Sensitive detection of platelet-derived and tissue factor positive extracellular vesicles in plasma using solid-phase proximity ligation assay

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Background
Extracellular vesicles (EVs) derived from various cell-types are promising biomarkers for various diseases. They constitute a heterogeneous population ranging from 0.1 to 1 µm in size. Due to their small size and low expression levels, they are difficult to measure accurately with flow cytometry.

Aim
To design a high-sensitive assay for platelet-derived and tissue factor (TF) positive EVs in plasma based on dual antibody recognition and the solid-phase proximity ligation assay (SP-PLA) technique.

Method
Lactadherin (binds to phosphatidylserine) and cholera toxin subunit B (CT-B, binds to ganglioside) were used to capture different types of EVs. Detection antibodies were conjugated with either of two oligonucleotides that are ligated and serve as a template for a qPCR reaction when antibodies are bound in proximity. We compared the results with high-sensitivity flow cytometry (platelet-derived) and a direct coagulation activity assay (TF-positive EVs).

Results
We demonstrate that platelet-derived EVs in plasma can be measured using SP-PLA with high sensitivity and specificity. The results correlate with high-sensitivity flow cytometry (Pearson $R= 0.65$ p$<0.0001$), with the difference that SP-PLA detects EVs also after filtration with a 0.2 µm filter, showing that also smaller EVs are detected. The assay, using CT-B or lactadherin as capture agents, furthermore allowed detection of the more rare population of TF+ EVs. Upon spike-in with relipidated TF, the SP-PLA for TF+EVs captured by lactadherin had a detection range of at least 4 logs and a Limit of Detection (LoD) score of 0.5 pg/ml.

Conclusions
These results demonstrate that different populations of cell-derived EVs can be detected sensitively with SP-PLA and that also the smaller EVs can be measured accurately. The SP-PLA technique is thereby a suitable tool for measuring EVs in plasma, and may prove useful when studying the role of EVs in several complex diseases.