

Cyclosporine A in Reperused Myocardial Infarction

The Multicenter, Controlled, Open-Label CYCLE Trial



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ABSTRACT

BACKGROUND Whether cyclosporine A (CsA) has beneficial effects in reperused myocardial infarction (MI) is debated.

OBJECTIVES This study investigated whether CsA improved ST-segment resolution in a randomized, multicenter phase II study.

METHODS The authors randomly assigned 410 patients from 31 cardiac care units, age 63 ± 12 years, with large ST-segment elevation MI within 6 h of symptom onset, Thrombolysis In Myocardial Infarction (TIMI) flow grade 0 to 1 in the infarct-related artery, and committed to primary percutaneous coronary intervention, to 2.5 mg/kg intravenous CsA ($n = 207$) or control ($n = 203$) groups. The primary endpoint was incidence of $\geq 70\%$ ST-segment resolution 60 min after TIMI flow grade 3. Secondary endpoints included high-sensitivity cardiac troponin T (hs-cTnT) on day 4, left ventricular (LV) remodeling, and clinical events at 6-month follow-up.

RESULTS Time from symptom onset to first antegrade flow was 180 ± 67 min; a median of 5 electrocardiography leads showed ST-segment deviation (quartile [Q]1 to Q3: 4 to 6); 49.8% of MIs were anterior. ST-segment resolution $\geq 70\%$ was found in 52.0% of CsA patients and 49.0% of controls ($p = 0.55$). Median hs-cTnT on day 4 was 2,160 (Q1 to Q3: 1,087 to 3,274) ng/l in CsA and 2,068 (1,117 to 3,690) ng/l in controls ($p = 0.85$). The 2 groups did not differ in LV ejection fraction on day 4 and at 6 months. Infarct site did not influence CsA efficacy. There were no acute allergic reactions or nonsignificant excesses of 6-month mortality (5.7% CsA vs. 3.2% controls, $p = 0.17$) or cardiogenic shock (2.4% CsA vs. 1.5% controls, $p = 0.33$).

CONCLUSIONS In the CYCLE (CYCLOsporinE A in Reperused Acute Myocardial Infarction) trial, a single intravenous CsA bolus just before primary percutaneous coronary intervention had no effect on ST-segment resolution or hs-cTnT, and did not improve clinical outcomes or LV remodeling up to 6 months. (CYCLOsporinE A in Reperused Acute Myocardial Infarction [CYCLE]; [NCT01650662](https://clinicaltrials.gov/ct2/show/study/NCT01650662); EudraCT number [2011-002876-18](https://eudra.europa.eu/CTD/CTDsearch/CTDsearch.jspx?search=2011-002876-18)) (J Am Coll Cardiol 2016;67:365-74)
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Manuscript received August 5, 2015; revised manuscript received October 23, 2015, accepted October 27, 2015.

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ABBREVIATIONS AND ACRONYMS

- CK** = creatine kinase
CsA = cyclosporine A
ECG = electrocardiogram
hs-cTnT = high-sensitivity cardiac troponin T
ITT = intention-to-treat
IV = intravenous
LV = left ventricle/ventricular
LV A/D = left ventricular akinetic/dyskinetic
LVEF = left ventricular ejection fraction
MI = myocardial infarction
mPTP = mitochondrial permeability transition pore
PCI = percutaneous coronary intervention
pPCI = primary percutaneous coronary intervention
PROBE = PRospective Open, Blinded Endpoint
Q = quartile
STEMI = ST-segment elevation myocardial infarction
TIMI = Thrombolysis In Myocardial Infarction

Early, successful restoration of myocardial perfusion after a ST-segment elevation myocardial infarction (STEMI) is the most effective way to reduce final infarct size and improve clinical outcome (1-3). However, experimental and clinical data have shown that reperfusion per se may have harmful effects, the “myocardial reperfusion injury,” identified as a target for cardioprotection (4-7). Mitochondria may play a key role in reperfusion injury by opening a nonspecific high-conductance channel called the mitochondrial permeability transition pore (mPTP), located in the inner mitochondrial membrane. Immediately after the onset of myocardial reperfusion, irreversible opening of the mPTP results in collapse of the membrane potential, uncoupling of the respiratory chain, and efflux of proapoptotic factors that may lead to cell death (8,9).

Experimental evidence suggests that different interventions designed to prevent mPTP opening may reduce myocardial infarct size by an additional 30% to 50% in various animal models (9). A single intravenous bolus of cyclosporine A (CsA) before reperfusion was reported to reduce creatine kinase (CK) release by 40% in 58 STEMI patients treated

with primary percutaneous coronary interventions (pPCI) (10), and to attenuate the impairment of left ventricular (LV) function over 6 months after myocardial infarction (MI) in the same patients (11). This proof-of-concept study created new enthusiasm in targeting reperfusion for cardiac protection, with CsA as prototypical agent (12,13), while also paving the way to a larger multicenter study to verify these very encouraging findings, before proceeding to a trial on “hard” clinical endpoints. However, a double-blind trial in 101 STEMI patients showed that CsA given right before thrombolysis neither reduced MI size nor improved clinical outcomes (14).

