We appreciate the comments of Marenzi and Bartorelli.\textsuperscript{10} The time between myocardial infarction and intracoronary infusion therapy was nearly identical in the BMC and placebo group (median of 4 days), thus any spontaneous improvement in left ventricular ejection fraction (LVEF) prior to intracoronary infusion therapy may have occurred to the same extent in both groups. As suggested by Marenzi and Bartorelli, we re-analysed a potential effect of time to reperfusion therapy and infarct location on treatment effect by BMC administration. Randomization to BMC remained significant associated with improved recovery of LVEF after adjusting for time to first reperfusion therapy ($P = 0.013$) as well as infarct location (anterior vs. inferior) ($P = 0.021$). There was no interaction between BMC treatment effect and infarct location ($P = 0.87$) or time to reperfusion ($P = 0.60$). Likewise, the beneficial effect of BMC administration on the combined clinical end point death, recurrent myocardial infarction, or revascularization procedures remained statistically significant in favour of BMC therapy, when adjusting for time to reperfusion therapy ($P = 0.018$) or infarct location ($P = 0.013$). Neither infarct location ($P = 0.37$) nor time to reperfusion (categorized according to the median of 4.5 h) ($P = 0.47$) was predictive for cardiovascular event rate. Thus, neither infarct location nor time to reperfusion had an impact on the results of the REPAIR-AMI trial, that intracoronary BMC administration favourably affects recovery of LVEF as well as clinical outcome.

References


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The REPAIR-AMI and ASTAMI trials: cell isolation procedures

In a recent article, Seeger et al.\textsuperscript{1} compare the methods for the preparation of the bone marrow mononuclear cells (BM-MNC) in the REPAIR-AMI\textsuperscript{2} and ASTAMI\textsuperscript{3} trials. In their paper, Seeger et al.\textsuperscript{1} claim to use Ficoll (Cambiax) for gradient centrifugation. Ficoll is a high-molecular-weight sucrose polymer, now rarely used on its own for cell separation procedures. Presumably, Seeger et al. mean Ficoll-Paque, which is Ficoll combined with sodium diatrizoate, density 1.077 g/mL. Ficoll-Paque or identical media from other producers is apparently the reagents used in their clinical studies, and they have identical constituents to the Lymphoprep density gradient medium used in the ASTAMI study.\textsuperscript{1,5}

In the experiments described in Figures 1 and 2 and Table 1, Seeger et al.\textsuperscript{1} use practically identical experimental conditions to compare BM-MNC isolated by their ficoll solution and Lymphoprep. They find significant differences between the cell recoveries obtained using the two products, a difference which in turn is the only discernible reason for the differences observed for the number of CD45$^+$CD34$^+$ BM-MNC, CFU, and MSC. Indeed, "...the overnight incubation protocols did not additionally affect the recovery of HSC, MSC and CFU (\ldots), indicating that the reduced cell recovery between the two protocols is predominantly caused by differences in the initial centrifugation steps.\textsuperscript{1} As this is a central observation in their study, we believe that Seeger et al. should discuss how they were able to get different cell recoveries using the same bone marrow aspirates and identical gradient centrifugation media. Alternatively, if they actually used Ficoll, they need to explain why the density gradient medium used in this study was different from that used in their previous studies.\textsuperscript{1,2}

