Immediate angioplasty versus standard therapy with rescue angioplasty after thrombolysis in the Combined Abciximab REteplase Stent Study in Acute Myocardial Infarction (CARESS-in-AMI): an open, prospective, randomised, multicentre trial

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Summary

Background **Thrombolysis remains the treatment of choice in ST-segment elevation myocardial infarction (STEMI) when primary percutaneous coronary intervention (PCI) cannot be done within 90 min. However, the best subsequent management of patients after thrombolytic therapy remains unclear. To assess the best management, we randomised patients with STEMI treated by thrombolysis and abciximab at a non-interventional hospital to immediate transfer for PCI, or to standard medical therapy with transfer for rescue angioplasty.**

Methods **600 patients aged 75 years or younger with one or more high-risk features (extensive ST-segment elevation, new-onset left bundle branch block, previous myocardial infarction, Killip class >2, or left ventricular ejection fraction ≤35%) in hospitals in France, Italy, and Poland were treated with half-dose reteplase, abciximab, heparin, and aspirin, and randomly assigned to immediate transfer to the nearest interventional centre for PCI, or to management in the local hospital with transfer only in case of persistent ST-segment elevation or clinical deterioration. The primary outcome was a composite of death, reinfarction, or refractory ischaemia at 30 days, and analysis was by intention to treat. This study is registered with ClinicalTrials.gov, number 00220571.**

Findings **Of the 299 patients assigned to immediate PCI, 289 (97·0%) underwent angiography, and 255 (85·6%) received PCI. Rescue PCI was done in 91 patients (30·3%) in the standard care/rescue PCI group. The primary outcome occurred in 13 patients (4·4%) in the immediate PCI group compared with 32 (10·7%) in the standard care/rescue PCI group (hazard ratio 0·40; 95% CI 0·21–0·76, log rank p=0·004). Major bleeding was seen in ten patients in the immediate group and seven in the standard care/rescue group (3·4% *vs* 2·3%, p=0·47). Strokes occurred in two patients in the immediate group and four in the standard care/rescue group (0·7% *vs* 1·3%, p=0·50).**

Interpretation **Immediate transfer for PCI improves outcome in high-risk patients with STEMI treated at a non-interventional centre with half-dose reteplase and abciximab.**

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Introduction

Primary percutaneous coronary intervention (PCI) is the preferred reperfusion strategy for patients with ST-segment elevation myocardial infarction (STEMI).[1,2] However, a review of consecutive admissions for STEMI in 365 US hospitals in 2005 found that most patients do not receive primary angioplasty within 90 min.[3] Even with an optimum network of community hospitals, tertiary referral centres with 24 h immediate PCI availability, and a technically advanced ambulance service using electrocardiogram (ECG) telediagnosis and helicopters, most patients from rural areas do not qualify for primary angioplasty.[4–6] The attempt to extend to these patients the benefit of mechanical revascularisation using initial thrombolysis followed by PCI has been hampered by a higher frequency of both bleeding and ischaemic events after the intervention.

One study showed a deleterious effect of early PCI after tenecteplase compared with primary angioplasty.[7] In most cases, patients are still managed conservatively at non-PCI centres, with initial thrombolytic therapy followed by transfer for PCI only if there is no evidence of reperfusion or the patient develops haemodynamic instability. We postulated that early pharmacological reperfusion at a non-PCI centre, addressing the need for a rapid and powerful platelet inhibition that overcomes the initial activation induced by thrombolytics, could be safely followed by immediate transfer for PCI. We expected this strategy to be better than the current standard management with selective late transfer for rescue PCI.

The Combined Abciximab Reteplase Stent Study in Acute Myocardial Infarction (CARESS-in-AMI) was a multicentre trial that randomly assigned high-risk patients with STEMI admitted to non-PCI hospitals to immediate

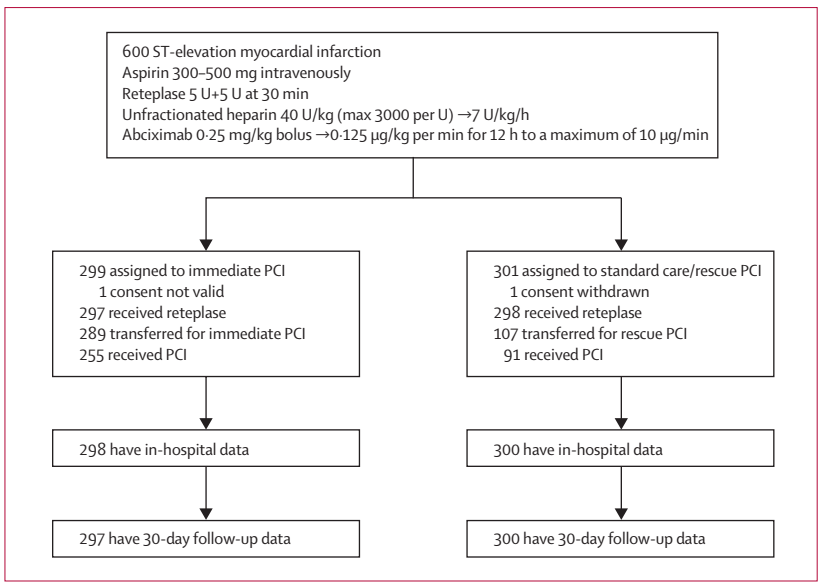

Figure 1: **Study flow chart**

transfer for PCI or to standard treatment with rescue PCI if needed.

Methods

Patients and procedures

The design features of CARESS-in-AMI have been published previously[8] and the amended protocol, modified in its sample size but with no change in endpoints or any other aspect, has been registered on the ClinicalTrials.gov website (number 00220571).

The study involved networks of non-PCI (so-called spoke) centres and specialist PCI (hub) centres in Poland (14 spoke and three hub sites), Italy (21 spokes and 12 hubs), and France (six spokes and five hubs) that worked together to manage patients in the trial. The median distance between hub and spoke was 31 km (IQR 18–70 km, range 8–110 km). The study protocol and informed consent was approved by the ethics committees of all the participating hospitals.

