

Identification of endothelial cells in peripheral blood. Principles of rare events analysis by flow cytometry.

Kideryová Linda, Pytlík Robert, Veselá Romana,
Rychtrmocová Hana

Laboratory of Experimental Cell Therapy
General Teaching Hospital and First Faculty of Medicine

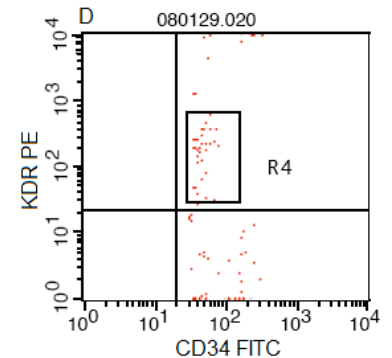
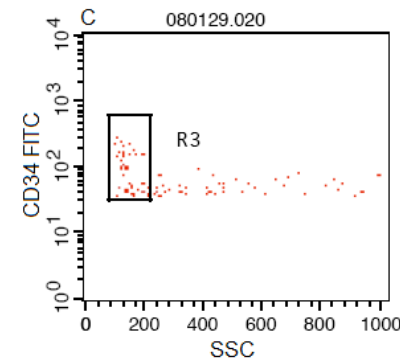
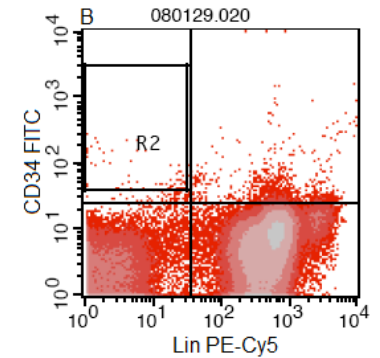
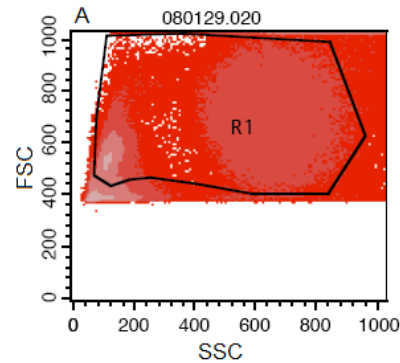
Charles University

Circulating Endothelial Cells (EPC) and matured Endothelial Cells (CEC)

Diagnostical and prognostical potential

Microvascular disorders

Cancer load



Pitfalls of FACS analysis

1. EPCs and CECs are extremely rare events (0,1 - 0,0001%)
2. Absence of standardized protocol
3. Lack of relevant markers

Journal of Immunological Methods 332 (2008) 31–40

Research paper

Quantification of circulating endothelial progenitor cells: A methodological comparison of six flow cytometric approaches

Emeline M.F. Van Craenenbroeck ^{a,*}, Viviane M.A. Conraads ^a, Dirk R. Van Bockstaele ^b,
Steven E. Haine ^a, Katrien Vermeulen ^b, Viggo F. Van Tendeloo ^b,
Christiaan J. Vrints ^a, Vicky Y. Hoymans ^a

Sample proceeding

Unspecific binding via Fc gamma receptors has been reduced by incubation with FcR blocking reagent (Miltenyi, Biotec).

Erythrocytes has been lysed with ammonium chloride based lysing solution (self prepared, **BD FACS Lysing Solution**, Dako Uti-lyse

Markers

Lineage specific Dump channel PE-Cy5

CD45 X CD3 + CD19 + CD14 + CD15

EPCs:

CD34 FITC/CD133 PE/ Lin PE-Cy5

CD34 FITC/VEGFR2 (KDR) PE/ Lin PE-Cy5

CECs:

