Early haemoglobin-independent increase of plasma erythropoietin levels in patients with acute myocardial infarction

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Received 21 July 2006; revised 26 February 2007; accepted 8 March 2007; online publish-ahead-of-print 5 April 2007

See page 1785 for the editorial comment on this article (doi:10.1093/eurheartj/ehm260)

Introduction

Erythropoietin (EPO) is the most important mediator of the renal response to hypoxia. According to the accepted paradigm, it is responsible for the proliferation and differentiation of erythroid progenitors in bone marrow, which is followed by an increase in circulating red cell mass and the recovery of adequate tissue oxygen supply.

This paradigm has been recently integrated by more complex actions that expand the biological role of EPO to tissues beyond the haematopoietic system.1 Together with vascular endothelial growth factor (VEGF) and other proteins involved in the adaptive response to hypoxia, EPO expression is up-regulated by an increase in intracellular transcriptional hypoxia-inducible factor-1 (HIF-1) levels2 and peaks very rapidly in vivo.3 This happens not only in renal peritubular fibroblasts, but also in neurons, hepatocytes, and cardiomyocytes. Furthermore, the expression of EPO receptors is not restricted to the haematopoietic lineage, but is also shared with other cells including neurons, astrocytes and microglia, endothelial cells and cardiomyocytes, all of which are candidate hormone targets.1,4–6

Two of the non-erythropoietic actions of EPO are involved in its expanded range of activities: its anti-apoptotic effect and its ability to mobilize endothelial progenitor cells (EPCs). Consistent experimental findings have documented the protective effects of EPO against hypoxic-ischaemic cell injury in vitro and in vivo in brain,7–9 limbs, and the myocardium,10–12 which seems to be due to the prevention of apoptosis. Regarding its role as cell mobilizer, it increases the number of circulating EPCs in animal models13 and in both healthy subjects and patients with renal failure.14 EPO can therefore be considered as one of the potential

KEYWORDS
Acute myocardial infarction; Erythropoietin; Vascular endothelial growth factor; Endothelial progenitors

Aims We studied plasma erythropoietin (EPO) levels and their relation with CD34+VEGFR-2+ (mature and progenitor endothelial cells) and CD34+CD133+VEGFR-2+, or CD34+CD117+VEGFR-2+ (early/imma-
ture endothelial progenitors) cells in patients with acute myocardial infarction (AMI).

Methods and results Fifty AMI patients undergoing percutaneous coronary intervention (PCI) within 6 h of symptom onset were enrolled. EPO, measured by ELISA, and cell subsets, by cytofluorimetric analysis, were evaluated before PCI, 24 h and 7 days afterwards. Forty-five healthy subjects (CTRLs) were studied. Plasma EPO levels were higher in AMI patients at admission, 24 h, and 7 days (P = 0.04, P = 0.0001, P = 0.001, respectively) than in CTRLs. No correlation was evidenced between EPO and haemoglobin (Hb) or haematocrit at admission or 24 h after AMI. Differently, both Hb and haematocrit inversely correlated with EPO at day 7 (P = 0.0016, P = 0.029, respectively). Plasma EPO levels correlated with CD34+CD133+VEGFR-2+ cells at day 7 (P = 0.03).

Conclusion AMI patients have increased plasma EPO levels until day 7. In the early phase, plasma EPO levels are Hb-independent; at day 7, an Hb-modulated increase of EPO correlates with the percentage of CD34+CD133+VEGFR-2+ cells.
actors in the defensive-reparative response of damaged tissues to ischaemic injury. A few papers have described increased plasma endogenous EPO levels in patients with acute myocardial infarction (AMI)\textsuperscript{15,16} or acute coronary syndromes,\textsuperscript{13} but no published study has yet thoroughly explored the behaviour of endogenous EPO and its relation with EPCs in the clinical setting of AMI.