Patents with STEMI admitted to a centre without PCI facilities within 12 h from onset of symptoms were regarded as eligible if they had one or more of the following high risk features: cumulative ST-segment elevation of more than 15 mm, new onset left bundle branch block, previous myocardial infarction, Killip class of 2 or more, or left ventricular ejection fraction of 35% or less. The main exclusion criteria were previous coronary artery bypass grafting or PCI with graft or stented vessel, cardiogenic shock, need for concomitant major surgery, severe chronic renal or hepatic impairment, myocardial infarction within the previous 2 weeks, and contraindications to thrombolytic therapy, abciximab, aspirin, or clopidogrel.

Patients eligible for inclusion were only enrolled in spoke hospitals. After consent was obtained, they were immediately started on the pharmacological treatment common to the two groups. Patients were randomly assigned in an open manner to either a strategy of immediate transfer to the hub site for PCI (immediate PCI group) or to continued care at the spoke site with transfer only for clinical deterioration (eg, persistent ST-segment elevation at 90 min greater than 50% compared with the baseline ECG, ongoing chest pain, or haemodynamic instability, in the standard care/rescue PCI group). Randomisation was done by telephone call to an automated service.

All patients received half-dose reteplase (5 units bolus followed by another 5 units after 30 min), intravenous aspirin 300–500 mg, unfractionated heparin (40 units/kg up to a maximum of 3000 units followed by 7 units/kg per h), and abciximab (0·25 mg/kg bolus followed by 0·125 µg/kg per min over 12 h, figure 1). In the immediate PCI group, heparin was maintained during the transfer period, activated clotting time was adjusted to 200–250 s during PCI, and heparin was stopped after the procedure. In the standard care/rescue PCI group, heparin was continued for 24 h unless a rescue PCI was done, in which case the protocol was the same as for the immediate PCI group. Clopidogrel (300 mg bolus) was started on arrival in the angioplasty centre and recommended for 1–12 months after stent implantation (75 mg once a day). β blockers, angiotensin converting enzyme inhibitors, and statins were administered to all patients unless contraindicated. A copy of all angiograms, as well as of the admission and post-reperfusion ECGs, were shipped to an independent core laboratory for analysis.

The primary outcome was a composite of all cause mortality, reinfarction, and refractory myocardial ischaemia within 30 days of randomisation. Reinfarction was defined as recurrent symptoms or signs of myocardial ischaemia lasting more than 30 min with new Q-wave or ST-T segment changes, or new-onset left bundle branch block and recurrent significant rise of cardiac enzyme concentrations. The increase in creatine kinase isoenzyme MB (CK-MB) concentration was considered significant when it occurred after at least a 25% decrease in CK-MB from a previous peak and was more than two times the upper limit of normal in the absence of coronary interventions, more than three times above the upper limit of normal after PCI, or more than five times above the upper limit of normal after bypass grafting.

Refractory ischaemia was defined as recurrent chest pain with ST-segment deviation or definite T-wave inversion occurring more than 12 h after randomisation persisting for at least 10 min despite administration of nitrates, β blockers, or calcium channel blockers and not fulfilling the diagnosis of myocardial reinfarction. The main safety outcomes were the incidence of intracranial bleeding (haemorrhagic stroke) and extracranial major bleeding at 30 days, including retroperitoneal or intraocular bleeds, bleeds requiring blood transfusion, or with a haemoglobin decrease of 50 g/L or more. Bleeds

were also classified according to the Thrombolysis in Myocardial Infarction (TIMI) criteria[9] as major (intracranial, overt bleeding with a decrease of haemoglobin >5 g or haematocrit >15%) and minor (spontaneous gross haematuria or haematemesis with a decrease of haemoglobin >30 g/L but with <15% decrease of haematocrit).[9]

An independent critical events committee screened and adjudicated all serious adverse events based on the review of the original source documents. The monitoring process included periodic visits to all the recruiting centres, with verification of data reported in the case record forms and particular attention to outcome results.

Statistical analysis

The sample size of 600 patients was based on an anticipated reduction in event rate from 13·2% in the standard care/rescue PCI group to 6·4% in the immediate PCI group with an estimated power of 80% at a two sided alpha level of 0·05. The initial sample size of 1800 patients, offering a statistical power of 95%, was reduced because of slower than expected enrolment.

Categorical variables were expressed as frequency (percentage), whereas continuous variables were expressed as mean (SD), with the exception of time intervals expressed as median (IQR). Continuous variables were compared between randomised groups using the Wilcoxon's rank sums test, whereas for binary variables the Fisher exact test was used. Estimation of the cumulative primary event rate was done with the Kaplan-Meier method on an intention-to-treat basis, and events over time were compared using the log rank test. The Cox proportional hazards model was used to estimate the treatment effect as unadjusted hazard ratio (HR) with 95% CIs. A two-sided p value less than 0·05 was considered significant. An independent data and safety monitoring board regularly reviewed unblinded events for safety and efficacy.

Role of the funding source

The Italian Society of Interventional Cardiology, sponsor of the study, approved the design and organisation of the trial. The writing committee had full responsibility for data analysis and interpretation and for this report. The corresponding author had full access to data in the study and bears final responsibility for the mansucript. Eli Lilly Italia and Biotronik Germany had no role in the study design, collection, analysis, interpretation of data, writing of the report and the decision to submit the paper for publication.

	Immediate PCI N=298	Standard care/rescue N=300
Demographic		
Age (years)	60·2 (10·2)	59·6 (9·7)
Sex (male)	232 (77·9)	238 (79·3)
Clinical		
Height (cm)	170·3 (8·0)	169·7 (7·9)
Weight (kg)	78·5 (13·3)	78·2 (13·9)
Blood pressure (mm Hg)		
Systolic	137·1 (22·3)	137·7 (25·2)
Diastolic	83·4 (13·3)	85·2 (13·9)
Heart rate (bpm)	74·8 (16·2)	75·1 (16·8)
Risk factors/previous medical conditions		
Hypercholesterolaemia	63 (21%)	97 (32%)
Hypertension	116 (39%)	140 (47%)
Current/previous cigarette smoker	159 (53%)	182 (61%)
Previous stroke	3 (1%)	6 (2%)
Family history	100 (34%)	116 (39%)
Diabetes mellitus	44 (15%)	44 (15%)
Diabetes, insulin treated	15 (5%)	16 (5%)
Previous myocardial infarction	35 (12%)	29 (10%)
Previous coronary artery bypass graft	1 (0·3%)	0 (0%)
Previous percutaneous transluminal coronary angioplasty	7 (2%)	8 (3%)
Previous congestive heart failure	2 (0·7%)	3 (1%)
Previous medication		
Aspirin	51 (17%)	65 (22%)
Angiotensin converting enzyme inhibitors	74 (25%)	68 (23%)
Statins	29 (10%)	40 (13%)
β blockers	39 (13%)	53 (18%)
Infarct location on ECG		
Anterior infarct	149 (50%)	130 (43%)
Inferior infarct	132 (44%)	156 (52%)
Left bundle branch block	1 (0·3%)	1 (0·3%)
Killip classification		
Class I	165 (55%)	172 (57%)
Class II	126 (43%)	125 (42%)
Class III	6 (2%)	3 (1%)
Ejection fraction (%)	44·9 (9·3)	46·8 (9·9)
Arrhythmic complications on presentation		
Cardiac arrest	1 (0·3%)	1 (0·3%)
Complete atrioventricular block	6 (2%)	10 (3%)
Ventricular fibrillation	7 (2%)	10 (3%)
Times		
Symptom onset to first admission (min)	120 (72–205)	120 (74–191)
Symptom onset to randomisation (min)	153 (99–245)	151 (100–226)
Symptom onset to thrombolysis (mins)	165 (115–254)	161 (120–245)

