ORIGINAL ARTICLE

Role of preprocedural glutathione concentrations in the prediction of major adverse cardiac events in patients with acute coronary syndrome treated with percutaneous coronary intervention

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KEY WORDS

coronary angioplasty, diabetes, glutathione, major adverse cardiac events

ABSTRACT

INTRODUCTION Poor antioxidant protection of cardiomyocytes due to cardiac ischemia and low serum levels of reduced glutathione (GSH) may be associated with enhanced risk of coronary restenosis after primary percutaneous coronary intervention (pPCI).

OBJECTIVES The aim of this study was to investigate whether preprocedural serum reduced GSH, reflecting the antioxidant status, may be predictive of major adverse cardiac events (MACE) in patients with acute coronary syndrome (ACS) treated with pPCI.

PATIENTS AND METHODS Preprocedural serum GSH level was evaluated in 141 patients with ACS treated with pPCI with bare-metal stent (BMS) deployment. During a 15-month follow-up, 30 patients (mean age, 61 ±10 years) experienced a MACE. The remaining 111 subjects constituted the non-MACE group (mean age, 63 ±10 years).

RESULTS The MACE group had significantly lower GSH levels compared with the non-MACE group (P <0.001); significant differences were also observed in a subgroup of type 2 diabetic patients (P <0.001). All patients were arbitrarily classified as having low (median, ≤1.39; 1.04–1.55 µmol/l) or high serum GSH (median, >2.26; 2.09–2.99 µmol/l; P <0.001). The Kaplan–Meier analysis showed a significantly longer MACE-free survival in patients with higher serum GSH (P <0.004). The Cox proportional hazards regression indicated that patients with lower GSH were 2.2 times more likely to experience MACE (95% confidence interval [CI], 1.2–3.9; P <0.02 for the whole group and 1.8–11.8 for diabetic patients; P <0.002).

CONCLUSIONS Preprocedural GSH levels may be useful in the prediction of MACE in patients with ACS scheduled for pPCI and BMS deployment, especially in diabetic subjects.

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INTRODUCTION Increased incidence and severity of ischemic heart disease in diabetic patients leads to an increased requirement for coronary revascularization, either percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG).[1,2] PCI is still regarded as less efficient in diabetic patients. Diabetic patients constitute 20% of he patients undergoing PCI, and this

TABLE 1 Baseline clinical and demographic characteristics of the study groups

Parameters	MACE n= 30	In-stent restenosis n = 15	Re-PCI n = 21	Non-MACE n = 111
age, y	61.3 ±10.3	58.3 ±10.3	59.8 ±10.7	63.2 ±9.9
sex, male/female	19/11	8/7	13/8	80/31
BMI, kg/m^2	27.4 ±3.5	28.5 ±3.9	27.1 ±3.7	28.3 ±3.6
HbA_{1c}, %	6.3 (5.8–7.4)	6.5 (5.5–8.3)	6.1 (5.6–7.9)	6.3 (5.7–6.8)
type 2 diabetes	17/30	9/15	10/21	58/111
with a history of disease[a]	10/30	6/15	7/21	21/111
newly diagnosed[b]	7/30	3/15	3/21	37/111
hypertension, n	19/30	11/15	14/21	60/111
total cholesterol, mmol/l	6.0 ±1.5	6.2 ±1.6	6.1 ±1.6	6.3 ±1.4
HDL cholesterol, mmol/l	1.2 (1.0–1.4)	1.2 (1.0–1.3)	1.2 (1.1–1.6)	1.3 (1.1–1.6)
LDL cholesterol, mmol/l	3.8 ±1.4	4.1 ±1.4	3.8 ±1.5	4.0 ±1.3
triglycerides, mmol/l	1.8 (1.2–2.3)	2.1 (1.6–2.5)	1.9 (1.2–2.3)	1.6 (1.1–2.5)
history of myocardial infarction[a], n	10/30	3/15	4/21	21/111
history of PCI, n	4/30	2/15	2/21	6/111
history of CABG, n	0/30	0/15	0/21	1/111
ejection fraction, %	52.6 ±9.2	53.6 ±8.6	54.8 ±8.6	51.4 ±10.5
restenosis-free survival, wk	16.0 (8.0–28.5)	20.0 (10.0–24.0)	20.0 (12.0–29.0)	–
overall survival, wk	57.5 (38.8–64.0)	48.0 (33.0–60.0)	59.0 (38.5–64.0)	64.5 (56.8–72.0)

Data are presented as mean ± standard deviation (SD) or median and interquartile range (from the lower to the upper quartile).

The significance of differences between the groups was estimated with the *t* test (with the Bonferroni correction for multiple testing), Kruskal–Wallis test, post-hoc all pairwise Conover–Inman test, or the test for proportions (with Bonferroni correction for multiple testing).

a one-sided $P = 0.0454$ for MACE vs. non-MACE, **b** one-sided $P = 0.0408$ for re-PCI vs. non-MACE

Abbreviations: BMI – body mass index, CABG – coronary artery bypass grafting, HbA_{1c} – hemoglobin A_{1c}, HDL – high-density lipoprotein, LDL – low-density lipoprotein, MACE – major adverse cardiac event, PCI – percutaneous coronary intervention, re-PCI – repeated PCI

number is expected to reach 30% by 2015.[1,3,4] Primary PCI (pPCI) with bare-metal stent (BMS) deployment plays a pivotal role in the treatment of acute coronary syndrome (ACS), but also has some limitations, restenosis being the major one. Even a more problematic issue, strictly connected to coronary angioplasty, is an early coronary reocclusion, the so called reperfusion injury.