This study describes the short-term pattern of plasma endogenous EPO levels in patients with AMI and compares the results with those observed in healthy subjects (CTRLs). It also evaluates the relation between EPO and cir-

The results of a previous paper\textsuperscript{15} which suggested that 35 patients and 35 controls could allow for >90% power to detect a 50% increase in EPO levels in patients with AMI after admission, compared with CTRLs. An overall population of 50 patients and 45 CTRLs resulted from the addition of subjects further enrolled to evaluate the CD34\textsuperscript{+}CD133\textsuperscript{+}VEGFR-2\textsuperscript{+} and mature endothelial cells (CD34\textsuperscript{+}VEGFR-2\textsuperscript{+}) and both more mature EPCs and mature endothelial cells (CD34\textsuperscript{+}VEGFR-2\textsuperscript{+})\textsuperscript{17–19}. We also assessed the plasma levels of granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), and VEGF which have recently been described to be potentially involved in EPC mobilization in patients with AMI\textsuperscript{20–22}

### Methods

#### Study population

We studied 50 patients out of a continuous series of 252 patients with ST-segment elevation AMI undergoing primary percutaneous coronary intervention (PCI) within 6 h of symptom onset (Table 1). AMI was defined as the presence of >30 min chest pain associated with persistent ST-segment elevation of >1 mm in two or more peripheral electrocardiographic leads or of >2 mm in two or more precordial leads. The exclusion criteria were: an age of >75 years; Killip classes 3–4; a history of MI, PCI, or coronary artery bypass graft; significant trauma, percutaneous or surgical interventions in the 6 months preceding the study; cancer; active or chronic inflammatory diseases; coagulative-haematological disorders; significant chronic obstructive pulmonary disease; renal or hepatic failure. Oxygen saturation was assessed both at admission and at 24 h by blood gas analysis. Venous blood samples were obtained upon admission (before PCI) and after 24 h and 7 days, in order to measure plasma EPO and VEGF levels and to perform flow cytometric analysis. The levels of total creatine-kinase (CK) and the CK-MB fraction were measured upon admission, every 6 h during the first day and then daily until normalization. Similar blood samples were obtained, during the same period of patients enrol-

| Table 1 Clinical, angiographic, and procedural findings |
|---|---|---|
| | CTRLs (n = 45) | AMI (n = 50) |
| Age (years) (mean ± SD) | 55 ± 4 | 56 ± 9 |
| Males (n) (%) | 35 (78) | 42 (84) |
| BMI (mean ± SD) | 26 ± 3 | 27 ± 3 |
| Cardiovascular risk factors | | |
| Family history (n) (%) | 16 (36) | 20 (40) |
| Hypertension (n) (%) | 12 (27) | 20 (40) |
| Smoking (n) (%) | 22 (49) | 33 (66) |
| Hypercholesterolaemia (n) (%) | 9 (20) | 18 (36) |
| Diabetes (n) (%) | 3 (7) | 4 (8) |
| Statins (n) (%) | 0 | 0 |
| ACE-inhibitors (n) (%) | 3 (7) | 7 (14) |
| Pre-infarction angina (< 48 h (n) (%) | 16 (32) |
| CAD | | |
| 1 vessel (n) (%) | – | 31 (62) |
| 2-3 vessels (n) (%) | – | 19 (38) |
| Anterior infarction (n) (%) | – | 26 (52) |
| Pre-PCI TIMI flow | | |
| 0 (n) (%) | – | 38 (76) |
| 1-2 (n) (%) | – | 12 (24) |
| Post-PCI TIMI flow | | |
| 0-2 (n) (%) | – | 11 (22) |
| 3 (n) (%) | – | 39 (78) |
| Time to balloon (min) (mean ± SD) | – | 228 ± 119 |
| LVEF (%) (mean ± SD) | – | 46 ± 7 |
| LVWMSI (mean ± SD) | – | 1.6 ± 0.4 |

CTRLs, healthy controls; AMI, acute myocardial infarction; BMI, body mass index; ACE, angiotensin-converting enzyme; CAD, coronary artery disease; PCI, percutaneous coronary intervention; TIMI, thrombolysis in myocardial infarction; LVEF, left ventricular ejection fraction; LVWMSI, left ventricular wall motion score index; –, not applicable.

### Sample size

A formal sample-size estimate was not performed, due to the exploratory nature of the study. However, the size of the study groups was determined \textit{a priori}, taking into account the preliminary results of a previous paper\textsuperscript{15} which suggested that 35 patients and 35 controls could allow for >90% power to detect a 50% increase in EPO levels in patients with AMI after admission, compared with CTRLs. An overall population of 50 patients and 45 CTRLs resulted from the addition of subjects further enrolled to evaluate the CD34\textsuperscript{+}CD133\textsuperscript{+}VEGFR-2\textsuperscript{+} circulating mononuclear cell subset.

