

Letters Regarding Article by Wojakowski et al, "Mobilization of CD34/CXCR4⁺, CD34/CD117⁺, c-met⁺ Stem Cells, and Mononuclear Cells Expressing Early Cardiac, Muscle, and Endothelial Markers Into Peripheral Blood in Patients With Acute Myocardial Infarction"

To the Editor:

We read with great interest the article by Wojakowski et al¹ on cell mobilization after myocardial infarction (MI) published recently in *Circulation*. The authors found that immature cells identified by several different surface markers were increased in patients with MI. In parallel, a higher amount of cytokines (such as granulocyte colony-stimulating factor, vascular endothelial growth factor, and others) was found, whereas stromal cell-derived factor-1 was decreased. The mRNA levels of cardiac-specific transcription factors GATA-4, MEF2C, and Nkx2.5/Csx were elevated in mononuclear cells in these patients.

Although we believe that this study adds important information to our knowledge of myocardial regeneration and stem cell mobilization, we would like to discuss a few aspects of it. With regard to the total cell number, which was given in absolute cells per microliter, the authors have found a surprisingly high number of CD34⁺ cells in peripheral blood (7 days after MI: 882 cells/ μ L). For example, Shintani et al² counted \approx 250 cells per million white blood cells, equaling 2.5 CD34⁺ cells/ μ L, with a white blood cell count of 10 000 cells/ μ L. In a previous study from our laboratory, we detected similar numbers.³ The usual estimates of cells in peripheral blood assume that \approx 0.01% to 0.1% of the cells in peripheral blood are CD34⁺, and on stimulation with granulocyte colony-stimulating factor, this number can be increased to \approx 1% (a similar fraction usually is found in bone marrow). Assuming a white blood cell count of 10 000/ μ L, the number given by Wojakowski et al equals a CD34⁺ cell fraction of \approx 8.8% at day 7 after MI.

In addition, we would like to discuss the mRNA measurements that were presented. Even in optimistic calculations, it is assumed that at most 0.1% of circulating cells represent a stem cell-like population. Thus, if the increase in GATA-4 expression, which is reported to be \approx 2-fold after MI, reflects cardiomyogenic differentiation of circulating stem cells, this increase would be accomplished by only 0.1% of the total cell population. For the total mRNA to increase by 2-fold, the mRNA of the stem cell population therefore must have been increased by 2000-fold. At the very least, this reflects a high induction of gene expression, which deserves a more detailed discussion than that provided by Wojakowski and colleagues.

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To the Editor:

Wojakowski et al¹ reported the mobilization of "tissue-committed stem cells" into peripheral blood (PB) in patients with acute myocardial infarction (AMI). We have serious concerns about the reliability of their findings. They found up to 1018 CD34⁺ cells/ μ L of PB in patients with AMI, as compared with 380 CD34⁺ cells in healthy controls. This would imply that in healthy individuals up to 10% of white blood cells express the hematopoietic progenitor cell-associated CD34 antigen. In fact, CD34⁺ cell counts in healthy individuals are at least a hundred times lower, and, even if donors of hematopoietic stem cell transplants are stimulated with recombinant granulocyte colony-stimulating factor for the mobilization of hematopoietic progenitor cells, the amount of CD34⁺ cells in PB is \approx 130 cells/ μ L.² In addition, the plasma levels of vascular endothelial growth factor in their control patients are 6-fold higher than reported previously.³ Performing quantitative reverse transcriptase-polymerase chain reaction for cardiac-, muscle-, and endothelial cell-associated genes with mRNA isolated from crude PB mononuclear cells is not a sufficient characterization of "tissue-committed stem cells." This should be done after fluorescence-activated cell sorting and in vitro characterization with well-chosen antigens, thus possibly supporting the disputed hypothesis that myocardial damage mobilizes progenitor cells into the circulation, perhaps from the bone marrow.

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Response

Dr Muller-Ehmsen and colleagues express concerns as to the validity of real-time reverse transcriptase-polymerase chain reaction results.¹ The mRNA increase was shown in the whole population of peripheral blood mononuclear cells (PBMNCs), not in the cardiomyogenic subpopulation; therefore, the 2-fold increase in GATA-4 expression corresponds to a 2-fold increase in mRNA copies detected in cell lysates from acute myocardial infarction (AMI) subjects. Dr Gunsilius and associates correctly suggest that the expression of tissue-specific markers in PBMNCs does not prove that these cells will actually transform into the cardiac lineage and that mRNA expression for these

markers should be assayed in a subpopulation of flow cytometer-sorted cells in patients with AMI. This concern, however, was addressed in our article.¹ Moreover, in another study, we observed a 20-fold increase in the expression of mRNA for Nkx2.5/Csx and GATA-4 in CXCR4⁺ stem cells isolated from the bone marrow-derived MNCs by a chemotactic isolation to stromal cell-derived factor-1 gradient, which coexisted with the immunocytochemically detectable presence of Nkx2.5/Csx and GATA-4 proteins in these cells.²

We also demonstrated that a mobile pool of CXCR4⁺ nonhematopoietic cells is found in both mouse and human bone marrow, which show expression of markers specific for different tissues and may be mobilized into PB in AMI.^{2,3} We believe that it is the same population of cells that is subsequently found in PB in patients with AMI. Again, we acknowledge that our findings were somewhat hypothetical when the manuscript was submitted, but they are consistent with our recent observations in murine models of AMI,² and with regard to the number of mobilized cells, similar to those recently published by Massa et al⁴ (absolute CD34⁺ counts range up to $\approx 700/\mu\text{L}$, CD34⁺CD33⁺ up to $\approx 1000/\mu\text{L}$). We agree, however, that if we had used immunomagnetic purification and International Society of Hematology and Graft Engineering guidelines, the number of CD34⁺ cells would have been lower in both control and AMI patient groups and that it should become a “gold standard” that potential discrepancies in total cell numbers between the studies be avoided.⁴

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