Free radicals play a major role in triggering a deleterious cascade of events after reperfusion. Increased lipid and protein oxidation products and decreased action of antioxidant enzymes contribute to increased oxidative stress and correlate positively with the severity of coronary artery disease (CAD).[5] Furthermore, antioxidative defense seems to be more impaired in type 2 diabetic patients and accounts for the development of macrovascular complications in diabetes.[6] A lowered serum concentration of reduced glutathione (GSH) is considered the predictor of coronary restenosis after pPCI, and its deficiency can lead to pronounced postreperfusion syndrome.[5]

This prospective study aimed at evaluating the effect of preprocedural serum GSH concentrations on the occurrence of major adverse cardiac events (MACE) in patients with or without type 2 diabetes, who underwent pPCI with BMS deployment in ACS. The serum GSH concentration was evaluated also in the context of a few selected MACE events, including restenosis and repeated PCI (re-PCI).

PATIENTS AND METHODS

Patients This was a single-center prospective cohort trial with a 15-month follow-up. The whole study lasted for 18 months (2004–2006). Of 397 patients hospitalized in the Department of Cardiodiabetology at the time of the study, 251 were primarily classified as eligible for PCI and stent deployment. Of these patients, 97 were excluded based on due to the inclusion and exclusion criteria (see below). Of the remaining 154 patients, 13 participants completed the treatment but were lost to follow-up (moved to other addresses). The remaining 141 patients completed the whole study (mean age, 62.8 ±10.0 years; 99 men [70.2%]; mean age, 61.3 ±10.4 years and 42 women [29.8%]; mean age, 66.1 ±8.3 years).

The inclusion criteria were ACS on admission and simultaneous coronary angioplasty with at least 1 BMS deployment. We deliberately enrolled only patients with BMS stents to ensure the homogeneity of the study group with regard to our complex primary endpoint. Of 397 patients with ACS who underwent urgent coronary angiography, 251 were scheduled for PCI and stent deployment; 46 patients were treated with plain old balloon angioplasty; 40 patients were scheduled

TABLE 2 Diabetes and elevated hemoglobin A_{1c} levels in the study groups

	All patients n = 141	MACE n = 30	In-stent restenosis n = 15	Re-PCI n = 21	Non-MACE n = 111
patients with a history of diabetes on admission					
HbA_{1c} >6.1%	30[a]	10	6	7	20
HbA_{1c} ≤6.1%	1[a]	0	0	0	1
patients without a history of diabetes on admission[b]					
HbA_{1c} >6.1%	44[a]	7	3	3	37
HbA_{1c} ≤6.1%	66	13	6	11	53

a the whole diabetic population comprised 75 patients (diagnosed based on the American Diabetes Association criteria of 2004)
b in patients without a history of diabetes on admission and HbA_{1c} levels >6.1%, diabetes was confirmed by a standard oral glucose tolerance test within a month after the diagnosis of acute coronary syndrome

Patients with a history of type 2 diabetes (n = 31, 22%) and newly diagnosed type 2 diabetes (n = 44, 31%) were all considered as one diabetic group (n = 75, 53%) and were compared with the remaining patients, enrolled as nondiabetic patients (n = 66, 47%).

Abbreviations: see **TABLE 1**

for CABG; and 60 patients received conservative treatment.

The trial exclusion criteria were as follows: clinical and laboratory signs of acute inflammatory process (C-reactive protein [CRP], >10 mg/l) and chronic inflammatory diseases, neoplastic diseases, immunosuppressive treatment, heart failure (ejection fraction, <30%) or renal failure (serum creatinine concentration, >1.5 mg/dl) on admission. Of the included patients with ACS, 79 individuals (56%) showed persistent ST-elevation myocardial infarction (STEMI), 27 patients (19%) had nonpersistent ST-elevation (NSTEMI), and 35 patients (25%) had unstable angina (UA). Patients with STEMI were scheduled for pPCI within 12 hours from the onset of chest pain, while those with NSTEMI and UA within 48 hours from the onset. The contributions of arteries responsible for ACS were as follows: left anterior descending (LAD) artery (37%), circumflex (Cx) coronary artery (23%), and right coronary artery (RCA) (40%). The identification of hemodynamically significant coronary stenosis exceeding 70% vessel diameter in 1, 2, or 3 coronary vessels among LAD, Cx, or/and RCA arteries were recorded in 64%, 58%, and 64.5% of the patients, respectively. Demographic and clinical characteristics of the patients enrolled in the study are presented in **TABLES 1** and **2**.

The composite primary endpoint in our study during a 15-month follow-up was MACE, defined either as re-PCI or CABG, myocardial infarction (MI), cardiac death, or cardiac rehospitalization, or a combination thereof. In patients with clinical symptoms of restenosis, urgent coronarography was performed. In the cases of angiographically proven restenosis (narrowing of the coronary artery lumen exceeding 50% of its diameter in relation to the referential coronary segment), patients were qualified either to re-PCI or CABG). Patients with a MACE were classified into 2 subgroups for the purpose of further analysis: 1) in-stent restenosis (ISR), defined as restenosis after previously deployed stent (15 patients); 2) pre-PCI, defined as the requirement for subsequent PCI during the follow-up period (21 patients) due to new changes in the same or other coronary vessels.

Mean times from pPCI to re-PCI (restenosis-free survival) and from pPCI to MACE were 20 and 16 weeks, respectively. There were no significant differences in the lengths and diameters of deployed stents between the examined groups (MACE, ISR, re-PCI, and non-MACE). In general, there were no differences between the ACS and CAD subgroups with regard to the lengths or diameters of BMS; however, in patients with NSTEMI, stents were significantly wider compared with those with STEMI or UA (3.5 mm vs. 3.1 mm or 3.0 mm, respectively, $P < 0.02$).