### Coronary angiography, primary percutaneous coronary intervention, and medical treatment

All of the patients underwent coronary angiography and primary PCI according to standard techniques. The procedural pharmacological protocol included intravenous aspirin, unfractionated heparin, and abciximab. Oral aspirin plus ticlopidine were administered for 1 month, followed by aspirin indefinitely. Low-molecular-weight heparin was administered subcutaneously during the 4 days following the procedure. Angiotensin-converting enzyme (ACE)-inhibitors were continued if already being taken (n = 7); otherwise, ACE-inhibitors were administered the day after the onset of AMI (n = 38) or delayed until after the end of the study (n = 5). Nitroglycerin, beta-blockers, and calcium antagonists were administered as indicated by the referring physician.

### Quantification of myocardial damage

The amount of myocardial necrosis in the AMI patients was evaluated on the basis of peak CK (CKp) and peak CK-MB (CK-MBp) levels and the area under the curve (AUC) of CK and CK-MB release. The echocardiographic left ventricular ejection fraction (LVEF) and left ventricular wall motion score index (LVWMSI, 16-segment model) were calculated within 24 h of admission in order to quantify the extent of myocardial damage during the acute phase.
High-sensitivity C-reactive protein concentrations

The assay for the high sensitive (hs)-C-reactive protein was conducted according to manufacturer’s instructions (Dada Behring, Dudingingen, Switzerland) on plasma samples obtained at admission, 24 h, and 7 days after AMI.

Cytokine plasma levels

Platelet-poor plasma samples of ethylenediaminetetraacetic acid (EDTA)-containing peripheral blood (PB) were stored at −70°C. Plasma EPO, G-CSF, GM-CSF and VEGF, levels were determined using commercially available enzyme-linked immunosorbent assays (R&D System and VEGF-duoset, R&D System, Minneapolis, MN, USA) according to manufacturer’s instructions; the lower detection limits were 2.5 mIU/mL, 39 pg/mL, 7.8 pg/mL, and 15.6 pg/mL, respectively.

Cytofluorimetric analysis

Fifty microlitres of fresh PB collected in EDTA-containing tubes were incubated for 30 min at 4°C with 20 μL fluorescein-conjugated (FITC)-anti-CD34 and biotin-conjugated-anti-VEGFR-2 (Sigma Chemical, St Louis, MO, USA) peroxidin-chlorophyll-protein (PerCP)-streptavidin (Becton Dickinson, Pharmingen, San José, CA, USA). Appropriate isotype controls were used. In 13 out of 50 patients, and in 13 out of 45 healthy subjects, PB cells were incubated with the monoclonal antibodies listed above associated with the phyceroerythrin-conjugated (PE)-anti-CD133 or the PE-anti-CD117 monoclonal antibody (Becton Dickinson). The isotype control was performed staining PB cells with FITC-anti-CD34 associated with both FITC and PE IgG1. After red cell lysis, the samples were centrifuged and the pellets resuspended in 300 μL phosphate buffer with 0.5% foetal calf serum. The cells (2 × 10^7) were acquired by a flow cytometer (FACSCalibur Becton Dickinson) and analysed using CellQuest software (BD Biosciences). The cytofluorimeter is validated according to the UKNECAS external quality control for leucocyte immunophenotyping.

The analysis of samples was performed by excluding cell debris in an SSC/FSC dot plot gating the PB mononuclear cells. The percentage of positive cells was obtained by subtracting the value of the appropriate isotype controls. The absolute number of positive cells per microlitre was calculated using the formula: percentage of specific positive cells × white blood cell count/100. The percentage of CD34+CD133+VEGFR-2+ or CD34+CD117+VEGFR-2+ circulating cells was evaluated as percentage of electronically gated CD34+ cells.