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Experimental (15) and clinical (10) data on CsA were deemed insufficient to justify a real phase III trial with hard clinical endpoints, such as the CIRCUS (Cyclosporine and Prognosis in Acute Myocardial Infarction [MI] Patients) trial, for which 1-year follow-up results have been published (16). Therefore, we planned and conducted a randomized, multicenter, pragmatic, phase II trial enrolling a medium-sized STEMI population undergoing pPCI with a 2-fold purpose: first, to verify, on a large scale, the efficacy of a single bolus of CsA in attenuating reperfusion

injury by detection of more complete resolution of ST-segment elevation, and by reduction of infarct size (measured as less release of biomarkers of myocardial injury and improvement of echocardiographically measured LV function); and secondly, to assess the safety of a single intravenous (IV) bolus of CsA given shortly before pPCI.

METHODS

The CYCLE (CYCLOsporine A in Reperused Acute Myocardial Infarction) trial (NCT01650662; EudraCT number 2011-002876-18) was a multicenter, randomized, open-label, controlled trial of CsA versus no CsA (control) in patients with STEMI undergoing pPCI with PRospective Open, Blinded Endpoint (PROBE) design for the evaluation of the primary endpoint. It was run according to the Declaration of Helsinki of Good Clinical Practice. Regulatory agencies and local ethics committees approved the study protocol. All patients gave written informed consent. CsA was purchased from a hospital pharmacy (Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy).

STUDY POPULATION. The study population comprised patients >18 years of age with a first STEMI (defined as nitrate-resistant chest pain ≥ 20 min and new ST-segment elevation ≥ 0.1 mV in 2 contiguous leads). In order to enroll patients with an area at risk large enough to benefit most from CsA, only patients with ST-segment elevation in ≥ 3 leads in anterior MI and ST-segment deviation in at least 4 leads in nonanterior MI were randomized. Additional inclusion criteria were presentation within 4 h of the onset of chest pain (subsequently extended to 6 h); occlusion of the culprit artery (Thrombolysis In Myocardial Infarction [TIMI] flow grade 0 to 1 at admission and before PCI); and the clinical decision to treat with PCI. Exclusion criteria were left bundle branch block in the presenting electrocardiogram (ECG) (not permitting evaluation of the resolution of ST-segment elevation); TIMI flow grade >1 in the culprit artery; coronary anatomy not suitable for PCI; previous MI and/or previous coronary artery bypass graft; thrombolytic therapy within 24 h before randomization; current treatment (<10 days) with CsA or contraindications to CsA; and severe renal or hepatic insufficiency.

EXPERIMENTAL PROCEDURES. After signing the informed consent form, eligible patients were randomly assigned in a 1:1 ratio to CsA or nothing by an automated web-based system, using a permuted block randomization scheme stratified by site of MI. The bolus of 2.5 mg/kg of CsA (Sandimmune IV, Novartis, Basel, Switzerland) was injected over 20 to 30 s into an

antecubital vein after coronary angiography, but just before passage of the wire into the culprit artery (thus avoiding any wire-related dissolution/fragmentation of the occlusive thrombus), and at least 5 min before PCI, to allow distribution of the drug. In the control group, patients received only recommended treatments according to STEMI European guidelines (3). Coronary angiography was then done using standard techniques to identify the culprit coronary artery and to check that reperfusion had not occurred before PCI (TIMI flow grade 0 to 1 at randomization).

Two readers blinded to treatment allocation analyzed the digitized angiograms for all stenoses, thrombus burden, collaterals before reperfusion, and TIMI flow grade and rate, as well as myocardial blush grade after reperfusion.

Essentials of the study design are shown in [Online Figure 1](#). Patient follow-up involved 4-week and 6-month (final) visits (at which serious adverse events were checked, with a cardiovascular examination and measurement of vital signs [heart rate and blood pressure]). A 12-lead ECG tracing was recorded at hospital admission and 60 min after the first evidence of antegrade flow following pPCI to assess the primary endpoint of the study, resolution of ST-segment elevation (see the following text).

Blood samples for central measurement of plasma high-sensitivity cardiac troponin T (hs-cTnT) (ECLIA Cobas 411, Roche Diagnostics, Rotkreuz, Switzerland) were collected before coronary angiography, the next morning, day 4, and 6 months after the study medication and stored in a central biobank at the Mario Negri Institute in Milan, Italy. Cardiac troponins and CK were assayed locally, recorded, and normalized by the upper limit of normal of each clinical chemistry laboratory for calculations.

A conventional 2-dimensional echocardiography examination was done in each patient on day 4 and at the 6-month visit, to assess global and segmental LV function. Measurements were taken in at least 3 consecutive cycles for patients in sinus rhythm and 5 for patients with atrial fibrillation, from the parasternal long-axis, mitral- and papillary-level short-axis, and apical 2- and 4-chamber views. LV volumes were calculated and normalized by body surface area, and the modified biplane Simpson's rule was applied to calculate left ventricular ejection fraction (LVEF). LV regional wall motion was evaluated using a 16-segment model (17). An index of the extent of LV damage was obtained by dividing the number of akinetic/dyskinetic segments by the total number of segments evaluated (up to 16) and expressed as a percentage. All echocardiographic data were stored digitally in Digital Imaging and Communications in

Medicine (DICOM) format for subsequent offline analysis. Quality was monitored by centrally reading 10% of randomly sampled echocardiographic examinations from each site.

