This editorial refers to ‘Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction’ by F.H. Seeger et al., on page 766.

Administration of adult stem cells for cardiac repair after myocardial infarction (MI) is a revolutionary new strategy that could become clinically feasible in the near term. Appears to be safe and relatively cost-effective, and offers tremendous potential to improve prognosis. Using diverse cell populations and strategies, several relatively small clinical studies have reported encouraging outcomes, with improvement in various measures of myocardial perfusion and left ventricular (LV) function. However, negative data have also emerged, generating controversy regarding the overall efficacy of cell therapy.

This controversy persists even after the recent publication of the three largest randomized studies to date of cell therapy for cardiac repair. Starting with the multicentre REPAIR-AMI study, 204 patients received an intracoronary infusion of autologous mononuclear bone marrow cells (BMCs) or placebo (medium) at 3–7 days after a reperfused acute MI. After 4 months, the absolute improvement in global LV ejection fraction (EF) was greater in patients treated with BMCs compared with control patients. After 1 year, BMC treatment was also noted in the TOPCARE-CHD trial among patients with an old MI. However, no benefit was found in the combined endpoints of death, recurrent MI, and revascularization and death, recurrent MI, and rehospitalization for heart failure. An improvement in LV function and clinical outcome after BMC treatment was also noted in the TOPCARE-CHD trial among patients with an old MI. However, no benefit was found in the other trial of acute MI (the ASTAMI trial), in which 100 patients with reperfused acute anterior MI received either intracoronary infusion of BMCs or no intervention at a median of 6 days after MI. After 6 months, there was no difference in LVEF, infarct size, or LV end-diastolic volume between the two groups. At first glance, these conflicting results might appear to portend a rather uncertain future for adult stem cell-based approaches for cardiac repair. Importantly, the ostensibly incongruent results may be construed by the opponents of adult stem cell therapy as evidence of lack of efficacy and inappropriately used to summarily dismiss this approach.

It is therefore important to understand the reason(s) for the discrepancy between the REPAIR-AMI and ASTAMI studies. Although REPAIR-AMI was larger (204 vs. 100 patients), utilized a higher dose of BMCs (median, 198×106 vs. 68×106), and had a more rigorous control protocol (unlike ASTAMI, control patients received bone marrow aspiration and intracoronary infusion of vehicle), it is unclear whether these differences can account for the different outcomes. A careful study by Seeger et al. provides important new insights into this issue. These investigators compared the methods for isolating BMCs in REPAIR-AMI and ASTAMI. Bone marrow was collected from healthy donors and patients with angiographically proven coronary artery disease (CAD) and tested for various parameters of phenotype and function. To obtain BMCs, aliquots from the same bone marrow aspirate were subjected to similar gradient centrifugation protocols using either Ficoll (Cambrex), as in REPAIR-AMI, or Lymphoprep (Axis-Shield), as in ASTAMI. Cells were stored overnight in either X-vivo 10 medium or 0.9% NaCl or autologous serum at room temperature (REPAIR-AMI protocol) or 0.9% NaCl + 20% heparin-plasma at 4°C (ASTAMI protocol). The results of the two protocols were quite different. Compared with Lymphoprep, the use of Ficoll yielded not only greater number of BMCs but also more CD45+/CD34+ and CD45+/CD133+ cells. Cells isolated with Ficoll yielded greater numbers of colony forming units, an in vitro indicator of the proliferative capacity and a surrogate measure of stemness. The number of mesenchymal stem cells (MSCs) was also greater after Ficoll separation. The authors also examined whether the overnight storage conditions might have affected the phenotype or functionality of the cells. In an in vitro assay, BMCs isolated with Lymphoprep (particularly from patients with CAD) showed reduced ability to migrate to the chemoattractant SDF-1, and this reduction was more pronounced after overnight storage. Consistent with these observations, the expression of CXCR4, the receptor for SDF-1, was significantly reduced after overnight storage in BMCs isolated with Lymphoprep. Following transplantation in a mouse model of hind-limb ischaemia, BMCs

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isolated with Ficoll produced greater improvement in blood flow compared with BMCs isolated with Lymphoprep, documenting the functional superiority of these cells.\(^7\)

Seeger et al. should be lauded for this careful methodological analysis. These results may explain, at least in part, the different outcomes of REPAIR-AMI and ASTAMI. Given the current explosion of clinical trials of cardiac repair with BMCs and other adult stem/progenitors, the importance of the observations of Seeger et al. is enormous. Because the bone marrow is a heterogeneous mixture of various progenitors and committed cells, some of which have been shown to be multipotent or even akin to embryonic stem cells,\(^8\) the cell populations obtained after density centrifugation consist of admixtures of various subsets with diverse phenotype and function. Therefore, every step in the isolation process is important, and even minor modifications may alter the composition of ‘mononuclear’ BMCs. Although the precise cell type(s) responsible for the salubrious effects of BMC therapy is unknown, CD34\(^+\) and CD133\(^+\) progenitors appear to play an important role,\(^9\) and MSCs have been shown to induce cardiac repair in several animal models.\(^9\) The results of Seeger et al. suggest that the isolation procedure used in REPAIR-AMI resulted in an enriched content of these cell types, which are important for cardiac repair.

Another important finding is that the use of X-vivo 10 medium with 20% serum was more effective in preserving CXCR4 expression when compared with NaCl with 20% heparin-plasma.\(^7\) The interaction of CXCR4 with SDF-1 is critical for BMC homing to injured tissues.\(^10\) SDF-1 is expressed in the infarcted myocardium and plays an important role in BMC homing and retention after MI. Therefore, isolation and infusion of BMCs enriched in CXCR4-expressing cells would be expected to increase myocardial homing and retention of BMCs after intracoronary delivery. In addition, expression of CXCR4 may serve as a surrogate marker of cells with greater differentiation potential.\(^11\)

The demonstration by Seeger et al. that the phenotype and function of mononuclear BMCs are profoundly affected by the isolation and storage conditions underscores the need to adopt optimal and standardized methods for BMC processing in future clinical trials. As the present study clearly shows, cell counts and viability are not enough. Characterization of cells in terms of phenotype and functional competence is also critical. For example, following intracoronary injection, BMCs first need to cross the vessel barrier to gain access to the myocardium. Therefore, \textit{in vitro} assays of migration of BMCs in response to SDF-1 would logically be expected to correlate with their beneficial effects after MI. This concept is supported by the current results, which demonstrate superior functional competence of BMCs isolated by Ficoll in improving flow in a hind-limb ischaemia model. Taken together, the current results not only identify the Ficoll gradient centrifugation as an effective method for procuring BMCs but also emphasize the importance of appropriate isolation methods and careful characterization of BMC populations prior to transplantation in future clinical trials. Unfortunately, to date, only a few studies have characterized the composition of the BMCs used for cardiac repair, and almost no clinical study has examined the function of these cells prior to transplantation. Establishing standard protocols for isolation of BMCs should eliminate some of the variability in the outcome of future clinical trials.

In conclusion, Seeger and colleagues\(^7\) have made an important contribution to the field of regenerative cardiology by carefully elucidating the influence of the isolation process on the phenotype and function of human BMCs. Interestingly, many of the differences in BMC isolation and preservation between REPAIR-AMI and ASTAMI\(^3,5\) might have resulted in conspicuous differences in outcomes. Future studies should be aimed at identifying the optimal isolation and storage conditions to preserve BMC number and function. These \textit{in vitro} analyses may not be as glamorous as the \textit{in vivo} studies, but are nevertheless fundamental to ensure both the reproducibility of the results and the overall progress of cell therapy.

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