Low plasma glutathione levels after reperfused acute myocardial infarction are associated with late cardiac events

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Objective **To clarify whether an altered redox state persists in the subacute phase of myocardial infarction and if specific redox patterns are associated with later cardiac events.**

Methods **Ninety-seven patients [80 men, median 63 (interquartile range, 53, 69) years] with a first acute myocardial infarction, with (53%) or without ST segment elevation, treated with successful percutaneous interventions, were tested at 5–6 days after admission for plasma α-tocopherol, ascorbic acid, total and reduced homocysteine, cysteine, glutathione, cysteinylglycine and blood-reduced glutathione, all assessed by high-pressure liquid chromatography. Free malondialdehyde was evaluated by gas chromatography. A subgroup of 14 patients had adjunctive blood samples within 1 h and at 72 h after angioplasty. Blood samples from 44 patients matched for age, sex, and risk factors served as controls. Patients were followed up for median 15 (interquartile range, 9, 17) months for cardiac events.**

Results **All plasma-reduced aminothiols, vitamins and plasma total glutathione were significantly lower in myocardial infarction at 5–6 days than in controls. In the 14 myocardial infarction patients sampled repeatedly, plasma-reduced glutathione, cysteinylglycine, total glutathione, and α-tocopherol significantly decreased, whereas blood-reduced glutathione, total homocysteine, and cysteine significantly increased over time. During follow-up, 20 of 97 (21%) patients had adverse cardiac events. Multivariate analysis revealed that only plasma-reduced glutathione was independently associated with events (hazard ratio 0.42, 95% confidence interval 0.18–0.99, $P=0.04$).**

Conclusions **Acute myocardial infarction patients have an altered redox state at 5–6 days after successful reperfusion with respect to controls. Low plasma levels of reduced glutathione at discharge are associated with cardiac events at follow-up.** ***Coron Artery Dis*** **18:77–82 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Patients with acute myocardial infarction (AMI) who undergo percutaneous coronary intervention (PCI) have a burst of reactive oxygen species generation [1]; inflammatory cell activity in the vulnerable plaques, ischemia, and the subsequent reperfusion by PCI are all sources of reactive oxygen species, contributing to myocardial injury [2–5]. Although a patent infarct-related artery after PCI with thrombolysis in myocardial infarction flow grade 2–3 is a major determinant of prognosis [6], a pro-oxidant environment persisting after successful revascularization may affect the late outcome.

Early evaluations of inflammatory and redox state markers have been assessed in patients with acute coronary syndromes and results are predictive of later cardiovascular events [7–9]. To our knowledge, data on redox state evaluation at discharge are lacking; the subacute phase of AMI is far from the burst of reactive oxygen species and their wide oscillations after PCI, and could be just the right time point to make up a multimarker profile of risk. Moreover, the maintenance of high antioxidant consumption in the subacute phase could conceivably lead to a persistent redox imbalance in the following months.

Measurements of plasma and blood aminothiols provide important insights on circulating redox state and are acknowledged to be accurate and not expensive. High plasma levels of cysteine and homocysteine (Hcy) favour a pro-oxidant milieu that has been associated with vascular disease [10,11]; conversely, glutathione (GSH) is one of the most important antioxidant compounds in blood with direct and indirect radical-scavenging properties [12]. The role of these aminothiols in predicting future events after AMI is still unknown.

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The aim of the present study was to test the hypothesis that in the subacute phase of reperfused AMI, an altered redox state persists and is associated with an adverse prognosis during mid-term follow-up.

Methods

Study population

We studied 97 patients [80 men, median 63 (interquartile range, 53, 69) years] consecutively admitted between May 2002 and December 2004 for a first AMI, with (53%) or without ST segment elevation. All patients were treated by PCI only and showed patent infarct-related coronary artery (thrombolysis in myocardial infarction flow grade 2 or 3) after angioplasty. Complete revascularization, which included the culprit lesion and the other possible lesions, was obtained in 61 patients (67%); the glycoprotein IIb/IIIa inhibitors were used during and after PCI in 71 patients (73%). Exclusion criteria were age >75 years, presence of known neoplastic disease, ongoing infection, disease affecting immune system, creatinine clearance <90 ml/min according to the Cockroft–Gault equation, type 1 diabetes mellitus, surgery or trauma within the previous month. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg on repeated measurements or chronic use of antihypertensive drugs [13]. Hypercholesterolemia was defined as the total cholesterol level >240 mgl/dl and/or low density lipoprotein (LDL) cholesterol level >160 mgl/dl or the need for lipid-lowering medication [14]. Diabetes mellitus was defined as fasting glucose levels ≥ 126 mg/dl or the need for insulin or oral hypoglycemic agents [15].

