

## Endothelial progenitor cells, defined by the simultaneous surface expression of VEGFR2 and CD133, are not detectable in healthy peripheral and cord blood

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Circulating endothelial cells (CEC) and their progenitors (EPC) are restricted subpopulations of peripheral blood (PB), cord blood (CB) and bone marrow (BM) cells, involved in the endothelial homeostasis maintenance. Both CEC and EPC are thought to represent potential biomarkers in several clinical conditions involving the endothelial turnover/remodeling. Although different flow cytometry methods for CEC and EPC characterization have been so far published, none of them have reached consistent outcomes, therefore consensus guidelines with respect to CEC and EPC identification and quantification need to be established. Here, we have carried out a deep investigation of CEC and EPC phenotypes in healthy PB, CB and BM samples, by optimizing a reliable polychromatic flow cytometry (PFC) panel. Results showed that the brightness of CD34 expression on healthy PB and CB circulating cells represents a key benchmark for the identification of CEC (CD45neg/CD34bright/CD146pos) respect to the hematopoietic stem cell (HSC) compartment (CD45dim/CD34pos/CD146neg). This approach, combined to a dual-platform counting technique, allowed a sharp CEC enumeration in healthy PB (n = 38), and CEC counts were consistent with previous reported data (median = 11.7 cells/ml). In parallel, by using rigorous PFC conditions, CD34pos/CD45dim/CD133pos/VEGFR2pos EPC were not found in any healthy PB or CB sample, since VEGFR2 expression was never detectable on the surface of CD34pos/CD45dim/CD133pos cells. Notably, the putative EPC phenotype was observed in all analyzed BM samples (n = 12), and the expression of CD146 and VEGFR2, on BM cells, was not restricted to the CD34bright compartment, but also appeared on the HSC surface. Altogether, our findings suggest that the previously reported EPC antigen profile, defined by the simultaneous expression of VEGFR2 and CD133 on the surface of CD45dim/CD34pos cells, should be carefully re-evaluated and further studies are needed to redefine EPC features in order to translate CEC and EPC characterization into clinical practice.

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### Keywords

Circulating endothelial cells; endothelial progenitor cells; polychromatic flow cytometry; vascular endothelial growth factor receptor 2; CD133.