CLINICAL RESEARCH
Acute coronary syndromes

Nitric oxide for inhalation in ST-elevation myocardial infarction (NOMI): a multicentre, double-blind, randomized controlled trial

Stefan P. Janssens[1,2], Jan Bogaert[3], Jaroslaw Zalewski[4], Attila Toth[5], Tom Adriaenssens[1,2], Ann Belmans[2], Johan Bennett[1], Piet Claus[2], Walter Desmet[1,2], Christophe Dubois[1,2], Kaatje Goetschalckx[1], Peter Sinnaeve[1,2], Katleen Vandenberghe[2], Pieter Vermeersch[2], Arpad Lux[5], Zsolt Szelid[5], Monika Durak[6], Piotr Lech[6], Krzysztof Zmudka[6], Peter Pokreisz[2], Pascal Vranckx[7], Bela Merkely[5], Kenneth D. Bloch[8], and Frans Van de Werf[2], for the NOMI investigators[†]

[1]The Department of Cardiovascular Diseases, University Hospitals Leuven, KU Leuven, Herestraat 49, 3000 Leuven, Belgium; [2]The Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium; [3]Division of Radiology, University Hospitals Leuven and Department of Imaging and Pathology, KU Leuven, Leuven, Belgium; [4]Department of Coronary Heart Disease, Jagiellonian University, Medical College, John Paul II Hospital, Pradnicka 80, 31202 Krakow, Poland; [5]Heart and Vascular Center, Semmelweis University, Varosmajor u. 68, 1122 Budapest, Hungary; [6]Department of Interventional Cardiology, Jagiellonian University, Medical College, John Paul II Hospital, Pradnicka 80, 31202 Krakow, Poland; [7]Heart Center Hasselt, Jessa Hospital, Stadsomvaart 11, 3500 Hasselt, Belgium; and [8]Department of Anesthesia, Critical Care, and Pain Medicine, and Cardiovascular Research Center, Department of Medicine, Massachusetts General Hospital, Fruit street 55, Boston, MA 02114 and Harvard Medical School, Boston, MA, USA

Aims	Inhalation of nitric oxide (iNO) during myocardial ischaemia and after reperfusion confers cardioprotection in pre-clinical studies via enhanced cyclic guanosine monophosphate (cGMP) signalling. We tested whether iNO reduces reperfusion injury in patients with ST-elevation myocardial infarction (STEMI; NCT01398384).
Methods and results	We randomized in a double-blind, placebo-controlled study 250 STEMI patients to inhale oxygen with (iNO) or without (CON) 80 parts-per-million NO for 4 h following percutaneous revascularization. Primary efficacy endpoint was infarct size as a fraction of left ventricular (LV) size (IS/LV$_{mass}$), assessed by delayed enhancement contrast magnetic resonance imaging (MRI). Pre-specified subgroup analysis included thrombolysis-in-myocardial-infarction flow in the infarct-related artery, troponin T levels on admission, duration of symptoms, location of culprit lesion, and intra-arterial nitroglycerine (NTG) use. Secondary efficacy endpoints included IS relative to risk area (IS/AAR), myocardial salvage index, LV functional recovery, and clinical events at 4 and 12 months. In the overall population, IS/LV$_{mass}$ at 48–72 h was 18.0 ± 13.4% in iNO (n = 109) and 19.4 ± 15.4% in CON [n = 116, effect size -1.524%, 95% confidence interval (95% CI) -5.28, 2.24; P = 0.427]. Subgroup analysis indicated consistency across clinical confounders of IS but significant treatment interaction with NTG (P = 0.0093) resulting in smaller IS/LV$_{mass}$ after iNO in NTG-naïve patients (n = 140, P < 0.05). The secondary endpoint IS/AAR was 53 ± 26% with iNO vs. 60 ± 26% in CON (effect size -6.8%, 95% CI -14.8, 1.3, P = 0.09) corresponding to a myocardial salvage index of 47 ± 26% vs. 40 ± 26%, respectively, P = 0.09. Cine-MRI showed similar LV volumes at 48–72 h, with a tendency towards smaller increases in end-systolic and end-diastolic volumes at 4 months in iNO (P = 0.048 and P = 0.06, respectively, n = 197). Inhalation of nitric oxide was safe and significantly increased cGMP plasma levels during 4 h reperfusion. The Kaplan–Meier analysis for the composite of death, recurrent ischaemia, stroke, or rehospitalizations showed a tendency toward lower event rates with iNO at 4 months and 1 year (log-rank test P = 0.10 and P = 0.06, respectively).

* Corresponding author. Tel: +32-16-344 235, Fax: +32-16-344 240, Email: stefan.janssens@kuleuven.be

Conclusions	Inhalation of NO at 80 ppm for 4 h in STEMI was safe but did not reduce infarct size relative to absolute LV_{mass} at 48–72h. The observed functional recovery and clinical event rates at follow-up and possible interaction with nitroglycerine warrant further studies of iNO in STEMI.
Keywords	Myocardial infarction • Cardioprotection • Inhaled nitric oxide • Left ventricular remodelling • Cyclic guanosine monophosphate • Reperfusion injury

Introduction

The extent of myocardial injury is an independent predictor of functional recovery and survival after ST-elevation myocardial infarction (STEMI).[1] Ischaemia reperfusion (I/R) injury accounts for up to 50% of myocardial damage in STEMI and has been a target for various pharmacological and non-pharmacological, 'conditioning' strategies, although with inconsistent clinical translation.[2]

NO-donor compounds have been extensively evaluated in animal models of cardiac I/R injury, with variable effects on MI size and left ventricular (LV) function (for a review, see reference 3). The therapeutic use of NO-donor compounds after MI has been hindered by the fact that these drugs dilate systemic vessels and lower blood pressure. Inhaled NO, in contrast, does not induce prohibitive hypotension and decreases neointima formation in rat carotid arteries subjected to balloon injury,[4] increases coronary artery patency after thrombolysis by decreasing platelet activation,[5] and improves blood flow with decreased leucocyte adhesion in a feline model of intestinal I/R.[6] Importantly, in all three experimental studies, breathing 80 ppm NO had beneficial systemic effects, whereas breathing 20 ppm did not, suggesting a dose–effect relationship.

Preclinical studies by our laboratory have shown that inhalation of NO at 80 ppm, during I/R reduces infarct size (IS) and improves functional remodelling in mice and in a more representative porcine model.[7,8] Inhalation of NO has been successfully administered in patients with pulmonary hypertension because of its selective pulmonary vasodilator capacity[9] and more recently in patients with cardiogenic shock due to right ventricular MI.[10]

We therefore investigated whether in patients with timely reperfused STEMI and successful percutaneous coronary intervention (PCI), inhaled NO reduces IS, and improves LV function and structure at 4 months' follow-up.