All patients underwent peripheral venous blood sample in fasting conditions for redox status evaluation 5–6 days after PCI. We first evaluated 14 patients with ST segment elevation AMI at three different times, 1 h, 72 h, and 5–6 days after primary PCI to assess the dynamics of redox parameters after reperfusion.

Blood samples were also obtained in a control group of 44 individuals matched for age, sex, and cardiovascular risk factors, as shown in Table 1. The enrolled participants, evaluated to assess their redox state and/or the presence of hyperhomocysteinemia, gave informed consent before entering the study.

Chemical analysis

Plasma samples for reduced and total thiols (Hcy, cysteine, GSH, cysteinylglycine), free malondialdehyde (MDA), vitamin E (α-tocopherol), and ascorbic acid were immediately centrifugated after blood collection to avoid any redox alteration, whereas the blood-reduced thiols were determined by prompt acidification. All samples were then frozen in liquid nitrogen and stored at -80°C until the analysis, which was performed within 2 days.

Table 1 Clinical and redox data in acute myocardial infarction (AMI) patients and controls

	AMI ($n=97$)	Controls ($n=44$)	P
Age (years)	63 (53, 69)	56 (51, 66)	NS
Male gender n (%)	80 (82)	34 (77)	NS
Smoking habit n (%)	50 (53)	22 (50)	NS
Diabetes n (%)	12 (12)	5 (11)	NS
Hypertension n (%)	44 (45)	25 (57)	NS
Hypercholesterolemia n (%)	43 (44)	22 (50)	NS
Total thiols			
Hcy	12.4 (9.7, 16.2)	11.6 (8.4, 16.2)	NS
Cysteinylglycine	37 (27, 37)	34 (28, 46)	NS
GSH	4.5 (3.0, 5.8)	5.4 (4.2, 6.4)	0.02
Cysteine	281 (205, 341)	269 (228, 308)	NS
Reduced thiols			
Hcy	0.13 (0.12, 0.18)	0.20 (0.12, 0.30)	0.007
Cysteinylglycine	2.27 (1.59, 3.01)	2.75 (2.14, 4.10)	0.002
GSH	1.19 (0.95, 1.37)	1.59 (1.11, 2.34)	0.006
Cysteine	6.21 (5.15, 7.95)	8.64 (6.30, 9.91)	0.0001
Ascorbic acid	22 (21, 26)	38 (20, 62)	0.0001
α-tocopherol	15 (12, 20)	20 (17, 28)	0.0001
MDA	0.76 (0.61, 1.03)	0.82 (0.43, 1.36)	NS
GSH_{bl}	645 (441, 808)	548 (337, 768)	NS

GSH, glutathione; GSH_{bl}, blood-reduced glutathione; Hcy, homocysteine; MDA, free malondialdehyde; NS, non significant.
Data are expressed as median and interquartile ranges (I, III) or number (percentage). Redox values are expressed in μmol/l.

Thiol and vitamin concentrations were determined by high-pressure liquid chromatography (HPLC; ProStar, Varian, Surrey, UK) according to methods described previously [16–18]. MDA levels were determined in stored plasma by gas chromatography–mass spectrometry with the isotope dilution technique [19]. Blood-reduced GSH (GSH_{bl}), an index of GSH concentrations in circulating cells, was determined as reported previously [20]and the values are expressed in μmol/l.

Troponin T at the admission and peak Creatine Kinase-MB mass levels were measured by the electro-immunochemiluminescent technique. High-sensitivity C-reactive protein (hs-CRP) at admission was determined by nephelometry (Image Immunochemistry Systems; Beckman Instrument, Fullerton, California, USA).