The primary efficacy endpoint was the incidence of $\geq 70\%$ resolution of ST-segment elevation 1 h after pPCI, as previously described (18,19). This was due to the assumption that in addition to being a validated tool to assess the efficacy of reperfusion therapy, ST-segment resolution would provide an indication of the outcome of PCI. ST-segment elevations in all 12 leads were measured 20 ms after the end of the QRS complex with an ad hoc-made electronic caliper on digitized ECG tracings by a reader blind to patient identification and treatment. ST-segment resolution was calculated as the difference between 60 min and baseline in the lead with maximal ST-segment elevation at baseline (18). Duplicate readings were taken on 10% of randomly chosen ECGs, with a third reader in case of disagreement.

The key secondary endpoint was the concentration of hs-cTnT measured on day 4 after pPCI. This easy, inexpensive, single-point measurement is closely related to infarct size (20), LV function (21), and clinical outcomes (22,23).

Other secondary endpoints (definitions provided in the [Online Appendix](#)) were regional and global LV function assessed on day 4 and 6 months, as well as rehospitalization for cardiovascular reasons, all-cause and cardiovascular death, heart failure, and cardiogenic shock at 6 months.

STATISTICAL METHODS AND POWER CALCULATIONS.

Because the main analysis was done according to the intention-to-treat (ITT) approach, all patients randomized in the study were included. Baseline characteristics by randomized treatment are presented as proportions for categorical variables and compared by the chi-square test; continuous variables are reported as mean \pm SD or median (quartile [Q]1 to Q3) and compared by a Student *t* test or the Wilcoxon rank sum test, as appropriate. The primary endpoint (complete ST-segment resolution [i.e., $\geq 70\%$, 60 min after PCI]) was analyzed with a logistic regression model. Adjustment was made by multivariable logistic regression for baseline characteristics (number of ECG leads with ST-segment deviation, ventricular tachycardia, and Rentrop score ≥ 2) unbalanced between the 2 groups.

On the basis of previous data (18) a 55% incidence of the primary endpoint was assumed in the placebo arm. We calculated that 444 patients (222 patients per group) were required for the study to have 80%

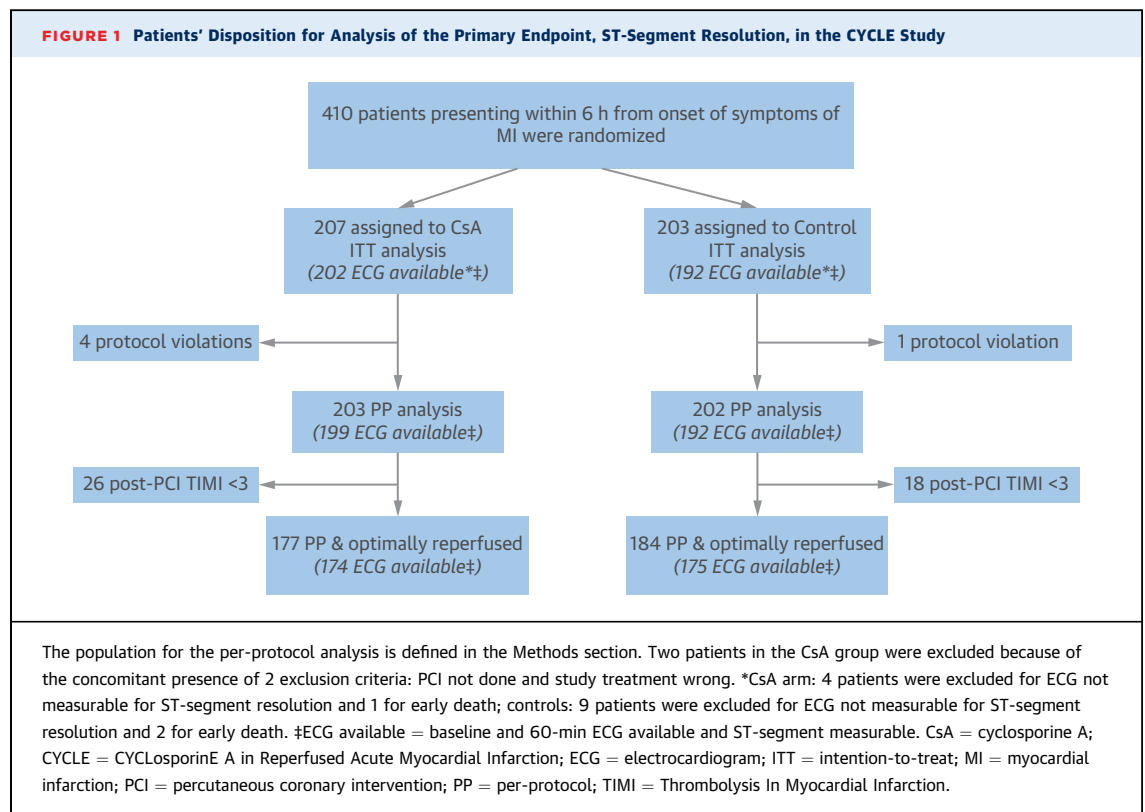
power to detect a 25% difference in the rates of ST-segment resolution with a 5% dropout rate at a 2-sided alpha of 5%. The size of the trial also allowed us to investigate the effects of treatment on circulating levels of hs-cTnT on day 4 after pPCI. Assuming a concentration of hs-cTnT of 2,700 ng/l on day 4 (SD: 2.1) in the control group, it would be possible to detect a 25% difference with 90% power, and a 2-sided alpha of 5%. The study was not powered to detect a treatment effect on the clinical events defined as secondary endpoints. Cumulative event rates for the secondary endpoints (i.e., events occurring over the 6-month follow-up) were estimated using the Kaplan-Meier method. Hazard ratios, 95% confidence intervals, and p values for comparison of the 2 groups were determined with the Cox proportional hazards regression model. Treatment effects on echocardiographic parameters related to ventricular remodeling and on log_e-transformed concentrations of centrally assayed hs-cTnT were examined by analysis of variance. All analysis of variance models included the interaction between study treatment and site of MI and the percentage of left ventricular akinetic/dyskinetic (LV A/D) segments. Between-group comparisons of area under the curves for the hs-cTnT concentrations measured

at admission, day 1, and day 4 were made with the Wilcoxon rank sum test.