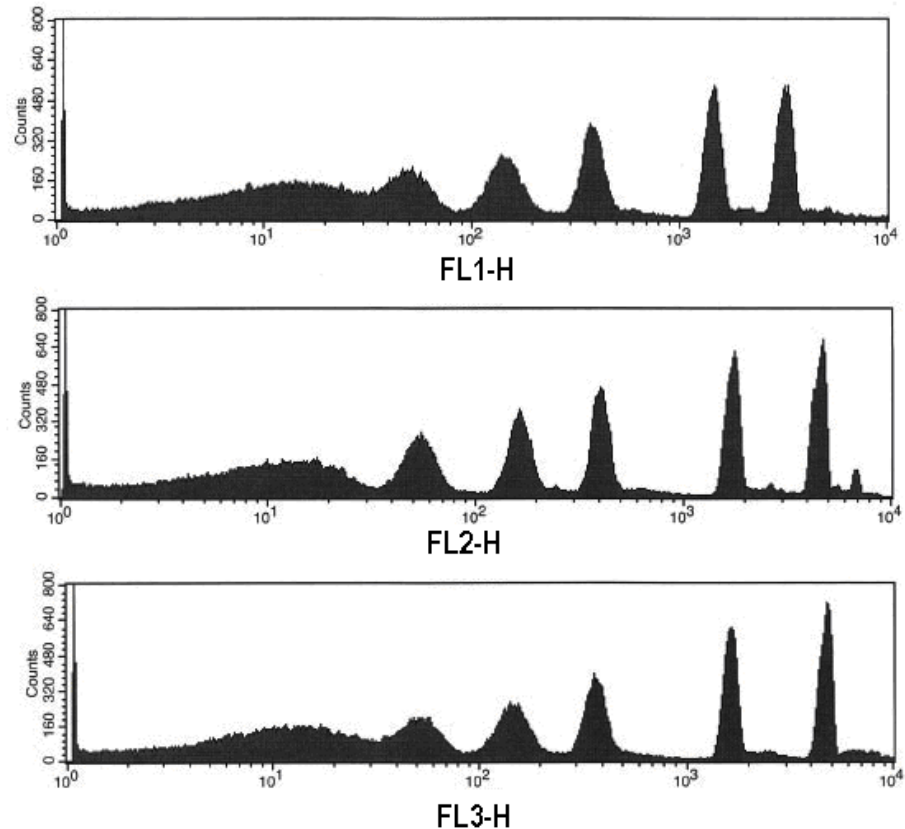
CD146 FITC/CD31PE/Lin PE-Cy5

Instrument set-up and Acquisition

FACSCalibur
(argon laser 488nm)

For PMT set-up has been
used Fluorospheres 8
Peaks

At least 500.000 events in
nucleated cells gate
should be acquired

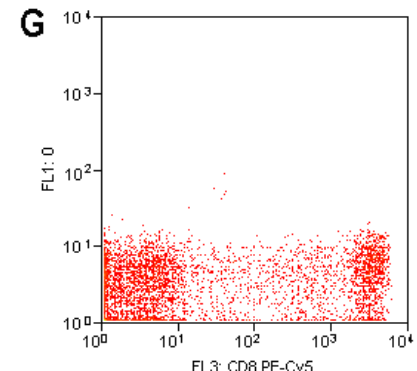
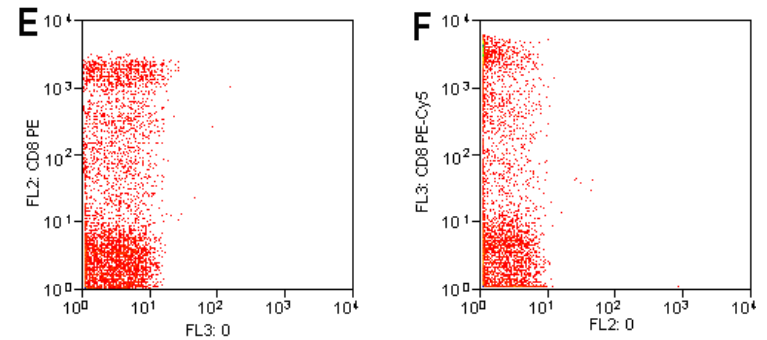
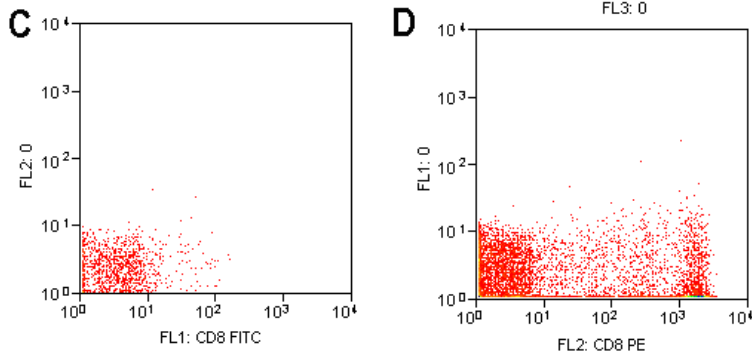
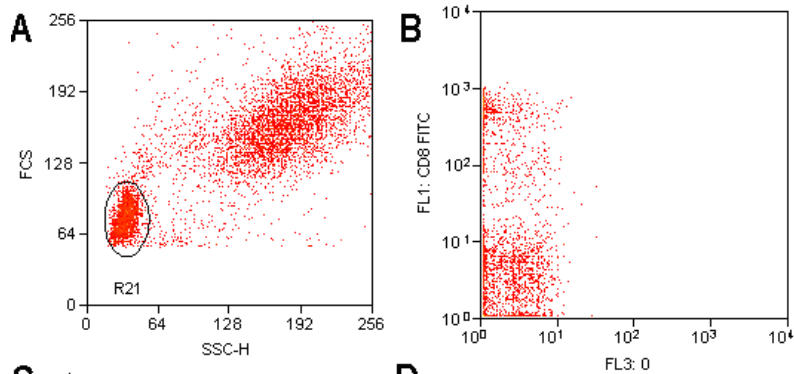