Follow-up

Patients were assessed for adverse cardiac events (cardiac death, nonfatal MI, and ischemia-driven revascularization procedures) by review of hospital records and/or by telephone interviews. The events were adjudicated by a panel of cardiologists blinded to the thiol levels. Follow-up information was obtained in all patients; median follow-up time was 15 (interquartile range, 9, 17) months.

Statistical analysis

Continuous variables are expressed as median and interquartile ranges (I, III). Differences between controls and AMI patients were compared using Student's t-test and Mann–Whitney's U-test when the distribution of the variable was asymmetrical; Fisher's exact test was used for categorical variables. Blood samples collected over the

three different times were compared using Friedman's nonparametric test. The Pearson correlation coefficient was calculated as a measure of association between variables. The individual effect of clinical characteristics, biochemical and redox data on outcome was evaluated by Cox's regression analysis; univariate hazard ratio was presented with 95% confidence interval per unit. Only the significant variables at univariate analysis entered into multivariate Cox's regression with the enter forward procedure, to avoid data overfitting. Statistical significance was settled at a P value of < 0.05. The statistical analyses were carried out with the Statistical Package for the Social Sciences release 10.0 for Windows (SPSS Inc, Chicago, Illinois, USA).

Results

Redox status in acute myocardial infarction and controls

Differences in redox aminothiols between AMI patients (blood sample at 5–6 days after admission) and controls are shown in Table 1. Moderate hyperhomocysteinemia, defined as plasma total Hcy falling between 12 and 30 μmol/l, was found in 51 AMI patients (52%) and 21 controls (48%) (P = NS). All plasma-reduced aminothiols, vitamins, and plasma total GSH were significantly lower in AMI patients than in controls.

Time course of redox parameters

In the 14 patients with ST segment elevation AMI sampled repeatedly after primary PCI, a significant increase in plasma total Hcy, cysteine, and GSH_{bl} was observed over time. In contrast, plasma-reduced cysteinylglycine and GSH, total GSH, and α-tocopherol significantly decreased over time; no changes in MDA were observed. The time course of the major significant redox measurements is shown in Fig. 1.

Redox status in patients with ST vs. without segment elevation acute myocardial infarction

Although all patients were treated with a successful percutaneous intervention, the two groups differed in the time between the onset of symptoms and the access to the cath lab [240 (180, 375) vs. 2320 (2285, 2410) min, respectively]. Furthermore, abciximab was administrated during and after coronary angiography in all ST segment elevation AMI patients, whereas a conventional antiplatelet and antithrombotic therapy was administrated in all non-ST segment elevation AMI patients. As the different treatment timing and the type of pharmacological treatment could affect redox pattern, we tested if ST and non-ST segment elevation AMI differed in redox state. Total plasma Hcy and cysteine were higher in ST segment elevation than in non-ST segment elevation AMI [12.9 (10.2, 18.2) vs. 12.0 (8.7, 14.6), $P = 0.04$; 294 (217, 366) vs. 254 (187, 306), $P = 0.02$, respectively]. The other redox parameters, including MDA, were not significantly different in the two subgroups.