Methods

Trial design

Nitric oxide for inhalation in ST-elevation myocardial infarction was a randomized, double-blind, placebo-controlled, parallel-group, multicentre, Phase 2 investigator-initiated study of NO for inhalation at 80 ppm vs. placebo in patients with STEMI. Patients were enrolled at four sites in three countries.

Selection of patients

Patients aged 18 years and older were eligible if they had ECG evidence of ST-segment elevation (sum > 0.6 mV in leads I, II, III, AVL, AVF, V1–6) with onset of infarction between 2 and 12 h, and if they had successful restoration of flow following PCI. Patients with heart failure (Killip Class 3) or requiring more than 2 L oxygen to maintain normal arterial saturation were excluded. Patients with prior MI, left bundle branch block, need for urgent coronary artery bypass grafting, or unable to tolerate magnetic resonance imaging (MRI) because of disallowed metallic implants or gadolinium contrast because of advanced renal failure were also excluded.

The Institutional Review Board of the University Hospitals Leuven, the local ethics committees, and the Federal Health Authorities in Belgium, Hungary, and Poland approved the protocol. All patients provided written informed consent. An independent data safety monitoring board (DSMB) assessed patients' safety.

Randomization and treatment masking

Randomization was done centrally at the Leuven Coordinating Center via a telephone interactive voice response system in a 1:1 ratio and blocked by site. All study drug cylinders maintained a blinded label and were stationed in the catheterization suite. Access to the randomization schedule was limited to 1 or 2 study personnel per site who were not involved in any other aspect of the study except for monitoring and reporting of safety parameters. Patients received a 4 h inhalation with 80 ppm NO or placebo (nitrogen gas) supplemented with oxygen through a snug-fitting facemask using a blinded version of the NO-A delivery system (version 1.2.1., Maquet, Ternat, Belgium), which allows continuous real time measurements of NO, O_2, and NO_2. Administration of study gas was started upon arrival in the catheterization laboratory with the intent to have study gas flow for 10 min while the interventional team was preparing the coronary intervention. All transmitted safety parameters were only accessible to the DSMB, but not recorded as adverse events to avoid unblinding.

Assessment of safety and efficacy

The primary efficacy objective was MI size as percentage of LV_{mass} at 48–72 h, measured using MRI. Secondary efficacy variables of infarct remodelling included IS as percentage of risk area, (or myocardial salvage index), microvascular obstruction, and myocardial haemorrhage at 48–72 h, and troponin T levels. Secondary efficacy endpoints of LV remodelling included global and regional LV function at 48–72 h and 4 months and changes in LV end-systolic and end-diastolic volumes and sphericity indices over time.

Safety assessments included serial measurements of methaemoglobin levels and NO_2 concentrations in inhaled gas. We simultaneously monitored plasma NOx levels, comprising nitrates, nitrites and S-nitroso compounds, and whole blood nitrite (NO_2^-) levels at baseline, and after 4 h inhalation using ozon-based chemiluminescence analysis (Sievers-NOA280, GE-Analytical Instruments, Boulder, CO, USA).[11] Because NO stimulates cyclic guanosine monophosphate (cGMP) synthesis, we measured plasma cGMP levels using a radio-immunoassay (Alfa-Esar, USA) at baseline and at 4, 24, and 48 h after iNO. We also measured plasma myeloperoxidase (MPO) levels, a marker of neutrophil activation using a Quantikine ELISA (#DMYE00, R&D, Abingdon, UK).

Exploratory safety endpoints included serious adverse events (SAEs) until hospital discharge, and non-SAEs during study drug administration. Exploratory clinical outcome at 4 and 12 months included the composite

of death, recurrent ischaemia requiring re-intervention, hospitalization for heart failure and stroke.

Cardiac magnetic resonance imaging acquisition and analysis

Cardiac MRI was performed at 48–72 h on a 1.5 T system to determine IS, presence of microvascular obstruction and intramyocardial haemorrhage, risk area and global LV function, as described.[12] In short, the myocardium at risk was defined by a T2-weighted short-tau inversion-recovery (STIR) fast spin-echo MRI in the cardiac short-axis and represents the sum of the area of myocardial oedema plus the infarct area. Global LV function was assessed using breath-hold steady-state free-precession (SSFP) cine MRI in the cardiac short-axis, vertical- and horizontal long-axis. The presence and extent of microvascular obstruction (MVO) was evaluated 2 to 5 min following contrast-injection of 0.15 mmol/kg of gadolinium diethylenetriamine pentaacetic acid and myocardial necrosis/fibrosis was measured 10 to 20 min later by inversion-recovery gradient-echo technique. To express LV remodelling, a sphericity index was measured by dividing the maximal longitudinal LV diameter (i.e. tip mitral valve to LV apex) by the maximal short-axis diameter, at end-diastole and end-systole, whereby values approaching 1 indicate increased sphericity.

Statistical analysis

Based on published data, we assumed that in the control group myocardial IS/LV$_{mass}$, would have a mean and standard deviation of $17.4 \pm 10\%$, with a similar variance in the treatment group. To have statistical power of 80% to detect a 25% reduction in relative IS using a t-test, with type 1 error of 0.05, 84 patients per group are required (PROC POWER in SAS). To allow for a 15% withdrawal rate and a bigger than expected rate of patients with missing MRI at 48–72 h, we randomized 250 patients, with the expectation of having at least 170 evaluable patients. Baseline characteristics and laboratory data are summarized as median (IQR) for continuous variables and number (%) for discrete variables.

The primary analysis set of interest was the full analysis set (FAS), which included all randomized patients who gave informed consent and were diagnosed with STEMI. In addition, analyses were performed on a per protocol set, excluding major protocol violators. Since performing a complete-cases (CC) analysis only might yield biased results,[13] multiple imputation of missing data was done for the analysis of the primary efficacy variable (in accordance with FDA guidelines to account for missing data), using the AregImpute function in R.

Continuous primary and secondary efficacy endpoints were analysed using analysis of variance (ANOVA) including factors for treatment and centre. In case of serious violations of the model assumptions, log-transformation was applied. If not appropriate, a Wilcoxon rank-sum test was used. Treatment effect of was estimated by the difference (or ratio, in case of log-transformation) between treatments and presented along with its associated 95% confidence interval (95% CI). For parameters with a baseline measurement, the baseline value was included as covariate in ANOVA.