Data are n (%), mean (SD), or median (IQR).

Table 1: **Baseline characteristics at randomisation**

Results

Between December, 2002, and February, 2007, 600 patients were randomly assigned to either immediate PCI (299 patients) or standard care/rescue PCI (301 patients, figure 1). Baseline clinical characteristics were well balanced between the two groups (table 1). Time from symptom onset to admission was 120 (IQR 75–196) min and time from admission to reteplase administration 42 (30–61) min. The distribution of time from symptom onset to administration of reteplase was well balanced between the two groups (figure 2).

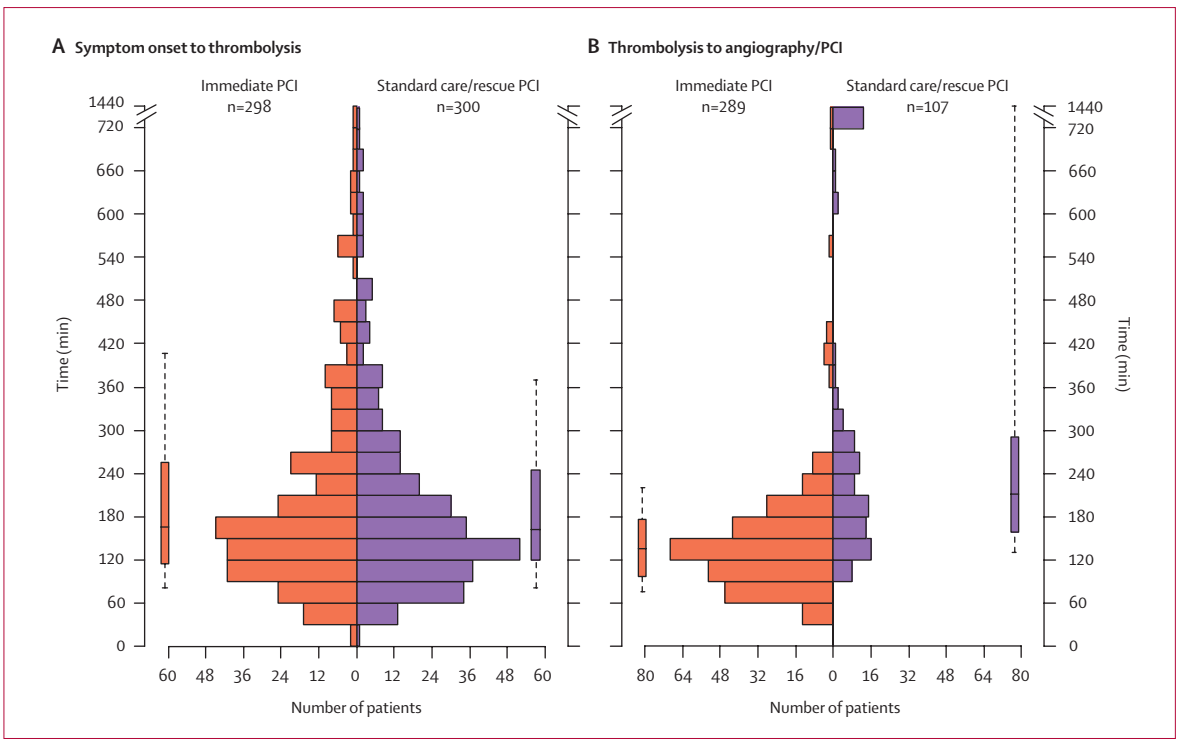

Figure 2: **Distribution of time (A) from symptom onset to thrombolysis and (B) from thrombolysis to PCI in the two groups**
Boxplots show median and IQR. Whiskers denote 10th and 90th percentiles.

In the immediate PCI group, 289 (97·0%) patients underwent angiography and 255 (85·6%) patients received PCI (table 2). The remaining patients had diameter stenosis of the infarct-related artery less than 50% (14·7%), had indications for surgical revascularisation and a patent culprit vessel (55·9%) or were technically unsuitable for PCI (29·4%). In the standard care/rescue PCI group, 107 patients (35·7%) were transferred for urgent clinically indicated angiography and 91 patients (30·3%) underwent rescue PCI. As expected, the time from reteplase to angiography or PCI was higher and the time distribution wider in the rescue group compared with the immediate PCI group (211 [IQR 157–290] *vs* 135 [96–175] min, p<0·0001, figure 2B).

In the immediate PCI group, TIMI 3 flow in the first angiogram assessed by the independent core laboratory was present in 145 patients (61·2%) and TIMI 2 flow was present in 54 (22·8%). The rate of TIMI 3 flow increased to 89·8% (212 patients) after the procedure with 16 patients (6·8%) having TIMI 2 flow. PCI resulted in a reduction of diameter stenosis from 73·3% (SD 14·3) to 17·6% (16·2). Stents were implanted in 245 patients, 96·1% of the patients undergoing PCI, 23·3% of which (57 patients) were drug-eluting.