In vitro cultures of endothelial progenitor cells

Mononuclear cells from PB were obtained by density gradient centrifugation (1077 g/mL) over Lymphoprep (Sentinel Diagnostic, Milano, Italy). After washing, the cells were plated on fibronectin-coated 30 mm Petri dishes in Endocult medium (StemCell Inc., Vancouver, Canada). After 24–48 h incubation, 5 × 10^6 non-adherent cells from PB were plated onto fibronectin-coated 24-well plates for early outgrowing endothelial colonies. After 10-day incubation at 37°C, 5% CO2, the number of colonies was scored by an inverted microscope. The endothelial origin of the cells was identified by their cobblestone-like morphology and ascertained by their immunophenotype profile, staining with anti-VE-cadherin, anti-CD31, anti-vWf (vonWillebrand factor), anti-CD45, and anti-CD14 monoclonal antibodies.

Statistical analysis

The continuous variables are described as mean ± SD of the mean or as median and interquartile range in the case of normal and non-normal values, respectively, and the categorical variables as numbers and percentages. The AUCs of CK and CK-MB release were calculated over 4 days, whereas the AUC of plasma EPO levels and hs-C-reactive protein levels over 7 days, using the trapezoidal rule; for single missing values during the observation time, a linear interpolation was used. Groups were compared by means of t-test for unpaired samples and Mann-Whitney U test, as appropriate.

Within patients with AMI, variables were analysed by means of ANOVA for repeated measures followed by post hoc Bonferroni test or by Friedman’s test, as appropriate. After Friedman’s test, three paired Wilcoxon tests were performed, setting the limit for significance (following Bonferroni’s correction) at P-value of 0.016. In all other cases, a P < 0.05 was considered significant. The correlations between the different variables were assessed using Spearman’s correlation coefficient. All of the computations were made using SPSS 12.1 software; the AUCs were calculated using R2.2.0 software for Windows package (R Foundation for Statistical Computing).

Results

Clinical, angiographic, and procedural findings

The demographic and epidemiological data of patients with AMI and CTRLs were comparable.

Sixteen patients (32%) complained of pre-infarction angina pectoris (the presence of transient spontaneous episodes of typical chest pain in the 48 h preceding the index event). Twenty-six patients (52%) had experienced an anterior MI, always associated with left anterior descending coronary artery occlusion; among the 24 inferior MIs (48%), the culprit vessel was the right coronary artery in 22 patients (44%) and the circumflex artery in the remaining two (4%). After primary PCA, the majority of patients (78%) achieved effective myocardial reperfusion (a TIMI 3 flow rate), 10 (20%) TIMI 2, and only one (2%) TIMI 0 due to a refractory no-reflow phenomenon (Table 1).

Plasma erythropoietin levels

The plasma EPO levels in patients with AMI were significantly higher than those in CTRLs (median 7.6 mIU/mL) upon admission (median 9.3 mIU/mL), 24 h (median 19 mIU/mL), and 7 days (median 10.3 mIU/mL) after AMI (Figure 1), whereas haemoglobin (Hb) concentration and haematocrit were within the normal range and comparable with those

![Figure 1](https://academic.oup.com/eurheartj/article-abstract/28/15/1805/474077/1807)
of CTRLs at all the time points. The red blood cell (RBC) count was significantly lower than that of CTRLs at both 24 h and 7 days after AMI (Table 2).

There was no correlation between plasma EPO levels and Hb concentrations in CTRLs ($R = -0.220, P = 0.25$) or AMI patients upon admission and at 24 h (Figure 2A and B). In addition, the increase in plasma EPO levels between admission and 24 h ($\Delta$EPO = EPO at 24 h – EPO upon admission) did not correlate with the decrease in Hb concentration during the same period ($\Delta$Hb = Hb at 24 h – Hb upon admission; Figure 2C). Similarly, no correlation was found between EPO levels and RBC or hematocrit at admission and 24 h.

Seven days after AMI, plasma EPO levels inversely correlated with Hb concentration (Figure 2D), RBC ($R = -0.45, P = 0.003$) and Hct ($R = -0.32, P = 0.029$).

The plasma EPO levels at admission were higher in AMI patients with pre-infarction angina pectoris ($n = 16$, median 10.6 mlU/mL, range 8.4–11.5) than in those without ($n = 34$, median 8.5 mlU/mL, range 5.7–11.5), but the difference was not statistically significant ($P = 0.10$). No relation was found between plasma EPO levels (upon admission, 24 h or 7 days) or the EPO-AUC and the echocardiographic LVEF or LVWMSI, or CKp, CK-MBp, or the AUCs of CK or CK-MB release (data not shown).