Compensation

MNC: singles

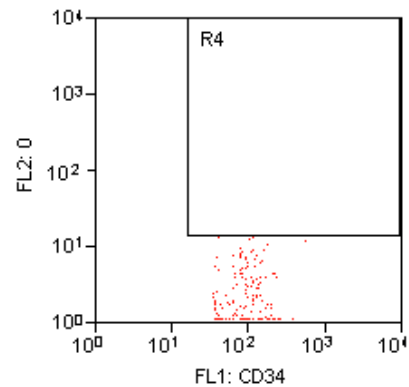
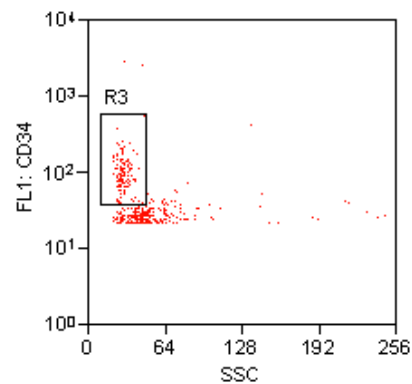
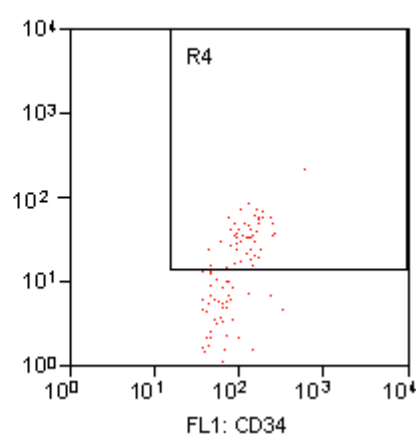
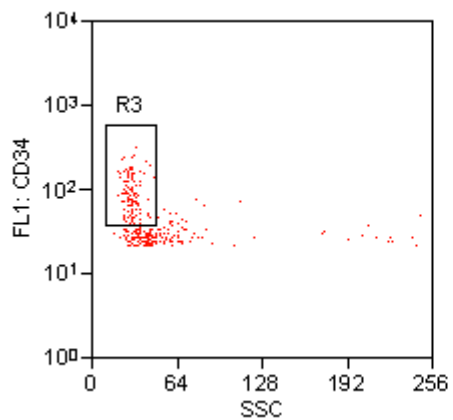
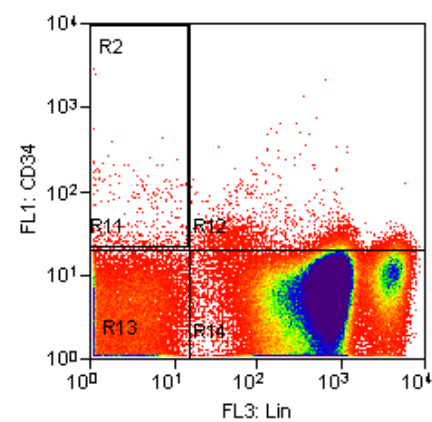
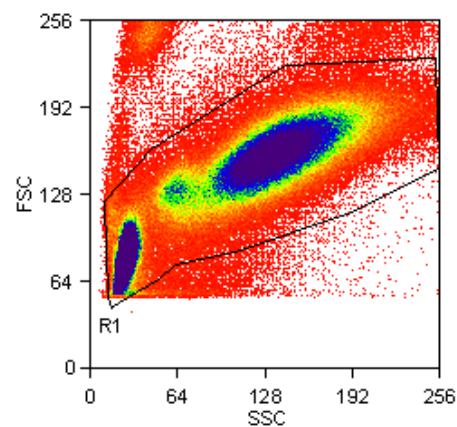
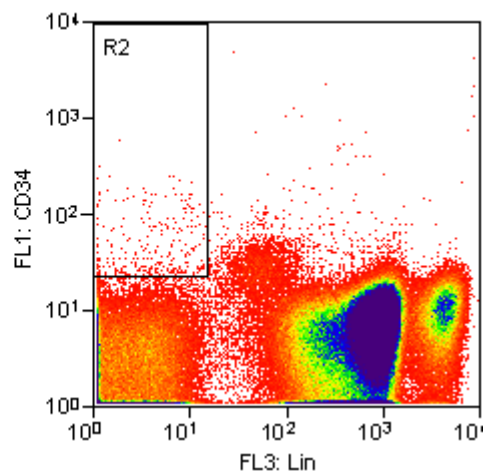
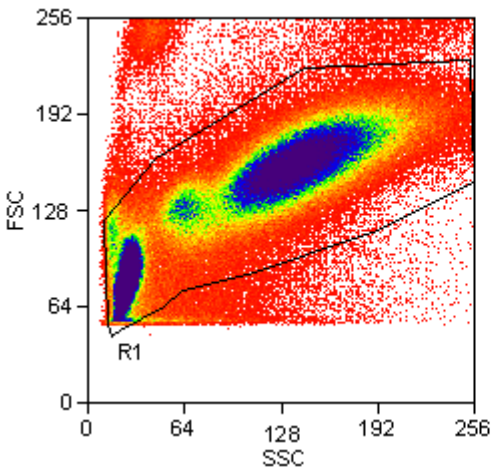
CD8FITC, CD8PE, CD8PE-Cy5