References


The REPAIR-AMI and ASTAMI trials: cell isolation procedures: reply

It obviously escaped the notion of Egeland and Brinchman that the protocols additionally differ with regard to the washing steps and buffer components used in the density gradient separation of the bone marrow. Our study has been conducted to elucidate factors in the preparation of bone marrow cells for intracoronary application that might influence the cell quality and hence the therapeutic benefit. Using split bone marrow samples, our side-by-side comparison of the REPAIR-AMI and ASTAMI protocols indicates that minor changes like washing steps or the use of sodium chloride solution containing heparinized plasma may result in reduced cell recovery and function. While these factors might in part explain the striking low cell recoveries reported in the ASTAMI trial, a productive bone marrow harvest may also be critical. Bone marrow usually contains $>20,000$ total nuclear cells ($\text{TNCs}$)/$\mu$L. Ficoll–Hypaque-based density gradient separations at a density of 1.077 g/L are reported to yield TNC recoveries of 20–30%. From 50 mL bone marrow, one would expect about $10^9$ TNCs and $2–3 \times 10^8$ TNCs after density gradient separation. With a mean cell number of 2.36 $\times 10^8$ TNCs, the REPAIR-AMI trial very much is in line with the expected cell recoveries, whereas only one-third of the cells (8.7 $\times 10^7$ cells) was recovered from identical volumes of bone marrow aspirates in the ASTAMI trial. We believe that it is mandatory to identify the underlying factors of such discrepancies and to establish the quality standards of cellular products for future clinical trials. Ultimately, this will lead to the provision of safe and effective investigational medicinal products to the patients benefit.

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doi:10.1093/eurheartj/ehm237

Online publish-ahead-of-print 2 August 2007

The REPAIR-AMI and ASTAMI trials: cell isolation procedures: reply

The Evaluation of the Methods and Management of Acute Coronary Events (EMMACE) risk model is a community-derived risk model for patients presenting with ST-elevation myocardial infarction. It is a simple model that uses patient age, admission heart rate, and systolic blood pressure to predict 30-day mortality [$C$-index = 0.76 (95% CI 0.72–0.79)]. The EMMACE model has good discriminatory performance because it relies on age and haemodynamic predictors. In the article by Yan et al., $^1$ RS which included these variables (GRACE and PURSUIT rather than TIMI) also offered improved discriminatory capacity. We have corroborated this using 100,686 patients from the Myocardial Infarction National Audit Project database (MINAP), an extensive community-based cohort of patients hospitalized in UK and Wales with ACS:$^2,^3$ C-index (95% CI): TIMI RS for 14-day mortality = 0.58 (0.57–0.59, $P < 0.001$), GRACE RS for in-hospital mortality = 0.80 (0.80–0.81, $P < 0.001$), GRACE RS for 6-month mortality = 0.80 (0.79–0.80, $P < 0.001$), and PURSUIT for 30-day mortality = 0.81 (0.78–0.81, $P < 0.001$).$^4$ A concern with the GRACE and PURSUIT RS is that they rely on the collection of multiple variables when it is known that secondary abstraction of difficult-to-obtain key clinical findings adds little to the predictive power of RS.$^5$ In the article, Yan et al.$^6$ have inclined that RS complexity may also be prohibitive to their use. Perhaps, RS that rely on a few easily recordable variables may be used more frequently by physicians and also allow as good a risk prediction as more complex scores?

Although the findings by Yan et al.$^7$ are important, a more appropriate external validation of the RS would have considered their performance over their original risk prediction periods (TIMI: 14 days;$^8$ PURSUIT, 30 days;$^9$ and GRACE, in-hospital$^{10}$ and 6 months$^{11}$) and outcomes (TIMI—composite of death, revascularization, and re-infarction at 14 days). Second, while the authors identified a significant correlation between the three RS, one would expect this because the authors have compared similar methods (i.e. RS designed to evaluate ACS mortality) over a range of values. The resultant tests of significance are therefore not relevant since it would be unusual that the RS were not related.

The article by Yan et al.$^1$ measured the discriminatory performances of the TIMI, PURSUIT, and GRACE risk scores (RS) and suggested that they offered better prediction of in-hospital and 1-year mortality than that of global risk assessment by physicians.$^1$ Moreover, the study revealed an inverse relationship between estimated risk and early invasive management when patients were stratified by RS, an important finding recently highlighted by Fox et al.$^1$ in the study by Yan et al.$^1$ revascularization was more frequently based on physicians’ global assessment, signifying that RS are not being used appropriately for estimating risk. Perhaps, this is because they are too complex.

Are acute coronary syndromes risk models too complex?

The article by Yan et al.$^1$ measured the discriminatory performances of the TIMI, PURSUIT, and GRACE risk scores (RS) and