In an attempt to minimize the risk of a false-negative result, we not only excluded patients who did not undergo PCI or who received the wrong dose of CsA (protocol violations) from the ITT population, thus defining the per-protocol population, but also those with incomplete revascularization of the culprit artery (i.e., TIMI flow grade <3 at the end of the procedure, thus defining an “optimally reperfed” group) (Figure 1). A 2-sided alpha level of 0.05 was considered to indicate statistical significance. All data analyses were conducted with SAS software (SAS Institute, Cary, North Carolina), version 9.2 or higher.

RESULTS

A total of 410 STEMI patients were enrolled from January 19, 2012, to April 30, 2014, in 31 Italian centers: 207 received an IV bolus of CsA, and 203, who served as controls, received conventional treatment. In all, 80% were male, 40% were active smokers, and 14% had diabetes. More than one-half of the population was hypertensive. Pre-infarction angina within 48 h before the index event was reported in approximately one-half of the patients (Table 1).



The 2 groups had a similar hemodynamic status, depicted by blood pressure, heart rate, and Killip class. They were also comparable for all major events occurring before randomization (i.e., defibrillation, cardiopulmonary resuscitation, shock, bradycardia, second- or third-degree atrioventricular block), except ventricular tachycardia, which was reported in 7 (3.5%) patients in the control group, but none in the CsA group ($p = 0.007$). Overall, nearly one-half of the patients had an anterior MI. Median pain-to-first antegrade flow time was 180 ± 67 min, with a negligible difference between the groups, showing that there was no delay in starting pPCI attributable to the CsA injection. The number of ECG leads with 1 mm or more ST-segment deviation was significantly ($p = 0.01$) higher in the CsA than in the control group. Among the angiographic characteristics, distribution of the culprit artery was comparable, with the left anterior descending coronary artery the site of occlusion in about one-half of the patients. The culprit artery was totally occluded (TIMI flow grade 0) in a large proportion of patients in both groups ($p = 0.09$). Technical details of the procedures, such as number and type of stents (drug-eluting vs. bare-metal stents) and maximum pressure of the balloon inflation, did not differ between the 2 groups (Online Table 1). PCI was not done in 4 patients (3 in the CsA group and 1 in the control group) for the following reasons: aortic dissection; unsuitable anatomy; no occlusion; and thrombus aspiration. CsA was not either given or the wrong dose was given to 3 patients. In all, 49 patients were excluded from the ITT population for the analysis limited to patients fulfilling protocol requirements and optimally reperfused: PCI not done in 4; wrong study treatment in 3 (2 patients with 2 criteria for exclusion); and incomplete reperfusion (TIMI flow grade <3) in 44 (Figure 1). There were 6 patients with Rentrop grade 2, corresponding to the presence of collateral flow, all in the control group (Online Table 1).

PRIMARY ENDPOINT. Centrally read ST-segment resolution of 70% or more at a median time of 61 min after pPCI (61 min in the CsA group and 62 min in the control group, $p = 0.73$) occurred at similar rates in CsA patients (105 of 202, 52.0%) and controls (94 of 192, 49.0%) (unadjusted $p = 0.55$). After adjustment for variables unbalanced for the randomized treatment, such as the number of leads with ST-segment deviation, Rentrop grade 2, and ventricular tachycardia, the difference was still not significant ($p = 0.76$). Analyzed as a continuous variable, median (Q1 to Q3) ST-segment resolution was 71% (50% to 90%) in CsA and 69% (47% to 88%) in controls