<http://www.drmmr.com/>

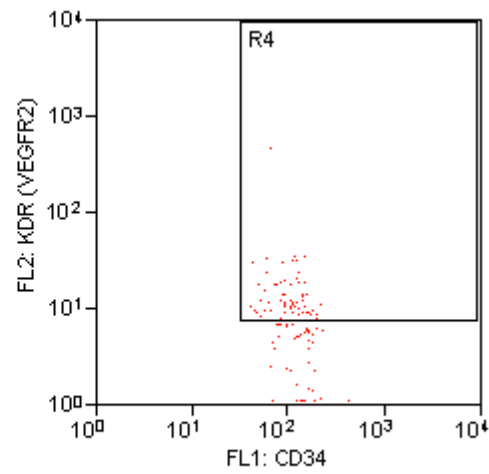
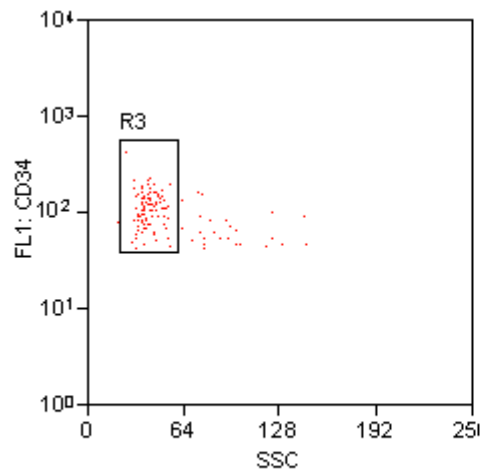
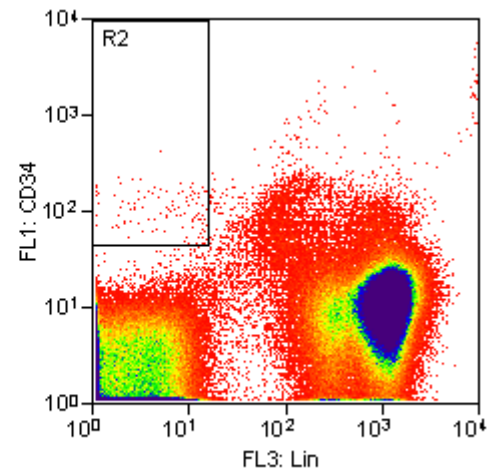
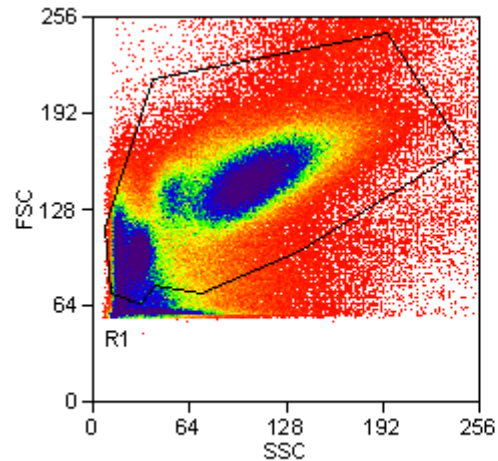


Gating Strategy

CD34 FITC/CD133 PE/Lin PE-Cy5

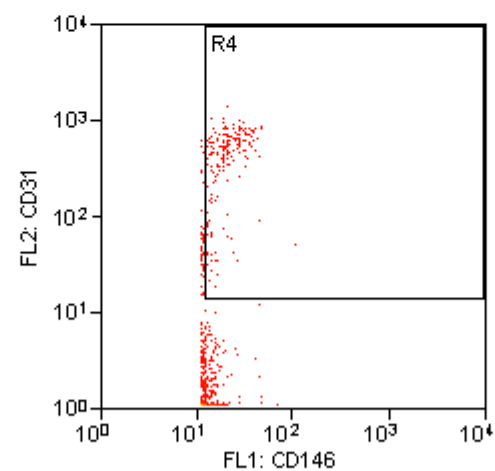
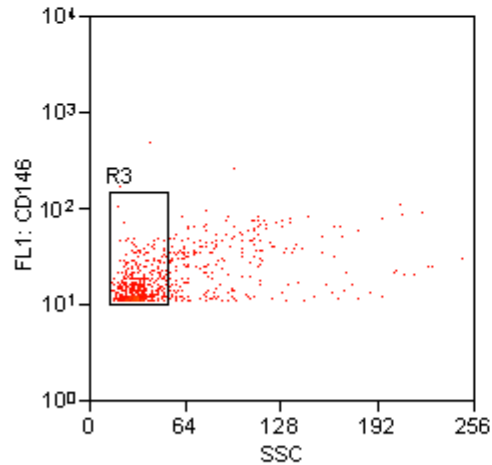
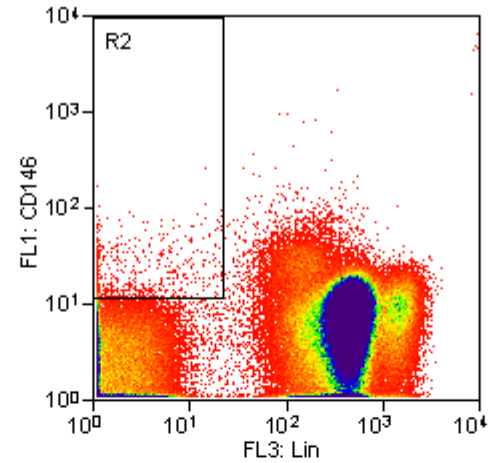
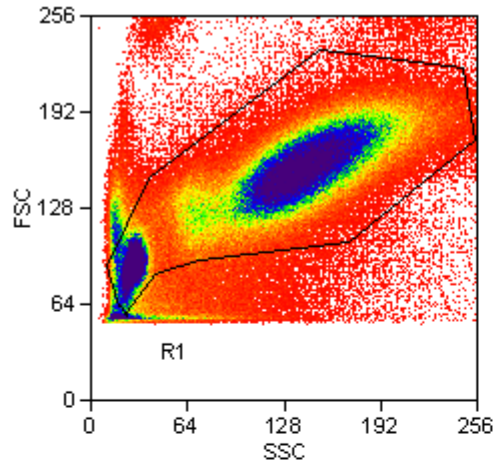


