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Circulating Endothelial Cell evaluation in peripheral blood from healthy subjects and diabetes mellitus patients by applying a single platform absolute count in a four-color flow cytometric assay

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Circulating endothelial cells, showing phenotypical markers from endothelial progenitors to mature endothelial cells, may have a profound impact on the course of a variety of pathological events, including cancer and cardiovascular disease. Although dramatic changes in the number of circulating endothelial cells in pathological conditions have been observed, a reliable identification and quantization of circulating endothelial cells still remains a technical challenge. Here, we report a novel methodology to identify and enumerate circulating endothelial cells at different stages of differentiation. This is characterized by a four-colour flow cytometric analysis using CD45, CD146, CD34 and CD117 as circulating endothelial cell markers, with single platform absolute count in a lyse/no wash procedure. We validated this technique with whole blood samples from normal volunteers as well as from type 2 diabetic patients, which are known to display a significant decrease in circulating endothelial cell number. Results clearly indicate that the methodology herein described is reliable and highly reproducible, thus providing an accurate estimate of differentiation stage and number of circulating endothelial cells. This technique may represent, therefore, a significant advance for the understanding of the pathobiology of circulating endothelial cells.

Key words

Absolute count; Circulating Endothelial Cells; Diabetes; Endothelial progenitors; Multicolour flow cytometry