Identification of common EV markers in plasma using high-resolution flow cytometry

Askeland, Anders; Botha, Jaco; Rasmussen, Rikke Wehner; Handberg, Aase

Published in:
Journal of Extracellular Vesicles

DOI (link to publication from Publisher):
10.1080/20013078.2019.1593587

Publication date:
2019

Document Version
Publisher's PDF, also known as Version of record

Journal of Extracellular Vesicles

ISSN: (Print) 2001-3078 (Online) Journal homepage: https://www.tandfonline.com/loi/zjev20

ISEV2019 Abstract Book

To cite this article: (2019) ISEV2019 Abstract Book, Journal of Extracellular Vesicles, 8:sup1, 1593587, DOI: 10.1080/20013078.2019.1593587

To link to this article: https://doi.org/10.1080/20013078.2019.1593587

Published online: 23 Apr 2019.

Oral with Poster Session 2
Chairs: Kazunari Akiyoshi; Muller Fabbri
Location: Level B1, Lecture Room

13:30–15:00

OWP2.01=PS08.08

Identification of common EV markers in plasma using high-resolution flow cytometry

Anders Askeland[a], Jaco Botha[b], Rikke Wehner Rasmussen[b] and Aase Handberg[b]

[a]Aalborg University Hospital, Aalborg, Denmark; [b]Department of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark

Introduction: Recent advancements in flow cytometry (FCM) have led to the development of high-resolution FCMs dedicated to the analysis of small particles (hFCM). hFCM studies have predominantly focused on the analysis of EVs expressing phosphatidylserine (PS). PS is enriched in microvesicles (MVs), wherein it is involved in lipid rearrangements responsible for MV budding. While PS also is expressed on exosomes, it is unknown whether it can be used as a universal marker for smaller EVs. In this study, we attempted to characterize proteins enriched in smaller EVs (CD9, CD63, CD81 and ADAM 10) and the relative co-expression of PS with each of these markers.
Methods: FCM analysis was performed on an Apogee A60 Micro-PLUS. In brief, platelet-poor plasma (PPP) from healthy individuals was stained with lactadherin-FITC (PS+) and one of several EV surface markers enriched in smaller EVs. To evaluate the precise differences in PS and specific EV marker expression, the analysis was performed twice, (1) triggering on lactadherin and (2) each EV marker (CD9-PE, CD81-PE, CD63-PE, ADAM10-PE), separately. All antibodies were matched with appropriate isotope controls and centrifuged at 17,000*g* for 10 min prior to antibody labelling. EVs were defined as lactadherin or EV surface marker positive events ≤1000 nm.
Results: Initial results indicate that CD9 is highly expressed on EVs and is not universally associated to PS. Triggering on PS revealed that 34.7% of all events were CD9 positive (CD9+|PS+). Conversely, triggering on CD9 resulted in a 2.1-fold increase in total events, where 17.0% of events were PS+ (CD9+|PS+). Inferring size from silica nanospheres, it appeared that populations containing CD9 (CD9+|PS+ and CD9+|PS-) were smaller (94.4–99.7% <180 nm) compared to populations that did not (PS+|CD9-; 85.6% <180 nm & 95.2% <300 nm). Interestingly, we did not detect CD81, CD63 or ADAM10 on EVs. We hypothesize that this is due to a low abundance of these markers in PPP from healthy individuals.
Summary/Conclusion: Our findings demonstrate that hFCM can be used for the characterization of smaller EVs in PPP. Furthermore, we find that CD9+EVs do not universally express PS. From this point on, we plan to study enrichment of these EV phenotypes following a number of EV purification protocols, and determine whether EV isolation enable a more extensive characterization of smaller EVs.

OWP2.02=PS08.09

Software to automate calibration and processing of flow cytometry data in clinical studies

Edwin van der Pol[a], Frank Coumans[b], Leonie de Rond[c], Aleksandra Gasecka[d], Najat Hajji[e], Rienk Nieuwland[b] and Ton van Leeuwen[f]

[a]Amsterdam UMC, University of Amsterdam, Department of Biomedical Engineering and Physics, Amsterdam, Netherlands; [b]Amsterdam UMC, University of Amsterdam, Laboratory of Experimental Clinical Chemistry, Amsterdam, Netherlands; [c]Amsterdam University Medical Centers, Amsterdam, USA; [d]1st Chair and Department of Cardiology, Medical University of Warsaw, Warsaw, Poland; [e]Amsterdam University Medical Centers, Amsterdam, Netherlands; [f]dAmsterdam UMC, University of Amsterdam, Department of Biomedical Engineering and Physics, Amsterdam, Netherlands

Introduction: In search of new biomarkers, flow cytometers are used in clinical studies to measure the concentration of specific extracellular vesicles (EVs). Flow cytometers measure light scattering and fluorescence of single EVs in a fluid stream. However, to realize data interpretation and comparison, light scattering and fluorescence signals and the flow rate require calibration. Moreover, flow cytometers generate large datasets. For example, a clinical study involving 60 patients, 30 controls, and 8 antibody labels covers 1224 data files, >33 gigabytes of data and >0.3 billion events. To manually calibrate and analyse such a dataset would take days if not weeks and is prone to human mistakes. Therefore, an urgent need exists for software to automate calibration and processing of flow cytometry data.
Methods: We have developed software (MATLAB R2018a) to automatically process multiple .fcs files and (1) relate two scatter signals to the diameter in nm and refractive index (RI) of EVs, (2) express