UNIVERSITA' DEGLI STUDI DI MILANO

PhD COURSE IN TRANSLATIONAL MEDICINE

PhD Thesis

EFFECTS OF STRESS HYPERGLYCEMIA ACCORDING TO DIABETIC STATUS IN PATIENTS WITH ST-ELEVATION MYOCARDIAL INFARCTION AND ITS RELATIONSHIP WITH CARDIAC CELL INJURY AND MITOCHONDRIAL DAMAGE: A TRANSLATIONAL APPROACHPhD

Student: Nicola COSENTINO

Matricola R12565

Tutor: Prof. Piergiuseppe AGOSTONI

Coordinator: Prof.ssa Chiarella SFORZA

XXXV Cycle

Academic year: 2021-2022

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1. ABSTRACT

Background: Acute hyperglycemia (AH) is common in ST-elevation myocardial infarction (STEMI) and predicts outcomes. AH is a more powerful prognostic predictor in patients without diabetes mellitus (DM) than with DM, emphasizing the role of an acute glucose rise compared to chronic elevations. Moreover, AH may exacerbate, thorough mitochondrial dysfunction, infarct size (IS). We investigated the association between AH and chronic glycemia, considered separately or in combination, with mitochondrial injury and myocardial IS in STEMI patients with or without DM.

Methods: We measured admission serum glucose (AH), cytochrome c and mitochondrial DNA levels (mitochondrial biomarkers), and estimated chronic glucose in all patients. We calculated the acute on chronic (A/C) glycemic ratio. The primary endpoint was IS at cardiac magnetic resonance. The composite of in-hospital mortality, acute-pulmonary-edema, and shock was the secondary endpoint.

Results: 100 STEMI patients with DM and 100 without were included. IS was 25gr and 19gr and the secondary endpoint occurred in 21% and 8% of patients with and without DM, respectively (p=0.02 and p=0.01, respectively). The A/C ratio only significantly correlated with cytochrome c and mitochondrial DNA levels in DM patients. However, at reclassification analyses, A/C glycemic ratio showed the best prognostic power in predicting the primary and secondary endpoints as compared to AH in DM (net-reclassification-index 28% and 31%, respectively) but not in non-DM patients (net-reclassification-index 1% and 2%, respectively). In DM patients, A/C glycemic ratio, but not AH, significantly predicted 1-year mortality, after adjustment for major confounders.

Conclusions: In STEMI patients with DM, A/C glycemic ratio seems to be a better predictor of IS and in-hospital and 1-year outcome than AH. This study highlights the prognostic role of A/C ratio, its impact on mitochondrial impairment and outcomes, and may pave the way to interventional trials targeting AH according to A/C ratio in DM patients with STEMI.

KEYWORDS: **ST-elevation myocardial infarction; diabetes mellitus; acute hyperglycemia; chronic glycemia; acute/chronic glycemic ration; mitochondrial injury; outcomes.**

2. ABBREVIATIONS

A/C = acute on chronic

AMI = acute myocardial infarction

AUC = area under the curve

CAD = coronary artery disease

CCU = coronary care unit

CKD = chronic kidney disease

CMR = cardiac magnetic resonance

DM = diabetes mellitus

HbA1c = glycated hemoglobin

MSI = myocardial salvage index

MVO = microvascular obstruction

mtDNA = mitochondrial DNA

PCI = percutaneous coronary intervention

ROC = receiver operating characteristic

STEMI = ST-elevation myocardial infarction

3. INTRODUCTION

3.1. Clinical relevance of diabetes mellitus in acute myocardial infarction

Diabetes mellitus (DM), in particular type 2 DM, constitutes one of the largest emerging threats to health in the 21st century. It is estimated that by 2030 as many as 360 million people world-wide will be affected (1). The cause of death in patients with DM is largely due to coronary artery disease (CAD), along with increased rates of stroke and peripheral vascular disease: the so called macro-vascular complications (2). Notably, at least two-thirds of deaths in DM patients are due to athero-thrombotic events and their sequelae (3,4).

Compared to individuals without DM, those with DM have a three-fold increased risk of acute myocardial infarction (AMI), which usually occurs 15 years earlier, as compared to their non-DM counterpart (5,6). Moreover, AMI may even represent the first clinical manifestation of DM (7). Indeed, in about 5-10% of AMI patients, the presence of DM, until then unknown, is detected during index hospitalization (8). Not only DM is a frequent comorbidity among AMI patients, but it also carries a significantly higher morbidity, mortality, and AMI recurrence risk than non-DM patients (9-11). This prognostic gap characterizes DM patients in the acute phase of AMI, as well as during the following years.

Diabetes mellitus has been for a long time a recognized risk factor for AMI. In the 1970s, the Framingham Study showed that DM conferred a two- to four-times greater risk for AMI (2). The Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (INTERHEART) study confirmed this greater risk for AMI on a global scale for DM patients (12).

Although in the last decades there was an almost 70% reduction in the rates of AMI in patients with DM, compared to a 30% reduction in those without DM (13), the AMI burden in the DM population continues to rise, as a result of the substantial increase in its prevalence. Thus, it is not surprising that the frequency of DM among AMI patients steadily increased from 18% in 1997 to 30% in 2016 (14-16). The latter figure is even higher when we consider pre-DM, as defined by glycated hemoglobin (HbA1c) between 5.7%-6.4% (39-46 mmol/mol), and unknown DM (8). Recent reports demonstrated that 25% of AMI patients has pre-DM (8), a metabolic profile that is also associated with adverse outcomes in this clinical setting (17). Moreover, the prevalence of previously unrecognized DM in the AMI population is reported to range between 4% and 22%, depending on the test used for its diagnosis (8,18,19). Taken together, these data clearly demonstrate that abnormal glucose metabolism is a frequent co-morbidity in AMI patients (Figure 1).

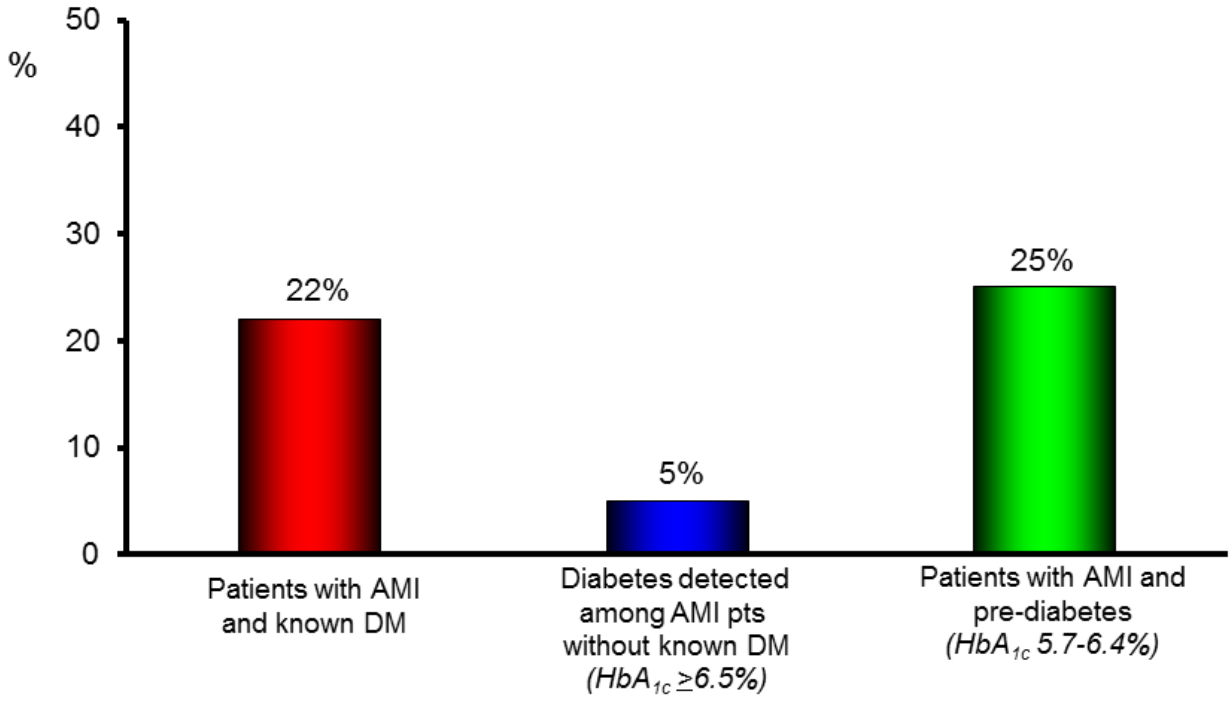


Fig 1. Prevalence of known and unknown diabetes mellitus among patients hospitalized with acute myocardial infarction.

