We believe this is unlikely for the following reasons:

(i) Comparable density gradient centrifugation techniques have been used for isolation of MNCs in the REPAIR-AMI and the ASTAMI studies. The density gradient solutions used for MNC separation, Lymphoprep™ and Ficoll-Hypaque (Ficoll-Paque™), contain ficoll and sodium diatrizoate at identical concentrations.

(ii) In the ASTAMI study, MNCs were kept at 4–8°C overnight in 0.9% NaCl and 20% autologous heparin plasma, cell concentration <10⁶ cells/mL. Cold saline-plasma storage of bone marrow or peripheral blood stem cells (PBSCs) for transplantation is being used worldwide for intercontinental transportation or prior to cryopreservation the following morning. To mention one of several examples: over the past 12 years, 163 multiple myeloma patients have received autologous PBSC transplantation in our hospital. PBSCs had been stored overnight at 4–8°C in saline-plasma prior to cryopreservation. Stem cell engraftment occurred in >90% of the patients within 3 weeks and for the rest within the next few weeks.

(iii) In the ASTAMI study, only MNCs with cell viability >90% were injected. The acridine orange/ethidium bromide technique was used for viability assessment; a sensitive, reliable, and reproducible method utilized for numerous viability tests, e.g. pre-transplant cell control and lymphocytoxicity cross-match analyses.

(iv) In parallel studies, functional assays on bone marrow MNCs from healthy controls, isolated and stored as for the ASTAMI patients, were shown to be normal by their numbers of the haematopoietic progenitor cell colonies CFU-GM and BFU-E (unpublished results).

(v) The relative number of isolated MNCs in the ASTAMI study was comparable to other studies. In addition, injected cell numbers have not been shown to correlate to changes in cardiac function.

Taken together, the cell-processing protocol in the ASTAMI study produces MNCs that are viable and functional. Whether intracoronary injection of autologous bone marrow MNCs has a beneficial effect still remains to be confirmed.

References


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Improved clinical outcome after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial

We read with great interest the recent article by Schächinger et al. showing improved 1 year clinical outcomes in patients with acute myocardial infarction receiving intracoronary administration of bone marrow-derived progenitors cells (BMCs) after successful reperfusion therapy. The authors attribute the improved clinical outcomes in the treatment group to the recovery of global leftventricular contractile function within 4 months, as recently reported in the same patient population. However, we believe that factors other than administration of BMCs may have influenced left ventricular ejection fraction (LVEF) recovery and, hence, clinical outcomes in their study. First, we can suppose that spontaneous LVEF recovery was already occurring in both groups before BMCs or placebo administration. Indeed, baseline LVEF was 46.7 ± 10 and 47.5 ± 10% in controls and BMC-treated patients, respectively—values higher than that used as a threshold for patient inclusion in the study (≤45%). This spontaneous recovery may be explained by the mean delay between enrolment and baseline LVEF measurement (4.3 ± 1.3 days). Secondly, and more importantly, two major determinants of LVEF recovery—time-to-reperfusion and infarct location—are possible confounders in this study. According to Sheiban et al., LVEF recovery is usually observed after primary angioplasty if coronary flow is restored ≥4 h from symptom onset, whereas no significant improvement occurs afterwards. This time “window” may be even narrower in anterior infarctions. Indeed, we have observed no significant recovery in LVEF after primary angioplasty despite an average shorter time-to-reperfusion (2.5 ± 1.4 h) when only anterior myocardial infarctions were considered. In the REPAIR-AMI study, however, the authors analysed anterior and inferior infarctions together, despite the fact that these two infarct locations differ in terms of acute left ventricular impairment severity, LVEF recovery and clinical outcome after reperfusion therapy. In BMC-treated patients, anterior infarctions were less represented (64 vs. 76%), which may explain the small difference in LVEF recovery between the placebo and treated groups (3.0 ± 6.5 vs. 5.5 ± 7.3%), and in clinical outcomes. Moreover, mean reperfusion time was ≥7 h, an interval usually not associated with LVEF improvement, particularly in anterior infarctions. The combination of different times to treatment and infarct locations may have a major influence on LVEF recovery.
and long-term event rate. It is surprising that these two critical factors were not taken into account among the variety of covariates considered by the authors. Thus, we believe that the LVEF changes and clinical outcomes should be re-analysed after adjustment for infarct location and time-to-reperfusion.