Prior to angioplasty, every patient received from 300 to 500 mg of acetylsalicylic acid (ASA) and a loading dose of clopidogrel, and was administered intravenous unfractionated heparin (100 U/kg body weight). None of the patients included in the study had been given any previous fibrinolytic treatment. Following the intervention, patients were treated with dual antiplatelet therapy (ASA + clopidogrel), administered according to the accepted standards. Any other medications were applied according to individual clinical indications.

The study was approved by the local Medical University Ethical Committee and followed the ethical principles for clinical research based on the Declaration of Helsinki.

Coronary angiography and angioplasty In all patients who did not undergo any previous fibrinolytic treatment, an urgent coronarography and simultaneous primary coronary angioplasty was performed (on average, 3 hours from admission) using the modified Seldinger's method (puncture of the femoral artery) or the modified Sones' method (puncture of the radial artery) on the Shimadzu angiograph with the Digitex 2400 system (Schimadzu, Florida, United States).

Laboratory tests The following parameters were measured in all patients: glycated hemoglobin

(HbA_{1c}; chromatographic ion-exchange low-pressure method, Fast HbA_{1c} Assay, Drew Scientific Limited, Cumbria, United Kingdom), lipid profile (total cholesterol, high-density lipoprotein cholesterol, total triglycerides, low-density lipoprotein cholesterol evaluated using the Friedewald's formula), high-sensitivity CRP (hsCRP), fibrinogen, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α; Milliplex-Cold, Merck Millipore, Darmstadt, Germany), N-terminal pro-B-type natriuretic peptide (immunochemical electroluminescence assay, Roche Diagnostics, Elecsys 1010 analyzer), and reduced GSH (Glutathione Assay Kit; Cayman Chemical Company, Ann Arbor, Michigan, United States). The samples of venous blood for laboratory tests were collected on admission (directly before coronarography and PCI).

Statistical analysis For all quantitative parameters, mean with a standard deviation (SD) or median and interquartile range (first to third quartiles, 1Q–3Q) were evaluated for normally and nonnormally distributed variables, respectively (Shapiro–Wilk's test). The *t* test was used to compare the data showing no departures from normality, and the nonparametric Mann–Whitney test was used for the remaining variables. The Bonferroni correction was employed for multiple testing (more than 2 groups).[7,8] Qualitative data were assessed with the χ^2 test or the exact Fisher's test. A logistic regression model, either unadjusted or adjusted according to selected demographic or clinical parameters, was used to assess how selected variables affected MACEs. The results of the logistic regression analysis are presented as the odds ratio (OR) with the 95% confidence interval (CI).

In the time-to-event analysis of data, the event of interest was disease-free survival, whereas the explanatory data were the factors believed to be associated with the event or to promote or inhibit its occurrence. The starting point was the date of the first PCI treatment, and in uncensored (complete data) the ending point was death or the occurrence/recurrence of the event of interest (MACE, ISR, re-PCI). Censored (incomplete) data included participants who remained alive at the termination of the study, died of causes unrelated to the disease of interest, or quitted the study for some reasons. The life-table method was used to estimate the survival rate and, for patients included in the study, the requirements of the Kaplan–Meier analysis have been met. For the purpose of the time-to-event analysis, all 141 patients with ACS were allocated to 1 of 2 groups according to serum GSH concentrations: rank 0 (group 2, GSH below or equal to the median) or rank 1 (group 1, GSH above the median). The log-rank test was used to compare 2 survival curves. The Cox proportional hazards regression analysis was used to assess the associations between explanatory variables and survival rate, with the assumption that a ratio risk greater than 1 (lower than 1) denotes an increased (decreased) risk for those with a given characteristic.

To estimate the minimal sizes of the groups, we used the standard statistical power-based algorithms. Briefly, our leading hypothesis was that in patients subjected to PCI procedures serum GSH concentration were characterized by large individual variability. We based this assumption on our preliminary observations which suggested that GSH is often binominally distributed in patients with ACS and that GSH distributions often showed platykurtic characteristics (with a negative value of kurtosis, i.e., with a flatter peak around its mean and thin tails within the distribution due to large variations within observations and the data being less concentrated around its mean). The sample sizes were estimated independently for 1 leading variable: 1) serum GSH concentrations in 2 groups of PCI

TABLE 3 Characteristics of the study groups in relation to the type of acute coronary syndrome, number of diseased coronary vessels, and basic parameters of bare-metal stents

Variable		All patients n = 141	MACE n = 30	In-stent restenosis n = 15	Re-PCI n = 21	Non-MACE n = 111
STEMI		79 (56)	12 (40)	7 (47)	8 (38)	67 (61)
NSTEMI		27 (19)	9 (30)	6 (40)	6 (29)	18 (16)
UA		35 (25)	9 (30)	2 (13)	7 (33)	26 (23)
CAD	1V	48 (34)	6 (20)	3 (20)	5 (24)	42 (38)
	2V	60 (43)	15 (50)	8 (53)	10 (48)	45 (40)
	3V	33 (23)	9 (30)	4 (27)	6 (28)	24 (22)
length of stent(s), mm		16.0 (13.0–22.0)	15.0 (12.8–22.8)	18.0 (15.0–27.0)	16.0 (14.0–22.5)	16.0 (13.0–22.0)
diameter of stent(s), mm		3.0 (3.0–3.5)	3.0 (2.8–3.5)	3.0 (2.9–3.5)	3.0 (2.8–3.5)	3.0 (3.0–3.5)

Data are presented as a number (percentage) or median and interquartile range.