3.2. In-hospital mortality of patients with acute myocardial infarction and diabetes mellitus

Prior to the advent of thrombolytic therapy, studies in AMI patients with DM showed a greater than two-fold in-hospital mortality rate in men, and an even higher rate in women, compared with their non-DM counterpart (20-26). In the thrombolytic era, observational, epidemiological, and randomized studies confirmed that in-hospital mortality is two times higher in patients with DM. This was mainly due to their higher rates of early re-infarction and congestive heart failure (9,27-32). In particular, in DM patients, acute heart failure or cardiogenic shock accounted for over 80% of in-hospital mortality, while arrhythmias and conduction defects for almost 20% of mortality (21). A subgroup analysis of the Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO)-1 trial demonstrated a significantly higher 30-day mortality in ST-elevation myocardial infarction (STEMI) patients with DM when compared with those without (10% vs. 6%) (33). Similarly, the Global Registry of Acute Coronary Events (GRACE) registry reported an almost twice-higher in-hospital case fatality rate for STEMI patients with DM (34). More recently, among 93,569 AMI patients (in most cases treated with percutaneous coronary intervention [PCI]) included in the National Cardiovascular Data Registry (NCDR) Acute Coronary Treatment and Intervention Outcomes Network-Get with the Guidelines (ACTION Registry-GWTG), the presence of DM was associated with a higher risk of in-hospital mortality, also after adjustment for major confounders (OR 1.17; 95% CI 1.07-1.27) (35). The adverse effect of DM on in-hospital mortality during AMI has been further confirmed in a recent cohort of more than 5,000 STEMI patients undergoing primary PCI (36). Again, DM was associated with an about two-fold higher in-hospital mortality, as compared to non-DM

patients. Notably, in this study, the different mortality rate was not related to differences in the extent of myocardial infarct size, as estimated by enzymatic peak value (36). Thus, despite evidence for improvement in outcomes in the general AMI population over the past 30 years, as well as in DM patients, a two-fold higher mortality in DM patients has been consistently reported across decades (Figure 2).

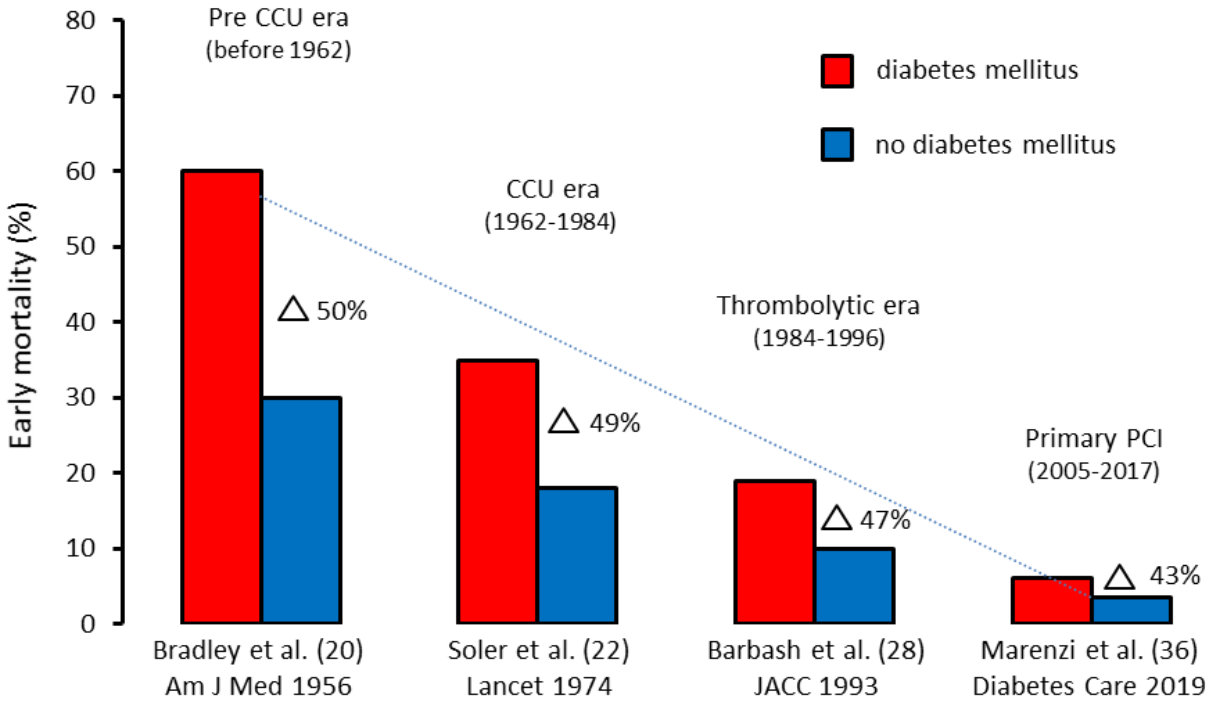


Fig 2. Difference (Δ) in early mortality rate in acute myocardial infarction patients with and without diabetes mellitus across decades, going from pre-coronary care unit (CCU) to primary percutaneous coronary intervention (PCI) era.

The reasons for the observed excess early mortality in AMI patients with DM have not been clearly defined, yet. Traditionally, several factors have been held responsible for their higher mortality (Figure 3): 1) the presence of other cardiovascular risk factors featuring DM subjects (hypertension, dyslipidemia, obesity, and kidney disease) (37); 2) a more diffuse and severe coronary atherosclerosis (37); 3) the higher incidence of painless infarction, possibly due to cardiac autonomic sensory neuropathy, and atypical symptoms, which may lead to delay in first medical contact and initiation of recommended therapies (38); 4) increased platelet activation and coagulation factors expression, and reduced intrinsic thrombolytic activity, which characterize DM, enhancing the ongoing pro-thrombotic and pro-coagulant state (39); 5) endothelial dysfunction associated with insulin resistance and metabolic syndrome, which may worsen coronary vasoconstriction (39); 6) the sub-clinical chronic inflammatory milieu, a typical feature of DM (39); 7) the impaired compensatory hyperdynamic response of the remaining non-ischemic myocardium, which has been shown in DM patients with AMI (40), possibly as a reflection of the diabetic systolic and diastolic cardiomyopathy.

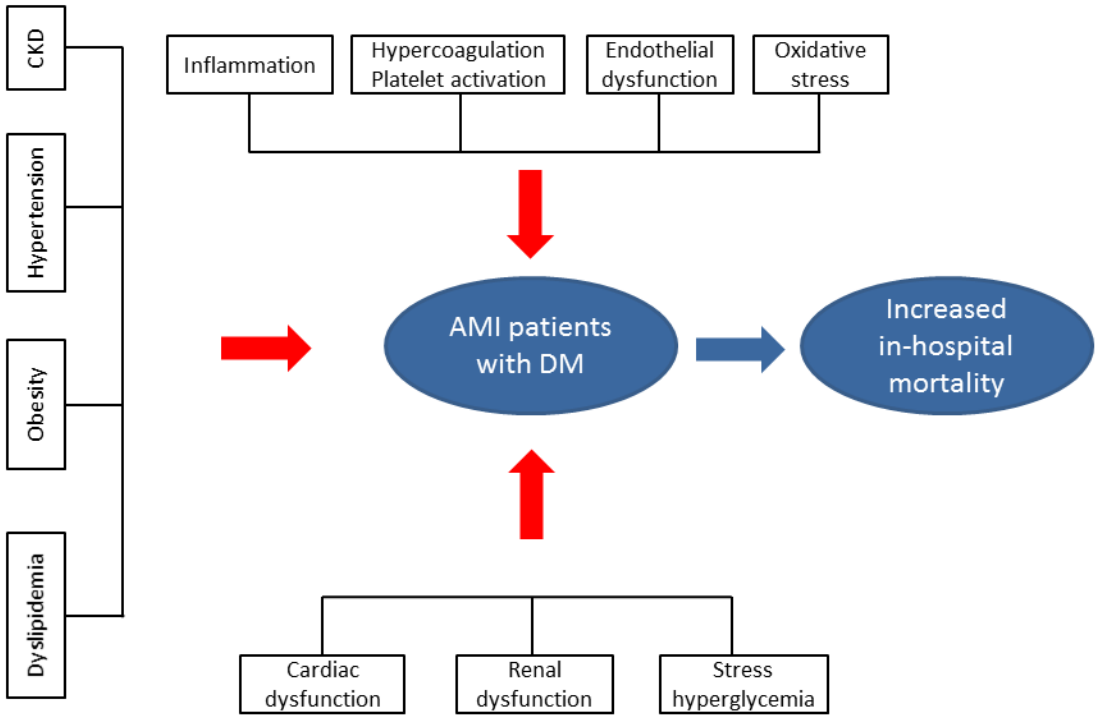


Fig 3. Possible mechanisms contributing to the increased in-hospital mortality of acute myocardial infarction (AMI) patients with diabetes mellitus (DM). CKD = chronic kidney disease.