### Correlation of plasma erythropoietin levels with endothelial progenitor cells

At admission, both the percentage and the absolute number of CD34$^+$VEGFR-2$^+$ cells were significantly higher than those of CTRLs; in addition, the number per microlitre of CD34$^+$VEGFR-2$^+$ cells was higher in AMI patients after 24 h and 7 days than in CTRLs (Table 3). In patients with AMI, at admission, we found that both the percentage and the number per microlitre of circulating CD34$^+$VEGFR-2$^+$ cells were significantly correlated with plasma EPO levels (Figure 3A and B, respectively). No difference was found in the percentage or number per microlitre of CD34$^+$VEGFR-2$^+$ cells at admission, 24 h, or 7 days after AMI in patients with or without pre-infarction angina pectoris. Owing to the possible influence of the treatment with ACE inhibitors on the percentage of circulating CD34$^+$VEGFR-2$^+$ cells, patients with AMI at admission were divided into those receiving ACE-inhibitors before AMI ($n = 7$, median 0.1%, 0.07–0.2), those receiving treatment 24 h after AMI ($n = 38$, median 0.2%, 0.07–0.5), and those who did not receive this treatment until 7 days after AMI ($n = 5$, median 0.3%, 0.1–0.6). The same evaluation was performed 7 days after AMI (ACE-inhibitors before the onset of AMI: median 0.1%, 0.1–0.2; 24 h after AMI: median 0.2%, 0.07–0.4; no treatment until 7 days after AMI: median 0.1%, 0.1–0.2). No difference was found among the groups of patients in the percentage or the absolute number per microlitre (data not shown) of CD34$^+$VEGFR-2$^+$ cells.

The percentage of early circulating EPCs, evaluated either as CD34$^+$VEGFR-2$^+$ co-expressing the CD133 or as CD117 antigen, was measured in 13 patients with AMI and in 13 CTRLs. The percentage of circulating CD34$^+$CD133$^+$VEGFR-2$^+$ cells in patients with AMI was not significantly different from that of CTRLs at all the time points tested (Table 3); however, in AMI patients, at 7 days, EPO plasma levels were significantly correlated with the percentage of circulating CD34$^+$CD133$^+$VEGFR-2$^+$ cells ($R = 0.60, P = 0.03$) (see Figure 4A and B for cyttofluorimetric analysis of one representative patient and Figure 4C for the correlation). The seven patients with EPO plasma levels $>10.2$ mlU/mL (the 75th percentile of CTRLs) at 7 days after AMI (Figure 4C) had a higher percentage of circulating CD34$^+$CD133$^+$VEGFR-2$^+$ cells (median 2.7% ($P = 0.043$, by Wilcoxon for matched pairs test) than that found at admission (median 1.1%). The percentage of circulating CD34$^+$CD117$^+$VEGFR-2$^+$ EPCs was always comparable with that of

### Table 2 Laboratory findings

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<th>CTRLs ($n = 45$)</th>
<th>AMI ($n = 50$)</th>
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<tr>
<td></td>
<td>Admission (T0)</td>
<td>24 h</td>
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<tr>
<td>Erythropoietin (mlU/mL)</td>
<td>7.6 (5.2–10.2)</td>
<td>9.3 (6.1–12.0)</td>
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<tr>
<td>Haemoglobin (g/dL)</td>
<td>14.2 (13.8–15.0)</td>
<td>14.5 (13.9–15.5)</td>
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<tr>
<td>Red blood cell count (10$^6$/µL)</td>
<td>4.6 (4.3–5.0)</td>
<td>4.6 (4.5–4.9)</td>
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<tr>
<td>Haematocrit (%)</td>
<td>41.1 (39.6–44.0)</td>
<td>42.4 (40.7–45.0)</td>
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<tr>
<td>White blood cell count (10$^3$/µL)</td>
<td>6.3 (5.9–7.3)</td>
<td>11.4 (9.3–14.1)</td>
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<tr>
<td>Arterial oxygen saturation (%)</td>
<td>&lt;39 (&lt;39 to &lt;39)</td>
<td>&lt;39 (&lt;39 to &lt;39)</td>
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<tr>
<td>Grumulocyte colony-stimulating factor (pg/mL)</td>
<td>&lt;39 (&lt;39 to &lt;39)</td>
<td>&lt;39 (&lt;39 to &lt;39)</td>
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<tr>
<td>Vascular endothelial growth factor (pg/mL)</td>
<td>&lt;15.6 (&lt;15.6–16.2)</td>
<td>&lt;15.6 (&lt;15.6–47.0)</td>
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Results are shown as median (inter-quartile range). $-$, not available.
CTRLs and no correlation was found with EPO plasma levels at all the time points tested (Table 3). No significant difference between these two cell subsets was present in patients with or without pre-infarction angina pectoris (data not shown).