Binary secondary endpoint data were assessed by means of a χ^2 test and treatment effect expressed as odds ratio with associated 95% CIs. Clinical endpoints were analysed in the FAS, whereby event rates are estimated using the Kaplan–Meier methodology and compared using the log-rank test. Comparisons between groups are made using the Pepe–Mori test.[14]

The following pre-specified subgroups and interactions were assessed: Thrombolysis-in-Myocardial-Infarction (TIMI) at baseline, culprit lesion, duration of symptoms, troponin T at baseline (positive vs. negative), and use of IC/IA nitroglycerine during procedure. All tests and CIs were two-sided and assessed at a significance level of 5%. The SAP was finalized prior to unblinding (see: https://lrd.kuleuven.be/onderzoekscentra/lcc/nomi/NOMISAP19May2014.pdf).

All analyses were done with SAS (version 9.2) and R2.25.2 for Windows. NOMI is registered with ClinicalTrials.gov (NCT01398384).

Results

Characteristics of the patients, procedural characteristics, and hospital course

We randomized 250 patients at four hospitals in three countries in NOMI, with 123 (49%) patients assigned to 4 h iNO and 127 (51%) assigned to CON (Figure 1). One iNO-treated patient was excluded because he was later diagnosed with Takotsubo syndrome. The FAS included 249 patients. Demographics are well balanced between groups (Table 1). Systolic blood pressure was lower in iNO at baseline and during study drug inhalation, but small absolute differences resolved after treatment cessation. Clinical confounders of IS were not different between groups (Table 1). More than two-thirds of patients were admitted within 6 h of symptom onset and 33% within the first 3 h. All patients received aspirin and clopidogrel or prasugrel. Concomitant use of nitro-vasodilators with potential interaction with iNO was similar for both groups and per local protocol (Table 1). Use of other pharmacological treatments in STEMI was similar between groups (Table 2).

Safety and efficacy

Methaemoglobin levels at 4 h were well below the 5% safety threshold ($1.06 \pm 0.41\%$ in iNO vs. 0.68 ± 0.31 in CON, $P < 0.0001$), and NO$_2$ levels never exceeded 3 ppm. Inhalation of NO did not reduce IS/LV$_{mass}$, the primary efficacy endpoint, ($18.0 \pm 13.5\%$ in iNO vs. $19.4 \pm 15.4\%$ in CON, estimated treatment effect (95% CI) -1.52 (-5.28; 2.24, $P = 0.427$; Figure 2A). In a complete-cases analysis ($n = 109$ iNO and $n = 116$ CON) without imputation for the missing primary measure, we observed an identical estimated treatment effect NO–CON (95% CI) of -1.48 (-5.25; 2.29, $P = 0.44$). The lack of IS reduction was consistent across pre-specified subgroups with potential impact on IS (Figure 3). We also did not observe differences between groups in total LV$_{mass}$ or myocardial area at risk (AAR). In patients randomized to iNO, we observed a trend ($P = 0.09$) towards lower IS/AAR ($n = 86$ for iNO and $n = 82$ in CON), which represented a secondary efficacy endpoint (Figure 2B). The resulting higher myocardial salvage index (MSI, Table 3) is consistent with more viable myocardial segments in iNO (18.4% of all segments with <50% infarct transmurality vs. 11.5% in CON, $P = 0.11$) and less segments with more than 75% transmurality (50.3% in iNO vs. 56.4% in CON, $P = 0.11$ using probit model with random intercept).

Cardiac necrosis markers [mean peak high sensitive troponin T concentrations 3.9 (1.5–9.4) ug/L in iNO vs. 3.8 (2.3–7.4) ug/L, in CON, $P = 0.96$] and incidence of microvascular obstruction at 48–72 h were similar between groups (Table 3). Plasma levels of NOx doubled after 4 h iNO but did not change in CON ($P < 0.0001$, Figure 4A) and plasma nitrite levels increased by 10% ($P < 0.08$, 4B). NO-based chemiluminescence confirmed comparable NO delivery between study sites (data not shown) and cGMP plasma levels were sustained during 4 h iNO. In contrast, CON patients manifest a swift decline in cGMP levels during early reperfusion ($P < 0.0001$ vs. iNO, Figure 4C) and lower levels at 48 h ($P = 0.0169$ vs. iNO).
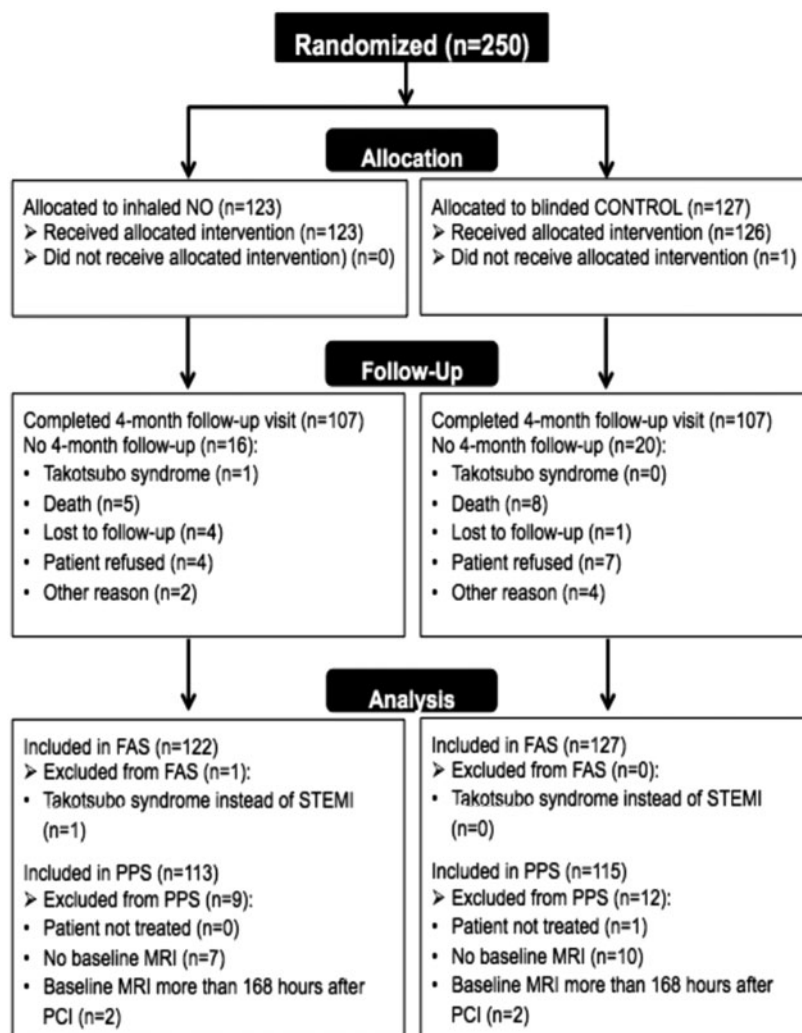
Figure 1 Randomization of patients and inclusion in the full analysis set and the per protocol set analysis. MRI, magnetic resonance imaging; NO, nitric oxide; PCI, percutaneous coronary intervention.