No significant differences in the lengths and diameters of stents between patients with and without MACE and those with ISR and re-PCI were observed as estimated with the Kruskal–Wallis test.

Abbreviations: CAD – coronary artery disease with 1 (1V), 2 (2V) or 3 (3V) diseased vessels, NSTEMI – non-ST elevation myocardial infarction, STEMI – ST-elevation myocardial infarction, others – see TABLE 1

TABLE 4 Reduced serum glutathione concentrations in the study groups

	All patients n = 141	MACE n = 30	In-stent restenosis n = 15	Repeated PCI n = 21	Non-MACE n = 111
all patients (141; 111, 30)	1.78 (1.39–2.28)	1.93 (1.49–2.37)	1.41 (0.92–1.87)	1.51 (0.71–2.05)	1.36 (0.73–1.93)
STEMI (79; 67, 12)	1.84 (1.35–2.35)	2.03 (1.38–2.36)	1.40 (1.20–1.60)	–	–
NSTEMI (25; 18, 9)	1.55 (1.39–2.08)	1.68 (1.44–2.10)	1.43 (0.92–1.70)	–	–
UA (35; 26, 9)	1.76 (1.39–2.37)	1.85 (1.49–2.44)	1.40 (1.06–2.05)	–	–
CAD 1V (48; 42, 9)	1.67 (1.17–2.31)	1.88 (1.38–2.36)	1.09 (0.77–1.49)	–	–
CAD 2V (60; 45, 15)	1.73 (1.45–2.33)	1.86 (1.55–2.40)	1.45 (1.15–1.77)	–	–
CAD 3V (33; 24, 9)	2.03 (1.39–2.23)	2.11 (1.64–2.33)	1.41 (1.37–2.05)		–
P[a]			0.0003	0.0071	0.0071
diabetic patients (75; 60, 15)	1.73 (1.36–2.26)	2.05 (1.48–2.50)	1.38 (0.89–1.52)	1.36 (0.72–1.79)	1.34 (0.72–1.66)
STEMI (18; 12, 6)	1.71 (1.37–2.36)	1.84 (1.41–2.49)	1.38 (1.36–1.43)	–	–
NSTEMI (41; 36, 5)	1.58 (1.38–2.13)	2.05 (1.60–2.49)	0.95 (0.82–1.35)	–	–
UA (16; 12, 4)	2.05 (1.49–2.26)	2.07 (1.55–2.37)	2.05 (1.40–2.07)	–	–
CAD 1V (25; 21, 4)	1.76 (1.07–2.41)	2.05 (1.25–2.64)	1.18 (0.80–1.68)	–	–
CAD 2V (30; 23, 7)	1.67 (1.43 – 2.14)	1.82 (1.54–2.38)	1.36 (1.15–1.42)	–	–
CAD 3V (20; 16, 4)	2.06 (1.43–2.14	2.08 (1.64–2.31)	1.71 (1.18–2.06)	–	–
P[b]			0.0002	0.0054	0.0015
nondiabetic patients (66; 51, 15)	1.82 (1.42–2.29)	1.85 (1.51–2.26)	1.56 (0.89–2.18)	1.69 (0.68–2.91)	1.41 (0.71–2.11)
STEMI (35; 29, 6)	2.18 (1.55–2.35)	2.19 (1.60–2.35)	1.61 (0.89–2.13)	–	–
NONSTEMI (9; 6, 3)	1.56 (1.51–1.82)	1.64 (1.45–1.80)	1.56 (1.54–1.84)	–	–
UA (21; 15, 6)	1.60 (1.38–2.44)	1.79 (1.51–2.45)	1.38 (1.14–1.65)	–	–
CAD 1V (25; 23, 2)	1.70 (1.32–2.16)	1.82 (1.39–2.26)	1.04 (0.87–1.20)	–	–
CAD 2V (27; 19, 8)	1.83 (1.50–2.39)	1.94 (1.58–2.41)	1.77 (1.29–2.61)	–	–
CAD 3V (14; 9, 5)	1.87 (1.41–2.34)	2.21 (1.79 3.04)	1.41 (1.40–1.56)	–	–
P[c]			0.1867	0.4236	0.1104

Data are presented as median and interquartile range and expressed in μmol/l; the overall number of patients and the numbers of patients with and without MACE are given in parentheses in the first column. Significance vs. patients without MACE was estimated by the Mann–Whitney test (corrected for multiple comparison testing).

a vs. patients without MACE, **b** vs. diabetic patients without MACE, **c** vs. nondiabetic patients without MACE

Abbreviations: see **TABLES 1** and **3**

patients diverged according to the GSH median (below or equal to the median and above the median) in the whole study group, and 2) restenosis-free survival times in these groups. In the first approach, we expected to reveal at least 50% difference with the significance of at least 0.01 and statistical power (i.e., the likelihood of rejecting false null hypothesis) of 0.8, assuming that individual variability (pooled SD) would not exceed 75% (based on our preliminary observations in ACS patients). Such an estimation gave at least 55 subjects in each of the 2 groups. In the second approach, we expected to find out the hazard ratio of at least 2 with the significance of at least 0.01 and statistical power of 0.8, assuming that the median restenosis-free survival time for patients with lower GSH levels would be 6 months at the most, and accepting the accrual time for recruitment of 6 months and the additional follow-up time after recruitment of 15 months. Relying on such group size estimates, we decided to include at least 66 to 70 patients in each group.

