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Cytometry PART A
Journal of the International Society for Advancement of Cytometry

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OMIP-011: Characterization of Circulating Endothelial Cells (CECs) in Peripheral Blood

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Additional Supporting Information may be found in the online version of this article.

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Received 15 June 2011; Revision Received 14 April 2012; Accepted 23 April 2012

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Published online 30 May 2012 in Wiley Online Library (wileyonlinelibrary.com)

DOI: 10.1002/cyto.a.22071

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International Society for Advancement of Cytometry

Purpose and Appropriate Sample Type

This panel was optimized for the evaluation of circulating endothelial cells (CECs) in peripheral blood (see Table 1). The combination of three different endothelial cell markers enables a reasonable analysis of CECs. The panel, so far tested on fresh human peripheral and cord blood, can be used to enumerate CECs in a dual platform method.

Background

CECs as well as bone-marrow-derived endothelial precursor cells (EPCs) are very rare events in the peripheral blood that have a high potential diagnostic value in different diseases which are characterized by cardiovascular problems and/or angiogenesis, e.g., cancer, ischemia, and diabetes.

Analysis of CECs is difficult because CECs are often discriminated using a combination of antigens with low, dull, or a continuum of cell surface expression (1). Since CECs cannot be characterized by a single marker, a combination of at least two markers is necessary. Therefore, different combination of several endothelial markers (CD31, CD34, CD146, KDR, and CD144) was used in order to get a more accurate discrimination of CECs. Such a test evidenced that KDR and CD144 were very weakly expressed on the CEC cell surface and could not be reliably analyzed, whereas CD31, CD34, and CD146 were largely detected and therefore chosen for the panel (see Figure 1 and supporting information). Dead cells, microparticles, and platelets were excluded from the analysis by using a DNA stain (Syto16) and a live/dead marker (NiRed) (see Table 2). Leucocytes were excluded by gating $CD45^-$ cells (2). The addition of the progenitor marker CD117 enables to distinguish between CECs and EPCs. CD106 is expressed on endothelial cells after stimulation with cytokines and allows analysis of activated subsets of CECs.

Since the number of cells acquired influences the detection sensitivity of CECs, large numbers of cells were acquired to reliably quantify very rare CECs (40–100 cells per ml blood) (3). Four milliliters whole blood, red blood cells (RBC)-lysed and washed, were stained and about 10 million events per sample were acquired in order to ensure sufficient numbers of CECs. The use of CECs as diagnostic marker demands the enumeration of absolute numbers of CECs and EPCs per ml blood. The number of these cells per ml peripheral blood was calculated by acquiring, in a dual platform procedure, an additional tube with TruCount beads (BD) and whole blood stained with Syto16 (4). The application of a dual platform method was necessary in order to allow the analysis of rare events. It must be noted that the possible cell loss occurring in such a procedure, which involves lyse/wash steps, is a random event and does not affect CEC subsets more than other nucleated events.

Similarity to published panels

None to date.

Table 1. *Summary table for application of OMIP-011*

Purpose	Characterization of CECs in peripheral blood
Species	Human
Cell types	Whole blood, RBC-lysed and washed
Cross references	n.a.