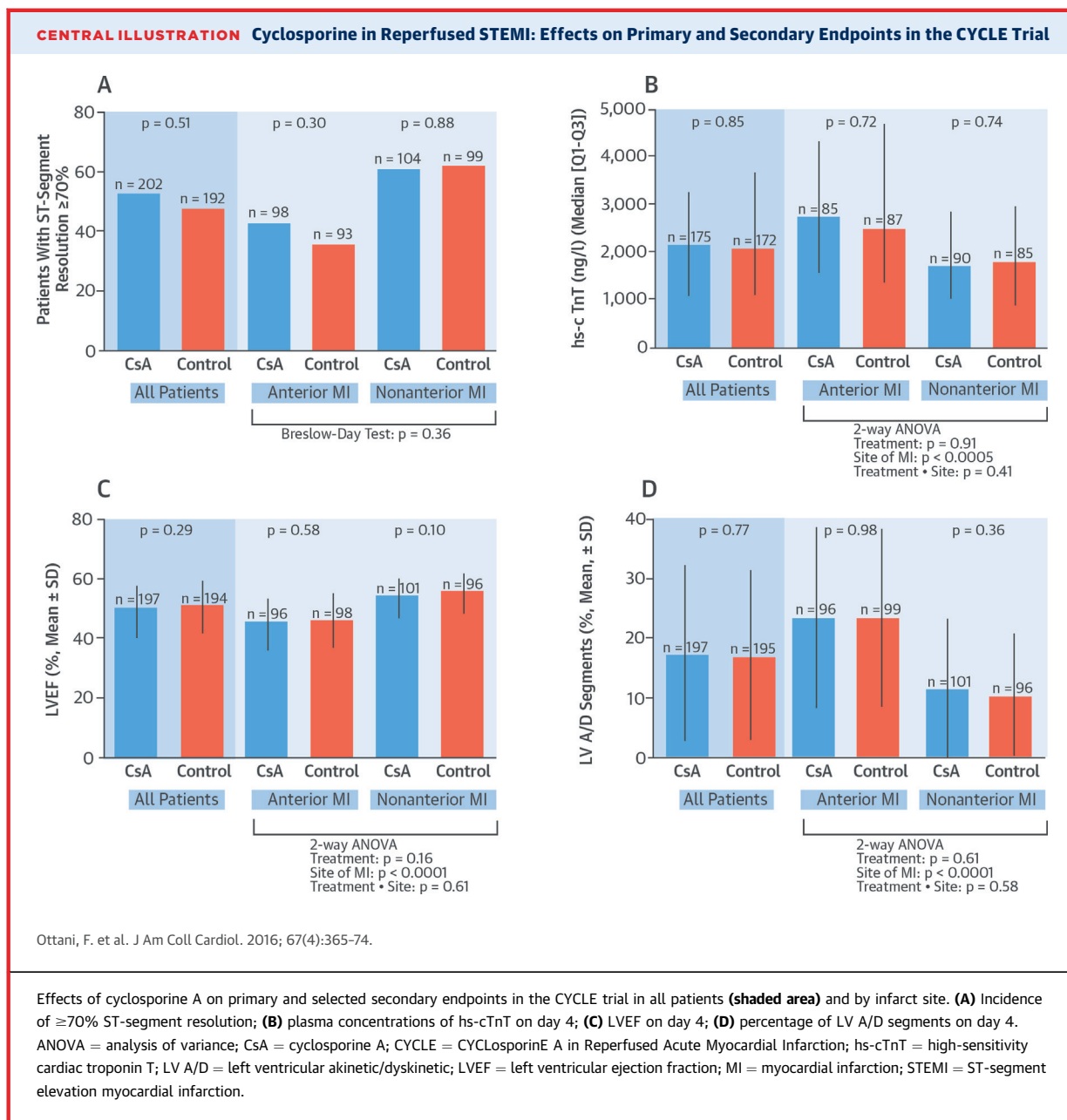
TABLE 1 Baseline Characteristics of the Study Population

	Total (N = 410)	CsA (n = 207)	Control (n = 203)	p Value
Age, yrs	62.9 ± 12	62.5 ± 12.4	63.2 ± 11.6	0.59
Male	327 (79.8)	167 (80.7)	160 (78.8)	0.64
BMI, kg/m ²	26.7 ± 3.9	27.1 ± 3.8	26.4 ± 3.9	0.06
Current smokers	165 (40.2)	84 (40.6)	81 (39.9)	0.83
Diabetes mellitus	58 (14.2)	27 (13.0)	31 (15.3)	
Type 1	4 (6.8)	2 (7.4)	2 (6.5)	0.52
Type 2	54 (93.2)	25 (92.6)	29 (93.5)	
Hypertension	227 (55.4)	114 (55.1)	113 (55.7)	0.90
Dyslipidemia	155 (37.8)	72 (34.8)	83 (40.9)	0.20
Pre-infarct angina	185 (45.1)	91 (44.0)	94 (46.3)	0.63
Heart rate, beats/min	75 ± 16	77 ± 16	74 ± 16	0.09
SBP, mm Hg	132 ± 26	132 ± 26	132 ± 26	0.98
DBP, mm Hg	78 ± 14	79 ± 14	78 ± 14	0.76
Killip class II-IV at entry	19 (4.6)	10 (4.8)	9 (4.4)	0.85
No. of ECG leads with ≥1-mm deviation	5 (4-6)	5 (5-7)	5 (4-6)	0.01
In-hospital therapies				
Aspirin	407 (99.3)	205 (99.0)	202 (99.5)	1.00*
Heparin	346 (84.4)	175 (84.5)	171 (84.2)	0.93
Thienopyridine	406 (99.0)	203 (98.1)	203 (100.0)	0.12*
Glycoprotein IIb/IIIa antagonist	182 (44.4)	90 (43.5)	92 (45.3)	0.71
Beta-blockers	331 (80.7)	164 (79.2)	167 (82.3)	0.44
Bivalirudin	99 (24.2)	46 (22.2)	53 (26.1)	0.36
ACEi/sartans	293 (71.5)	147 (71.0)	146 (71.9)	0.84
Statins	365 (89.0)	183 (88.4)	182 (89.7)	0.69
Symptoms to first antegrade flow time, min	180 ± 67	178 ± 69	183 ± 65	0.52
Anterior infarct	203 (49.5)	102 (49.3)	101 (49.7)	0.92
TIMI flow before PCI				
0	342 (83.4)	179 (86.5)	163 (80.3)	0.09
1	68 (16.6)	28 (13.5)	40 (19.7)	
Stent implantation, 1-4	393/406 (95.9)	198/204 (97.0)	195/202 (97.0)	0.70

Values are mean ± SD, n (%), median (interquartile range), or n/N (%). *Fisher exact test.
ACEi = angiotensin-converting enzyme inhibitor; BMI = body mass index; CsA = cyclosporine A; DBP = diastolic blood pressure; ECG = electrocardiogram; PCI = percutaneous coronary intervention; SBP = systolic blood pressure; TIMI = Thrombolysis In Myocardial Infarction.

