the manifestation of any variations in erythrocyte morphology. The significant correlation observed between CRP levels and the TIBC levels confirms that this protein is a negative "acute phase reactant". The direct correlation between CRP and ferritin levels may be partly due to increased iron stores and partly to the behavior of this protein, which is considered an acute phase protein[9]; in this study, ferritin levels were significantly higher in patients with inflammation and with the related increase in CRP and serum amyloid A levels than in those without. The iron/TIBC ratio was found to be less affected by inflammation and the correlation with the CRP levels was weak although statistically significant: a very low value of the %TS would have identified patients with iron deficiency[10]. This type of patient was not included in this study. The strongest correlation observed was between CRP and serum iron levels. High levels of CRP are the pivotal factor that influences the iron status during acute inflammation[11]. At the site of inflammation iron is bound by almost one leukotriene excreted by activated neutrophils; these leukotrienes transfer iron from the circulation into the tissues[12]. CRP has recently been localized directly within atheromatous plaques where it mediates monocyte recruitment[13]. While a prognostic role for CRP and serum amyloid A in unstable angina has now been largely demonstrated[1,14], data regarding a similar capability for serum iron are less numerous and are conflicting[15,16]. During the clinical course, patients who had serum iron levels < 70 ng/dl had more events than those who had higher serum iron values. A limitation of the present study is the large number of soft events (i.e. ischemic episodes) versus the hard ones. In establishing a correlation between serum iron levels and prognosis in unstable angina, it is important to rule out the presence of factors that can influence biochemical measurements of iron status. The response of serum iron levels is due to substances activated at the site of inflammation such as leukotrienes, interleukin-8 and cytokines[7]. The potential activation sites are the atherosclerotic obstruction sites in the coronary vessel and the microcirculatory interface with injured myocardium[8]. Activation of inflammatory cells may promote acute complications by increasing procoagulant activity[17] and the risk of plaque dissection and hemorrhage. In conclusion, we suggest that iron serum levels are a marker of inflammation. The study confirms the role of CRP and demonstrates that iron levels are also associated with a worse outcome in patients with unstable angina.

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