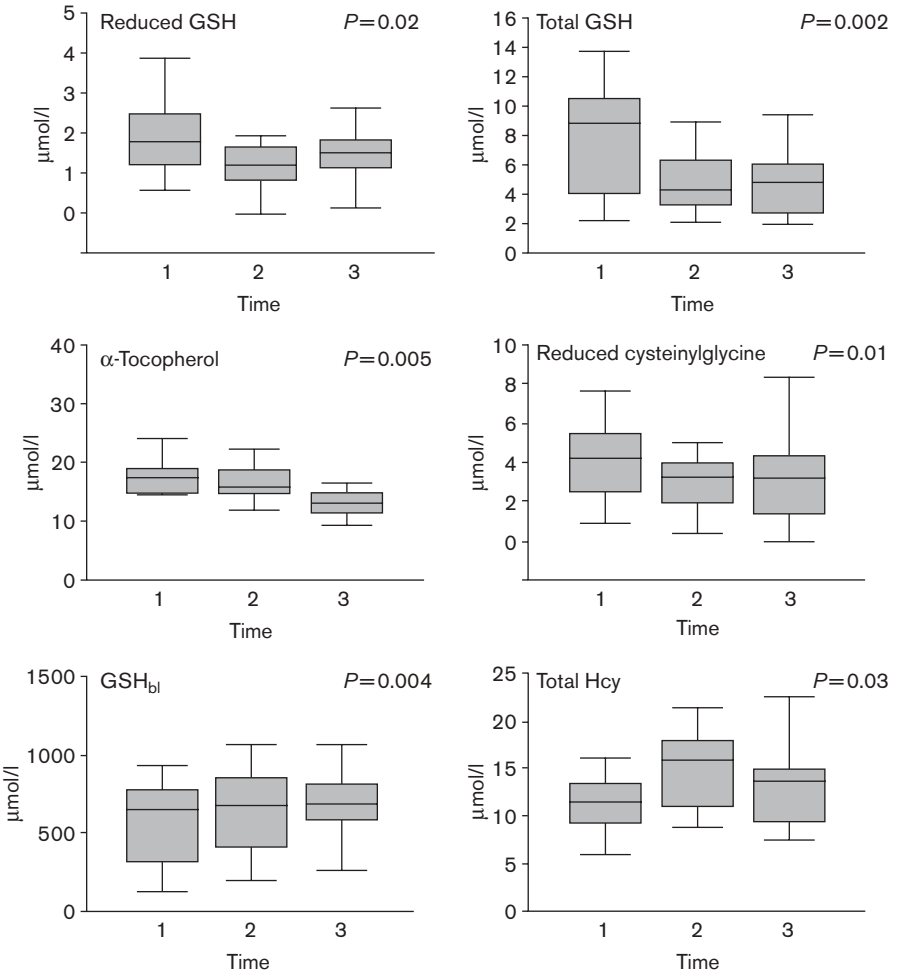
Redox status and late outcomes

During follow-up, 20 (21%) patients had adverse cardiac events as follows: two patients died of cardiac cause, two suffered from reinfarction and 16 patients underwent myocardial revascularization procedures driven by clinical worsening. No one in the subgroup of patients who underwent repeated blood sampling had cardiac events. Univariate analysis among clinical, biochemical and redox data showed that hypercholesterolemia ($P = 0.03$) plasma-reduced GSH ($P = 0.01$), and ascorbic acid ($P = 0.001$) were significantly associated with the outcome (Tables 2 and 3). Nevertheless, at multivariate analysis only plasma-reduced GSH was an independent predictor of cardiac events (hazard ratio 0.42, 95% confidence interval 0.18–0.99, $P = 0.04$).

Discussion

The main findings in this study are: first, a different redox state characterizes AMI patients in subacute phases after successful PCI with respect to control participants, matched for age, sex, and cardiovascular risk factors. No previous studies have performed a complete aminothiol evaluation in this clinical setting and, consequently, there are no reports on the comparison with control participants. All plasma-reduced aminothiols, vitamins, and plasma total GSH were significantly lower in AMI than in controls, confirming an abnormal redox status after an acute coronary event, which led to an imbalance in antioxidant defence. Interestingly, AMI and control participants, matched for cardiovascular risk factors, except Hcy, had similar values of Hcy, ruling out that elevated plasma levels of this thiol may cause redox state differences between the two groups. Moreover, the different redox state might also be related to its time course after PCI; in fact, plasma-reduced cysteinylglycine and GSH, total GSH, and α-tocopherol significantly decreased over time. The decrease in plasma GSH values probably reflects the scavenger role of this tripeptide against ischemia and reperfusion injury; this depletion involved both reduced and total plasma GSH forms, excluding a relative increase in the oxidized form and suggesting a reduced transport flow from the intracellular to the extracellular space. Consequently, intracellular GSH (GSH_{bl}) significantly increased over time and was slightly higher in AMI than in controls, confirming a successful buffer of intracellular free radical formation. Furthermore, the progressive fall in α-tocopherol levels reflected its role as the major lipid phase chain-breaking antioxidant and its consumption is confirmed by the significant lower level in AMI patients with respect to controls. The effectiveness of the antioxidant protection of GSH_{bl} and α-tocopherol is proved by the fact that MDA, a marker of lipid peroxidation, did not increase after PCI. This is in contrast with previous reports [4,5,21], in which significant variations in MDA during ischemia/reperfusion injury have been found. In these studies, however, the time scale of blood samples

Fig. 1



Changes in plasma and blood glutathione (GSH) forms, plasma Hcy, cysteinylglycine, and α-tocopherol after primary PCI in 14 patients. Box-plots show values (µmol/l) expressed in median, interquartile ranges (box), and extreme values (whiskers). Time 1, 2, 3: at 1 h, 72 h, and 5–6 days after PCI, respectively; *P* for time. GSH, glutathione; GSH_{bl}, blood-reduced glutathione; Hcy, homocysteine; PCI, percutaneous coronary intervention.