($p = 0.55$). The primary endpoint could not be calculated in 16 patients: 8 because of idioventricular rhythm; 1 because of the presence of a pacemaker; 3 died before the second ECG could be recorded; and 4 for other reasons. Of the 16 patients with a missing primary endpoint, 5 were in the CsA group and 11 were in the control group ($p = 0.12$) (Figure 1). The lack of effect of CsA on ST-segment resolution was confirmed when data were analyzed by MI site (Central Illustration, panel A).

SECONDARY ENDPOINTS. The time course of centrally assayed hs-cTnT did not differ in the CsA and control groups (Figure 2). This was true on day 4, even after stratification by MI site (Central Illustration, panel B). Median (Q1 to Q3) areas under the hs-cTnT concentration versus time (entry to day 4) were,



respectively, 268 (151 to 446) and 250 (141 to 444) $\text{ng}\cdot\text{ml}^{-1}\cdot\text{h}$ in CsA and controls ($p = 0.66$). The results of the centrally assayed hs-cTnT were consistent with those of cardiac troponins and CK in terms of peak levels (Online Figure 2) and time courses (Online Figure 3), assayed locally, and normalized by the upper limit of normal for each laboratory. Analysis of the echocardiographic assessment of LV remodeling was in full agreement with these biomarkers with respect to CsA effects. The LVEF was lower, as expected in anterior MIs, but there was no difference between the CsA and control

groups (p for treatment = 0.16). The percentage of LV A/D segments was higher in anterior MIs, and decreased significantly from day 4 to month 6, although there was no difference between the 2 study groups ($p = 0.61$) (Central Illustration, panels C and D, Online Table 2).

Consistent with the findings on ST-segment resolution, myocardial blush grades 2 to 3 at the final angiography (a marker of successful reperfusion) did not show any difference by study treatment: 77.3% in CsA and 80.8% in controls ($p = 0.82$) (Online Table 1).

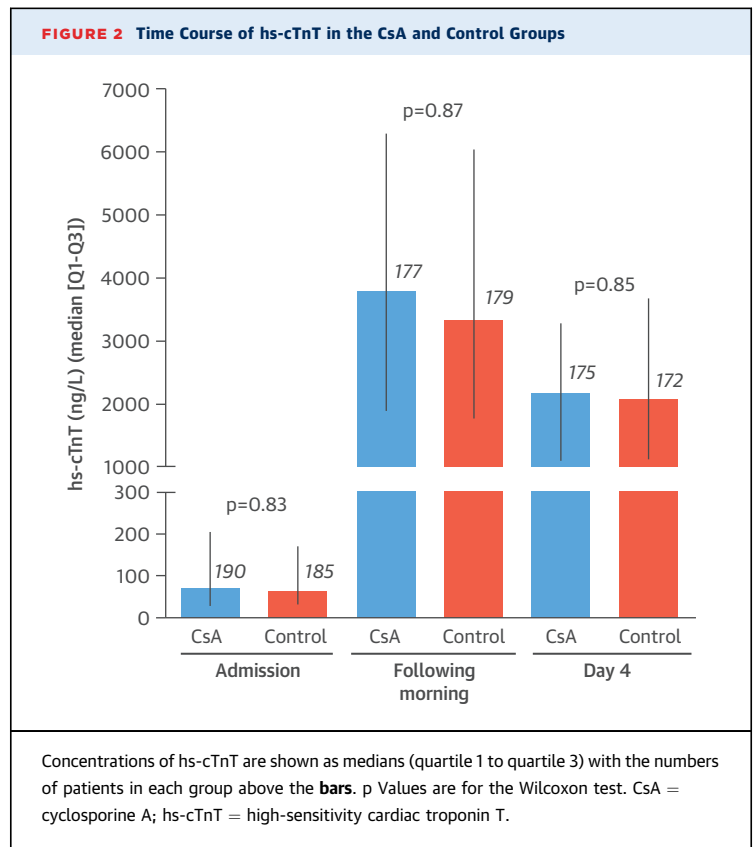
Clinical events over the 6-month follow up are reported in **Table 2**. Of the 18 patients who died, 12 (5.7%) in the CsA group and 6 (3.2%) controls ($p = 0.17$), a cardiovascular cause was adjudicated in 13, of whom 9 (4.4%) were in the CsA group and 4 (2.2%) were controls ($p = 0.18$). Six patients (2.4%) in the CsA group and 3 (1.5%) in the control group had cardiogenic shock ($p = 0.33$), whereas hospitalization for cardiovascular reasons and episodes of heart failure were equally distributed across the 2 groups. The incidence of the combined endpoint of all-cause mortality, heart failure, or cardiogenic shock was similar: 29 (13.9%) in the CsA group and 28 (13.4%) in the control group, respectively ($p = 0.91$).

