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REVIEW ARTICLE

Recent developments in the nomenclature, presence, isolation, detection and clinical impact of extracellular vesicles

E. VAN DER POL,*† A. N. BÖING,* E. L. GOOL*† and R. NIEUWLAND*
*Laboratory of Experimental Clinical Chemistry; and †Department of Biomedical Engineering and Physics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

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Summary. The research field of extracellular vesicles (EVs), such as microparticles and exosomes, is growing exponentially. The goal of this review is to provide an overview of recent developments relevant to the readers of the *Journal of Thrombosis and Haemostasis*. We will discuss nomenclature, the presence of EVs in fluids, methods of isolation and detection, and emerging clinical implications. Although research on EVs has been performed within the ISTH for over a decade, most of the recent research on EVs has been brought together by the International Society on Extracellular Vesicles (ISEV). To achieve an overview of recent developments, the information provided in this review comes not only from publications, but also from latest meetings of the ISEV (April 2015, Washington, DC, USA), the International Society on Advancement of Cytometry (June 2015, Glasgow, UK), and the ISTH (June 2015, Toronto, Canada).

Keywords: cell-derived microparticles; exosomes; flow cytometry; limit of detection; secretory vesicles; terminology.

Introduction

Cells release extracellular vesicles (EVs), such as microparticles and exosomes, into blood. Because the concentration and composition of EVs change with disease, EVs have potential clinical applications, including the diagnosis of thrombosis [1]. However, the clinical applications of EV have not yet been realized, because EV detection and isolation are challenging. The currently used detection techniques lack the sensitivity to detect the majority of EVs, because EVs are: (i) 30–1000 nm in diameter; (ii) heterogeneous; and (iii) optically dim [2–5]. Isolation of EVs is difficult, owing to the biological complexity of body fluids, which often contain particles within the size range of EVs [6]. The inter-related difficulties in EV detection and isolation explain the differences in nomenclature and contradictions in clinical insights. Here, we will summarize recent developments in the nomenclature, presence, isolation, detection and clinical implications of EVs relevant to the readers of the *Journal of Thrombosis and Haemostasis*.

Correspondence: Edwin van der Pol, Department of Biomedical Engineering and Physics, Academic Medical Center of the University of Amsterdam, Meibergdreef 9, PO Box 22660, 1100 DD Amsterdam, the Netherlands.
Tel.: +31 20 5664386; fax: +31 20 5669569.
E-mail: e.vanderpol@amc.uva.nl

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Nomenclature

EV is an umbrella term for all types of vesicle released from prokaryotic and eukaryotic cells. The most well-known types of EV are probably microparticles and exosomes, but many more types of EV have been classified on the basis of various criteria. Figure 1 shows a list of common EV terms. As this plethora of terms is based on different criteria, *in vitro* studies, and outdated isolation and detection techniques, the single term 'EV' was introduced to avoid confusion and to improve the exchange of information between investigators and societies.

New information obtained with rapidly improving isolation and detection techniques has led to a consensus on the existence of several cellular pathways for the production and release of different EV types. New technology also shows, however, that these different EV types show more similarities than previously expected. For example, the term 'exosomes' is still commonly used to describe small EVs secreted by eukaryotic cells when membranes of multivesicular endosomes fuse with the plasma membrane. Throughout the literature, exosomes are reported to have a diameter between 30 nm and 100 nm, and exosomes are supposed to have a characteristic subset of proteins, including tetraspanins such as CD63 and CD81.

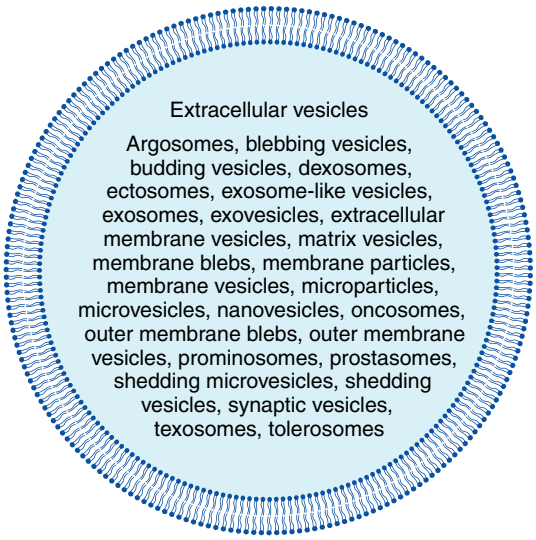

Fig. 1. Nomenclature of extracellular vesicles (EVs). Throughout the literature and in different medical and scientific societies, different terms are used for EVs. For example, the term 'microparticles' is commonly used within the ISTH, but not within the International Society on Extracellular Vesicles. Because many previously introduced identification criteria have proven either false or not useful, we propose use of the term 'extracellular vesicles' to bring ISTH research on EVs in line with other societies.