Gating Strategy CD34/VEGFR2/Lin



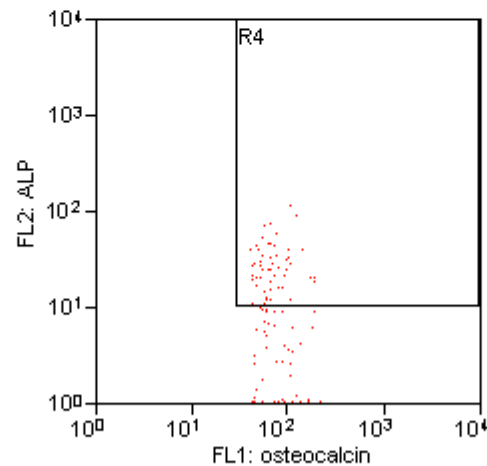
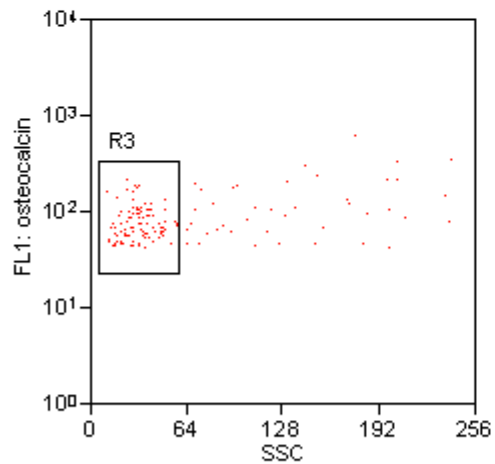
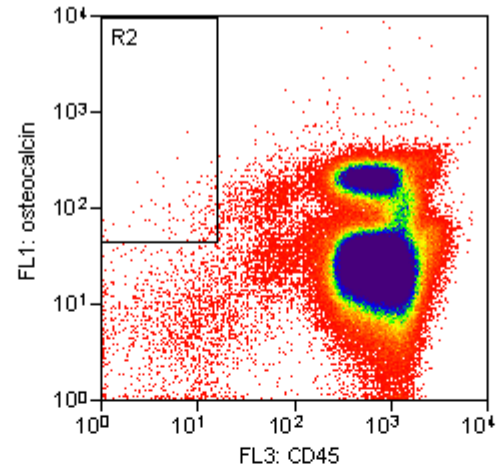
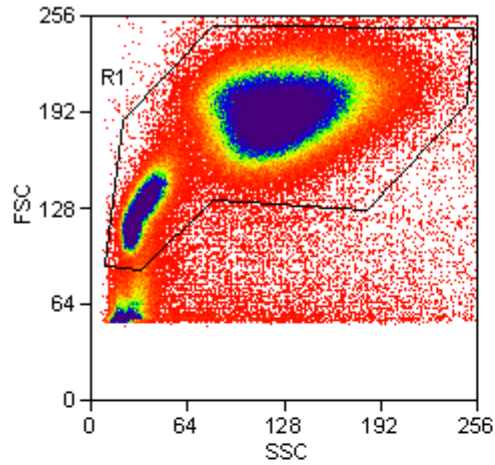
Gating Strategy

CD146 FITC/CD31 PE /Lin PE-Cy5



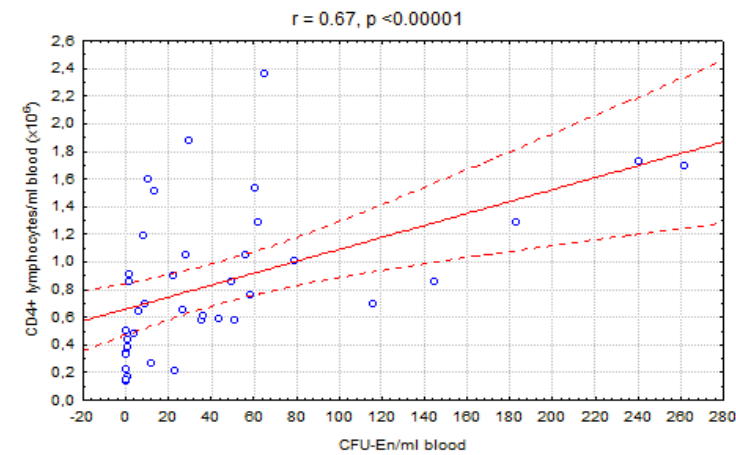
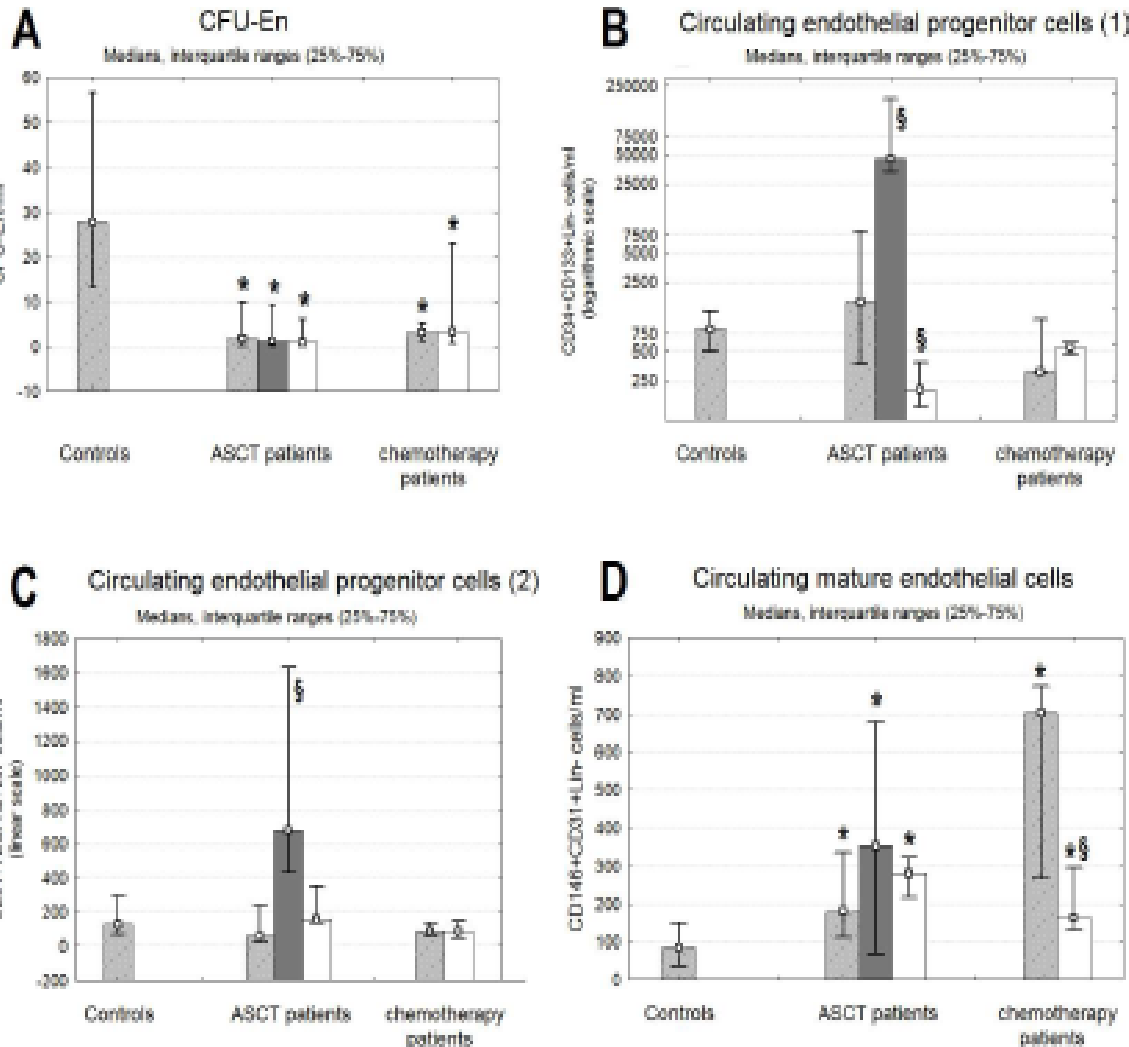
Gating Strategy