Finally, a very recent study, focusing on STEMI patients treated with primary PCI, specifically investigated the impact of cardiac (as evaluated by admission left ventricular ejection fraction) and renal (as assessed by admission estimated glomerular filtration rate) function on in-hospital mortality in DM patients (36). Interestingly, the higher in-hospital mortality rate of STEMI patients with DM (6.1% vs. 3.5%) was mainly driven by their more frequent cardio-renal dysfunction, as the prognostic power of DM was no longer confirmed after adjustment for cardiac and renal function. These findings cannot be considered fully unexpected, since cardiac and renal functions are the two most important predictors of in-hospital mortality in AMI (36). Notably, cardiac function evaluated at hospital admission in AMI patients incorporates several clinical information, including pre-existing cardiac dysfunction, extent of the ongoing ischemic process, and the related hemodynamic effects. Similarly, the evaluation of renal function reflects a variable combination of acute (hemodynamic impairment) and chronic (underlying co-morbidities) information. These data do not allow clarifying whether and to what extent the more likely cardio-renal impairment observed in DM patients is due to a pre-existing dysfunction or whether it is the acute consequence of a more severe AMI. Possibly, the evaluation not only of renal function but also of admission microalbuminuria might help to discriminate between these two possibilities (41). Indeed, a body of evidence has shown that microalbuminuria is associated with an increased risk of mortality, beyond that yielded by renal function in patients with AMI (42) and, particularly, in those with DM (43). The mechanisms underlying the adverse prognosis associated with microalbuminuria are not well known. Microalbuminuria may represent an index of generalized vascular damage because it has been

correlated with markers of endothelial dysfunction and inflammation that are directly involved in atherogenesis (44,45).

More studies are needed to confirm this intriguing cardio-renal hypothesis and to further investigate the mechanisms underlying this association in AMI patients with DM. This research might pave the way to novel therapeutic strategies, aiming at reducing the mortality gap still existing between DM and non-DM patients.

3.3. In-hospital clinical relevance of admission glycemia in acute myocardial infarction patients

Elevated levels of plasma glucose at hospital admission (acute hyperglycemia) are common among patients with AMI, occurring in up to 50% of all AMI patients, according to the considered glycemic threshold (46-49). Although there is currently no uniform definition of hyperglycemia in the setting of AMI, as prior studies used various hyperglycemia cut-off values ranging from 110 to 200 mg/dL. A threshold of 200 mg/dL is usually considered based on prior large studies in patients with AMI (46-49). Moreover, admission glucose has been identified as a major independent predictor of both in-hospital morbidity and mortality in AMI for DM and non-DM patients (49,50). Of note, for every 18 mg/dL (1 mmol/L) increase in glucose level above 200 mg/dL, it has been reported a 4% and a 5% increase in hospital mortality risk in patients without and with DM, respectively (51). For the same increase in glucose level, an adjusted increase in mortality risk of 10% has been reported for STEMI patients undergoing primary PCI (48). Among studies showing that blood glucose levels predict the outcome of patients with AMI, most of them relied on the blood glucose level detected at hospital admission (52,53), whereas others used fasting blood glucose (54) or average glucose levels during the admission period (55-57). Importantly, patients with both elevated admission and next day fasting glucose levels have a three-fold increase in hospital mortality, as compared to those with admission hyperglycemia only (56).

During AMI, counter regulatory hormones, like catecholamine, growth hormone, glucagon and cortisol, are released in proportion to the degree of cardiovascular stress and may cause hyperglycemia and an elevation of free fatty acids, both of which lead to an increase in hepatic gluconeogenesis and a decrease in insulin-

mediated peripheral glucose disposal (58). In addition to reflect the ongoing cardiovascular stress associated with AMI, acute hyperglycemia may directly contribute to a poor outcome through several adverse effects. They include suppression of flow-mediated vasodilatation, increased production of oxygen-derived free radicals (58,59), and activation of pro-inflammatory factors (58). Importantly, the degree of oxidative stress has been shown to correlate most closely with acute, rather than chronic, glucose fluctuations (60). Finally, acute hyperglycemia has been shown to have pro-thrombotic effects (enhanced thrombin formation, platelet activation, and fibrin clot resistance to lysis), which may amplify the risk of thrombotic complications in this clinical setting (61,62). From a clinical point of view, it has been reported that acute hyperglycemia in AMI patients is independently associated with lower rate of Thrombolysis In Myocardial Infarction (TIMI) flow grade 3 before primary PCI, with impairment of epicardial coronary flow after primary stent implantation, and with "no-reflow" phenomenon (63,64). Moreover, acute hyperglycemia is associated with increased left ventricular dysfunction, larger infarct size, and higher risk of acute heart failure, cardiogenic shock, acute kidney injury, and in-hospital mortality (48-50,63,65). Noteworthy, glucose normalization after admission in hyperglicemic patients hospitalized with AMI seems to be associated with better survival (66).

The close association between admission high glucose levels and poor outcome in AMI has been shown to be particularly robust in patients without DM when compared with those with DM (67-69). Moreover, this association is detectable at lower glycemic values in non-DM patients. Indeed, in them, mortality risk progressively increases when blood glucose is higher than 120 mg/dL. Conversely, in patients with DM, a blood glucose >200 mg/dL has been associated with a poor

outcome (70-72). This emphasizes the role of an acute rise of glucose level, compared to its chronic elevation, in predisposing AMI patients towards a worse prognosis. In fact, in DM patients, elevated glucose levels at hospital admission may not indicate the occurrence of a stress hyperglycemia, but they may reflect a poor chronic glycemic control. Thus, in DM patients, the evaluation of acute glycemia, considered not in absolute terms but in relation to chronic glycemia, may better reflect "true" stress hyperglycemia and help physicians to accurately discriminate high-risk from low-risk AMI patients. Moreover, it could help to customize treatment for intensive glucose control during the acute phase of AMI. Indeed, the detrimental effects of the glycemic disorder are not limited to stress hyperglycemia but they also include fluctuations of glycemic values, with acute glucose changes in both directions (66). In line with this, it has been shown that acute variability of glucose values, as assessed by measuring the mean amplitude of glycemic excursion with a continuous glucose monitoring system, negatively correlated with the myocardial salvage index (i.e., the proportion of reversibly injured tissue that does not progress to infarction) in AMI (73). Moreover, in a very recent study, in which measurement of glycemic variability was evaluated during AMI in DM patients, a glucose variability of >2.70 mmol/L (49 mg/dl) was demonstrated to be the strongest independent predictive factor for mid-term (mean follow-up time was 17 months) major adverse cardiac events (death for cardiac cause, new-onset AMI, acute heart failure) (74).

Future studies are needed to investigate whether in AMI patients with DM a strategy based on glucose normalization only in patients with a high acute/chronic glycemic ratio, an accurate index of stress hyperglycemia, and/or with an elevated glycemic

variability, may be more beneficial on infarct size and outcomes than an approach centered on the treatment of admission hyperglycemia alone.

4. MATERIALS AND METHODS: STUDY DESIGN

4.1. Background and rationale

Elevated levels of plasma glucose at hospital admission (acute hyperglycemia) are common among patients with STEMI. Acute hyperglycemia has been recognized as an independent determinant of adverse outcomes, both in patients with and without DM (54,75). Although STEMI patients with DM have a worse outcome than those without DM (51,76), acute hyperglycemia has been shown to be a powerful predictor of poor prognosis particularly in non-DM patients (51). This emphasizes the role of an acute rise of glucose level, as compared to its chronic elevation, in predisposing patients hospitalized with a STEMI towards a worse prognosis (69,77-79). Moreover, this may have potential clinical implications. Indeed, the role of a tight control of hyperglycemia as a strategy for improving prognosis in STEMI patients and, in particular in those with DM, is still under debate, and controversial data have been provided, thus far (80,81). Notably, in the setting of cardiothoracic surgery, intensive glycemic control improved outcomes in patients without known DM but not in those with a previous diagnosis of DM (81). This may be probably due to the fact that patients with DM often show high glycemic levels at admission that are not always associated with acute hyperglycemia. In them, an intensive lowering of glucose levels may not be beneficial, as the detrimental effects of the glycemic disorder are not limited to stress hyperglycemia but they also include fluctuations of glycemic values, with acute glucose changes in both directions. Accordingly, previous studies indicated that glucose variability in DM patients has a more pronounced effect on oxidative stress, platelet activation and aggregation, and on macrovascular and microvascular complications, than chronically elevated glucose

levels (60,82-85). In particular, acute variability of glucose values, assessed by measuring the mean amplitude of glycemic excursion with a continuous glucose monitoring system, negatively correlated with the myocardial salvage index, a well-known prognostic predictor, in STEMI patients (73). Thus, identification of true stress hyperglycemia in DM patients, beyond high glucose levels at hospital admission, may have a critical role both for risk stratification and treatment strategy. Chronic elevation of glucose levels cannot be determined in patients admitted with STEMI, but it can be estimated by assessing the HbA1c value (86). Therefore, in STEMI patients, the combined information provided by acute (measured at hospital admission) and chronic (estimated by HbA1c) glycemic value assessment may be a better prognostic predictor than glycemic value at admission or DM status alone. Indeed, it may represent the "true" acute glycemic increase. This may be particularly relevant in DM patients, in whom elevated glucose levels at admission do not necessarily indicate the occurrence of acute hyperglycemia.