Left ventricular dimensions at 48–72 h were comparable between groups (*Table 3*) but LV sphericity indices measured in long- and short-axis at end-systole were higher in iNO ($P = 0.024$ and $P = 0.006$ vs. CON, respectively). At 4 months, LV dimensions were smaller in iNO ($P = 0.048$, *Table 3*).

Mechanism of action, subgroup analysis

The effect of iNO on the primary efficacy endpoint appears to be modulated by peri-procedural use of NTG (*P*-value for interaction 0.0093, *Figure 3*). We documented a statistically significant heterogeneity of the treatment effect of iNO in patients receiving supplemental NTG, administered via the radial artery (up to 500 µg NTG) or the infarct-related coronary artery (200 µg to 700 µg NTG). Nitroglycerine-naive patients ($n = 140$ or 56%) had a greater than 5% reduction of IS/LV$_{mass}$ after iNO ($16 \pm 14\%$ vs. $23 \pm 18\%$ in CON, $P = 0.03$). In contrast, patients who received NTG in combination with iNO did not benefit ($20 \pm 13\%$ vs. $15 \pm 9\%$ without iNO, $P = 0.06$). In the same exploratory analysis in NTG-naive patients, IS/

AAR was also significantly smaller after iNO ($49.8 \pm 28.1\%$, $n = 49$ vs. $62.0 \pm 28.7\%$ in CON, $n = 45$, $P = 0.027$), and was associated with a tendency toward less myocardial haemorrhage ($n = 6/60$ or 10.0% in iNO vs. $n = 14/63$ or 22.2% in CON, $P = 0.088$). Finally, in NTG-naive patients the increases in LV-ESVi and LV-EDVi between baseline and 4 months were markedly smaller in iNO than in CON ($P = 0.03$ and $P = 0.04$, respectively). The significant interaction was unexpected and prompted additional analyses. Patients who received the combination of NTG and iNO showed significantly greater myocardial risk areas (measured as oedema volume, Supplementary material online, *Figure S1*, $P = 0.02$ for interaction), and this was not attributable to differences in plasma myeloperoxidase levels, a surrogate for neutrophil activation in STEMI[15] (Supplementary material online, *Figure S2*).

Clinical outcome

There were three deaths in iNO and six in CON during hospital stay (all-cause mortality of 2.5 and 4.7%, respectively), but none were reported to be study treatment-related. After study drug

Table 1 Baseline characteristics of study patients

	iNO (n = 122)	CON (n = 127)
Demographics		
Age (mean, SD)	63 (13)	60 (11)
Female, n (%)	44 (36)	33 (26)
Medical History		
Hypertension, n (%)	75 (61)	72 (57)
Hyperlipidaemia, n (%)	55 (45)	60 (47)
Type 2 diabetes, n (%)	15 (12)	13 (10)
Current smoker, n (%)	57 (47)	56 (44)
Peripheral vascular disease, n (%)	7 (6)	5 (4)
Stroke, n (%)	3 (2)	5 (4)
COPD, n (%)	5 (4)	4 (3)
Clinical characteristics at randomization		
Weight (mean, SD) (kg)	79 (15)	80 (15)
Body-mass index (mean, SD) (kg/m^2)	27 (4)	28 (4)
Systolic Blood Pressure (mean, SD) (mmHg)	129 (24)	135 (21)
Mean arterial pressure (mean, SD) (mmHg)	95.7 (17.9)	99.6 (15.1)
Oxygen saturation (%)	98 (3)	98 (2)
Timings and procedural characteristics		
Time from symptom to Tx, (median, IQR) (h)	3.7 (2.7; 5.6)	3.5 (2.6; 6.0)
TIMI Grade 0–1 pre PCI, n (%)	83 (68)	89 (70)
PCI performed, n/total	120/122	125/127
TIMI Grade 0–1 post PCI, n (%)	1 (0.83)	2 (1.60)
TIMI Grade 2–3 post PCI, n (%)	119 (99.2)	123 (98.4)
Anterior location, n (%)	52 (43)	55 (43)
Positive troponin T at baseline, n (%)	85 (70)	79 (62)
IA/IC NTG use, n (%)	52 (43)	57 (45)
GP IIb/IIIa use, n (%)	48 (39)	54 (43)

Data are shown as numbers (percentage) unless otherwise indicated.
COPD, chronic obstructive pulmonary disease; GP, glycoprotein IA, intra-arterial; IC, intracoronary; NTG, nitroglycerine; SD, standard deviation; TIMI, Thrombolysis-in-Myocardial-Infarction.

Table 2 Pharmacological treatment of study patients

	iNO (n = 122)	CON (n = 127)	P-value
Concomitant medication during study drug administration			
Nitroglycerine IA/IC	52 (43)	57 (45)	0.90
GP IIb/IIIa antagonists	48 (39)	54 (43)	0.70
Unfractionated heparin	92 (75)	99 (78)	0.66
LMW heparin	8 (7)	10 (8)	0.81
Direct thrombin inhibitor	20 (16)	25 (20)	0.52
Anti-arrhythmic drugs	10 (8)	7 (6)	0.46
Diuretics	11 (9)	15 (12)	0.54
Vasopressor agents	7 (6)	5 (4)	0.56
Nitroprusside	2 (1.6)	3 (2)	1.0
Concomitant medication at 4 months	(n = 113)	(n = 114)	
Aspirin	108 (95)	111 (98)	0.28
Clopidogrel	71 (62)	70 (62)	1.0
Prasugrel	34 (30)	36 (32)	0.77
β-blocker	103 (90)	104 (92)	0.82
Angiotensin-converting enzyme inhibitor	92 (81)	96 (85)	0.48
Angiotensin receptor blocker	12 (11)	11 (10)	1.0
Aldosterone antagonists	14 (12)	11 (10)	0.67
Anti-arrhythmic drugs	6 (5)	3 (3)	0.50
Diuretics	12 (11)	18 (16)	0.25
Statins	110 (97)	109 (97)	1.0
Calcium channel blockers	13 (11)	6 (5)	0.15
Oral antidiabetic drugs	12 (11)	13 (12)	0.84
Insulin	4 (4)	10 (9)	0.11