osteocalcin FITC/ALP PE/CD45 PE-Cy5



ENDOTHELIAL CELLS (EC) AND ENDOTHELIAL PRECURSOR CELLS (EPC) KINETICS IN HEMATOLOGICAL PATIENTS UNDERGOING CHEMOTHERAPY OR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

Kideryová Linda¹, Pytlík Robert¹, Benešová Kateřina², Veselá Romana¹, Karban Josef¹, Rychtmocová Hana¹, Goettsch Winfried³, Morawietz Henning³, and Trněný Marek¹

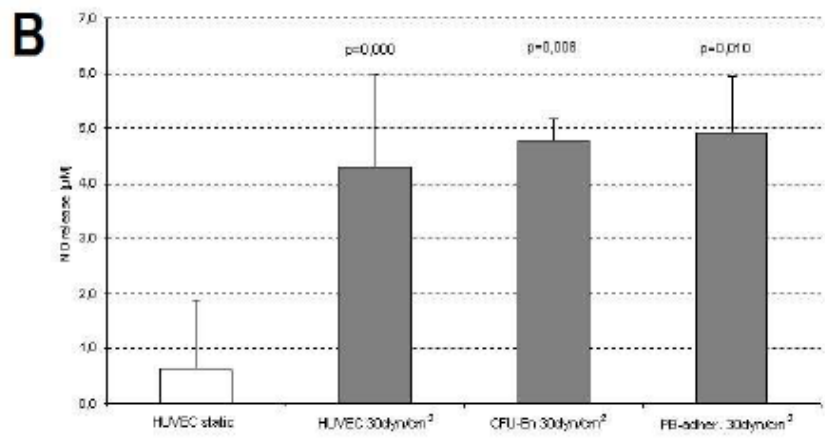
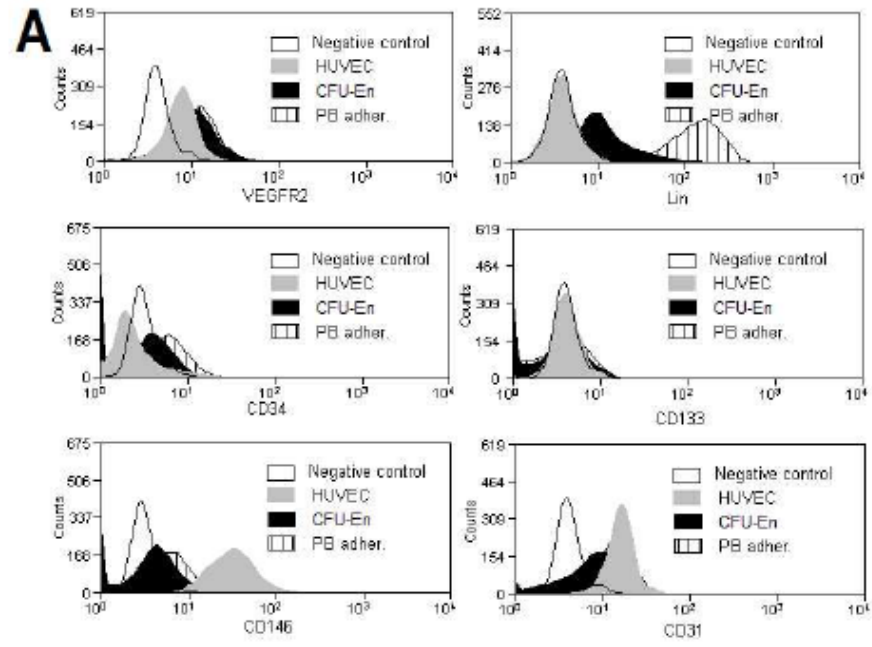
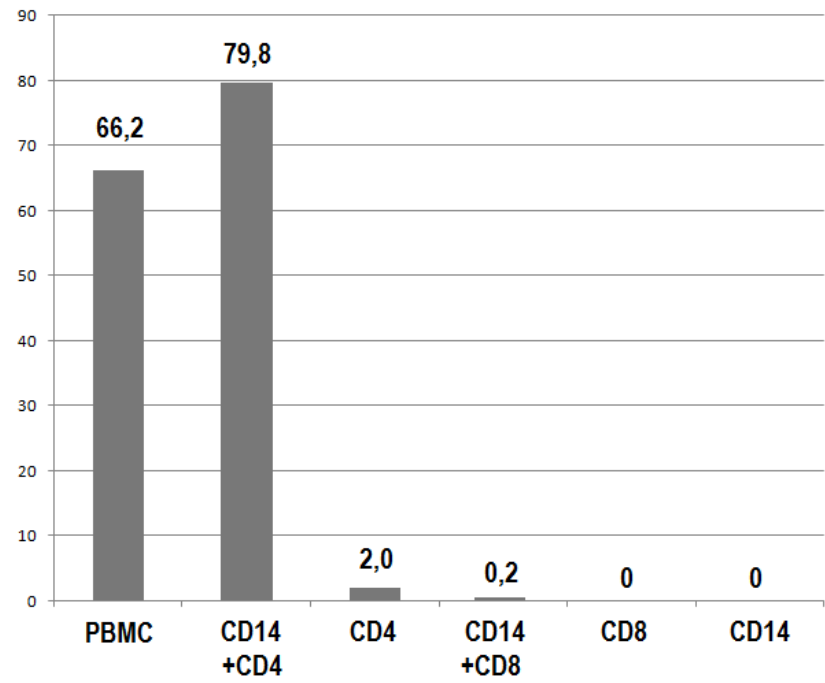