Plasma granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, and vascular endothelial growth factor levels

The plasma G-CSF levels in patients with AMI were comparable to those of CTRLs (median <39 pg/mL) upon admission (median <39 pg/mL), and 7 days after AMI (median <39 pg/mL). At 24 h after the onset of AMI, the median plasma G-CSF levels (median 66.0 pg/mL) were significantly higher than those of CTRLs (median 39 pg/mL, P=0.0016) (Table 2). In patients with AMI, the G-CSF AUC over 7 days was directly correlated with the hs-C-reactive protein AUC over 7 days (n=13, R=0.686, P=0.013). No relation was found between plasma G-CSF levels (upon admission, 24 h, or 7 days) or the G-CSF AUC and the echocardiographic LVEF or LVWMSI, or CKp, CK-Mbp, or the AUCs of CK or CK-MB release (data not shown). Importantly, plasma G-CSF levels did not correlate with EPC cell subsets at any time point (data not shown).

In all the plasma samples of patients with AMI and CTRLs, GM-CSF levels were under the detection limit of the assay (data not shown).

Plasma VEGF levels in the AMI patients were not higher than CTRLs at all time points tested, possibly because a wide scattering of data was present (Table 2). However, a positive relation was observed between EPO and VEGF plasma levels upon admission (Figure 3C).

Endothelial progenitor cell-derived colonies

As a functional study of circulating EPCs, we evaluated the number of endothelial colonies/10⁶ PBMCs (CFU-E) in 13 AMI patients and 13 CTRLs. The number of CFU-E obtained after culture of PBMC from patients with AMI at admission (median 5 CFU-E/10⁶ MNC, 2.5–13), 24 h (median 6 CFU-E/10⁶ MNC, 0–14), and 7 days (median 12 CFU-E/10⁶ MNC, 7–31) was not statistically different from that of CTRLs; no significant correlation was found between the CFU-E number and either the EPO levels or the circulating EPC cell subsets.

Discussion

We found that plasma EPO levels were significantly higher in patients with AMI at admission, 24 h (2.4-fold increase with respect to CTRL), and 7 days after the onset of the symptoms than in CTRLs.
The increase at day 7 was associated with an inverse cor-
relation between EPO and Hb concentration, RBC, and Hct,
thus documenting the importance of a renal modulation of
EPO expression in this phase, whereas the increase at admi-
sion and at 24 h was not related to a direct renal modula-
tion of EPO. In fact, the Hb plasma levels of patients with AMI
both at admission and at 24 h from the onset were compar-
able with those of CTRLs, and there was no correlation be-
 tween EPO and Hb levels. In addition, the early increase in
plasma EPO levels in patients with AMI was not secondary
either to renal or to peripheral hypoxia/hyperperfusion due
to myocardial failure or to blood loss during the PCI pro-
cedure. In fact, we excluded from enrolment AMI patients
in an advanced Killip class, and among the included patients,
the LVEF was only slightly depressed and the arterial oxygen
saturation was not reduced. A significant blood loss during
PCI was excluded by the comparability of plasma Hb levels
of patients with AMI at admission or at 24 h from the onset
with those of CTRLs.