PER-PROTOCOL ANALYSIS AND OPTIMALLY REPERFUSED PATIENTS. In the per-protocol analysis, 102 (51.3%) patients in the CsA group and 94 (49.0%) in the control group ($p = 0.89$, adjusted) had a ST-segment resolution of at least 70%. Of the 349 patients who were optimally reperfused, fulfilling study protocol and with ST-segment resolution measured, 94 (54.0%) CsA and 88 (50.3%) control patients ($p = 0.69$, adjusted) showed a ST-segment resolution $\geq 70\%$. The analysis showed a 6-month all-cause mortality of 3.4% versus 2.2% ($p = 0.49$) and cardiovascular mortality of 2.8% versus 1.1% ($p = 0.25$). As with ITT, the combined endpoint of all-cause mortality, heart failure, or cardiogenic shock was not different in the 2 groups: 21 (11.9%) in the CsA group and 23 (12.5%) in the control group ($p = 0.88$). Echocardiography indicated larger LV volumes both on day 4 and at 6 months with CsA, reaching statistical significance in the per-protocol analysis (**Online Table 3**).

SAFETY. Great care was taken to monitor patients in the first few hours after the index event for rare acute reactions to intravenous CsA, which have been reported and attributed to 1 of the excipients, ricinoleic acid (24-26). In fact, no anaphylactoid reactions were reported during the CYCLE trial. Only 1 serious adverse drug reaction was reported in the whole trial, a patient in the CsA group who died after surgery for myocardial rupture 23 days after the index MI (**Table 3**).

DISCUSSION

The present multicenter, controlled clinical trial does not confirm the previous findings of a marked cardiac protective effect of CsA in the setting of timely reperfusion acute MI by means of pPCI (10). All selected trial endpoints, such as ST-segment resolution, circulating levels of troponins, and LV remodeling, with clinical outcomes, consistently showed that an IV bolus of CsA



just before recanalization of an occluded coronary artery did not enhance the beneficial effect of reperfusion. The slight (but not statistically significant) excess in all-cause and cardiovascular mortality with CsA, but not in nonfatal clinical events, and the tendency towards larger LV volumes might even suggest a harmful effect. Our findings provide strong support and expand previous neutral or negative pre-clinical (15,27) and clinical (14,16) studies with CsA.

The representativeness of the large number of cardiology centers involved in the trial protects

Patients With Events at 6 Months (Adjudicated by the Event Validation Committee)	Total (N = 410)	CsA (n = 207)	Control (n = 203)	HR (95% CI) CsA vs. Control	p Value
Death	18 (4.3)	12 (5.7)	6 (3.2)	1.98 (0.74-5.27)	0.17
CV death	13 (3.3)	9 (4.4)	4 (2.2)	2.24 (0.69-7.26)	0.18
Heart failure	44 (10.1)	21 (9.7)	23 (10.4)	0.91 (0.50-1.64)	0.75
Cardiogenic shock	9 (2.2)	6 (2.4)	3 (1.5)	1.99 (0.50-7.95)	0.33
All-cause mortality or HF or cardiogenic shock	57 (13.5)	29 (13.9)	28 (13.4)	1.03 (0.61-1.73)	0.91
Rehospitalization for CV reasons	58 (14.3)	28 (14.3)	30 (14.6)	0.95 (0.57-1.59)	0.84

Values are n (%), unless otherwise noted. Percentages are product-limit survival estimates derived by the Kaplan-Meier method.
 CI = confidence interval; CsA = cyclosporine A; CV = cardiovascular; HF = heart failure; HR = hazard ratio.

TABLE 3 Adverse Drug Reactions Reported in CYCLE

	Total (N = 410)	CsA (n = 207)	Control (n = 203)	p Value
Patients with adverse reactions to the study drug	4 (1.0)	4 (1.9)	0 (0.0)	0.12
Allergic reaction	2 (0.5)	2 (1.0)	0 (0.0)	
Acute renal failure	—	—	—	
Hypertensive crisis	—	—	—	
Other	2 (0.5)	2 (1.0)	0 (0.0)	
SADR	1 (0.2)	1 (0.5)	0 (0.0)	

Values are n (%).
CsA = cyclosporine A; SADR = serious adverse drug reactions.

against bias that could originate from the lack of blinding of the investigators. Moreover, readers unaware of patients' characteristics or allocation to study treatment centrally analyzed ECGs and troponin by (PROBE design).

Because reperfusion injury starts in the very early phase of recanalization of an infarct-related artery, patients in a network of coronary care units of the Italian National Health Service were enrolled as soon as possible in this pragmatic trial, so that average time from symptom onset to first antegrade flow was only 3 h, compared with 4.5 h in the CIRCUS trial (16). This very short interval between self-reported onset of symptoms and reperfusion of the culprit artery was similar in both study groups, ensuring that the CsA injection time did not delay successful recanalization of the culprit artery.

To increase the chance of showing a beneficial effect of CsA, "small" infarcts, defined by the number of leads with ST-segment elevation on the presenting ECG tracing, were excluded. Slightly bigger, ECG-defined infarcts in the CsA group than in the control group could have instead maximized the beneficial effect. Additionally, the infarct-related artery had to be a large, occluded coronary artery (i.e., TIMI flow grade 0 to 1 before randomization), and the CsA bolus had to be given before reopening the culprit vessel (not even the passage of the guidewire was allowed before drug injection) in order to have the CsA distributed in the bloodstream at the time of recanalization.