Whether STEMI patients with similar acute hyperglycemia have different risk profiles, both at short- and long-term outcomes according to their chronic glycemic values, has never been investigated. In addition to the extent of myocardial necrosis, as reflected by infarct size assessed by cardiac magnetic resonance (CMR), several functional changes may occur during the early phase of STEMI contributing to myocardial cell injury and ventricular dysfunction, thus increasing morbidity and mortality. Among them, multiple lines of evidence suggest that mitochondrial dysfunction has a pivotal role in the pathogenesis of cell injury (87-89). Indeed, the contraction of the heart is an energy-dependent process requiring large amounts of adenosine triphosphate generated by mitochondrial oxidative metabolism (90-92). During ischemia and reperfusion, oxygen-dependent mitochondrial processes are

damaged and this may impair cellular homeostasis and adversely affect cardiomyocyte contraction. Thus, as mitochondrial dysfunction has been identified as a central mechanism underlying myocardial ischemia-reperfusion injury, it can be hypothesized that stress hyperglycemia may exacerbate myocardial damage in STEMI thorough mitochondrial dysfunction. Of note, experimental models demonstrated that prolonged ischemia and reperfusion cause mitochondrial injury and subsequent release of cytochrome c and mitochondrial DNA (mtDNA) into the cytosol and into the blood stream. Under physiologic conditions, cytochrome c and mtDNA localizes within the mitochondria, and they are not detectable in the blood of healthy human subjects (89-92). Conversely, elevated levels of circulating cytochrome c and mtDNA have been reported in association with cardiac arrest, pulmonary embolism, sepsis, and acute myocardial infarction (89-92). Therefore, cytochrome c and mtDNA can be considered biomarkers of mitochondrial dysfunction in patients presenting with acute myocardial infarction patients, and they may provide prognostic information complementary to that provided by infarct size.

4.2. Study objectives.

Thus, the purpose of this study will be to investigate the possible association between acute and chronic glycemic values, considered separately or in combination, and the extent of myocardial cell and mitochondrial injuries in a cohort of STEMI patients with or without DM. In particular, the study hypothesis is that the combined assessment of acute and chronic glycemia, as compared to admission glycemic value alone, is more closely associated with ischemic and/or myocardial reperfusion injury and, therefore, with the final extent of mitochondrial injury and infarct size. In order to investigate ischemic and reperfusion injury, all patients will undergo CMR imaging before hospital discharge, aiming at assessing infarct size, cardiac area at risk, myocardial salvage index, and microvascular obstruction (93). In order to investigate mitochondrial impairment, cytochrome c and mtDNA will be measured in all patients at hospital admission. In all patients, we will also measure blood glucose at admission (acute glycemia) and we will estimate chronic glucose levels (chronic glycemia) by admission HbA1c determination. Acute and chronic glycemia will also be combined to calculate the acute on chronic (A/C) glycemic ratio. The association between acute and chronic glycemia, considered separately or in combination, and all clinical and CMR imaging variables of interest, along with the association with the two investigated mitochondrial biomarkers (cytochrome c and mtDNA) will be assessed in the whole population and will be compared in patients with and without DM.

4.3. Study protocol and study population

This was a prospective, observational study. The final study population included 200 consecutive patients (100 with DM and 100 without DM) with a first STEMI, undergoing primary PCI, admitted to the Intensive Cardiac Care Unit of the Centro Cardiologico Monzino in Milan (Italy). Patients with a prior myocardial infarction (in whom myocardial necrosis may occur in an area already damaged by a previous infarction and the two infarct sizes cannot not be distinguished at CMR imaging), those with a contraindication to CMR imaging (renal failure with glomerular filtration <30 ml/min, claustrophobia, and pace-makers), those experiencing STEMI as a complication of elective PCI (Type 4a acute myocardial infarction), those with a history of hemoglobinopathy, and those with severe anemia (hemoglobin <8 g/dl), that are known to interfere with HbA1c levels, were excluded (Figure 4). The Ethics Committee (n. R520-CCM549) approved the study and informed consent was obtained in all patients enrolled.

STUDY POPULATION

INCLUSION CRITERIA

- STEMI patients undergoing primary PCI

EXCLUSION CRITERIA

- Patients with a prior STEMI
- Patients with a contraindication to CMR imaging
- Patients experiencing STEMI as a complication of elective PCI
- Patients with a history of hemoglobinopathy
- Patients severe anemia (hemoglobin <8 g/dl)

Fig. 4. Inclusion and exclusion criteria

Blood glucose and HbA1c levels were measured in all STEMI patients at hospital admission. A diagnosis of DM was made if this disease and/or anti-diabetic treatment, including oral agents or insulin, are recorded in the admission history (80). A diagnosis of unknown DM was made when patients have HbA1c ≥6.5% (48 mmol/mol) despite no previous history of the disease; these patients were considered as having DM (80). Acute hyperglycemia was defined as a blood glucose at admission >198 mg/dl (>11 mmol/l) according to the definition more frequently used in previous studies focusing on STEMI patients (51,54,75,76). Average chronic glucose levels was estimated by HbA1c, expressed as percent value, according to the following validated formula (86):

$$\text{Estimated chronic glucose levels (mg/dl)} = 28.7 \times \text{HbA1c (\%)} - 46.7$$

In all patients, we measured blood glucose at admission (acute glycemia) and we estimated chronic glucose levels (chronic glycemia). They were combined to calculate the A/C glycemic ratio (Figure 5, Panel A). Moreover, in all patients, cytochrome c (ng/mL) was measured in the serum at hospital admission by ELISA, using a commercially available enzyme-linked immunosorbent assay (Quantikine, R&D System Inc., Minneapolis, MI) (91). The lowest detection limit was 0.04 ng/mL. Circulating levels of mtDNA in plasma were assessed by measuring the copy number of the NADH dehydrogenase 1 gene using quantitative real-time PCR (92) (Figure 5, Panel A).

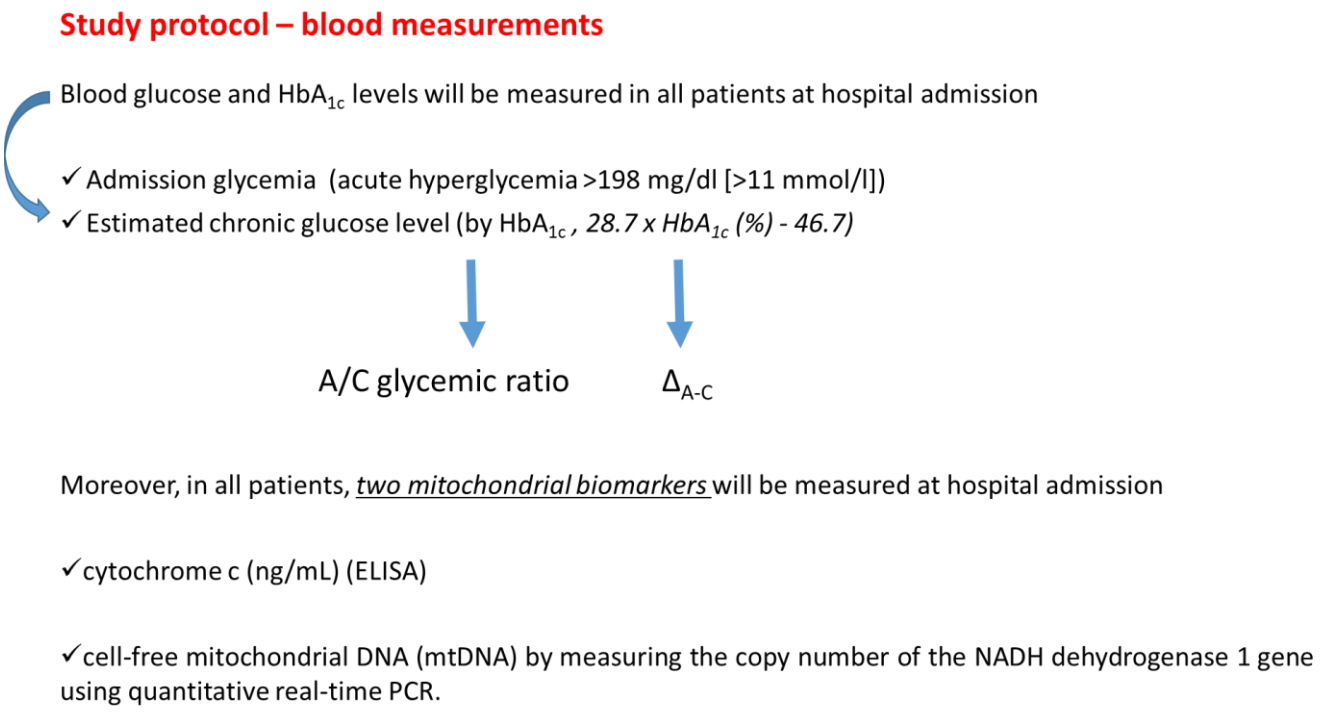


Fig. 5 (Panel A). Study Protocol.