Our findings are in contrast to the data recently published
by Namiuchi et al., who described that high EPO levels in
AMI patients after primary PCI (10 h after the onset of symp-
toms) are inversely correlated with plasma Hb levels. More-
over, whereas Namiuchi reported that high-concentration
EPO group of AMI patients showed less CK-release than the
low-concentration EPO group, we observed neither a rela-
tion between EPO levels or the EPO-AUC and a number
of indexes of myocardial damage or necrosis (i.e. LVEF,
LVWMSI, peak CK and peak CKMB, CK-AUC and CKMB-AUC),
nor a relation with the presence/absence of a pre-infarction
angina. With respect to the inverse correlation between Hb
and EPO after PCI, a different method of assessment of
plasma EPO levels may play a role; alternatively, a larger
blood loss during PCI in the Namiuchi’s group of patients
could account for this difference. With respect to the corre-
lation between high plasma EPO levels and the infarct size,
it should be noted that the correlation was weak and that it
could be hampered by the amount of blood loss. We have
no data showing which are the mechanisms that drive the
increase in plasma EPO levels in our patients. However,
one may hypothesize that MI results in an up-regulation of
factors known to regulate EPO gene transcription such as
HIF-1α, in agreement with the data published by Lee et al.

A number of recently published papers have suggested a
role for EPO as a mobilizer of EPCs from bone marrow in
different disease contexts emphasizing its possible role
in tissue/vascular repairing processes. When we analysed
circulating CD34+CD133+VEGFR-2+ cells (pure EPCs,
devoided of contaminating mature endothelial cells), we
found that the percentage of these cells directly correlated
with the plasma EPO levels of AMI patients 7 days after AMI.
Moreover, we found the highest percentage of EPCs among
those patients who had the highest values of plasma EPO
levels. The fact that in some patients with increased
plasma EPO levels we did not observe an increase of EPCs
could be explained either by the possibility that a number
of patients may belong to a ‘non-mobilizer’ fraction of sub-
jects or by the need of higher amounts of EPO to induce
mobilization. Interestingly, the requirement of a prolonged
increase in EPO plasma levels to mobilize EPCs is reminis-
cent of the mechanism of action of G-CSF used for CD34+
haematopoietic progenitor mobilization, which requires
3–5 days of continuous subcutaneous administration of
G-CSF to take place. In keeping with all these consider-
ations, a recent pilot study describes the effect of a single
bolus (300 μg) of darbepoetin (a long-term acting formu-
lation of EPO) in non-anaemic patients with AMI before
primary coronary intervention. This procedure induced
an increase in circulating EPCs (assessed as CD34+CD45−
cells) 72 h after the administration of the drug, suggesting
that even pharmacological doses of EPO take days to
induce cell mobilization. We also found that both the per-
centage and the absolute number per microlitre of CD34+
VEGFR-2+ cells, a subset of circulating cells that has been
for long time considered representative of circulating
EPCs, was significantly increased in AMI patients at admis-
sion. However, it is difficult to discriminate between an
increase of CD34+VEGFR-2+ due to mature endothelial
cells detachting from vessel walls and an EPO-induced
mobilization of this cell subset. We cannot exclude that
other cytokines, such as G-CSF and VEGF, might also
act as mobilizing agents partially overlapping and/or rein-
forcing the action of EPO, at least in the early phase of
AMI. With respect to G-CSF, we actually observed a
significant although slight increase of this cytokine 24 h
after AMI onset. However, the absence of a direct corre-
lation with the percentage of any EPC subset seems to rule

### Table 3 Circulating progenitor endothelial cells

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<th>n</th>
<th>CTRLs</th>
<th>AMI</th>
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<tr>
<td></td>
<td>n</td>
<td>Admission (T0)</td>
<td>24 h</td>
</tr>
<tr>
<td>CD34+VEGFR-2+ (% PB)</td>
<td>45</td>
<td>0.09 (0.05–0.20)</td>
<td>50</td>
</tr>
<tr>
<td>CD34+VEGFR-2+ (n/μL)</td>
<td>45</td>
<td>6 (3–10)</td>
<td>50</td>
</tr>
<tr>
<td>CD34+CD133+VEGFR-2+ (% CD34+ cells)</td>
<td>13</td>
<td>0.36 (0–2.2)</td>
<td>13</td>
</tr>
<tr>
<td>CD34+CD117+VEGFR-2+ (% CD34+ cells)</td>
<td>13</td>
<td>5.3 (2.7–13.1)</td>
<td>13</td>
</tr>
</tbody>
</table>