Table 2 Clinical and biochemical data at univariate analysis in acute myocardial infarction (AMI) patients with or without events

	No event (*n*=77)	Event (*n*=20)	HR	95%CI
Age (years)	63 (55, 70)	61 (51, 64)	0.97	0.94–1.01
Male sex *n* (%)	63 (82)	17 (85)	1.17	0.34–4.04
BMI (kg/m^2)	26.1 (23.7, 28.6)	25.7 (24.0, 28.2)	0.99	0.86–1.15
Smoking habit *n* (%)	39 (51)	11 (58)	1.23	0.49–3.09
Diabetes *n* (%)	8 (10)	3 (16)	1.51	0.44–5.24
Hypertension *n* (%)	28 (39)	7 (44)	1.37	0.51–3.71
Hypercholesterolemia *n* (%)	25 (35)	11 (61)	2.80	1.08–7.25
Anterior AMI *n* (%)	32 (42)	12 (60)	2.11	0.86–5.20
ST segment elevation *n* (%)	40 (53)	10 (56)	1.22	0.48–3.09
Vessel I (%)				
1	39 (52)	6 (30)	1.00	–
2	21 (28)	8 (40)	1.83	0.63–5.28
3	15 (20)	6 (30)	2.10	0.68–6.55
Incomplete revascularization n (%)	22 (29)	8 (40)	1.71	0.65–4.48
Hs-CRP (µg/l)	1.10 (0.63, 3.18)	0.70 (0.30, 4.30)	1.00	0.94-1.06
Admission troponin T (µg/l)	0.21 (0.08, 2.24)	0.29 (0.10, 5.81)	1.01	0.99-1.001
Peak CK MB (µg/l)	130 (34, 336)	144 (51, 261)	1.00	0.99-1.002

BMI, body mass index; hs-CRP, high sensitivity C-reactive protein; HR, hazard ratio with their 95% confidence interval (CI, per unit); Data are expressed as median and interquartile ranges (I, III) or number (percentage).

Table 3 Redox data at univariate analysis in acute myocardial infarction (AMI) patients with or without events

	No event ($n=77$)	Event ($n=20$)	HR	95%CI
Total thiols				
Hcy	12.5 (9.7, 16.2)	11.9 (9.4, 17.8)	1.02	0.98–1.07
Cysteinylglycine	37 (27, 55)	38 (23, 45)	0.99	0.97–1.02
GSH	4.5 (3.1, 5.9)	4.5 (2.8, 5.7)	0.88	0.69–1.12
Cysteine	274 (206, 340)	182 (294, 342)	1.01	0.99–1.01
Reduced thiols				
Hcy	0.13 (0.12, 0.17)	0.13 (0.09, 0.24)	1.91	0.02–163
Cysteinylglycine	2.27 (1.75, 3.06)	2.26 (1.21, 2.46)	0.70	0.43–1.03
GSH	1.19 (1.02, 1.43)	1.16 (0.43, 1.25)	0.31	0.12–0.80
Cysteine	6.21 (5.43, 7.95)	6.21 (2.89, 7.60)	0.89	0.73–1.10
Ascorbic acid	22 (17, 23)	26 (22, 49)	1.05	1.02–1.08
α-tocopherol	14 (12, 20)	16 (13, 21)	1.02	0.94–1.11
MDA	0.76 (0.60, 0.90)	0.84 (0.76, 1.36)	1.34	0.91–1.95
GSH_{bl}	635 (447, 817)	681 (393, 753)	1.00	0.99–1.002

GSH, glutathione; GSH_{bl}, blood-reduced glutathione; Hcy, homocysteine; MDA, free malondialdehyde; HR, hazard ratio with their 95% confidence interval (CI, per unit). Values (μmol/l) are expressed as median and interquartile ranges (I, III).

was shorter than 2 days after reperfusion, involving just the phases with a great burst of reactive oxygen species. Furthermore, the laboratory techniques used, that is, the HPLC method and the thiobarbituric acid colorimetric assay, have been proved to be less accurate than gas chromatography–mass spectrometry with the isotope dilution technique [19]. Nevertheless, according to a previous report [22] an increase, we found in total Hcy levels in AMI patients sampled repeatedly, likely owing to its increased binding with acute phase proteins [23].