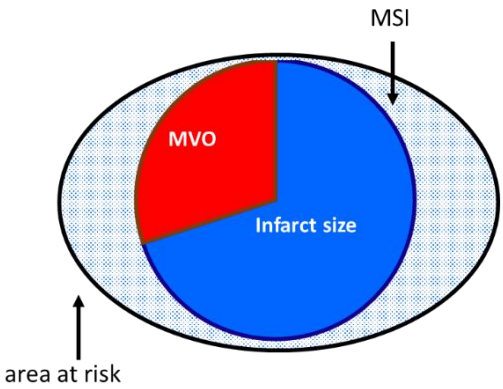
Study patients received standard medical treatment and coronary revascularization (primary PCI) according to the current standards of care recommended by published guidelines. In all patients with DM, anti-diabetic medications were withheld at hospital admission. In patients with acute hyperglycemia, insulin was administered with a glucose level target range of 140-180 mg/dl, according to our clinical protocol. Demographical, clinical, biochemical, echocardiographic, and angiographic data were obtained. Left ventricular ejection fraction was measured with echocardiography in all patients within 24 hours from hospital admission.

In all patients, CMR was performed before hospital discharge in order to determine the main variables of interest and known to strongly impact on prognosis (infarct size, area at risk, myocardial salvage index, and microvascular obstruction) (Figure 5, Panel B) (93).

Study protocol – CMR measurements

All patients will undergo CMR imaging before discharge.
The following parameters will be assessed

- Area at risk (index of the ischemic injury)
- Infarct size
- Myocardial salvage index (MSI)
- Microvascular obstruction (MVO) (index of reperfusion injury)



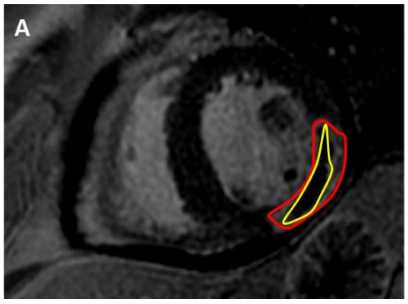


Fig. 5 (Panel B). Study Protocol.

The following in-hospital clinical outcomes were recorded: in-hospital death, acute pulmonary edema, and cardiogenic shock. Acute pulmonary edema was defined as severe respiratory distress, tachypnea, and orthopnea with rales over the lung fields and arterial oxygen saturation <90% on room air prior to treatment with oxygen. Cardiogenic shock was defined as prolonged hypotension (systolic blood pressure <85 mmHg) with evidence of decreased organ perfusion caused by severe left ventricular dysfunction, right ventricular infarction, or mechanical complications of infarction requiring intra-aortic balloon pump and/or inotropic agents.

4.4. Study endpoints

The primary endpoint of the study was the infarct size determination, as assessed by CMR. The secondary endpoints of the study were: 1) cardiac area at risk, an index of the ischemic injury, myocardial salvage index and microvascular obstruction (indexes of reperfusion injury), as assessed by pre-discharge CMR; 2) the association with cytochrome c and mtDNA levels measured at hospital admission; 3) the composite of in-hospital mortality, acute pulmonary edema, and cardiogenic shock; 4) 1-year mortality. The association between acute and chronic glycemia, considered separately or in combination, and primary and secondary endpoints were assessed in the whole population and were compared in patients with and without DM.

4.5. Statistical analysis

Continuous variables are presented as mean±SD, and they were compared using the t-test for independent samples. Variables not normally distributed are presented as median and interquartile ranges, and were compared with the Wilcoxon rank-sum test. Categorical data were compared using the chi-square test or the Fisher exact test, as appropriate. Spearman correlation was used to detect possible correlations between cytochrome c and mtDNA and admission glycemia and A/C glycemic ratio.

The association between admission glycemia and A/C glycemic ratio and the clinical endpoint was assessed by logistic regression analysis and odds ratio (for the in-hospital clinical endpoint) and hazard ratio (for 1-year mortality) were adjusted for age and gender in patients with and without DM. Then, we tested the independence in determining the main CMR variables of interest (infarct size; area at risk, myocardial salvage index, and microvascular obstruction) of both admission glycemia and /AC glycemic ratio in patients with and without DM, by using a multivariable linear regression model.

Receiver Operating Characteristic (ROC) curves were calculated and the areas under the ROC curves with 95% confidence interval were used to measure the ability of the considered variables to predict infarct size, major CMR variables of interest, and the composite clinical outcome (in-hospital death, acute pulmonary edema, and cardiogenic shock). AUCs were be compared as recommended by DeLong. Net reclassification improvement was also used to identify the possible additional prognostic value of A/C glycemic ratio when added to acute glycemia.

A P value <0.05 was considered statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina).

5. RESULTS

In total, 100 consecutive STEMI patients with DM (mean age 64±14 years, 75 men) and 100 consecutive STEMI patients without DM (mean age 67±11 years, 72 men), treated with primary PCI, were enrolled. Median admission glycemic value was 197±75 mg/dl and 145±35 mg/dl in patients with and without DM, respectively ($p<0.001$). Median estimated chronic glucose level was 158±35 mg/dl and 113±15 mg/dl in patients with and without DM, respectively ($p<0.001$).

Table 1 shows the baseline characteristics and outcomes of patients stratified according to DM status. As expected, patients with DM tended to be older, more likely to have lower estimated creatinine value and LVEF than patients without DM. They also had a more complicated in-hospital clinical course. Moreover, mitochondrial biomarker levels were significantly higher at hospital admission in patients with DM as compared to those without (Table 1).

Table 1. Baseline clinical characteristics and in-hospital and 1-year outcomes of the study patients according to the presence of diabetes mellitus (DM).

	Non-DM (n=100)	**DM** (n=100)	**P value**
Age (years)	67±11	64±14	0.11
Male sex, n	72	75	0.63
Body mass index (kg/m^2)	27±4	27±5	0.87
Hypertension, n	49	69	0.004
Smokers, n	53	48	0.48
Dyslipidemia, n	33	52	0.007
Prior PCI, n	18	28	0.10
Anterior AMI	44	38	0.38
LV ejection fraction (%)	49±10	45±10	0.19
TIMI pre pPCI 0-1	98	97	0.65
TIMI post pPCI 0-1	2	4	0.41
Time to presentation	3 (1.5-5)	3 (2-5)	0.49
Laboratory values at hospital admission			
High-sensitivity-C reactive protein (mg/L)	7.6 (1.8-24.4)	9.9 (2.6-32.7)	0.03
Blood glucose (mg/dl)	145±35	197±75	<0.001
Chronic glycemia (mg/dl)	113±15	158±35	<0.001
HbA1c (%)	5.6±0.3	7.1±1.2	<0.001
A/C glycemic ratio	1.28±0.40	1.29±0.32	0.84
Serum creatinine (mg/dl)	1.0±0.3	1.1±0.4	0.28
Hemoglobin (g/dl)	14±2	13±2	0.12
Total cholesterol	186±45	168±47	0.007
Admission High-sensitivity Troponin I (ng/L)	106±43	114±62	0.29
Peak High-sensitivity Troponin I (ng/L)	63,806±12,151	84,125±10,241	<0.001
Cytochrome *c* (ng/mL)	0.29±0.50	0.47±0.63	0.02
Mitochondrial DNA (copies/µl)	918 (283-1645)	1331 (467-3968)	0.02
Therapy at admission			
Statins , n	5	18	<0.001

Aspirin, n	12	38	<0.001
Betablockers, n	23	32	0.006
ACE/ARB-in, n	26	36	0.13
Therapy at discharge			
Statins , n	93	92	0.60
DAPT, n	100	100	0.98
Betablockers, n	80	76	0.49
ACE/ARB-in, n	60	69	0.18
In-hospital complications			
Death, n (%)	1	2	0.38
Cardiogenic shock, n (%)	4	11	0.10
Acute pulmonary edema, n (%)	7	14	0.16
Composite clinical endpoint, n (%)	8	21	0.01
Mechanical ventilation, n (%)	3	8	0.12
Renal replacement therapy, n (%)	1	2	0.38
Acute kidney injury, n (%)	2	8	0.05
Major bleedings, n (%)	2	4	0.41
VT/VF, n (%)	7	8	0.78
New-onset atrial fibrillation, n (%)	9	13	0.37
Advanced AV block, n (%)	3	4	0.90
1-year outcome			
1-year mortality, n (%)	3	7	0.33

A/C=acute/chronic; ACE/ARB-in= angiotensin-converting enzyme/angiotensin receptor blocker inhibitors; AMI=acute myocardial infarction; AV=atrio-ventricular; DAPT=dual antiplatelet therapy; LV=left ventricular; PCI=percutaneous coronary intervention; TIMI=Thrombolysis in Myocardial Infarction; VT/VF=ventricular tachycardia/ventricular fibrillation.

