Quantification of Circulating Endothelial Cells by Flow Cytometry

To the Editors: We read with great interest the article by Mancuso et al. (1) in the January edition of Clinical Cancer Research. In this article, the authors describe the validation of a novel multicolor flow cytometrical assay that allows the quantification of both circulating endothelial cells (CEC) and circulating endothelial progenitor cells (CEP), considered promising monitoring tools for vascular damage (vascular-derived CEC) or angiogenesis (bone marrow–derived CEP) in a large number of diseases with vascular involvement. Therefore, the development and validation of an assay by which these cells can be reliably detected are definitely warranted. Nevertheless, we feel that several issues deserve further attention.

First, this article adds yet another reference value for CEC and CEP in healthy donors, namely 140 CEC/mL and 181 CEP/mL. These numbers are in sharp contrast to reference range CEC and CEP numbers reported in earlier studies from this group (mean, 7,900 CEC/mL and <500 CEP/mL, respectively; ref. 2), whereas the most recent article from this group reports on average 14,500 CEC/mL and 740 CEP/mL (3). Strikingly, all assays by Mancuso et al. (4, 5) largely exceed CEC numbers typically reported in literature, namely up to 20 CEC/mL. It is also remarkable that CEC counts greatly exceeded CEP counts in their initial study (2), whereas on the contrary, their current study reveals the opposite (1). In view of the large number of clinical studies that have been performed using the assay first published in Blood in 2001 (2) or modifications thereof (3), it is our opinion that the authors should elaborate on the discrepancy in CEC and CEP numbers between their assays, as well as on the effect this difference could have on the overall interpretation of the results.

Second, the exact quantification of CEC and CEP is very confusing (1). Both EDTA and CPT tubes are drawn, but it is not indicated which tube is used for absolute counting and how these counts were obtained. The gold standard for obtaining absolute cell counts in flow cytometry is by using fluorescent counting beads, but use of these beads is not reported in the Materials and Methods section (1). Furthermore, counting cells with a prevalence as low as CEC and CEP requires a large volume of blood, typically 1 to 4 mL, to assure a low coefficient of variation. Mancuso et al. (1) do not state the volume used; however, it is mentioned that at least 1 × 10⁶ cells are acquired, which reportedly yielded 300 to 400 events, suggesting that CEC constitute 0.03% to 0.04% of nucleated cells. Based on these numbers, a normal blood sample, with a leukocyte count of 5 × 10⁹/mL, would have 5 × 10⁶ × 0.03% = 1,500 CEC, yet the authors report a mean CEC count of 140/mL. How would the authors explain this?

Finally, we have some concerns about the validation by electron microscopy. Based on our own experience on this approach, we know that to obtain a cell pellet suitable for electron microscopy analysis, at least 800,000 cells are required to have a cell pellet large enough for preparing pellet sections. Given the reported mean CEC counts (140/mL) and recovery (average of 39%) after Ficoll, the authors would have needed 800,000/(140 × 0.39)/1,000 = 14.7 liters of blood to obtain enough cells for such a pellet. Even in the theoretical case of maximum CEC isolation, i.e., 100% recovery after Ficoll, 5.7 liters would be necessary. This issue needs clarification.

Up to now, several assays enabling the detection of CEC and CEP have been described and others are likely to be published in the near future. In addition to establishing the true value of CEC and CEP enumeration in cancer and other diseases, general consensus on the best way to enumerate these cells is now definitely required.

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References


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