Results are shown as median (inter-quartile range).
out the possibility that G-CSF could act as an EPC mobilizer in the early phase of AMI, in agreement with previously reported data. On the contrary, the correlation between G-CSF AUC and hs-C-reactive protein AUC over 7 days suggests that G-CSF could act as a protein involved in the acute phase response, as previously reported. Regarding VEGF, we found a significant direct correlation between plasma EPO and VEGF levels in patients with AMI at admission. Although the VEGF plasma levels were never significantly higher than those of CTRLs, it is well known that the circulating levels of a cytokine may not reflect those produced at the site of injury. Both the detachment of CD34⁺CD133⁺VEGFR-2⁺ cells from the vessel walls and the production of yet undefined cytokines could be related to a local hypoxic environment, an event that cannot be easily explored in humans.
We also performed in vitro cultures as a functional evaluation of EPCs in AMI patients. Although we found that the median number of colonies obtained 7 days after AMI was the double of that at admission, the colony number did not correlate either with plasma EPO levels or with CD34⁺CD133⁺ VEGFR-2⁺ cells. In this regard, it must be considered that we used an in vitro assay which assesses only a subset of EPCs, in particular those which give rise to early outgrowing colonies, representing endothelial progenitors endowed with limited proliferative potential and more mature than the ‘early’ endothelial progenitors. The latter, which can be identified as CD34⁺CD133⁺ VEGFR-2⁺ cells, are endowed with greater proliferative potential and generate late outgrowing endothelial colonies in vitro. We could not assess the number of this subset of progenitors by cell culture, because a large number of PB-derived mononuclear cells, and in turn a large amount of blood, is required to set up the assay for growing these cells in vitro. However, we believe that the detection of early outgrowing endothelial colonies at all time points tested as well as their increase 7 days after AMI could be considered as an evidence of the presence of a functional population of circulating EPCs in AMI patients.

In summary, our data show that AMI patients have increased plasma EPO levels from admission up to 7 days after the onset of symptoms. This increase in the early phase (up to 24 h from symptoms onset) is Hb-independent, whereas 7 days after AMI, it is driven by Hb concentration and Hct. Our data also show that increased plasma EPO levels correlate with EPC concentration at day 7, suggesting that sustained EPO levels above the normal range can favour EPC mobilization. Although we did not observe any correlation between plasma EPO levels and indexes of cardiac damage or function, further studies are needed to explore the possibility that EPO could play a role in modulating the amount of myocardial damage in AMI patients.

Acknowledgement

This study was supported by grant Ricerca Corrente 80520 from Fondazione IRCCS Policlinico San Matteo, Pavia.

Conflict of interest: none declared.

References

Clinical vignette

Thirty-seven-year follow-up of a 'less known' aortic valve prosthesis

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A 60-year-old woman with anginal symptoms and effort dyspnoea was referred for coronary arteriography. She had a history of rheumatic heart disease and had aortic valve replacement 37 years ago in South Africa for aortic valve regurgitation. Now she was found to have moderately severe mitral valve stenosis with a calculated orifice of 1.2 cm² and good function of the mechanical aortic valve with a 30 mmHg gradient and no incompetence on echocardiography.

Chest X-ray and fluoroscopy during cardiac catheterization revealed a University of Cape Town (UCT) aortic prosthesis (Panels A and B) and aortography (Panel C) showed competent function.

The UCT aortic prosthesis was a modification of the lenticular mitral prosthesis by Barnard. It was first used in the early 60s and consisted of a fixed and mobile portion (Panel D). The fixed portion was a stainless-steel ring covered with Teflon cloth. It carried two arms, the one above projecting into the aorta and the one below projecting into the left ventricular outflow area, each ending in a small ring. The mobile portion was hemispherical on the ventricular aspect and cone-shaped on its aortic aspect, with flexible guide rods moving through the two rings and holding it in position. The first five patients with this aortic prosthesis with a follow-up of a few months were reported by Barnard et al. (Lancet 1963;2:856-859). In our patient, the UCT aortic prosthesis was functioning perfectly well 37 years after implantation.

Panel A. Cranial PA view of the valve.
Panel B. LAO view of the valve.
Panel C. Aortography without any valve incompetence.
Panel D. Fixed portion (left), mobile portion (middle), and assembled prosthesis (right) (reprinted from Barnard C, Shrire V, Goosen C. Total aortic valve replacement. Lancet 1963;2:856-859, copyright with permission from Elsevier).