The composite clinical endpoint incidence was 21% in DM patients and 8% in those without (p=0.01). In non-DM patients, both admission glycemia and A/C glycemic ratio predicted the primary endpoint, in DM patients, only A/C glycemic ratio independently predicted the primary endpoint (Figure 6).

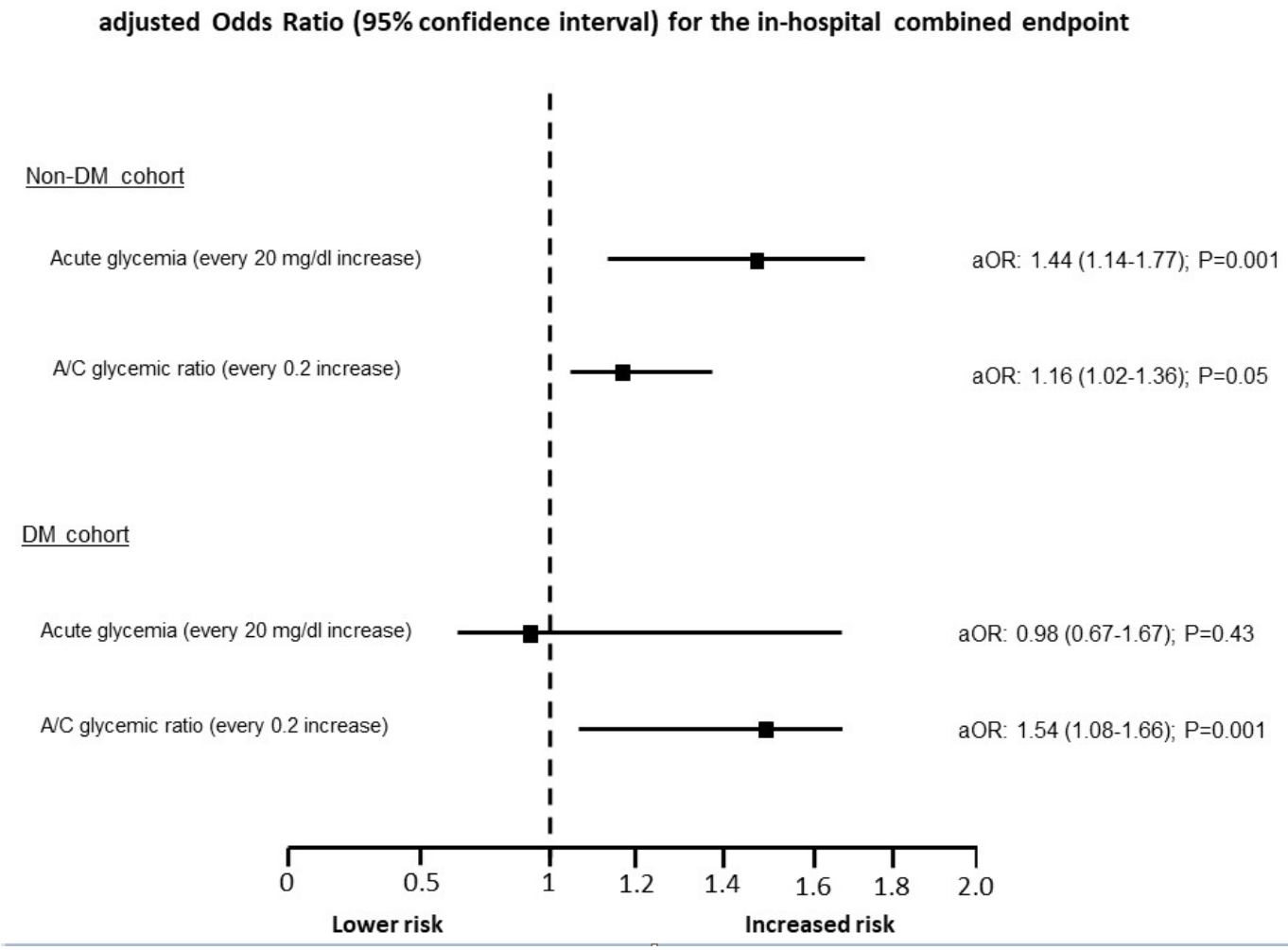


Fig. 6. Adjusted odds ratio (aOR) and 95% confidence interval of the composite clinical endpoint for admission glycemia and acute/chronic glycemic ratio in patients with and without diabetes mellitus (DM). ORs were adjusted for age and gender.

When considering CMR data (Table 2), infarct size (the primary endpoint) was 25±19 and 19±17 gr in patients with and without DM, respectively (p=0.02). Of note, while area at risk was similar between patients with and without DM (p=0.58), myocardial salvage index was significantly lower in patients with DM than in those without (0.46±0.27 vs. 0.55±0.32, p=0.03) and microvascular obstruction extent was higher in DM patients than in those without (p<0.001).

Table 2. Cardiac magnetic resonance parameters of the study patients, stratified according to the presence of diabetes mellitus.

Variables	non-DM cohort	DM cohorts	P value
LV end-diastolic volume (mL)	83±21	89±23	0.05
LV end-systolic volume (mL)	43±22	48±20	0.09
LV ejection fraction (%)	48±9	43±11	0.001
LV mass (grams)	118±41	123±36	0.20
RV end-diastolic volume (mL)	67±14	69±15	0.33
RV end-systolic volume (mL)	29±9	30±11	0.48
RV ejection fraction (%)	58±9	58±10	0.98
Area at risk (grams)	39±25	41±26	0.58
Infarct size (grams)	19±17	25±19	0.02
Myocardial salvage index	0.55±0.32	0.46±0.27	0.03
MVO (grams)	5±2	7±3	<0.001
MVO (yes, %)	6	14	0.06
Myocardial hemorrhage (yes, %)	5	9	0.41

LV=left ventricular; MVO=microvascular obstruction; RV=right ventricular.

At general linear model, A/C glycemic ratio, but not admission glycemia, independently predicted infarct size, myocardial salvage index and microvascular obstruction in DM patients (Table 3).

Table 3. Independent predictor value of acute glycemia and A/C glycemic ratio for the main variables of interest assessed at cardiac magnetic resonance.

Acute glycemia						
	DM cohort			*non-DM cohort*		
	Estimate	SE	P value	Estimate	SE	P value
AAR	0.05	0.02	0.78	0.19	0.01	0.04
IS	0.05	0.03	0.67	0.18	0.04	0.01
MSI	0.04	0.08	0.96	0.22	0.02	0.01
MVO	0.08	0.01	0.44	0.17	0.03	0.01
A/C glycemia value						
	DM cohort			*non-DM cohort*		
	Estimate	SE	P value	Estimate	SE	P value
AAR	0.18	0.03	0.01	0.37	0.08	0.01
IS	0.43	0.05	<0.01	0.21	0.04	0.01
MSI	0.22	0.04	0.01	0.16	0.07	0.02
MVO	0.32	0.05	0.02	0.18	0.08	0.02

A/C=acute/chronic; AAR=area at risk; DM=diabetes mellitus; IS=infarct size; MSI=myocardial salvage index; MVO=microvascular obstruction; SE=standard error.

Correlations among admission glycemia, A/C glycemic ratio, mitochondrial biomarkers, and CMR data in patients with and without DM are shown in Table 4. In particular, the A/C glycemic ratio more closely correlated with mitochondrial markers and CMR features than admission glycemia only in DM patients.

Table 4. Correlations between admission glycemia and acute/chronic glycemic ratio with mitochondrial biomarkers and variables of interest assessed at cardiac magnetic resonance in the study population stratified according to the presence of diabetes mellitus (DM).