References


Recently, Osterziel raised a question about the appropriateness of the clinical endpoints used in the REPAIR-AMI trial. Primary endpoint of the trial was the change in left ventricular ejection fraction, assessed by LV angiography. As such, the REPAIR-AMI trial was designed as a proof-of-concept trial, and intracoronary infusion of bone marrow-derived progenitor cells (BMC) is associated with improved left ventricular contractile function. Therefore, we are rather puzzled about the disappointment expressed by Osterziel, given that the trial was obviously not powered to and did never claim to detect differences in single clinical endpoints. It is universally accepted in phase II trials, such as the REPAIR-AMI, to assess safety aspects using a combined clinical endpoint, which may also generate hypotheses to be tested in subsequent larger clinical outcome trials. It might have escaped the attention of Osterziel that we had already presented the detailed analysis of the location of the recurrent myocardial infarctions in the supplement of our original article. Nevertheless, even when recurrent myocardial infarctions are limited to the target vessel, the combined endpoint of cardiovascular death, target vessel-related recurrent myocardial infarction, or rehospitalization for heart failure remains significantly reduced in patients receiving BMC compared with placebo \( n = 11 (11\%) \) vs. \( n = 2 (2\%) \), \( P = 0.011 \). Thus, these results are indeed encouraging to test the hypothesis, that intracoronary infusion of BMC improves clinical outcome in patients after an acute myocardial infarction.

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Improved clinical outcome after intracoronary administration of bone marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial: reply

Ad 1: As recently published, comparison of different isolation protocols revealed a vastly reduced recovery of mononuclear cells from identical volumes of bone marrow aspirates when using the ASTAMI protocol. In addition, the cells’ functional capacity, as measured by migratory capacity, colony forming unit capacity as well as in vivo recovery of blood flow in a hindlimb ischemia model were profoundly impaired, when the ASTAMI cell isolation protocol was used. Most importantly, the in vivo neovascularization capacity of patient-derived cells was completely abrogated, when the ASTAMI cell isolation/storage protocol was used. Thus, cell processing protocols profoundly interfere with functional capacity of bone marrow-derived progenitor cells.

Ad 2: The effects of different storage conditions and medium on progenitor cell functional parameters have extensively been studied and published. Obviously, the functional properties of progenitor cells mediating improved cardiac function after intracoronary administration are most likely different from the cell properties required to repopulate and reconstitute the bone marrow. In fact, it is well established that single cell transplantation is sufficient to reconstitute the bone marrow. Moreover, different integrins are used for homing of BMC to the bone marrow compared with the heart.

Ad 3: Even with respect to cell viability, which does not reflect functional activity of the cells, there are major differences between the different cell processing protocols. Median viability of injected cells was 95% in ASTAMI compared with 99% in the REPAIR-AMI trial. Notably, careful reading of the original report of the ASTAMI trial reveals that two of the 47 bone marrow-derived cell preparations were contaminated by bacteria.

Ad 4: Assessing colony forming unit capacity of bone marrow-derived cells from healthy controls is insufficient. At least in our view, it is absolutely mandatory to assess the functional capacity of patient-derived cells in experimental models, and this has to be done prior to embarking on a clinical trial.

Ad 5: The statement by Egeland and Brinckmann is incorrect. Of all the four controlled, randomized trials assessing the effects of BMC administration in acute myocardial infarction, ASTAMI stands out as the single trial with by far the lowest number of mononuclear cells infused \( (68 \times 10^6) \) compared with the Leuven trial \( (172 \times 10^6) \), REPAIR-AMI \( (198 \times 10^6) \) (all median) and the BOOST trial (mean of \( 246 \times 10^6 \)). Moreover, experimental studies clearly documented a dose-response relationship, at least with respect to CD34+ cells.