Hospital stay was longer in the standard care/rescue PCI group compared with the immediate PCI group (9 [IQR 7–11] days *vs* 7 [6–9] days, p<0·0001). β blockers, statins, and angiotensin converting enzyme inhibitors were used in most patients, with treatment evenly balanced across both groups (table 3). Clopidogrel was prescribed at discharge in 249 (85·9%) in the immediate PCI group compared with 164 (57·1%) in the standard care/rescue PCI group (p<0·0001), indicating the higher rate of stent implantation in the immediate group. Aspirin was also less frequently prescribed at discharge (288 patients (99·3%) in the immediate PCI group versus 271 (94·4%) in the standard care/rescue PCI group, p=0·0006) because clopidogrel was used as monotherapy in patients with poor tolerance of aspirin in the standard care/rescue group.

	Immediate PCI N=298	Standard care/ rescue N=300	p*
Characteristic			
Received angiography	289 (97·0%)	107 (35·7%)	
Times			
Thrombolysis to admission to PCI centre (min)	110 (80–141)	180 (130–253)	<0·0001
Transfer to PCI centre (min)	55 (35–80)	60 (35–90)	0·48
Admission to PCI centre to angiography (min)	15 (10–30)	20 (10–40)	0·39
Duration of angiography/PCI (min)	50 (30–62)	44 (30–64)	0·32
Complications during transfer†	10 (3·5%)	7 (6·5%)	0·26
Cardiogenic shock	1 (0·4%)	3 (2·8%)	0·25
Major arrhythmias	6 (2·1%)	2 (1·9%)	0·33
Major bleeding	0 (0·0%)	1 (0·9%)	0·41
Other	3 (1·0%)	1 (0·9%)	0·60

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The primary endpoint rate at 30 days was significantly higher in the standard care/rescue PCI group compared with the immediate PCI group (32 patients, [10·7%] *vs* 13 patients, [4·4%], HR 0·40, 95% CI 0·21–0·76; log rank p=0·004, figure 3). Consistent trends in favour of the immediate PCI group were present for each of the components of the primary endpoint (table 3). Refractory ischaemia showed the largest difference, driving the outcome for the composite endpoint. Although almost all deaths occurred during the initial-hospital admission, the incidence of reinfarction and recurrent ischaemia showed a progressive increase throughout the first 3 weeks in the standard care/rescue PCI group. The rate of subsequent revascularisation was significantly higher in the standard care/rescue group, with 92 patients (30·7%) receiving PCI after day 1 and up to day 30 from enrolment versus 19 patients (6·4%) in the immediate group. Heterogeneity of treatment effects was only observed for age groups (interaction p=0·044), with a greater benefit from immediate PCI seen in younger patients (figure 4).

Stroke occurred in two patients (0·7%) in the immediate group and four patients (1·3%) in the standard care/rescue group (p=0·50). The rate of cerebral haemorrhage was 0·7% (two patients) in the immediate group and 1·0% (three patients) in the standard care/rescue group (table 3). The rate of major bleeding according to the protocol definitions, which included all blood transfusions, was 3·4% (ten patients) in the immediate PCI and 2·3% (seven patients) in the standard care/rescue group, a 47·8% proportional increase which was not significant because of the low absolute incidence of events (p=0·47). TIMI major or minor bleeding rates were also greater in the immediate PCI group but no significant differences were observed. Patients undergoing immediate PCI had a higher rate of minor bleeding (32 [10·8%] *vs* 12 [4·0%] in the standard care/rescue PCI group, p=0·002) and TIMI minimal bleeding (23 patients [7·7%] *vs* 7 [2·3%], p=0·002), with the difference mainly driven by the higher rate of puncture site bleeding in the immediate PCI group.

Discussion

Our study shows that in patients 75 years or younger with large STEMI admitted to centres without PCI facilities, a strategy of immediate transfer for PCI after a combination of half-dose reteplase plus abciximab is better than continuing standard management at the same centre. The driving component of the composite endpoint was refractory ischaemia, since death and reinfarction were lower but not significantly different in the immediate PCI group. The late rise in reinfarction and refractory ischaemia beyond the 9 days of median hospital admission in the standard care/rescue PCI group suggests that a more aggressive policy of pre-discharge angiography and PCI might have avoided some of these adverse events.

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Angiographic characteristics			
Number of vessel disease			
1-vessel disease	130 (45·0%)	53 (49·5%)	0·43
2-vessel disease	92 (31·8%)	30 (28·0%)	0·54
3-vessel disease	61 (21·1%)	17 (15·9%)	0·32
No lesions with ≥50% diameter stenosis	6 (2·1%)	7 (6·6%)	0·05
Access site			
Femoral	277 (95·8%)	100 (93·5%)	0·30
Radial	12 (4·2%)	6 (5·6%)	0·59
Brachial	0 (0·0%)	1 (0·9%)	0·27
Culprit lesion			
Left main artery	3 (1·0%)	3 (2·8%)	0·35
Left anterior descending artery	143 (49·5%)	54 (50·5%)	0·91
Left circumflex artery	34 (11·8%)	16 (15·0%)	0·40
Right coronary artery	105 (36·3%)	31 (29·0%)	0·19
Graft	1 (0·3%)	0 (0·0%)	1
Not identified	3 (1·0%)	3 (2·8%)	0·35
TIMI flow (adjudicated by investigators)			
0	33 (11·4%)	25 (23·4%)	0·004
1	19 (6·6%)	11 (10·3%)	0·28
2	51 (17·6%)	14 (13·1%)	0·36
3	186 (64·4%)	57 (53·3%)	0·06
TIMI flow (adjudicated by QCA core laboratory)	N=237	N=77	
0	29 (12·2%)	24 (31·2%)	0·0003
1	9 (3·8%)	4 (5·2%)	0·53
2	54 (22·8%)	13 (16·9%)	0·34
3	145 (61·2%)	36 (46·8%)	0·03
Pre-PCI vessel diameter stenosis (%)	73·3 (14·3)	80·4 (17·1)	0·001
PCI done	255 (88·2%)	91 (85·0%)	0·40
Thrombectomy, filters/distal protection	9 (3·5%)	6 (6·6%)	0·23
Balloon angioplasty only	10 (3·9%)	6 (6·6%)	0·38
Bare metal stents	188 (73·7%)	78 (85·7%)	0·02
Drug eluting stents	57 (22·4%)	7 (7·7%)	0·002
Intraortic balloon pump	5 (2·0%)	9 (9·9%)	0·003
Post-PCI			
TIMI flow post-PCI (adjudicated by investigators)			
0	5 (2·0%)	1 (1·1%)	1
1	2 (0·8%)	1 (1·1%)	1
2	11 (4·3%)	2 (2·2%)	0·53
3	237 (92·9%)	87 (95·6%)	0·46
TIMI flow post-PCI (adjudicated by QCA core laboratory)	N=236	N=75	
0	6 (2·5)	1 (1·3%)	1
1	2 (0·9)	2 (2·7%)	0·25
2	16 (6·8%)	6 (8·0%)	0·8
3	212 (89·8%)	66 (88·0%)	0·67
Post-PCI vessel diameter stenosis (%)	17·6 (16·2)	19·5 (14·7)	0·34

Data are n (%), mean (SD), or median (IQR) unless otherwise stated. *Continuous variables were compared between randomised groups using the Wilcoxon's rank sums test. Binary variables were compared using Fisher's exact test. †Number (% of total complications).