Non-DM cohort				
	Admission glycemia		**acute/chronic glycemic ratio**	
	R	**P**	**R**	**P**
Cytochrome *c*	0.27	0.001	0.24	0.002
Mitochondrial DNA	0.12	0.05	0.13	0.08
Area at risk	0.31	<0.001	0.26	0.001
Infarct size	0.19	0.004	0.17	0.01
Myocardial salvage index	-0.24	0.003	-0.25	0.001
Microvascular obstruction	0.16	0.001	0.18	0.002
DM cohort				
	Admission glycemia		**acute/chronic glycemic ratio**	
	R	**P**	**R**	**P**
Cytochrome *c*	0.06	0.12	0.25	0.0001
Mitochondrial DNA	0.12	0.10	0.19	0.03
Area at risk	0.06	0.43	0.21	0.01
Infarct size	0.04	0.51	0.16	0.02
Myocardial salvage index	-0.06	0.38	-0.15	0.03
Microvascular obstruction	0.09	0.09	0.16	0.04

Table 5 shows AUC for acute glycemia and A/C glycemic ratio in predicting the clinical endpoint /the secondary endpoint) and the extent of infarct size (the primary endpoint), myocardial salvage index, and microvascular obstruction, as assessed by CMR, in patients with and without DM. At reclassification analysis, the A/C glycemic ratio provided the best prognostic power compared to acute glycemia in patients with DM (Table 5).

Table 5. Area under the curve of acute glycemia and the ratio of acute to chronic (A/C) glycemic values for the prediction of the composite clinical endpoint and of infarct size, myocardial salvage index, and microvascular obstruction at cardiac magnetic resonance, and reclassification statistics comparisons of A/C glycemic ratio added to acute glycemia in the study population grouped according to diabetes mellitus (DM) status.

Variables	**AUC (95% CI)**	**P value***	**NRI (95% CI)**	**P value***
In-hospital clinical endpoint				
Non-DM cohort				
Acute glycemia	0.67 (0.62-0.79)			
A/C glycemic ratio	0.68 (0.62-0.78)	0.15	2% (-3-9)	0.09
DM cohort				
Acute glycemia	0.58 (0.44-0.71)			
A/C glycemic ratio	0.71 (0.60-0.82)	<0.001	31% (16-43)	<0.001
Infarct size (above the overall median value)				
Non-DM cohort				
Acute glycemia	0.67 (0.61-0.76)			
A/C glycemic ratio	0.68 (0.62-0.75)	0.21	1% (-6-7)	0.14
DM cohort				
Acute glycemia	0.61 (0.53-0.68)			
A/C glycemic ratio	0.73 (0.65-0.879)	0.01	28% (11-38)	<0.001
Myocardial salvage index (above the overall median value)				
Non-DM cohort				
Acute glycemia	0.69 (0.62-0.75)			
A/C glycemic ratio	0.68 (0.63-0.76)	0.18	2% (-5-10)	0.10
DM cohort				
Acute glycemia	0.61 (0.52-0.67)			
A/C glycemic ratio	0.73 (0.64-0.80)	<0.001	26% (12-39)	<0.001

Microvascular obstruction (above the overall median value)				
Non-DM cohort				
Acute glycemia	0.65 (0.59-0.71)			
A/C glycemic ratio	0.67 (0.61-0.71)	0.11	3% (-3-9)	0.08
DM cohort				
Acute glycemia	0.59 (0.50-0.69)			
A/C glycemic ratio	0.71 (0.61-0.79)	<0.001	28% (9-38)	<0.001

AUC=area under the curve; CI=confidence intervals; NRI=net reclassification improvement.

*p values refer to the comparisons with acute glycemia.

One-year mortality rate was 3% in non-DM patients and 7% in DM patients (p=0.33). In non-DM patients, both admission glycemia and A/C glycemic ratio predicted 1-year mortality; while in DM patients, only A/C glycemic ratio independently predicted the primary endpoint (Figure 7).

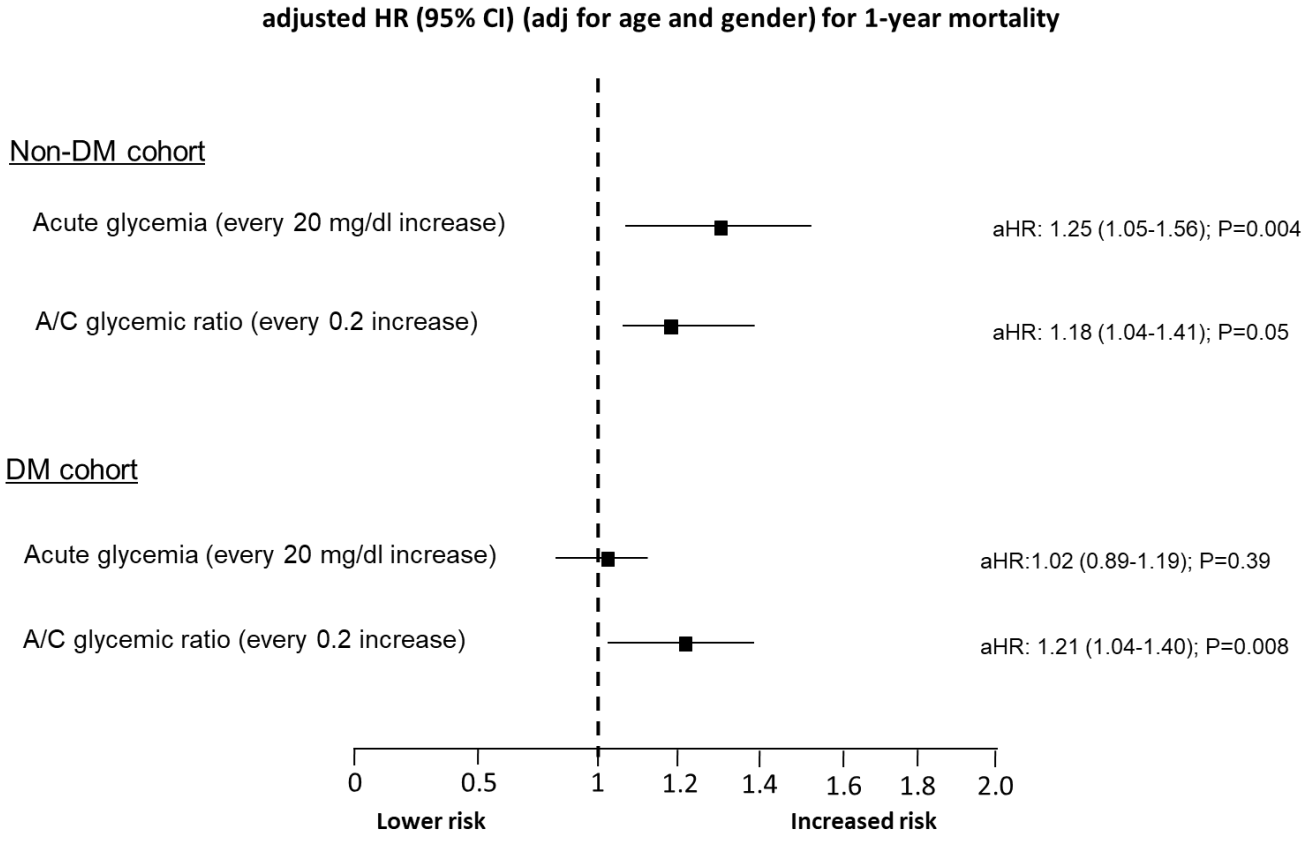


Fig 7. Adjusted hazard ratio (HR) and 95% confidence interval of 1-year mortality for admission glycemia and acute/chronic glycemic ratio in patients with and without diabetes mellitus (DM). HRs were adjusted for age and gender.

6. DISCUSSION

In this study, we showed that the ability of glycemia measured at admission to predict infarct size, in-hospital mortality, morbidity, and 1-year mortality in STEMI patients improves when the average chronic glucose level, as estimated by HbA1c, is taken into account. This is particularly true in DM patients, in whom a high glucose value at admission is not always an index of an acute glycemic rise.