However, at the latest International Society on Extracellular Vesicles (ISEV) meetings: (i) EVs indistinguishable from exosomes were shown to be released directly from the plasma membrane; (ii) diameters of exosomes up to 250 nm were reported; and (iii) proteins such as tetraspanins were shown not to be unique to exosomes (C. Théry, ISEV Meeting 2015, Washington, DC, USA). In summary, identification criteria have led to confusion rather than consensus, and it is strongly recommended that the term 'EV' be used also within the ISTH.

Presence

Thus far, most studies have focused on the presence of eukaryotic EVs in human body fluids. However, there are also > 500 scientific publications on EVs released by bacteria. Until 2014, it was unknown that ocean water contains high concentrations of EVs from bacteria, which are thought to protect these bacteria against the abundantly present bacteriophages by acting as decoys [7]. These bacterial EVs facilitate communication between bacteria by transferring their biomolecules, such as messenger molecules and genetic information [1]. Because bacterial EVs can exchange biomolecules with eukaryotic cells, such EVs may contribute to inter-kingdom communication [8,9]. Given the growing interest in the role of endogenous bacteria in human health and disease, novel roles of bacterial EVs may have to be explored.

In addition, beverages were recently shown to contain EVs. For example, beer contains high concentrations of yeast-derived and thus eukaryotic EVs [10]. Thus, EVs are common and widely distributed, indicating that EVs underlie conserved biological mechanisms rather than being an epiphenomenon.

Isolation and purification

To explain the latest insights into isolation, purification, and detection, a measured size distribution of EVs will be used. Figure 2 shows the concentration versus diameter of EVs from human plasma or urine fitted by a power-law function [3]. Figure 2 also shows that other plasma particles, such as lipoprotein particles, overlap in size with EVs. Moreover, plasma has high density and viscosity [11–14], which, together with the lack of a decent isolation method, has led to: (i) numerous published artefacts; (ii) overinterpretation and extrapolation of unreliable results; and (iii) non-comparable measurement results between laboratories. In the following sections, recent progress made in EV isolation will be discussed.

Isolation by centrifugation

In most studies, EVs are separated from cells and isolated by differential centrifugation [15–17]. In this procedure, cells are removed by centrifugal accelerations of typically 200–1500 $\times$ *g* to prepare cell-free supernatant containing EVs. Figure 2 shows that platelets and large EVs, such as oncosomes, overlap in size, and may therefore be removed during this first centrifugation step. Because EVs may also bind to cells or be phagocytosed, the removal of cells may also result in the removal of EV (sub)populations. Consequently, perfect separation of EVs from cells in body fluids, particularly blood, will never become feasible. Not only cells, but also other 'membrane structures', are difficult to separate completely from EVs by centrifugation. For example, Brisson *et al.* recently prepared platelet-depleted plasma according to the ISTH-developed criteria, but they still encountered large membrane structures up to 8–9 μm [2].

In most protocols, EVs are subjected to subsequent high-speed centrifugation and ultracentrifugation steps to concentrate and pellet EVs. Evidence is now available that high-speed centrifugation of plasma induces aggregation of platelet-derived EVs, whereas EVs of erythrocyte origin have less tendency to aggregate [18,19]. Figure 2 shows that the concentration of EVs decreases because of centrifugation losses and aggregation of EV (sub)populations. Sensitive single-particle detection devices, such as tunable resistive pulse sensing (TRPS) and nanoparticle tracking analysis (NTA), therefore detect a lower EV concentration than expected. However, insensitive particle detection devices, such as standard flow cytometers, will detect these larger aggregates, and therefore detect a higher particle concentration. Thus, although differential centrifugation protocols can be easily applied, the results