As our study population included both ST and non-ST segment elevation AMI patients, we verified whether a difference in redox status occurred, accounting for the effect of time between onset of symptoms and access to the cath lab and type of treatment. AMI patients with ST segment elevation had higher total plasma Hcy and cysteine than non-ST segment elevation AMI patients. These findings are in agreement with the increase of Hcy concentrations after tissue damage [23], owing to the acceleration of specific methylation reactions. The significant correlation between Hcy and cardiac necrosis markers, which were higher in ST segment elevation AMI patients (data not shown), reinforces this hypothesis.

Our data differ from previous studies in which inflammatory and redox status was assessed in the acute phases, that is, at the admission or after revascularization with a short blood samples time scale [4,5,7,8]. In fact, in the population as a whole, blood samples were performed in the subacute phases to minimize the burst of reactive oxygen species and their wide oscillations after PCI, which are peculiar to the ischemic–reperfusion injury; furthermore, discharge could be the right time point to define the patient's risk profile. Nevertheless, the similar prognostic value of the inflammatory markers (i.e. myeloperoxidase) [24,25] sampled in acute phases and our redox variables may point out the link between leucocyte activation, which generates oxidative stress, and consumption of antioxidant and reducing molecules.

The outcome analysis revealed plasma-reduced GSH as the only marker independently associated with cardiac events at a mid-term follow-up. The evidence of high antioxidant consumption, in terms of low plasma-reduced GSH, could lead to the persistence of oxidative stress and, conceivably, to a persistent inflammation in the following months. This finding is of significant clinical impact, identifying patients at high risk of active atherosclerosis and, thus, of future coronary events.

GSH and GSH-related enzymes were reported to play a crucial role in myocardial protection from free radicals [26], which, in turn, are able to increase the atherogenicity of lipoproteins. Furthermore, GSH may enhance the bioavailability of nitric oxide [27], and inhibit platelet aggregation [28]; its deficiency has been found in atherosclerotic plaques [12]. The prognostic role of GSH peroxidase-1 activity, sampled at admission in patients with suspected or known coronary artery disease, has been recently assessed [9,29].

In our patients, hs-CRP did not correlate with outcomes, although it is considered the easier currently available inflammatory biomarker [30] and an excellent specific predictor of poor outcome after acute coronary syndromes [31]. We have almost half of patients with hs-CRP < 1.0 mg/l and the small sample size, combined with the short follow-up, did not allow us to detect any prognostic value. Nevertheless, GSH levels may intrinsically reflect the balance between the inflammatory burden and the efficacy of antioxidant response in the subacute phases after AMI.

Study limitations

Redox measurements at hospital admission are lacking. Therefore, redox state changes between admission and discharge could not be assessed. Furthermore, we did not perform measurements of GSH-related antioxidant enzymes, such as GSH peroxidase, because the study design took into account the feasibility (real-time

measurements) and the economic implications of redox analyses. In fact, a single run determination of plasma aminothiols by HPLC provides a cost-saving assessment of redox status. On the other hand, accurate GSH peroxidase determinations are costly, time consuming and usually not performed in real time.

Conclusions

In the present study, reperfused AMI patients showed an altered redox state in the subacute phase in terms of lower values of reduced forms of thiols and vitamins, with respect to controls matched for age, sex, and cardiovascular risk factors. Conversely, lipid peroxidation and intracellular GSH content in AMI patients were similar to controls, suggesting an effective antioxidant defence at 5–6 days after AMI. Patients with lower plasma-reduced GSH at the discharge exhibit major risk of cardiac events at a mid-term follow-up, indicating that GSH may be a potential biomarker for outcome prediction. These results support the clinical relevance of assessing circulating thiols following an acute ischemic event, to identify patients with persistent inflammation and, thus, at high risk for future coronary events.

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