Statistical calculations were made with Statistica for Windows (StatSoft Inc., Tulsa, Oklahoma, United States) and Stats Direct (StatsDirect Ltd., ver. 2.7.7., Cheshire, United Kingdom).

RESULTS **Study outcomes** At 15 months of follow-up, 30 patients (21.3%) experienced MACEs. Requirement for re-PCI occurred in 21 patients with MACE (70%). The most frequent adverse events was ISR, which occurred in 15 patients (50% of the MACE patients). Other cardiac events occurred in 14 patients (46.7%) and included: re-PCI not associated with a previously deployed stent (6 patients), CABG (1 patient), cardiac death (4 patients), and cardiac hospitalizations (4 patients). Patients in the MACE group did not differ significantly from controls (non-MACE) with regard to applied pharmacological treatment and to the results of serum biochemical tests evaluated on admission. Duration of diabetes in patients with MACE was longer compared with those without MACE. The compared subgroups of patients (STEMI, NSTEMI,

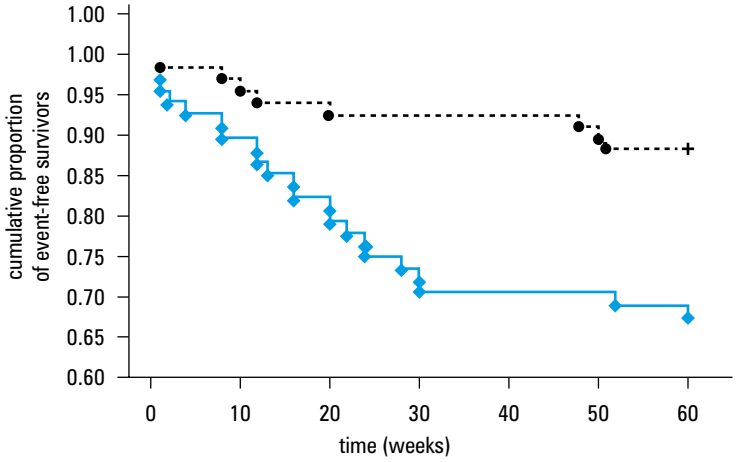

FIGURE 1 Kaplan–Meier curves of major adverse cardiac events (MACE)-free survivors in the study groups divided according to serum glutathione (GSH) concentrations; the step function of the estimated cumulative proportions of MACE-free survivors are given for patients with either high (higher than the median of 1.78 μmol/l, dashed line; group 1) or low (lower than or equal to the median, solid line; group 2) serum GSH concentrations; complete observations are marked with circles or triangles for groups 1 and 2, respectively; censorships are marked by "+"; the significance of differences between survival curves was $P = 0.0037$ (log-rank test)

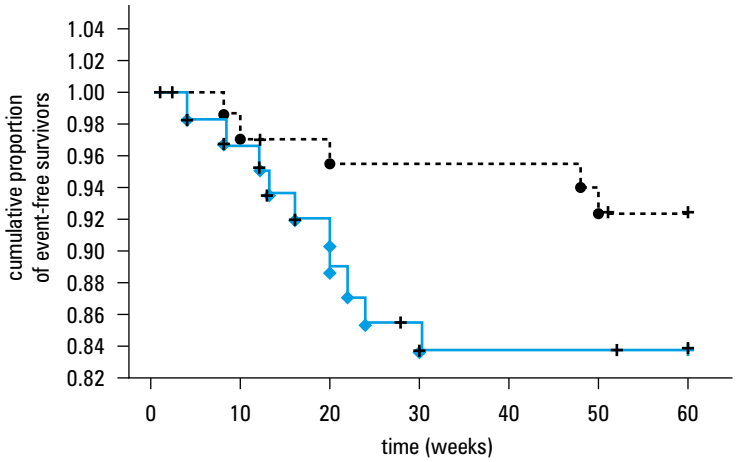

FIGURE 2 Kaplan–Meier curves of in-stent restenosis-free survivors in the study groups divided according to serum glutathione (GSH) concentrations; the estimated cumulative proportions of restenosis-free survivors are given for patients with either high (higher than the median of 1.78 μmol/l, dashed line; group 1) or low (lower than or equal to the median, solid line; group 2) serum GSH concentrations; complete observations are marked with circles or triangles for groups 1 and 2, respectively; censorships are marked by "+"; differences between the groups were nonsignificant ($P = 0.121$; log-rank test)

UA) did not differ with respect to the ejection fraction (EF) (TABLE 1). Also, we did not demonstrate a significant effect of the number of narrowed coronary vessels on EF (data not shown).

Serum GSH concentrations in the MACE and non-MACE groups The serum GSH concentration was significantly lower in the MACE compared with the non-MACE group. The same tendencies were observed in the ISR and re-PCI groups, showing a lower serum GSH concentration compared with the non-MACE group. The trend of reduced GSH in MACE patients was significant in diabetic patients, and remained beyond significance among nondiabetic individuals (TABLE 4). Regardless of the occurrence of diabetes, we did not show significant differences in GSH concentrations between the subgroups of patients with STEMI, NSTEMI, and UA and between the subgroups with different numbers of occluded coronary vessels (TABLE 4).