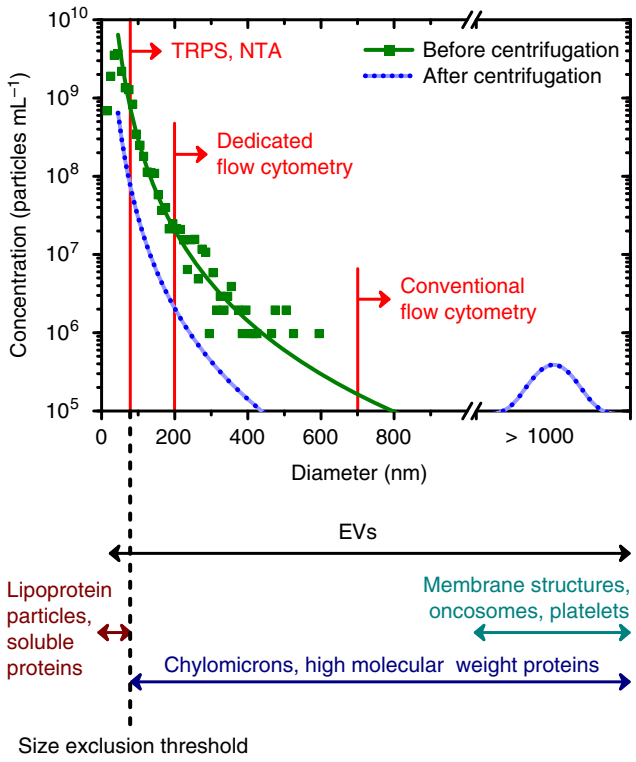

Fig. 2. Measured concentration versus diameter for extracellular vesicles (EVs) from human cell-free urine fitted by a power-law function (solid curve) [3]. The data were obtained with tunable resistive pulse sensing (TRPS) and transmission electron microscopy without high-speed centrifugation, and are indicative for particles in plasma. The vertical lines show the smallest particles detectable by conventional flow cytometers, flow cytometers dedicated to the detection of submicrometer particles, TRPS, and nanoparticle tracking analysis (NTA). The dashed curve indicates the effect of high-speed centrifugation on the concentration of platelet microparticles [19]. After centrifugation, the concentration of relatively small EVs (as detected by TRPS and NTA) is reduced by losses and aggregate formation. In turn, owing to the formation of aggregates, the concentration of particles of > 1000 nm (as detected by conventional flow cytometry) is increased. The arrows below the plot indicate the size range of EVs, lipoprotein particles, soluble proteins, membrane structures, oncosomes, platelets, chylomicrons and high molecular weight proteins relative to the size exclusion threshold of size-exclusion chromatography (dotted vertical line) with a Sepharose CL-2B column [27].

obtained may be unreliable and not easy to interpret. Some isolation problems can be overcome by diluting the plasma in saline or phosphate-buffered saline prior to centrifugation. This procedure was reported in 1967, and its effectiveness for EV isolation was confirmed recently [12,20]. Owing to dilution, both the viscosity and the density of plasma decrease, thereby increasing the efficacy of EV isolation or depletion at lower centrifugal accelerations.

A different approach to isolate EVs is the use of density gradient ultracentrifugation [14,21–23]. Apart from the involved time and requirements, the applied gradients are hyperosmotic [24], and EVs are subjected to extreme *g*-forces, leading to disruption and loss of biological activity (M. Wauben, ISEV Meeting 2013, Budapest, Hungary). Moreover, when plasma is used as a starting material, the isolation of EVs leads to coisolation of HDL, because EVs and HDL have similar densities [13,14]. This coisolation has led to confusion regarding the contributions of EVs and HDL as carriers of microRNA (miRNA) in human plasma [6,25,26]. Whereas HDL contains miRNA after removal of plasma EVs [25], the presence of miRNA in plasma EVs after removal of HDL remains controversial (see also 'Separation of EVs from miRNA by SEC').

Isolation by size-exclusion chromatography (SEC)

In 2014, the METVES project rediscovered SEC for the isolation of EVs from plasma and other biological fluids [27–29]. At present, over 170 research groups are testing or using SEC, because this method: (i) is relatively easy and fast; (ii) has an EV recovery rate up to 80%; and (iii) perhaps most importantly, results in the almost complete removal of soluble proteins and most types of lipoprotein from plasma [27–29]. When SEC is applied to isolate EVs from plasma, the isolated EVs remain biologically active and are sufficiently pure to be used as the starting material for applications such as electron microscopy, proteomics, and small-angle X-ray scattering. An instruction movie of our SEC protocol is available at www.metves.eu. This protocol utilizes a stationary-phase material that separates particles of < 75 nm from larger particles, but other materials are available to match the separation size to the needs of the user.