ENDOTHELIAL CELLS (EC) AND ENDOTHELIAL PRECURSOR CELLS (EPC) KINETICS IN HEMATOLOGICAL PATIENTS UNDERGOING CHEMOTHERAPY OR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

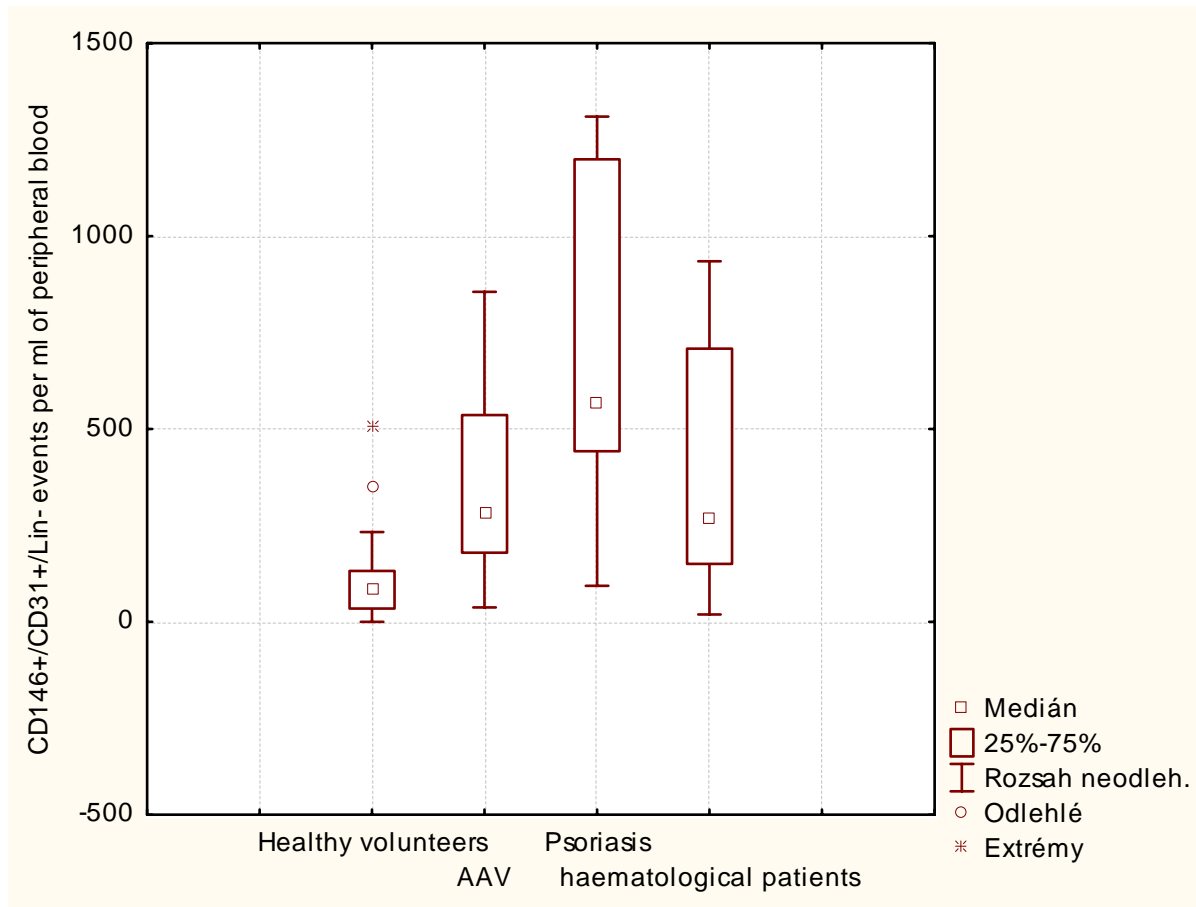
Kideryová Linda¹, Pytlík Robert¹, Benešová Kateřina², Veselá Romana¹, Karban Josef¹, Rychtrmccová Hana¹, Goettsch Winfried³, Morawietz Henning³, and Trněný Marek¹