Data are shown as numbers (percentage).

administration, one iNO and three CON patients suffered from re-infarction during index hospitalization. Three CON developed recurrent ischaemia and one CON suffered a thrombotic stroke. During out-of-hospital follow-up, two patients in each group died over the next 4 months, and two more died in CON at 1 year vs. one in iNO. The Kaplan–Meier analysis of adverse cardiac events showed lower rates for composite clinical endpoints up to 120 days in iNO than in CON, but the differences did not reach statistical significance (Figure 5, log-rank test: P = 0.10). Extended follow-up to 1 year confirmed these trends (Supplementary material online, Figure S3, log-rank test: P = 0.063).

Discussion

The findings of this randomized double-blind, controlled trial indicate that in timely reperfused STEMI, inhalation of 80 ppm NO for 4 h is safe but did not reduce IS relative to LV$_{mass}$, the primary efficacy endpoint. With respect to secondary efficacy endpoints, the absolute troponin levels, extent of microvascular obstruction and myocardial haemorrhage did not differ by randomized treatment. In contrast, inhaled NO showed a tendency to reduce IS when normalized to LV risk area and was associated with significantly smaller increases in LV dimensions at 4 months' follow-up. Finally, major adverse event rates during hospital stay were similar in both treatment groups and the Kaplan–Meier analysis for the composite of death, recurrent ischaemia, or rehospitalizations showed trends towards lower event rates in iNO at 4 and 12 months' follow-up (log-rank test P = 0.10 and P = 0.06, respectively).

In pre-specified subgroup analysis, we observed a marked heterogeneity in the response to inhalation therapy. While the action of iNO was not modified by determinants of IS, we measured a highly statistically significant interaction between iNO and peri-procedural use of the pharmacological NO-donor nitroglycerine (NTG). In NTG-naive patients, comprising 56% of the total population, iNO significantly reduced IS irrespective of the denominator (LV$_{mass}$ or LV risk area), and showed beneficial effects on LV remodelling at 4 months' follow-up. Plasma NOx and nitrate concentrations after 4 h
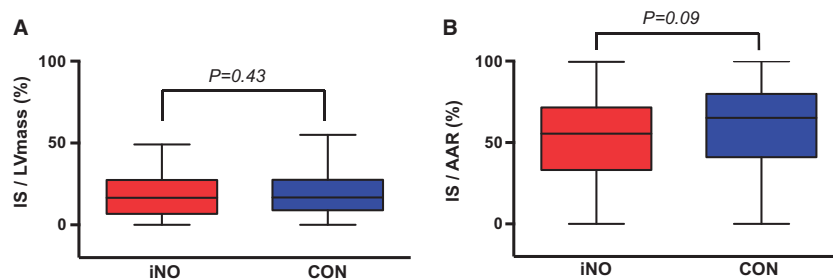
Figure 2 Infarct size as a fraction of left ventricular mass (*A*) and infarct size as a fraction of the myocardial area at risk (*B*).

Figure 3 Forest plot for the treatment effect of inhaled nitric oxide on infarct size as a fraction of left ventricular mass in pre-specified subgroups. IA/IC, intra-arterial/intracoronary; PCI, percutaneous coronary intervention.

iNO were almost two times as high as baseline levels and associated with significantly increased cGMP levels, an established marker of cardioprotection during ischaemic stress.[16,17] In contrast, peri-procedural use of NTG in CON failed to increase plasma cGMP levels, but as it was not randomized, no conclusions can be made regarding potential cardioprotection by NTG in CON. Our data are consistent with previous observations that iNO in addition to its well-known selective pulmonary vasodilator action[18] may affect remote vascular beds and ischaemic tissues,[19] in part via cGMP-mediated effects on cardiac myocytes and circulating cells.[5,8,20]

In this investigator-initiated trial, we translated for the first time a therapeutic NO-based inhalation strategy with established cardioprotection in preclinical models of myocardial reperfusion injury to STEMI patients.[7,8,21] So why did NOMI fail to translate the observed reduction in IS of iNO in experimental models to STEMI patients?

A first potential explanation could be an imbalance in clinical determinants of IS between treatment groups, insufficient IS, suboptimal metrics to test ancillary therapies, or recruitment of patients beyond the 'golden 3 h window of opportunity'[22] for myocardial salvage. However, in NOMI major clinical modifiers of IS and the extent of LV territory at risk and pre-existing collaterals were similar in iNO and CON patients (*Table 1* and *Figure 2B*) and IS was almost twice the 10% threshold of LV_{mass} necessary to explore novel ancillary therapies for STEMI.[1] Whether normalizing IS to LV risk area (*Table 3*) might represent a more sensitive metric of reperfusion injury, frequently referred to as myocardial salvage index in contemporary clinical trials, is unknown. We also evaluated our data separately in STEMI of less than 3 h duration, but numbers were low (33% of the population) and did not affect the primary efficacy endpoint.

Table 3 Cardiac magnetic resonance imaging at 4872 hours and at 4 months

	NO	CON	Effect size: difference (NO-CON) or ratio (NO/CON) (95% CI)	P-value
Primary endpoint				
Infarct size/LV$_{mass}$ (%)[a]	18 (13) (n = 109)	19 (15) (n = 116)	-1.52 (-5.28, 2.24)*	0.427
Secondary endpoint				
Infarct size/area at risk (%)	53 (26) (n = 86)	60 (26) (n = 82)	-6.80 (-14.80, 1.30)	0.098
Area at risk (g)	42 (39)	39 (37)	2.70 (-3.96, 9.35)	0.425
Myocardial salvage index (%)	47 (26) (n = 86)	40 (26) (n = 82)	6.80 (-1.30, 14.80)	0.098
Myocardial haemorrhage, n (%)	14 (13)	23 (21)	0.58 (0.28, 1.20)	0.141
Infarct transmurality (%)	86 (17)	90 (14)	-3.90 (-8.16, 0.38)	0.074
Microvascular obstruction, n (%)	60 (57)	71 (64)	0.7 (0.41; 1.24)	0.232
Microvascular obstruction (g)	4.4 (7.3)	5.9 (9.1)	-1.51 (-3.72; 0.69)	0.179
LV function and dimension at 48–72 h	(n = 115)	(n = 117)		
LV-ESVi (mL/m^2)	41 (14)	44 (18)	0.93 (0.85, 1.02)	0.105
LV-EDVi (mL/m^2)	79 (16)	82 (19)	0.97 (0.91, 1.02)	0.214
LVEF (%)	49 (11)	47 (10)	1.18 (-1.44, 3.81)	0.376
LV function and dimension at 4 months	(n = 98)	(n = 99)		
LV-ESVi (mL/m^2)	41 (16)	46 (21)	0.90 (0.81, 1.00)	0.048
LV-EDVi (mL/m^2)	84 (18)	90 (22)	0.94 (0.89, 1.00)	0.063
LVEF (%)	53 (10)	51 (10)	2.21 (-0.60, 5.03)	0.123