Preprocedural serum GSH as the prognostic factor for MACE All patients were classified into group 1 or 2 according to preprocedural serum GSH concentrations. Group 1 included patients showing serum GSH lower than or equal to the median (1.39; 1.04–1.55 μmol/l; n = 68), while group 2 included those with GSH above the median (2.26; 2.09–2.99; μmol/l, n = 68; $P < 0.001$). As shown in FIGURE 1 for the MACE group, the Kaplan–Meier estimates of the 12-month event-free survival rate were 69.1% (95% CI: 56.7%–78.6%) for group 1 (lower serum GSH) and 88.2% (95% CI: 77.8%–93.9%) for group 2 (higher GSH) ($P < 0.004$ by the generalized Wilcoxon–Gehan–Breslow and log-rank tests). The mean survival time was 55.9 weeks (95% CI: 52.5–59.2 wk) for group 1 (high GSH) and 46.2 weeks (95% CI: 41.3–51.7 wk) for group 2 (low GSH). Correspondingly, a 10-percentile survival time was 49.4 weeks for group 1 (high GSH) and 7.4 weeks for group 2 (low GSH), which means that the estimated 10% of the patients with low serum GSH will experience MACE within 7.4 weeks after the starting point, while the other 90% will either not have MACE or will experience the event later than 7.4 weeks after the starting point. In contrast, 10% of the patients with high GSH will not have MACE within 49.4 weeks, that is, they will survive free of MACE for nearly the overall follow-up period (1 year). The Cox proportional hazards regression analysis, which controlled for the effects of the variables significantly differing MACE and non-MACE patients (history of MI and type 2 diabetes), indicated that patients with lower GSH were about 2.2 times more likely to experience MACE than those who had higher GSH levels (the hazard [HR] or risk ratio = 2.16; 95% CI: 1.19–3.92; $P < 0.015$). The 100 nmol/l increment in the GSH concentration was associated with a 7.99% risk reduction in MACE (95% CI: 1.7%–14.6%; $P < 0.02$). The significant HR was also noted in the subgroup of patients with diabetes (HR = 4.56; 95% CI: 1.76–11.84, controlled for the effect of previous MI; $P < 0.002$), but not in nondiabetics.

Preprocedural serum GSH as the prognostic factor for restenosis The Kaplan–Meier curves for the ISR-free survival rates showed that patients with low GSH had a lower 12-month rate of restenosis-free survival (83.8%; 95% CI: 71.9%–90.9%; n = 68) compared with those characterized by high GSH (92.5%; 95% CI: 82.8%–96.8%; n = 68) ($P = 0.117$ by the Wilcoxon–Gehan–Breslow test and $P = 0.121$ by the log-rank test) (FIGURE 2). The 10-percentile survival time was 69.6 weeks for the group with high GSH (mean survival time, 57.5 wk,; 95% CI: 54.9–60.2 wk)

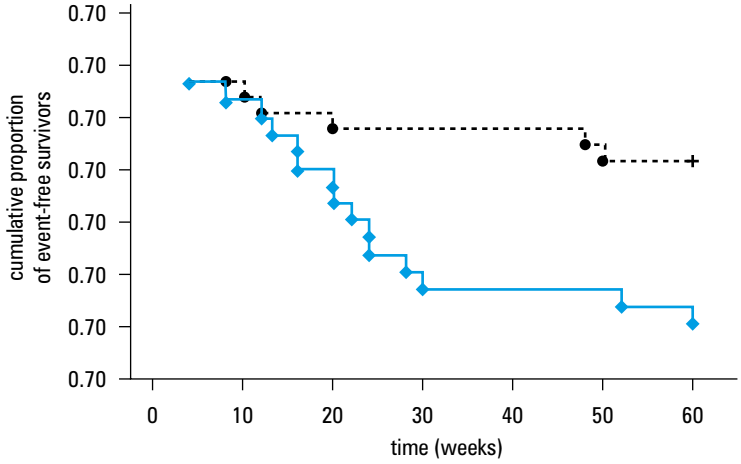

FIGURE 3 Kaplan–Meier curves of repeated percutaneous coronary intervention (re-PCI)-free survivors in the study groups divided according to serum glutathione (GSH) concentrations; the estimated cumulative proportions of re-PCI-free survivors are given for patients with either high (higher than the median of 1.78 µmol/l, dashed line; group 1) or low (lower than or equal to the median, solid line; group 2) serum GSH concentrations; complete observations are marked with circles or triangles for groups 1 and 2, respectively; censorships are marked by "+"; the significance of differences between survival curves was $P = 0.0206$ (log-rank test)

and 19.5 weeks for the group with low GSH (mean survival time, 54.1 wk; 95% CI: 50.1–58.2 wk). The Cox proportional hazards regression analysis (controlled for the effects of MI and history of type 2 diabetes) indicated that in the whole group, patients with lower GSH levels were over 2-fold more likely to experience restenosis than those with higher GSH levels (HR = 2.50; 95% CI: 0.83–7.51; $P = 0.102$). In the subgroup of diabetic patients, the risk was nearly 5-fold higher (HR = 4.78; 95% CI: 1.14–11.53; $P < 0.03$).

Preprocedural serum GSH as the prognostic factor for repeated revascularization FIGURE 3 shows the Kaplan–Meier curves indicating the estimated cumulative proportions of participants free of re-PCI in the course of a 15-month follow-up. The 12-month re-PCI-free survival rates were 79.4% (95% CI: 67.7%–87.3%) for the group with lower GSH concentrations (n = 68) and 91.2% (95% CI: 81.4%–95.9%) for the group with higher GSH levels (n = 68) ($P = 0.0226$ by the generalized Wilcoxon–Gehan––Breslow test, $P = 0.0206$ by the log-rank test). The 10-percentile survival time was 51.6 weeks for patients with high GSH levels (mean survival time, 56.9 weeks; 95% CI: 53.9–59.8 wk) and 17.5 weeks for patients with low GSH levels (mean survival time, 51.9 wk; 95% CI: 47.8–56.0 wk). The standardized Cox proportional hazards regression analysis showed that patients with lower GSH levels were over 3.1 times more likely to require re-PCI than those with higher GSH levels (HR = 3.13; 95% CI: 1.19–8.26; $P < 0.025$). The 100 nmol/l increment in GSH concentration was associated with a 7.25% risk reduction of re-PCI (95% CI: 0.5%–14.6%; $P < 0.04$). In the subgroup of diabetic patients, the risk of re-PCI was nearly 6 times higher in patients with lower GSH levels (HR = 5.97, 95% CI: 1.28–28.01; $P < 0.025$; controlled for previous MI events).

