Link between erythropoietin release and mobilization of endothelial progenitor cells in acute myocardial infarction

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This editorial refers to 'Early haemoglobin-independent increase of plasma erythropoietin levels in patients with acute myocardial infarction' by M. Ferrario et al., on page 1805

Myocardial infarction (MI) is associated with the increase in plasma levels of inflammatory and haematopoietic cytokines and mobilization of a heterogenous population of cells which consists predominantly of committed lineages (monocytes, polymorphonuclear granulocytes, and lymphocytes), as well as numerous types of stem/progenitor cells [endothelial progenitor cells (EPCs), haematopoietic stem cells (HSCs), and mesenchymal stem cells (MSCs)]. The number of circulating EPCs may have a prognostic value; however, this concept remains to be proved in large prospective studies. The number of circulating EPCs and mature endothelial cells (ECs) supposedly reflects the vascular endothelial injury and the repair mechanisms activated to restore the endothelial integrity.

The article by Ferrario et al. investigates the haemoglobin-independent increase of the plasma levels of erythropoietin (Epo) in patients with acute MI and seeks to confirm the hypothesis that changes of Epo levels are associated with the mobilization of EPCs and ECs from the bone marrow.

The paper by Ferrario et al. confirms previous work by the same group, published in 2005, describing the mobilization of heterogenous population of progenitor cells in patients with acute MI. In the present paper the authors identify two populations of immature EPCs (CD34+CD133+VEGFR2+ and CD34+CD117+VEGFR2+), more mature EPCs, and mature ECs (CD34+VEGFR2+). This study continues the search for humoral factors involved in EPC mobilization following acute myocardial ischaemia and vascular injury. One of the important issues when comparing numerous studies involving EPCs is the lack of a unified definition of an EPC.

Many authors use only flow cytometry to define the EPC, and others combine fluorescence-activated cell sorting (FACS), in vitro culture assays, immunochemistry, and expression of endothelial markers to validate the cell identity. EPCs represent only a fraction of circulating mononuclear cells (MNCs) and share some membrane markers with mature ECs, therefore it is possible that the cell populations described in one study may be different from those of other studies.

Ferrario et al. use a modification of the original method of Ashahara, in which after initial pre-incubation of MNCs to deplete the monocytes and ECs on fibronectin-coated dishes with Endocult medium, the non-adherent cells are replated to obtain the early outgrow of colony-forming unit ECs (CFU-ECs). The presence of cobblestone-like morphology and endothelial markers (VE-cadherin, CD31, and von Willebrand factor) confirms the endothelial commitment of circulating cells. The authors also use staining with anti-CD45 antibodies, which is appropriate because the presence of CD45 can distinguish between the haematopoietic (CD45+ ) and non-haematopoietic subpopulations of MNC-derived progenitor cells. This is important, because in humans both EPCs and HSCs express CD34 and CD133. The use of cultures to isolate and enumerate CFU-ECs from circulating MNCs may yield different results compared with FACS, because the pool of MNCs is heterogenous which means that subsets of progenitors other than those assayed by FACS may contribute to the number of CFU-ECs, such as monocytes which are depleted when this particular culture method is used.

The significance of another population of EPCs defined by the presence of CD34, CD117, and VEGFR2 still needs to be defined in terms of its capacity for endothelial differentiation. Also, in contrast to the data of Ferrario et al., some other studies showed significant mobilization of these cells following acute MI. The CD117 (c-kit) marker, which is a receptor for the chemoattractant stem cell factor (SCF), was identified on bone marrow-derived stem and progenitor cells (haemangioblasts, HSCs, and EPCs) and resident cardiac stem cells. In addition to vascular endothelial growth factor (VEGF), stroma-derived factor 1 (SDF-1), leukaemia inhibitory factor (LIF), and their receptors,
the SCF/CD117 axis is important in mobilization of stem/progenitor cells from the bone marrow. CD117-knock-out mice are poor mobilizers of HSCs. Additionally, the majority of circulating murine VEGFR2\(^+\) progenitor cells also express the CD117 receptor. In the setting of experimental MI the cardiac population of CD117\(^+\) cells is significantly enriched, at least in part due to the SCF/CD117 axis-dependent mobilization of bone marrow cells.\(^9\)

Another important aspect of measurement of circulating EPCs is their similarity to myelomonocytic cells with regard to the presence of markers and some functional properties. As a matter of fact, the population of CD14\(^+\) monocytes may be a source of EPCs, and CD34\(^+\) haematopoietic progenitors may express the monocyte marker CD14. Interestingly, monocytes sorted from peripheral blood have several properties attributed to EPCs, such as expression of mRNA for endothelial markers, uptake of dil-Ac-LDL, binding of BS-1 lectin, and expression of CD31, CD105, and CD144, that contribute to CFU-ECs. Moreover, the expression of EPC markers is downregulated during transformation of monocytes to EPCs, which may suggest that the angiogenic milieu in culture may be suboptimal for production or incorporation into generated vascular networks.\(^6,7\) Therefore, according to some authors, the term EPC should be used to describe the cell which is the progeny of a haemangioblast.\(^11-13\)

Ferrario et al. reported a significant correlation between plasma Epo levels and the number of CD34\(^+\)CD133\(^+\)VEGFR2\(^+\) EPCs, and concluded that Epo is one of the humoral factors involved in EPC mobilization. Numerous cytokines and hormones involved in EPC mobilization were identified (VEGF, angiopoietin-1, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), SDF-1, hepatocyte growth factor (HGF), LIF, and interleukin-8 (IL-8)]. The findings of the present study have expanded the knowledge about the mobilization of progenitor cells. It must, however, be interpreted with some caution, and in a broader context of known factors associated with changes in the number of EPCs.

Some patients had poor mobilization of EPCs despite high levels of Epo, and the authors state that the possible explanation is that higher levels of Epo or longer time of exposure to increased Epo levels might be necessary to evoke significant EPC output. This explanation is interesting and consistent with the kinetics of Epo release in acute MI. One can speculate, however, that other factors may be involved, in particular the patient’s age. Different studies showed that advanced age is associated with a lower number of progenitor cells in the bone marrow, and elderly patients have impaired infarction-related cell mobilization.\(^9,14\) Also the levels of haematopoietic and inflammatory cytokines are measured in the majority of studies in samples of peripheral blood. This may be misleading, however, because in the setting of acute MI the myocardium itself may be the source of cytokines, such as SDF-1. Therefore, more valuable data can be derived from studies in which the samples are obtained from the coronary sinus, which may better reflect the local concentration of these factors.\(^15\)

Also, the mere presence of statistical correlations is not hard proof of a real causal relationship between the various cytokines and circulating cells, given the high individual variability in cytokine levels, multiple and complex reciprocal interactions between cytokines, and co-existing diseases and medications, all of which may contribute to the fluctuations in the plasma levels.

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References