Table 2: **Angiographic and procedural characteristics**

Still, revascularisation of all patients before discharge in the absence of demonstrable ischaemia is not recommended by the present guidelines[1,2] and, for totally

occluded vessels, is in conflict with the outcome of the recent large randomised Occluded Artery Trial.[10]

CARESS-in-AMI was specifically designed to address optimum treatment in patients for whom primary PCI is not readily available. Unlike ASSENT-4 PCI[7] and the recently concluded FINESSE trial,[11] CARESS-in-AMI was not a trial of facilitated angioplasty opposed to primary angioplasty. It was a comparison between the general application of a combined pharmaco-invasive approach and the standard thrombolysis plus selective rescue PCI approach in patients who do not qualify for primary angioplasty. The trial does not question the general consensus concerning the superiority of timely primary PCI over thrombolysis,[12] and does not address the controversial issue of pre-treatment with thrombolytics or IIb-IIIa inhibitors in patients otherwise eligible for primary angioplasty.[13,14]

CARESS-in-AMI defined the best treatment strategy for STEMI patients admitted to hospitals without PCI facilities or collected by mobile units far from PCI facilities. Even the best possible coordination between ambulance service, community hospitals, and PCI centres cannot make primary PCI available to all patients with STEMI because of the relative lack of primary PCI facilities in non-densely populated areas with long travel times to PCI centres.[15]

The most recent guidelines on STEMI treatment confirm that thrombolytics should be used for patients admitted within 3 h from symptom onset unless primary angioplasty can be done within 90 min.[16] The PRAGUE-2 and DANAMI trials[17,18] have challenged this indication and suggested that transfer for primary angioplasty is preferable to local thrombolysis. These trials, however, included patients admitted to both PCI and non-PCI centres, discouraged cross-over to rescue angioplasty, and were done in small countries with established networks and short distances between community hospitals and PCI centres. Both in PRAGUE 2 and CAPTIM,[17,19] patients randomised early after symptom onset showed a trend towards mortality benefit from immediate thrombolysis.

The REACT trial has convincingly shown that emergency rescue PCI is warranted in case of failed thrombolysis,[20] leading to a class IA indication for rescue PCI.[16] The control group in our study was similar to the active treatment group in REACT, with a more liberal use of rescue angioplasty and a shorter transfer time. Still, this aggressive strategy of early transfer for rescue PCI proved inferior to routine immediate transfer of all patients for PCI. Other trials (GRACIA-1, SIAM III, and CAPITAL-AMI)[21–23] advocated a strategy of routine transfer within 24 h of admission of all STEMI patients treated with full-dose thrombolysis. Their conclusions were, however, hampered by the sparing use of rescue angioplasty and the inclusion of late (6–12 months) ischaemic endpoints such as target lesion revascularisation and unstable angina.

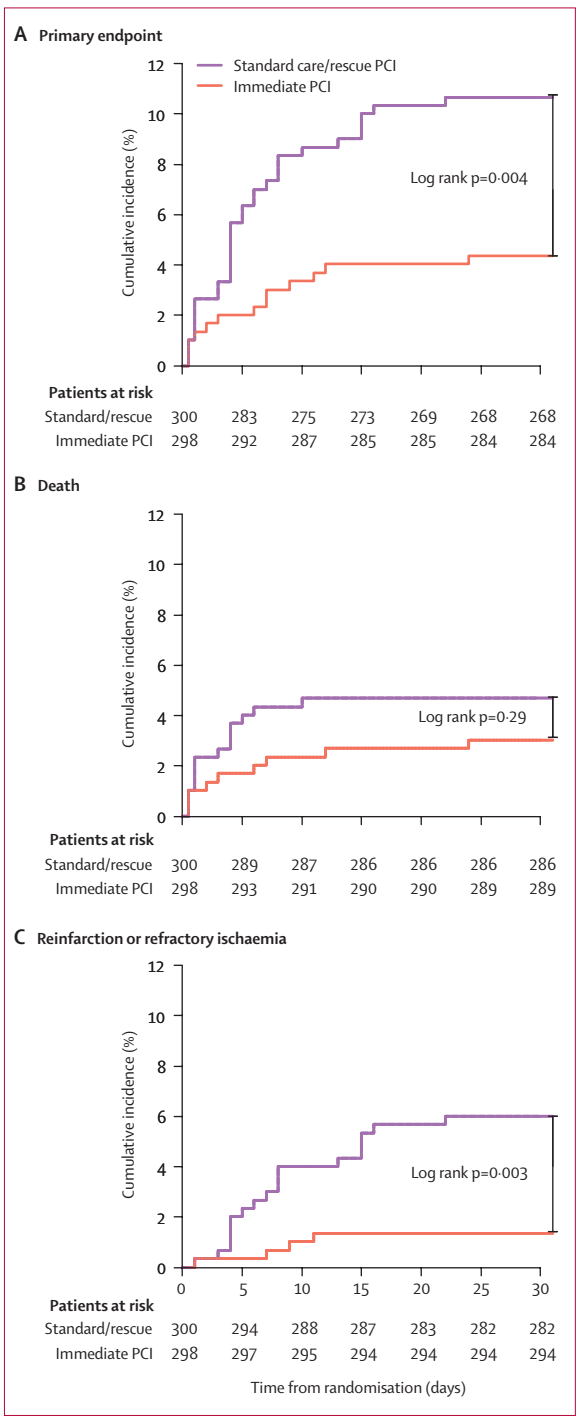

Figure 3: **Kaplan Meier event curves (A) for the primary outcome (with 95% CI), (B) for death, and (C) for reinfarction, refractory ischaemia, or both**

The delay between thrombolytic administration and PCI (mean 19·6 h in the largest of these trials),[21] denies to the routine PCI strategy any real possibility of myocardial salvage in case of failed thrombolysis. The results of the CARESS-in-AMI trial confirm and expand the practice of routine transfer in patients after

thrombolysis for STEMI, suggesting that immediate transfer and angioplasty is safe in terms of bleeding risk and procedure outcome, and avoids the additional delay of a conventional rescue strategy.