Acute hyperglycemia is frequently observed in the early phase of STEMI, irrespective of DM presence, and it has been constantly associated with a poor outcome and a larger infarct size (51,54,75,76). The impact of acute hyperglycemia seems to be more pronounced in patients without DM than in those with DM, suggesting that the magnitude of the acute glycemic rise from chronic levels, rather than the absolute admission glycemic level per se, can be detrimental (69,77-79). From a practical point of view, STEMI patients with similar acute hyperglycemia may have different risk profiles according to their chronic glycemic values. Therefore, we hypothesized that the assessment of the A/C glycemic ratio could better identify true stress hyperglycemia than the glycemic value measured at hospital admission. In order to estimate the average chronic glycemia, we utilized the formula proposed by Nathan and colleagues (86). To the best of our knowledge, this is the first study exploring the A/C glycemic ratio in STEMI patients and specifically focusing on the combined assessment of CMR data and mitochondrial injury. Indeed, while the prognostic impact of glycemia at admission has been widely evaluated in STEMI, the clinical relevance of the A/C glycemic ratio has never been fully investigated so far, especially in relationship to myocardial infarct size, as assessed by CMR, and mitochondrial injury. A recent study by Yang et al. (78) analyzed the parameter of relative hyperglycemia (defined in their study as stress

hyperglycemia ratio) in a large registry of patients undergoing PCI. They found that this ratio is a strong predictor of short-term and long-term major adverse cardiovascular and cerebrovascular events. Differently from our study focusing on STEMI patients only, they included all spectrums of coronary artery disease, with only 30% of which involved acute myocardial infarction. Moreover, owing to the retrospective design of the study, their index was based not on admission glycemia but, rather, on the first-measured random glycemia during hospitalization. Another study, by Fujino et al. (79), considered the possible additional role of acute and chronic hyperglycemia in acute myocardial infarction and showed that patients with acute hyperglycemia had worse in-hospital outcome. However, in patients with chronic hyperglycemia who showed acute hyperglycemia at admission, mortality was significantly lower. A limitation of this study was that chronic glycemia is defined in a dichotomic way, according to HbA1c value (<6.5% or ≥6.5% [< or ≥48 mmol/mol]) indicating DM status. Thus, they could not detect acute glycemic rise, i.e., the occurrence of true stress hyperglycemia, and were not able to evaluate its magnitude.

In our study, the prognostic power of the A/C glycemic ratio seems to be particularly robust in patients with DM, in whom it allowed to reclassify properly about 30% of patients for the primary endpoint and for the in-hospital composite clinical endpoint. Conversely, in patients without DM, A/C glycemic ratio and acute glycemia had a similar prognostic accuracy. These findings are not unexpected as the magnitude of acute glycemic elevation may be small in patients with DM and an impaired chronic glyco-metabolic profile. In these patients, the A/C glycemic ratio may better identify the presence of a true stress hyperglycemia. It is unclear whether an acute rise of glucose level directly contributes to myocardial injury, thus

affecting patient outcome, or is only a marker of disease severity. Although no causal link can be inferred from our data, the relationships between acute glycemic rise, infarct size at CMR, and worse clinical outcome remained significant after adjustment for major clinical confounders. In agreement with this hypothesis, experimental and clinical evidence have shown that an acute increase of plasma glucose triggers oxidative stress, inflammation, and endothelial dysfunction, activates coagulation, and abolishes ischemic preconditioning (62,82,94). All these factors may further increase myocardial damage in the setting of acute ischemia. Indeed, acute hyperglycemia has been associated with a lower myocardial salvage index evaluated by cardiac CMR (73). Interestingly, this association was not found in acute myocardial infarction patients with acute hyperglycemia and DM. This may be due to the ≥180 mg/dL glycemic threshold that was used to define acute hyperglycemia, a value that seems low in patients with DM with chronically elevated glycemic levels. On the other hand, in our study, the A/C glycemic ratio closely correlated with the extent of myocardial salvage index in the DM cohort.

Another novelty of our study was the evaluation of mitochondrial markers. The mitochondria are fundamental elements of cardiac function, as they supply the cell with essential biologic energy, through adenosine-three-phosphate production (95). Several experimental models have recognized that mitochondrial dysfunction is a critical factor in causing myocardial ischemic and reperfusion injury, directly contributing to reducing cardiac contractility and increasing infarct size (87,88). Mitochondrial biomarkers, such as cytochrome c and cell-free mtDNA have been studied in various clinical settings and elevated circulating levels have been associated with poor prognosis, including mortality, after cardiac arrest and in other critical conditions (92,96-99). In particular, in the setting of coronary artery disease,

it has been demonstrated that the prognostic power of cytochrome c and mtDNA is particularly evident in STEMI patients, and it is independent of clinical variables that are known to affect AMI outcome (89,91). Notably, a 10-fold higher in-hospital mortality in STEMI patients has been reported when cytochrome is detected, despite similar baseline risk profile, drug treatment, and mechanical reperfusion rate between patients with or without detectable cytochrome c (91). In NSTEMI patients in whom cytochrome c was detected, a 2-fold higher in-hospital mortality rate was observed (91). Indeed, ischemia and reperfusion phenomena cause intracellular calcium overload and generation of reactive oxygen species, which predispose to mitochondrial dysfunction, and may contribute, in addition to cell necrosis, to myocardial contractility impairment (87,88). In our study, we investigated the potential association between acute hyperglycemia and A/C glycemic ratio with mitochondrial biomarkers, measured in all patients at hospital admission. We, interestingly, found that cytocrome c and mtDNA levels are significantly higher in DM patients as compared to those without. Moreover, while in non-DM patients, both acute hyperglycemia and A/C glycemic ratio correlated with cytochrome c and mtDNA levels; in DM patients, only A/C glycemic ratio correlated with both mitochondrial biomarkers. This, again, suggests that, in DM patients, A/C glycemic ratio, rather than acute hyperglycemia, better reflects the ongoing myocardial injury during STEMI. However, experimental data are required in suitably designed models to explore the specific mechanism(s) inducing cytochrome c and mtDNA release, as well as their biological meaning. In addition, we cannot exclude that cytochrome c and mtDNA measurement performed also after the procedure (primary PCI), instead of at hospital admission only, could have increased its prognostic potential by incorporating mitochondrial damage

associated with mechanical reperfusion injury. Moreover, because this was an observational study, a cause-effect relationship between detection of mitochondrial biomarkers and study endpoints cannot be established

Our findings may have some, relevant potential clinical implications. In STEMI patients without DM, high glucose levels at admission will reflect stress hyperglycemia, and may be used to guide intensive glycemic control. Conversely, in patients with DM and high glycemic levels at admission, the combined assessment of acute and chronic glycemia may identify true stress hyperglycemia and may help physicians to better discriminate high-risk from low-risk STEMI patients and, more importantly, to tailor treatment. Of note, Kosiborod et al. (66) have shown that glucose normalization after admission is associated with better survival in hyperglycemic patients hospitalized with acute myocardial infarction. However, in patients undergoing cardiothoracic surgery, intensive glycemic control improved outcomes in patients without known DM but not in those with a previous diagnosis of DM (81). Thus, the role of a tight control of hyperglycemia as a strategy for improving prognosis in AMI patients, and in particular in those with DM, is still under debate. Patients with DM often show high glycemic levels at admission that are not always associated with acute hyperglycemia. In these patients, an intensive lowering of glucose levels may not be beneficial, as the detrimental effects of the glycemic disorder are not limited to stress hyperglycemia but, rather, also include fluctuations of glycemic values, with acute glucose changes in both directions (60). Accordingly, previous studies have indicated that glucose variability in patients with DM has a more pronounced effect on oxidative stress (60), platelet activation, and aggregation (100), and on macrovascular and microvascular complications (84), than chronically elevated glucose levels. Moreover, acute variability of glucose

values, assessed by measuring the mean amplitude of glycemic excursion with a continuous glucose monitoring system, negatively correlated with the myocardial salvage index in patients with acute myocardial infarction (85). Therefore, future multicenter studies are needed to confirm our results and to investigate whether a strategy based on glucose normalization in patients with a high A/C glycemic ratio may have a greater impact on myocardial infarct size and mitochondrial injury reduction and, hence, outcome improvement than an approach centered on the treatment of hyperglycemia at admission only in DM patients hospitalized with STEMI.

7. STUDY STRENGHTS AND LIMITATIONS

The strengths of the current study include the prospective design, a well-characterized population, adjustment for a variety of risk factors, the estimation in all patients of chronic glycemia, a special focus on STEMI patients undergoing CMR, and the investigation of mitochondrial biomarkers. Some limitations warrant mention. Firstly, we evaluated STEMI patients admitted to a single center and treated, in all cases, with primary PCI. As this therapeutic strategy may have influenced the results of our study, the overall applicability of our findings to acute myocardial infarction patients not undergoing coronary revascularization needs to be clarified. Secondly, because this was an observational study, a cause-effect relationship between plasma glucose, infarct size, mitochondrial injury, and outcomes cannot be established. Thirdly, the impact on outcomes of in-hospital glycemic fluctuations, therapeutic management of acute hyperglycemia, glycemic target choice, and diabetes type (1 vs. 2) was not investigated and should be taken into account as a possible bias. Finally, the A/C glycemic ratio was calculated on the average chronic glycemic value estimated from HbA1c. Thus, we cannot exclude that the calculated ratio does not fully reflect acute glycemic changes occurring during the index event. Furthermore, in patients with low admission hemoglobin value, average chronic glucose level might have been underestimated.

8. CONCLUSIONS

In conclusion, we have demonstrated that the A/C glycemic ratio in STEMI patients is closely associated with in-hospital morbidity and mortality. Use of the A/C glycemic ratio may be particularly valuable in patients with DM with chronically elevated glycemic levels because it may identify true stress hyperglycemia, which has been associated with a larger infarct size, a greater mitochondrial injury, and a worse outcome.

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