Effect of hemoglobin A_{1c} and other coexplaining variables on serum GSH and survival rates No significant effects of HbA_{1c}, the occurrence of type 2 diabetes, the history of type 2 diabetes or MI on the event (MACE, ISR, or re-PCI)-free survival was evidenced by the Cox proportional hazards regression analysis ($P > 0.5$) in ACS patients with different serum GSH concentrations. However, there was a tendency (though beyond significance), for the effect of sex on the survival rates in MACE, ISR, and re-PCI (with a tendency for lower survival in women, P values from 0.07 to 0.09). EF was not significantly associated with serum GSH ($R_S = 0.079$, nonsignificant). We found, however, a significant negative correlation with serum TNF-α ($R_S = –0.201$, $P < 0.01$) and a borderline significant negative association with serum IL-6 ($R_S = –0.122$, $P = 0.079$), although neither of these variables differed significantly between the groups with lower and higher serum GSH levels. The multivariate logistic regression analysis demonstrated a significant effect of serum GSH concentration on MACE (OR = 2.09; 95% CI: 1.12–3.89; $P < 0.02$), also after adjusting for angiographic parameters of implanted stents (length, diameter), number of occluded coronary vessels, and the subgroups of ACS (STEMI, NSTEMI, UA) (OR = 2.29, 95% CI: 1.17–4.48; $P < 0.02$). Importantly, neither of these adjusting variables affected the risk of MACE to a significant extent.

DISCUSSION Our study clearly showed that GSH levels are significantly decreased in the whole MACE group and in the subgroups of the patients with MACE compared with those withhout MACE. Higher serum GSH concentrations were associated with a lower risk of experiencing a MACE in general, and ISR or re-PCI in particular. More importantly, the analysis of the results in the subgroups of patients with and without diabetes led to a conclusion that a statistically significant effect of GSH on the occurrence of monitored cardiac events resulted mainly from the differences between diabetic and nondiabetic patients. This finding was further validated by the outcomes of our analysis showing that lowered GSH levels and poorer antioxidant status were closely related to diabetes itself. On the other hand, we did not observe significant effects of either age or sex on the rate of MACEs, which is not consistent with other reports that demonstrated the significance of age and sex in the incidence of complications after cardiovascular interventions.[9]

This was a preliminary study with a relatively low number of subjects. Due to this limitation, we observed a relatively low number of MACEs during follow-up. The preliminary design of our study implied that it was not very "broad" (did not include a lot of variables and tests to monitor antioxidant defense) and not very "deep" (a relatively small population studied). This was

because our aim was to detect a trend and a possible association between one particular marker of antioxidant defense, GSH, and the clinical outcome within a well-characterized population of patients with ACS. Second, we used preprocedural GSH concentrations as a discriminative variable, and we did not monitor the fluctuations in the GSH concentration over time, to use it as a harmonic (time-dependent) variable. It resulted from the fact that none of the patients were hospitalized for such a long time and any therapeutic decisions were based on the facts observed at the time of periodical consultation.

The frequency of restenosis, which is the most common MACE, varies depending on follow-up; however, it is still higher when compared with drug-eluting stents.[10-12] ISR is considered a crucial problem in diabetic patients undergoing interventional treatment.[13,14] In our study, over 20% of the patients with ACS experienced MACE after pPCI and BMS deployment, and half of them developed ISR. Among the affected individuals, diabetic patients with restenosis comprised 60%. Of all the patients in whom MACE or re-PCI occurred, diabetic individuals constituted 57% and 52%, respectively. However, due to a relatively small number of patients, we deliberately included only patients with BMS to reduce heterogeneity. First, considering the higher risk of restenosis in BMS patients, we were mostly interested in the effect of serum GSH levels in such a high-risk group. Second, in a relatively small cohort, we were more likely to record a considerable incidence of restenosis.

Unfortunately, we failed to identify a single and definite predictor of restenosis. The most common factors underlying its pathogenesis include chronic inflammatory subclinical process and reduced serum antioxidant capacity, both leading to proliferative phases of vascular response and subsequent restenosis.[15,16] On one hand, poor antioxidant defense can be the result of acute ischemia after coronary artery occlusion.[5] On the other hand, poor antioxidative protection and its relation to prolonged hyperglycemia have been recognized to worsen the outcome after interventional procedures in diabetic patients.[6] In our study, the basic characteristics of the patients were homogenous. The main characteristics that were different between patients were the presence of diagnosed diabetes, metabolic control of diabetes (HbA_{1c}), or serum GSH concentration. Lowered GSH levels and a poorer antioxidant status were found to be closely related to diabetes itself. In this respect, our finding of GSH as a risk factor for MACE, especially in patients with diabetes, may be quite a novel finding.