A major determinant of myocardial salvage and of the long-term outcome after MI is the extent of reperfusion. In the CYCLE trial, the similarity between study groups in the rate of ST-segment resolution, TIMI flow grade 2 or 3, and myocardial blush post-pPCI strengthens the conclusions of CsA's lack of efficacy. Even when effects were analyzed by MI site (i.e., anterior or nonanterior), circulating hs-cTnT, ST-segment resolution, and echocardiography

consistently showed no changes attributable to CsA. The results of the anterior MI subgroup are consistent with those of the CIRCUS trial, while expanding the findings to patients with other infarct locations. Also consistent with these results, intravenous CsA conferred no advantages in terms of ST-segment resolution in the per-protocol analysis and in a further analysis that excluded 5 patients with protocol violations and 44 patients nonoptimally reperused.

In order to avoid possible confounding with different pharmaceutical preparations, the CYCLE study (unlike the CIRCUS trial) used Sandimmune (Novartis), as in the earlier pilot study that showed benefit from CsA (10). In the CIRCUS trial, a formulation without ricinoleic acid was used, with the aim of reducing the risk of acute anaphylactoid reactions.

As in previous studies on cardioprotection, we paid special attention to circulating levels of cardiac troponins measured by a contemporary high-sensitivity assay, rather than to CKs, assayed either locally or centrally: the results failed to show any potential beneficial effect of CsA (Central Illustration, panel B, Figure 2, Online Figures 2 and 3).

The present study is not the first to show the failure of agents targeted to reperfusion injury for cardioprotection (28). In other cases, some acute protective action was apparent, which did not appear to lead to substantial long-term benefits (29,30). Cardioprotective effects leading to long-term benefit on cardiac function have been shown only for ischemic conditioning (31-33) and for a beta-blocker, metoprolol (34,35). The differences between pre-clinical studies conducted by different groups in the field of cardioprotection have recently triggered the establishment of the National Heart, Lung, and Blood Institute-sponsored CAESAR consortium (Consortium for preclinical assessment of cardioprotective therapies) (36,37).

The question may be "Does reperfusion injury have an impact on cardiac function that can be influenced by specifically-targeted interventions?" (38-40). First, considering the overall results of the latest trials in which highly promising agents/maneuvers were tested, it appears that reperfusion either accounts for a much lower fraction of overall ischemia/reperfusion injury or may be extremely difficult to effectively target in real-world clinical practice. Atar et al. (28) reported no LV function improvement in terms of end-diastolic and end-systolic volume, evaluated by 2-dimensional echocardiography on days 3 to 5 and 30 days after hospital admission. These results match the 90-day lack of LVEF improvement reported in exenatide-treated

patients, although the drug increased myocardial salvage by 15% (29).

Finally, although of limited clinical relevance, the safety profile of intravenous CsA in acute MI, assessed in the CYCLE and CIRCUS trials, can exclude the risk of allergic reactions (24-26). The nonstatistically significant excess of mortality in CYCLE with CsA was not confirmed by the larger CIRCUS study (16).

STUDY LIMITATIONS. The main limitation of the present trial is the use of echocardiography instead of magnetic resonance, which is the preferred imaging technique for studies of cardioprotection, given the possibility of estimating a myocardial salvage index. However, the limited accessibility of this technique in the 31 participating Italian Centers of the National Health Service and its high cost hampered its use in a multicenter, investigator-driven clinical trial, such as the CYCLE trial. Therefore, echocardiography with a stringent protocol, central validation, and quality control was adopted.

The study was slightly underpowered because the calculated target of 444 patients was not reached and 410 patients were enrolled. However, it is unlikely that a positive finding was missed because of insufficient power, given the lack of evidence even for only a positive trend in any of the variables measured. The potential bias originating from the lack of blinding was mitigated by use of central randomization and the PROBE design.

CONCLUSIONS

The consistent results on ST-segment resolution, cardiac biomarkers, echocardiography, and clinical events strongly support our observation that CsA does not reduce ischemia/reperfusion injury when given intravenously just before pPCI in acute MI

treated within 6 h from the onset of symptoms. Further research is needed to gain additional insight into the nature of reperfusion injury as a potential therapeutic target.

ACKNOWLEDGMENTS The authors thank the participants in the study and the cardiologists, nurses, ethics committee members, hospital pharmacists, and administrative staff in hospitals who assisted in the study. The authors also thank Daniela Bellavista, Roche Diagnostics Italy, for providing local support. The authors greatly benefited from Maylene Wong's suggestions on the design of the echocardiographic protocol. The authors thank Judy Baggott for checking the English language of the whole manuscript.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Intravenous administration of cyclosporine A immediately before primary coronary angioplasty within 6 h from the onset of symptoms in patients with STEMI did not improve markers of myocardial injury, ventricular remodeling, or clinical outcomes, indicating that this strategy did not prevent or reduce reperfusion-related injury.

TRANSLATIONAL OUTLOOK: Additional research is needed to identify more effective adjunctive strategies to attenuate ischemia/reperfusion injury in patients with STEMI undergoing pPCI.

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KEY WORDS acute myocardial infarction, echocardiography, left ventricular function, troponins

APPENDIX For an expanded Methods section and list of contributors, as well as supplemental figures and tables, please see the online version of this article.