Data are mean ± standard deviation unless otherwise indicated.
EDVi, end-diastolic volume index; EF, ejection fraction; ESVi, end-systolic volume index; LVEF, left ventricular ejection fraction.
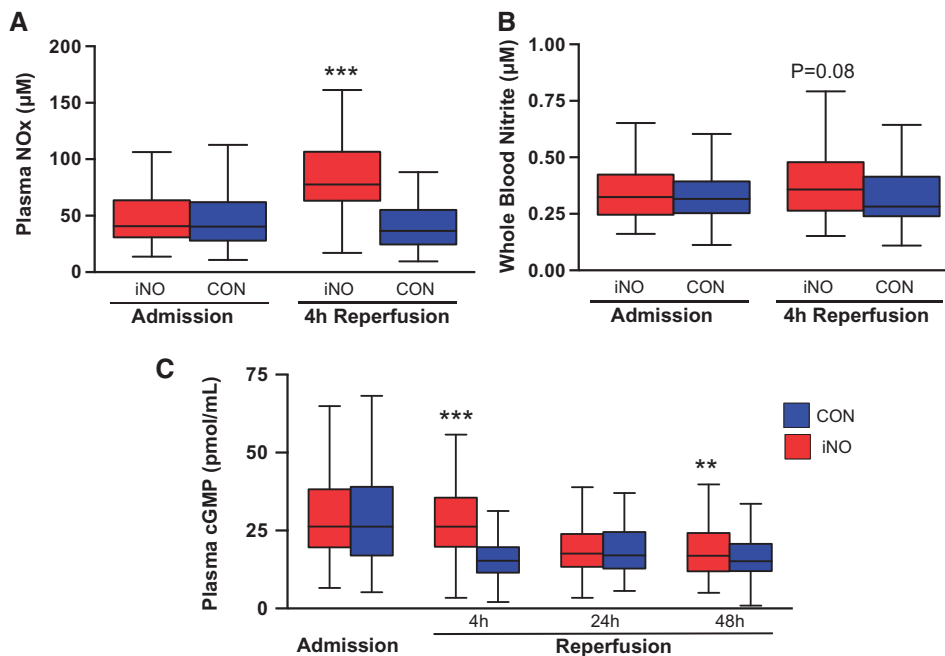[a]After multiple imputation.

Figure 4 Measurements of stable end-oxidative products of nitric oxide in plasma (A), whole blood nitrite (B) and the second messenger cyclic guanosine monophosphate (C). ***P < 0.001 vs. CON; **P < 0.01 vs. CON.
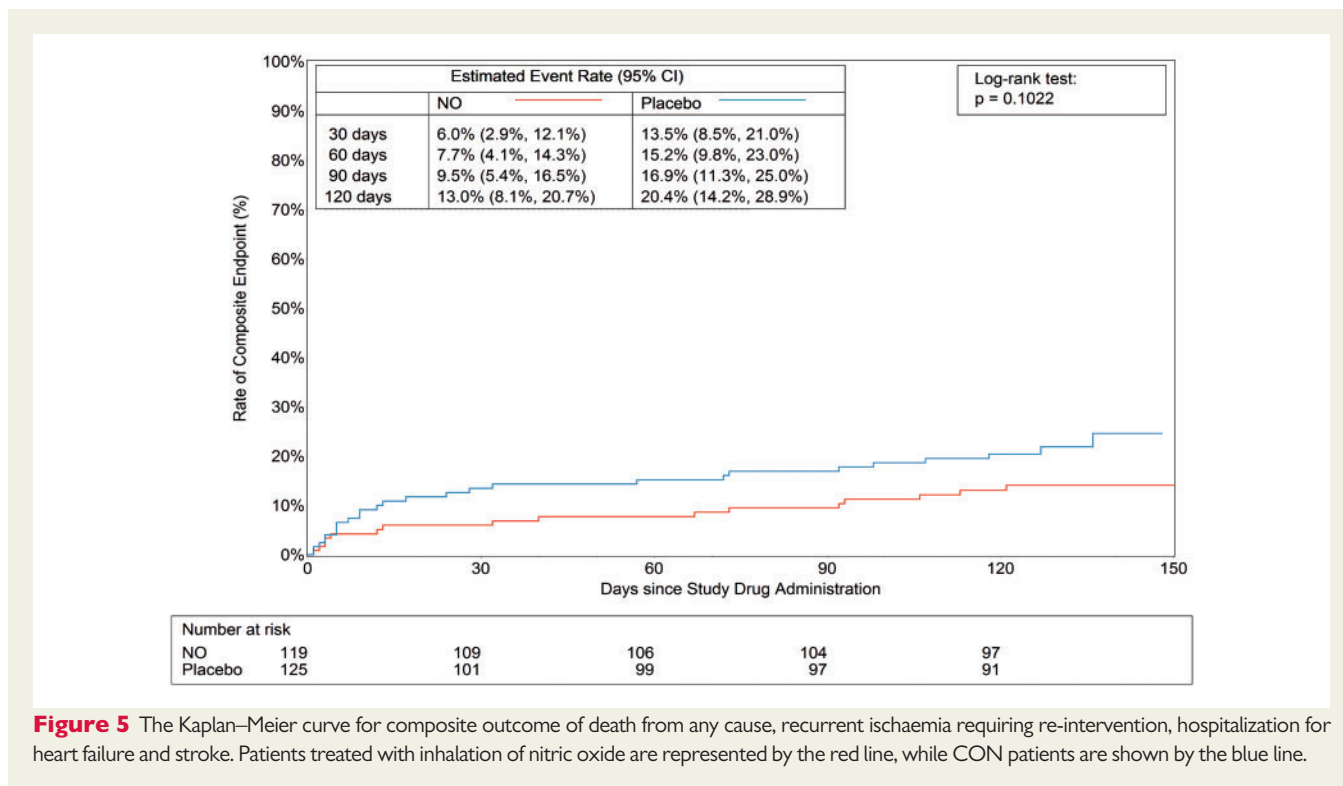
Figure 5 The Kaplan–Meier curve for composite outcome of death from any cause, recurrent ischaemia requiring re-intervention, hospitalization for heart failure and stroke. Patients treated with inhalation of nitric oxide are represented by the red line, while CON patients are shown by the blue line.