Hematological Oncology

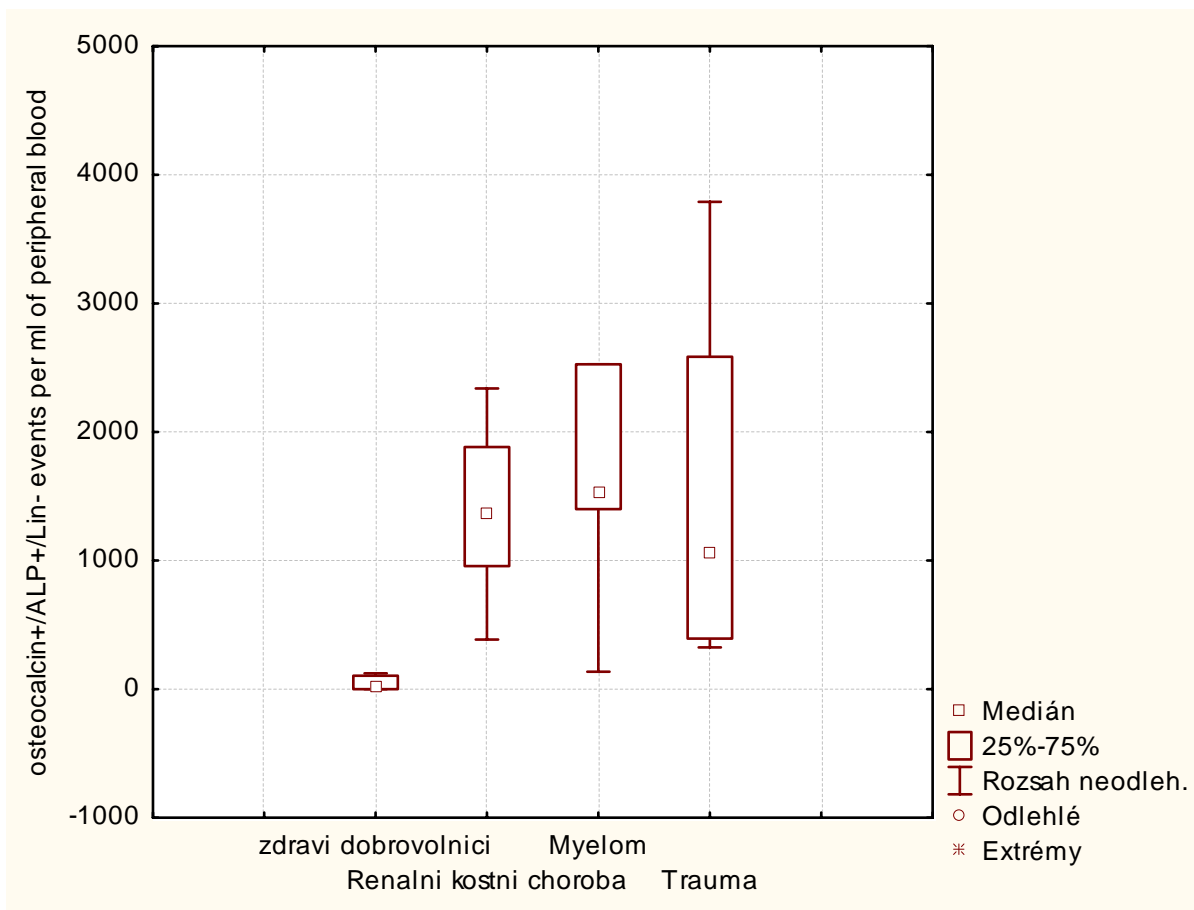
CFU-En/ml peripheral blood



Numbers of CECs were significantly higher in all groups of patients in compare to healthy volunteers



Numbers of circulating osteoblasts were significantly higher in all groups of patients in compare to healthy volunteers



Summary

We have established a reproducible, high sensitive method for rare nonhaematological events detection, namely CECs, EPCs and osteoblasts

Sample proceeding

Markers + Lin

Instrument set-up

Compensation

Acquisition

Gating Strategy

This method were applied to patients with disorders of microcirculation, haematological malignities, psoriasis, metabolic bone disease or trauma

A large group of cartoon blue characters with glasses, smiling and waving, set against a dark blue background with a large moon. The characters are of various sizes and are scattered throughout the scene, creating a sense of a large gathering or celebration. The overall mood is cheerful and friendly.

Thank you for attention