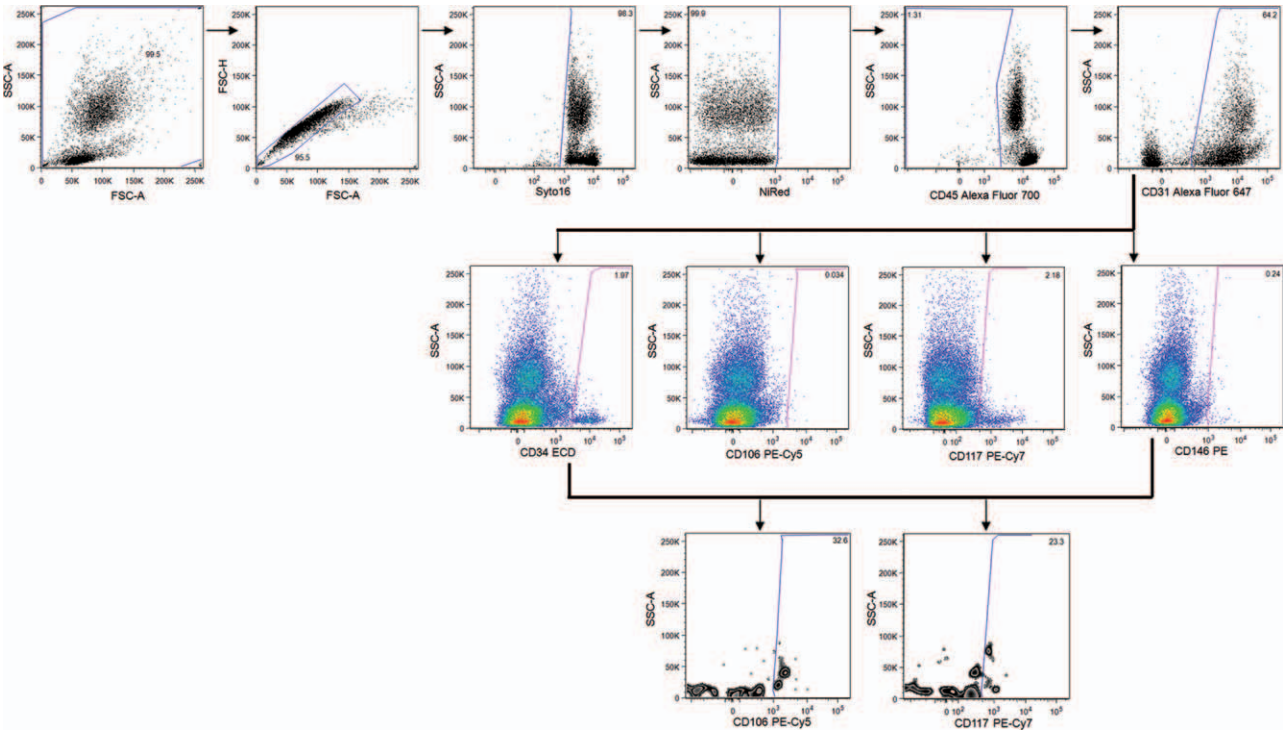

Figure 1. Example of staining and gating. Cells were not gated on morphological parameters, indeed only artifacts in the upper left and lower right corner were excluded. Aggregates were gated out on a FSC-Area (FSC-A) versus FSC-High (FSC-H) dot plot. Debris, dead cells, microparticles, and platelets were excluded by gating Syto16$^+$ cells and NiRed$^-$ cells. Leucocytes were excluded by gating CD45$^-$ and CD45dim cells. CD31$^+$ cells were then identified; CD34$^+$ and CD146$^+$ cells were separately gated and analyzed by boolean gating. CD106 and CD117 gates are shown as well. Live CD45$^-$CD31$^+$CD34$^+$CD146$^+$ CECs are displayed in a CD106 versus SSC and a CD117 versus SSC low resolution zebra plot. Dot plots show 8,000 events each, while pseudocolor plots and low resolution zebra plots show all analyzed events. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 2. *Reagents used for OMIP-011*

SPECIFICITY	FLUOROCHROME/REAGENT	AB CLONE	PURPOSE
Dead cells	NiRed		Exclusion of dead cells
CD45	Alexa Fluor 700	HI30	Exclusion of leucocytes
DNA	Syto 16		DNA marker
CD31	Alexa Fluor 647	M89D3	
CD34	ECD	581	Endothelial cell marker
CD146	PE	P1H12	
CD117	PE-Cy7	104D2	Progenitor marker
CD106	PE-Cy5	51-10C9	Activation marker

APC - allophycocyanin, Cy - cyanine, ECD - energy-coupling dye, FITC - fluorescein isothiocyanate, NiRed - LIVE/DEAD fixable Near - infrared dead cells stain, PE - R-phycoerythrin.

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