Second, the neutral results in the overall NOMI population may relate to pharmacodynamic limitations, insufficient duration of gas flow prior to reperfusion, and insufficient duration of cGMP rise to effectively impact mediators of myocardial injury. All of these may contribute to poor translation of experimental data in the clinic and similar pharmacodynamic limitations likely account for the neutral results following single bolus infusions of nitrite[23] or the mitochondrial pore stabilizing mitofusin compound.[24]

Third, the interaction between iNO and nitroglycerine was unexpected and needs to be interpreted in the context of a pre-specified subgroup analysis. In the absence of specific recommendations in the latest ESC guidelines on STEMI,[25] we left the use of intra-arterial NTG at the discretion of the investigator (see Supplementary material online for NOMI study Protocol). A relatively large number of patients were given high doses NTG (44%, most of whom received 500 µg NTG per protocol in one centre during radial access), and manifested a significantly greater risk area and absolute IS (Supplementary material online, *Figure S1*). Whether such combinatorial treatment increases cardiotoxic peroxynitrite intermediates, vascular permeability, and interstitial oedema in the infarct core with more extensive haemorrhage needs further study. Of note, a recent multicentre MRI study in 247 STEMI patients testing powerful vasodilator strategies for I/R injury showed greater IS and worse clinical outcome with adenosine and significantly more microvascular obstruction with intracoronary nitroprusside, a NO-releasing vasodilator.[26]

We recognize the limitations of NOMI. First, we studied only a single duration and dose of iNO and cannot exclude different results with extended inhalation or varying dose regimens. A recent double-blind, randomized intervention trial in patients undergoing extracorporeal cardiac bypass for multiple valve surgery reported significant kidney protection in patients receiving 24 h inhalation with 80 ppm NO.[27] Second, peri-procedural intra-arterial use of vasodilatory NO-donor compounds will need to be carefully controlled in future studies. Third, we cannot exclude that oxygen delivery may constitute an independent source of free radicals during reperfusion with potential impact on IS, as recently suggested.[28] Finally, our study was not powered for clinical endpoints of death, stroke, recurrent ischaemia, and revascularizations. Adequately powered clinical outcome studies are indispensable, as recently emphasized by the neutral results of the Phase 3 cyclosporine trial in anterior STEMI, in striking contrast with the 58 patient Phase 2 study.[29]

In conclusion, inhalation of 80 ppm NO for 4 h in timely reperfused STEMI was safe, effectively increased plasma cGMP levels but did not reduce IS relative to absolute LV_{mass} at 48–72 h. Promising signals on functional LV remodelling warrant additional pharmacodynamic studies and careful control for the unexpected inhibitory interaction with intra-arterial NO-donor compounds. Ultimately, the impact of inhaled NO on clinical outcome in STEMI must be assessed in adequately powered prospective follow-up studies.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