As reported by Nojiri et al.,[17] the total antioxidant status in patients with CAD correlates negatively with the number of diseased coronary vessels, which further validates our hypothesis of a possible involvement of oxidative damage in the atherosclerotic process. Hence, serum antioxidants and their efficiency in neutralizing reactive oxygen species (ROS) seems promising in the context of the above-mentioned complications after PCI.[18] As far as the endothelial cells are considered the target cells for ROS,[19] free radicals may be also responsible for myocardial damage connected with reperfusion following ischemia after transluminal coronary angioplasty due to subsequent lipid peroxidation that is likely to cause endothelial dysfunction. In line with this argument is the finding that the detrimental effect of ROS can be diminished by antioxidant enzymes such as GSH peroxidase.[19,20]

Various reports are generally consistent regarding the possible detrimental effect of increased oxidative stress and decreased antioxidant defense on the severity of atherosclerosis and progress of CAD.[5,21,22] Also, the role of oxidative stress in postangioplastic restenosis is considered to be prominent, as far as we are aware that oxidative stress may be related to the degree of vascular injury.[23]

Despite extensive research into various antioxidant systems, no attempts have been made to modulate these systems, i.e., by external supplementation of antioxidants such as GSH.[24] The GSH concentration in erythrocytes extracted from the blood of patients with acute MI appeared significantly lower compared with healthy controls, thus implying the impaired oxidative status after MI.[25] Also Ohsawa et al.[26] reported a significantly lowered GSH/GSSG ratio in patients with MI and postinfarct left ventricular dysfunction and impaired contractile function, and interpreted these findings as due to ROS overproduction.[26]

Our findings encourage to consider the effect of reduced serum GSH levels on the occurrence of MACE in patients with diabetes. In our study, neither diabetes nor metabolic control (HbA_{1c}) appeared to be significant explanatory variables contributing to the reduced survival rate. However, we report that diabetic patients experiencing a MACE had significantly decreased serum GSH levels compared with diabetics who did not experience a MACE, which may support the hypothesis of impaired antioxidative protection caused by diabetes.[13]

Overall, the effect of ROS on the occurrence of MACE should not be underestimated, as they may lead to subsequent restenosis and postreperfusion myocardial injury. Preprocedural GSH supplementation may thus be a novel treatment to prevent restenosis and postreperfusion injury in patients with ACS. Serum GSH levels may also become a reliable marker of antioxidant system efficiency in our efforts to enhance risk stratification in patients with ACS undergoing pPCI.

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Note The preliminary results were partly presented during Transcatheter Cardiovascular Therapeutics in Washington, D.C., USA, Oct 22–26, 2006 (Pietruszynski et al. The Role of Pre-Procedural Glutathione Concentrations in Major Adverse Cardiac Event (MACE) Prediction in Patients with Acute Coronary Syndrome Treated with Percutaneous Coronary Intervention) and published in an abstract form in *The American Journal of Cardiology*, 98 Suppl. S1, #372.

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ARTYKUŁ ORYGINALNY

Znaczenie osoczowego stężenia glutationu jako czynnika ryzyka występowania zgonu i poważnych zdarzeń sercowych u pacjentów z ostrymi zespołami wieńcowymi poddawanych zabiegom przezskórnej angioplastyki wieńcowej

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SŁOWA KLUCZOWE

angioplastyka wieńcowa, cukrzyca, glutation, poważne niekorzystne zdarzenia sercowe

STRESZCZENIE

WPROWADZENIE Słaba ochrona antyoksydacyjna kardiomiocytów na skutek niedokrwienia oraz niskie stężenie zredukowanego glutationu (GSH) w surowicy krwi mogą wiązać się ze zwiększonym ryzykiem nawrotów zwężenia tętnicy po zabiegach pierwotnej przezskórnej angioplastyki wieńcowej (*primary percutaneous coronary intervention* – pPCI).

CELE Celem tego badania było określenie czy przedzabiegowe stężenie zredukowanego GSH w surowicy krwi, odzwierciedlającego stan antyoksydacyjny w organizmie, może być predyktorem poważnych niekorzystnych zdarzeń sercowych (*major adverse cardiac events* – MACE) u pacjentów z ostrym zespołem wieńcowym (OZW) leczonych pPCI.

PACJENCI I METODY Stężenie GSH w surowicy przed zabiegiem pPCI oceniano u 141 pacjentów z OZW z zaimplantowanymi stentami metalowymi (*bare-metal stents* – BMS). Podczas 15-miesięcznej obserwacji u 30 chorych (średni wiek 61 ±10 lat) wystąpiło MACE. Pozostałych 111 pacjentów zaliczono do podgrupy bez MACE (średni wiek, 63 ±10 lat).

WYNIKI W grupie z MACE stwierdzono istotnie niższe stężenia GSH w porównaniu z grupą bez MACE ($p < 0.001$); różnice były znamienne także w podgrupie pacjentów z cukrzycą typu 2 ($p < 0.001$). Pacjentów przydzielono losowo do grupy z niskim stężeniem GSH (mediana: ≤1.39; 1.04–1.55 mmol/l) lub wysokim stężeniem GSH (mediana: >2.26; 2.09–2.99 mmol/l; $p < 0.001$). Analiza Kaplana–Meiera wskazała na istotnie dłuższy czas przeżycia bez MACE wśród pacjentów z wyższym stężeniem GSH ($p < 0.004$). Analiza regresji Coxa wykazała, że pacjenci z niższym stężeniem GSH byli 2.2 raza bardziej narażeni na wystąpienie MACE (95% CI, 1.2–3.9; $p < 0.02$ dla całej grupy oraz 1.8–11.8 dla pacjentów z cukrzycą; $p < 0.002$).

WNIOSKI Pomiar stężenia GSH w surowicy przed zabiegiem może być użytecznym czynnikiem predykcji wystąpienia MACE u pacjentów z OZW, przygotowywanych do zabiegu pPCI z BMS, zwłaszcza wśród pacjentów z cukrzycą.

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