The thrombolytic regimen (half-dose reteplase, abciximab, and low-dose heparin) used in the present study is not recommended by current STEMI guidelines,[1,2,6] since the large GUSTO V study did not show a mortality benefit of this regimen compared with standard thrombolytic therapy (full-dose reteplase and standard-dose heparin), and showed a slight excess in bleeding with a significantly higher rate of intracranial haemorrhage in elderly patients.[24,25] Consequently, in the trial reported here, only patients younger than 75 years and with intermediate high risk of events were recruited.

The extremely slow enrolment in CARESS-in-AMI indicates the stringent inclusion and exclusion criteria, as well as the expansion of primary angioplasty in the participating countries, with some spoke centres starting a programme of primary angioplasty and others bypassed by a different organisation of the ambulance service taking patients directly to the hub centres.

Systematic reporting of the screening process was done in 18 spoke centres, with a total of 884 patients screened and 207 randomised (23·4%). The two main reasons for exclusion from enrolment were an age of 75 years or older (29·8%) and an absence of ST-elevation of more than 15 mm and other criteria of high risk (52·6%). We acknowledge, therefore, that the conclusions of this study are applicable only to a high-risk subgroup of STEMI patients with no access to primary angioplasty facilities.

The abciximab-reteplase combination selected for treatment of these patients in the present study has two characteristics that render it attractive for use in high-risk STEMI patients undergoing long-distance transfer for early PCI.[26,27] First, the combination of half-dose reteplase and abciximab is highly effective in providing early patency in the infarct-related artery, as confirmed by the present study, in which 199 (84·0%) of 237 patients were found to have TIMI 2 or 3 flow, adjudicated by the QCA core laboratory, at the time of initial angiography. Second, the profound platelet inhibition achieved by using abciximab has the potential to counteract platelet activation secondary to the release of fibrinogen degradation products after thrombolytic therapy which, in the ASSENT-4 PCI study,[7] has been deemed responsible for the excess in ischaemic events following PCI early after tenecteplase. Using different pharmacological regimens in the two groups negates the possibility to test the difference between the two strategies (immediate transfer *vs* watchful waiting). Conversely, using thrombolytics alone in both groups would have resulted in duplication in the interventional arm of the poor outcome observed in the ASSENT-4 PCI trial.[7]

As expected, there was a higher rate of bleeding in patients in the immediate PCI group and the main driver of this was puncture site bleeding. Radial approach was used in less than 5% of patients, precluding a meaningful subgroup analysis. Results of previous non-randomised trials, however, suggest that avoiding femoral puncture leads to a major reduction of bleeding events in primary angioplasty and can attenuate the excess bleeding in the immediate PCI group in these patients heavily loaded with thrombolytic, antiplatelet, and antithrombotic drugs.[28,29]

The 47·8% proportional increase in major bleeds and the more than two-fold increase in minor bleeds did not translate into a prolonged hospital stay or increased mortality, which were both lower in the immediate PCI group than in the standard care/rescue PCI group. However, since bleeding has been shown to predict late mortality,[27] we must wait for the 1-year results before discarding the increased incidence of minor bleeding events as a benign unavoidable consequence of immediate PCI. The rate of cerebral haemorrhage (0·8%) was low and well within the expected range,[24,25] confirming the safety of combination treatment with reduced dose reteplase and abciximab in patients well-screened for contraindications to thrombolytics and younger than 75 years.

CARESS-in-AMI was designed at a time when the clinical benefit of clopidogrel[30,31] and enoxaparin[32] were not

	Immediate PCI	Standard care/rescue	p*
Length of stay in hospital (days)	7 (6–9)	9 (7–11)	<0·0001
Discharge medication			
Aspirin	288 (99·3%)	271 (94·4%)	0·0006
Angiotensin converting enzyme inhibitors	260 (89·7%)	247 (86·1%)	0·22
Statins	263 (90·7%)	266 (92·7%)	0·36
β blockers	249 (85·9%)	246 (85·7%)	0·95
Clopidogrel	249 (85·9%)	164 (57·1%)	<0·0001
1-to-30-day revascularisation			
PCI	19 (6·4%)	92 (30·7%)	<0·0001
Coronary artery bypass graft	13 (4·4%)	8 (2·7%)	0·28
30-day bleeding events			
Major	10 (3·4)	7 (2·3%)	0·47
Minor	32 (10·8)	12 (4·0%)	0·002
TIMI bleeding classification			
Major	8 (2·7)	7 (2·3%)	0·80
Minor	10 (3·4)	4 (1·3%)	0·11
Minimal	23 (7·7)	7 (2·3%)	0·002
30-day cerebrovascular events	2 (0·7)	4 (1·3%)	0·50
Ischaemic	0 (0·0)	1 (0·3%)	1
Haemorrhagic	2 (0·7)	3 (1·0%)	1
30-day endpoints	N=297	N=300	
Primary endpoint	13 (4·4%)	32 (10·7%)	0·005
Death	9 (3·0%)	14 (4·7%)	0·40
Reinfarction	4 (1·3%)	6 (2·0%)	0·75
Refractory ischaemia	1 (0·3%)†	12 (4·0%)	0·003

Data are n (%) or median (IQR). *Continuous variables were compared between randomised groups using the Wilcoxon's rank sums test. Binary variables were compared with Fisher's exact test. †One patient had refractory ischaemia followed by reinfarction.