References

1. Gibbons RJ, Valeti US, Araoz PA, Jaffe AS. The quantification of infarct size. *J Am Coll Cardiol* 2004;**44**:1533–1542.
2. Ibanez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. *J Am Coll Cardiol* 2015;**65**:1454–1471.
3. Schulz R, Kelm M, Heusch G. Nitric oxide in myocardial ischemia/reperfusion injury. *Cardiovasc Res* 2004;**61**:402–413.
4. Lee JS, Adrie C, Jacob HJ, Roberts JD Jr, Zapol WM, Bloch KD. Chronic inhalation of nitric oxide inhibits neointimal formation after balloon-induced arterial injury. *Circ Res* 1996;**78**:337–342.
5. Adrie C, Bloch KD, Moreno PR, Hurford WE, Guerrero JL, Holt R, Zapol WM, Gold HK, Semigran MJ. Inhaled nitric oxide increases coronary artery patency after thrombolysis. *Circulation* 1996;**94**:1919–1926.
6. Fox-Robichaud A, Payne D, Hasan SU, Ostrovsky L, Fairhead T, Reinhardt P, Kubes P. Inhaled NO as a viable antiadhesive therapy for ischemia/reperfusion injury of distal microvascular beds. *J Clin Invest* 1998;**101**:2497–2505.
7. Hataishi R, Rodrigues AC, Neilan TG, Morgan JG, Buys E, Shiva S, Tambouret R, Jassal DS, Raher MJ, Furutani E, Ichinose F, Gladwin MT, Rosenzweig A, Zapol WM, Picard MH, Bloch KD, Scherrer-Crosbie M. Inhaled nitric oxide decreases infarction size and improves left ventricular function in a murine model of myocardial ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 2006;**291**:H379–H384.
8. Liu X, Huang Y, Pokreisz P, Vermeersch P, Marsboom G, Swinnen M, Verbeken E, Santos J, Pellens M, Gillijns H, Van de Werf F, Bloch KD, Janssens S. Nitric oxide inhalation improves microvascular flow and decreases infarction size after myocardial ischemia and reperfusion. *J Am Coll Cardiol* 2007;**50**:808–817.
9. Korkmaz S, Radovits T, Barnucz E, Hirschberg K, Neugebauer P, Loganathan S, Veres G, Páli S, Seidel B, Zöllner S, Karck M, Szabó G. Pharmacological activation of soluble guanylate cyclase protects the heart against ischemic injury. *Circulation* 2009;**120**:677–686.
10. Inglessis I, Shin JT, Lepore JJ, Palacios IF, Zapol WM, Bloch KD, Semigran MJ. Hemodynamic effects of inhaled nitric oxide in right ventricular myocardial infarction and cardiogenic shock. *J Am Coll Cardiol* 2004;**44**:793–798.
11. Yang BK, Vivas EX, Reiter CD, Gladwin MT. Methodologies for the sensitive and specific measurement of S-nitrosothiols, iron-nitrosyls, and nitrite in biological samples. *Free Radic Res* 2003;**37**:1–10.
12. Ganame J, Messalli G, Dymarkowski S, Rademakers FE, Desmet W, Van de Werf F, Bogaert J. Impact of myocardial haemorrhage on left ventricular function and remodelling in patients with reperfused acute myocardial infarction. *Eur Heart J* 2009;**30**:1440–1449.
13. Little RJ, D'Agostino R, Cohen ML, Dickersin K, Emerson SS, Farrar JT, Frangakis C, Hogan JW, Molenberghs G, Murphy SA, Neaton JD, Rotnitzky A, Scharfstein D, Shih WJ, Siegel JP, Stern H. The prevention and treatment of missing data in clinical trials. *N Engl J Med* 2012;**367**:1355–1360.
14. Pepe MS, Mori M. Kaplan-Meier, marginal or conditional probability curves in summarizing competing risks failure time data? *Stat Med* 1993;**12**:737–751.
15. Koch C, Henrich M, Heidt MC. Sequential analysis of myeloperoxidase for prediction of adverse events after suspected acute coronary ischemia. *Clin Cardiol* 2014;**37**:744–749.
16. Inserte J, Garcia-Dorado D. The cGMP/PKG pathway as a common mediator of cardioprotection: translatability and mechanism. *Br J Pharmacol* 2015;**172**:1996–2009.
17. Garcia-Dorado D, Agullo L, Sartorio CL, Ruiz-Meana M. Myocardial protection against reperfusion injury: the cGMP pathway. *Thromb Haemost* 2009;**101**:635–642.
18. Rimar S, Gillis CN. Selective pulmonary vasodilation by inhaled nitric oxide is due to hemoglobin inactivation. *Circulation* 1993;**88**:2884–2887.
19. Griffiths MJ, Evans TW. Inhaled nitric oxide therapy in adults. *N Engl J Med* 2005;**353**:2683–2695.
20. Sato Y, Walley KR, Klut ME, English D, D'Yachkova Y, Hogg JC, van Eeden SF. Nitric oxide reduces the sequestration of polymorphonuclear leukocytes in lung by changing deformability and CD18 expression. *Am J Respir Crit Care Med* 1999;**159**:1469–1476.
21. Nagasaka Y, Fernandez BO, Garcia-Saura MF, Petersen B, Ichinose F, Bloch KD, Feelisch M, Zapol WM. Brief periods of nitric oxide inhalation protect against myocardial ischemia-reperfusion injury. *Anesthesiology* 2008;**109**:675–682.
22. Gersh BJ, Stone GW, White HD, Holmes DR Jr. Pharmacological facilitation of primary percutaneous coronary intervention for acute myocardial infarction: is the slope of the curve the shape of the future? *JAMA* 2005;**293**:979–986.
23. Siddiqi N, Neil C, Bruce M, MacLennan G, Cotton S, Papadopoulou S, Feelisch M, Bunce N, Lim PO, Hildick-Smith D, Horowitz J, Madhani M, Boon N, Dawson D, Kaski JC, Frenneaux M, Siddiqi N, Neil C, Bruce M, MacLennan G, Cotton S, Dawson D, Frenneaux M, Singh S, Schwarz K, Jagpal B, Metcalfe M, Stewart A, Hannah A, Awsan N, Broadhurst P, Hogg D, Garg D, Slattery E, Davidson T, McDonald A, McPherson G, Kaski J-C, Lim PO, Brown S, Papadopoulou SA, Gonzalvez F, Roy D, Firoozi S, Bogle R, Roberts E, Rhodes J, Hildick-Smith D, de Belder A, Cooter N, Bennett L, Horowitz J, Rajendran S, Dautov R, Black M, Jansen E, Boon N, Struthers A, Toff W, Dargie H, Lang C, Nightingale P. Intravenous sodium nitrite in acute ST-elevation myocardial infarction: a randomized controlled trial (NIAMI). *Eur Heart J* 2014;**35**:1255–1262.
24. Atar D, Arheden H, Berdeaux A, Bonnet JL, Carlsson M, Clemmensen P, Cuvier V, Danchin N, Dubois-Rande JL, Engblom H, Erlinge D, Firat H, Halvorsen S, Hansen HS, Hauke W, Heiberg E, Koul S, Larsen AI, Le Corvoisier P, Nordrehaug JE, Paganelli F, Pruss RM, Rousseau H, Schaller S, Sonou G, Tuseth V, Veys J, Vicaut E, Jensen SE. Effect of intravenous TRO40303 as an adjunct to primary percutaneous coronary intervention for acute ST-elevation myocardial infarction: mITOCARE study results. *Eur Heart J* 2015;**36**:112–119.
25. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2018;**39**:119–177.
26. Nazir SA, McCann GP, Greenwood JP, Kunadian V, Khan JN, Mahmoud IZ, Blackman DJ, Been M, Abrams KR, Shipley L, Wilcox R, Adgey AA, Gershlick AH. Strategies to attenuate micro-vascular obstruction during P-PCI: the randomized reperfusion facilitated by local adjunctive therapy in ST-elevation myocardial infarction trial. *Eur Heart J* 2016;**37**:1910–1919.
27. Lei C, Berra L, Rezoagli E, Yu B, Strelow S, Nordia F, Bonventre J, Xiong L, Zapol WM. Prevention of acute kidney injury by nitric oxide during and after prolonged cardiopulmonary bypass. A double blind randomized controlled trial. *Circulation* 2015;**132**:2274–2275.
28. Stub D, Smith K, Bernard S, Nehme Z, Stephenson M, Bray JE, Cameron P, Barger B, Ellims AH, Taylor AJ, Meredith IT, Kaye DM; AVOID Investigators. Air versus oxygen in ST-segment-elevation myocardial infarction. *Circulation* 2015;**131**:2143–2150.
29. Cung TT, Morel O, Cayla G, Rioufol G, Garcia-Dorado D, Angoulvant D, Bonnefoy-Cudraz E, Guerin P, Elbaz M, Delarche N, Coste P, Vanzetto G, Metge M, Aupetit JF, Jouve B, Motreff P, Tron C, Labeque JN, Steg PG, Cottin Y, Range G, Clerc J, Claeys MJ, Coussement P, Prunier F, Moulin F, Roth O, Belle L, Dubois P, Barragan P, Gilard M, Piot C, Colin P, De Poli F, Morice MC, Ider O, Dubois-Rande JL, Unterseeh T, Le Breton H, Beard T, Blanchard D, Grollier G, Malquarti V, Staat P, Sudre A, Elmer E, Hansson MJ, Bergerot C, Boussaha I, Jossan C, Derumeaux G, Mewton N, Ovize M. Cyclosporine before PCI in patients with acute myocardial infarction. *N Engl J Med* 2015;**373**:1021–1031.