Table 3: **Times, drugs at discharge, and 30-day events**

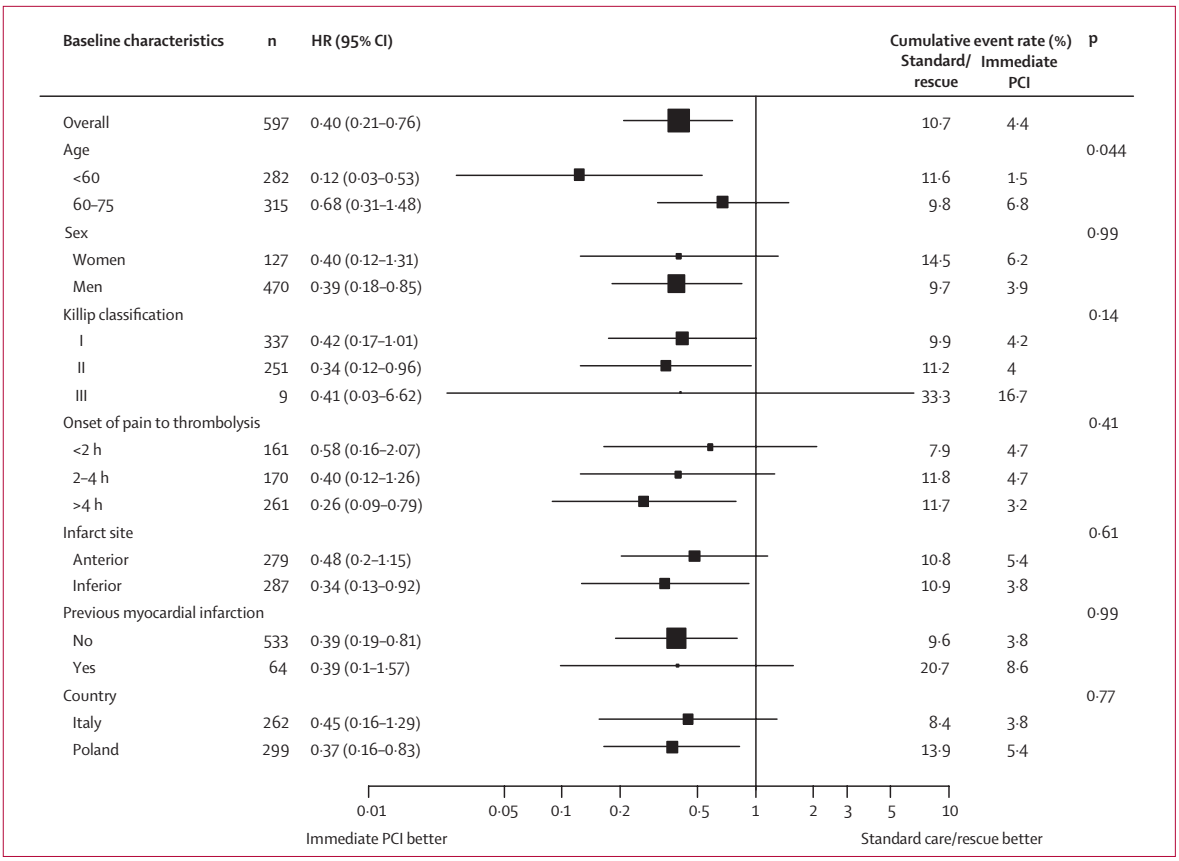

Figure 4: **Cumulative primary event rate in subgroups**
Chart shows the hazard ratios (squares, size proportional to sample size) and 95% CIs on a logarithmic scale. Interaction p values also shown. PCI=percutaneous coronary intervention.

shown in acute myocardial infarction. Only one of six patients enrolled in CARESS-in-AMI received upfront clopidogrel. The remaining patients followed the original protocol, as described in the Methods section, and received clopidogrel in the catheterisation suite, more frequently in the immediate PCI group (85·9% *vs* 57·1%, p<0·0001).

The benefit conferred by clopidogrel in terms of relative risk reduction of the combined 30-day endpoint of death, reinfarction, and recurrent ischaemia in the CLARITY TIMI 28 study[31] was 17·7%, smaller than the 58·9% seen in our study. In our view, the reason for the greater efficacy of the CARESS strategy is the immediate maximal platelet inhibition achieved with abciximab, which cannot be matched by any loading dose of clopidogrel, as shown in other acute coronary syndromes (non-STEMI and unstable angina) in the ISAR REACT 2 trial.[33] In EXTRACT TIMI 25,[30] the relative risk reduction of death, non-fatal myocardial infarction and urgent revascularisation at 30 days with the use of enoxaparin versus unfractionated heparin was 19·3%, again smaller than the benefit conferred by immediate PCI in our study. In fact, the immediate PCI group had a negligible incidence of reinfarction and refractory ischaemia, the only endpoints significantly improved, but not eliminated, by the use of enoxaparin.

In conclusion, the CARESS-in-AMI trial shows that after treatment with a combination of half-dose reteplase plus abciximab, urgent transfer for immediate PCI is a better strategy than standard therapy with clinically indicated rescue PCI. Our study provides evidence suggesting that all high risk STEMI patients receiving thrombolysis should be routinely and immediately transferred for PCI. These data further support the need for established networks of PCI and non-PCI centres to allow rapid transfer of appropriate STEMI patients for urgent PCI.

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Conflict of interest statement

CDM, DD, SS, and FP had minor financial revenues from consultancies, speaker's bureau honoraria, and received travel grants from Eli Lilly Italia SpA, Eli Lilly UK, Eli Lilly Critical Care Europe and Biotronik GmbH Germany. MF has received research grants from Eli Lilly Critical Care Europe for other studies, and travel grants to attend scientific meetings. All other authors declare that they have no conflict of interest.

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References

1 Antman EM, Anbe DT, Armstrong PW, et al. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction. *Circulation* 2004; **110:** e82–293.

2 van der Werf F, Ardissino D, Betriu A, et al. Management of acute myocardial infarction in patients presenting with acute ST elevation. The task force on the management of acute myocardial infarction of the European Society of Cardiology. *Eur Heart J* 2003; **24:** 28–66.

3 Bradley EH, Herrin J, Wang Y, et al. Strategies for reducing the door-to-balloon time in acute myocardial infarction. *N Engl J Med* 2006; **355:** 2308–20.

4 Henry TD, Sharkey SW, Burke MN, et al. A regional system to provide timely access to percutaneous coronary intervention for ST-elevation myocardial infarction. *Circulation* 2007; **116:** 721–28.

5 Ting HH, Rihal CS, Gersh BJ, et al. Regional systems of care to optimize timeliness of reperfusion therapy for ST-elevation myocardial infarction: the Mayo Clinic STEMI protocol. *Circulation* 2007; **116:** 729–36.

6 Bassand JP, Danchin N, Filippatos G, et al. Implementation of reperfusion therapy in acute myocardial infarction. A policy statement from the European Society of Cardiology. *Eur Heart J* 2005; **26:** 2733–41.

7 van der Werf F, Ross A, Armstrong P, Granger C. Primary versus tenecteplase-facilitated percutaneous coronary intervention in patients with ST-segment elevation acute myocardial infarction (ASSENT-4 PCI): randomised trial. *Lancet* 2006; **367:** 569–78.

8 Di Mario C, Bolognese L, Maillard L, et al. Combined abciximab reteplase stent study in acute myocardial infarction (CARESS in AMI). *Am Heart J* 2004; **148:** 378–85.

9 Rao AK, Pratt C, Berke A, et al. Thrombolysis in myocardial infarction (TIMI) trial—phase I: hemorrhagic manifestations and changes in plasma fibrinogen and the fibrinolytic system in patients treated with recombinant tissue activator and streptokinase. *J Am Coll Cardiol* 1988; **11:** 1–11.

10 Hochman JS, Lamas GA, Buller CE, et al. Coronary intervention for persistent occlusion after myocardial infarction. *N Engl J Med* 2006; **355:** 2395–407.

11 Ellis S, for the FINESSE Investigators. The FINESSE trial. http://spo.escardio.org/CongressPresentation/ESC2007/20070903/1760/ellis_1760_esc2007_slides.pdf (accessed Jan 9, 2008).

12 Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. *Lancet* 2003; **361:** 13–20.

13 Stone GW, Gersh BJ. Facilitated angioplasty: paradise lost. *Lancet* 2006; **367:** 543–46.

14 Keeley EC, Boura JA, Grines CL. Comparison of primary and facilitated percutaneous coronary interventions for ST-elevation myocardial infarction: quantitative review of randomised trials. *Lancet* 2006; **367:** 579–88.

15 Nallamothu BK, Bradley EH, Krumholz HM. Time to treatment in primary percutaneous coronary intervention. *N Engl J Med* 2007; **357:** 1631–38.

16 Silber S, Albertsson P, Aviles FF, et al. Guidelines for percutaneous coronary interventions. The Task Force for Percutaneous Coronary Interventions of the European Society of Cardiology. *Eur Heart J* 2005; **26:** 804–47.

17 Widimsky P, Budesinsky T, Vorac D, et al. Long distance transport for primary angioplasty vs immediate thrombolysis in acute myocardial infarction: Final results of the randomized national multicentre trial—PRAGUE-2. *Eur Heart J* 2003; **24:** 94–104.

18 Andersen HR, Nielsen TT, Rasmussen K, et al. A comparison of coronary angioplasty with fibrinolytic therapy in acute myocardial infarction. *N Engl J Med* 2003; **349:** 733–42.

19 Steg PG, Bonnefoy E, Chabaud S, et al. Impact of time to treatment on mortality after prehospital fibrinolysis or primary angioplasty: data from the CAPTIM randomized clinical trial. *Circulation* 2003; **108:** 2851–56.

20 Gershlick AH, Stephens-Lloyd A, Hughes S, et al. Rescue angioplasty after failed thrombolytic therapy for acute myocardial infarction. *N Engl J Med* 2005; **353:** 2758–68.

21 Fernandez-Aviles F, Alonso JJ, Castro-Beiras A, et al. Routine invasive strategy within 24 hours of thrombolysis versus ischaemia-guided conservative approach for acute myocardial infarction with ST-segment elevation (GRACIA-1): a randomised controlled trial. *Lancet* 2004; **364:** 1045–53.

22 Le May MR, Wells GA, Labinaz M, et al. Combined angioplasty and pharmacological intervention versus thrombolysis alone in acute myocardial infarction (CAPITAL AMI study). *J Am Coll Cardiol* 2005; **46:** 417–24.

23 Scheller B, Hennen B, Hammer B, et al. Beneficial effects of immediate stenting after thrombolysis in acute myocardial infarction. *J Am Coll Cardiol* 2003; **42:** 634–41.

24 Topol EJ. Reperfusion therapy for acute myocardial infarction with fibrinolytic therapy or combination reduced fibrinolytic therapy and platelet glycoprotein IIb/IIIa inhibition: the GUSTO V randomised trial. *Lancet* 2001; **357:** 1905–14.

25 Savonitto S, Armstrong PW, Lincoff AM, et al. Risk of intracranial haemorrhage with combined fibrinolytic and glycoprotein IIb/IIIa inhibitor therapy in acute myocardial infarction. Dichotomous response as a function of age in the GUSTO V trial. *Eur Heart J* 2003; **24:** 1807–14.

26 Antman EM, Gibson CM, de Lemos JA, et al. Combination reperfusion therapy with abciximab and reduced dose reteplase: results from TIMI 14. The Thrombolysis in Myocardial Infarction (TIMI) 14 Investigators. *Eur Heart J* 2000; **21:** 1944–53.

27 The SPEED Investigators. Trial of abciximab with and without low-dose reteplase for acute myocardial infarction. Strategies for Patency Enhancement in the Emergency Department (SPEED) Group. *Circulation* 2000; **101:** 2788–94.

28 Philippe F, Larrazet F, Meziane T, Dibie A. Comparison of transradial *vs* transfemoral approach in the treatment of acute myocardial infarction with primary angioplasty and abciximab. *Catheter Cardiovasc Interv* 2004; **61:** 67–73.

29 Valsecchi O, Musumeci G, Vassileva A, et al. Safety, feasibility and efficacy of transradial primary angioplasty in patients with acute myocardial infarction. *Ital Heart J* 2003; **4:** 329–34.

30 Chen ZM, Jiang LX, Chen YP, et al. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: placebo-controlled trial. *Lancet* 2005; **366:** 1607–21.

31 Sabatine MS, Cannon CP, Gibson CM, et al. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med* 2005; **352:** 1179–89.

32 Antman EM, Morrow DA, McCabe CH, et al. Enoxaparin versus unfractionated heparin with fibrinolysis for ST-elevation myocardial infarction. *N Engl J Med* 2006; **354:** 1477–88.

33 Kastrati A, Mehilli J, Neumann FJ, et al. Abciximab in patients with acute coronary syndromes undergoing percutaneous coronary intervention after clopidogrel pretreatment: the ISAR-REACT 2 randomized trial. *JAMA* 2006; **295:** 1531–38.