Although Fig. 2 shows that EVs cannot be separated from other plasma particles by size, a size exclusion threshold of ~ 75 nm is advantageous for EV isolation. At this threshold, the eluate fraction of particles of < 75 nm will contain the bulk of soluble proteins, HDL, LDL, the majority of VLDL, and EVs. As the concentration of soluble proteins and lipoprotein particles will be greater than that of EVs [30], and because single EVs in this size range cannot yet be detected in suspension [3], the eluate fraction is usually discarded. The void fraction of particles of > 75 nm then contains EVs that are enriched relative to soluble proteins and most types of lipoprotein. However, the void fraction may also still contain some VLDL particles, chylomicrons, and high molecular weight proteins, e.g. von Willebrand factor. Although the coelution of von Willebrand factor is difficult to avoid, coelution of, especially, chylomicrons can be limited by collection of blood from overnight fasting subjects. Table 1 summarizes the properties of size exclusion chromatography, density gradient centrifugation, and differential centrifugation.

The use and application of SEC to isolate EVs has led to new and important insights, which will be illustrated by two examples. The first example involves EV research, whereas the second example may even affect the

conclusions from previous studies on the composition and functional properties of platelets.

Separation of EVs from miRNA by SEC

Hitherto, > 800 articles have been published on the presence of miRNA in EVs. The great interest in miRNAs in EVs is attributable to the fact that some miRNAs may be useful for diagnosis, although one of the most studied miRNAs, miR-141, was recently shown not to be disease-specific [31]. In most miRNA studies, EVs have been isolated by ultracentrifugation. When EVs are isolated by SEC, most miRNAs elute in the fraction of < 75 nm, which also contains other carriers of miRNA, such as argonaute-2 protein and HDL (S. El Andaloussi, ISEV Meeting 2014, Rotterdam, The Netherlands) [32]. Consequently, it is tempting to speculate that, after ultracentrifugation, the pelleted EVs from plasma may be contaminated by other carriers of miRNA [25,33]. Moreover, miRNA in plasma of cancer patients was recently shown to remain soluble after ultracentrifuation, suggesting that no or hardly any plasma miRNA may be present in EVs. In fact, the main conclusion from this study was as follows: 'We propose revised models to reconcile the exosome-mediated, miRNA-based intercellular communication hypothesis with the observed stoichiometry of miRNA associated with exosomes' [34]. Together, these findings make it clear that the role of EVs as carriers of miRNA may be less prominent than previously expected.

Platelet isolation

Although SEC is very new in the field of EVs, it is precisely the same method as previously used to prepare 'gel-filtered' platelets. Because both platelets and EVs will elute in the void volume (Fig. 2), earlier studies on gel-filtered platelets are likely to have involved a mixture of platelets and EVs. In contrast, procedures for preparing 'washed' platelets involve centrifugation and washing, and, in these procedures, most EVs will be removed. To what extent the different platelet isolation protocols have affected the outcomes of earlier platelet studies is unclear, but ongoing discussions on the presence of tissue factor (TF) or mRNA in isolated platelets may be attributable to the presence or absence of EVs.

Detection and standardization

There are many new developments to be reported with regard to detection and standardization of EV measurements. Since 2010, detection methods with the capability of studying single particles of < 200 nm in suspension have become available, such as TRPS and NTA. Meanwhile, limitations of flow cytometry have been discovered, flow cytometers with improved sensitivity have become available, standardization of EV measurements has been initiated, and techniques are being combined to simultaneously study multiple physical properties of EVs [29,35,36].

Recent methods for detecting single EVs

TRPS and NTA are two recent methods that can measure the size distribution and concentration of particles in solution with < 10% measurement error, except for concentration measurements with NTA, which can have errors up to 70% [3]. Figure 2 shows that both methods are capable of detecting EVs with a diameter of 70–100 nm [3], and even smaller EVs may be detected with the latest technology and software. The combination of TRPS, NTA, transmission electron microscopy and flow cytometry has substantially improved our insights into the relationship between the concentration and size of EVs in biological fluids. For example, Fig. 2 shows that the concentration–size relationship follows a power-law function in normal human plasma and urine [3], meaning that small EVs with a diameter of < 100 nm outnumber larger EVs. Consequently, the detected EV concentration strongly depends on the smallest detectable EV. An important consequence is that future EV concentration measurements will require a statement of the lower detection limit of the applied method.

Apart from fundamental research, NTA and TRPS are of limited use for studying clinical samples. First, both methods are slow and laborious. For example, the analysis of 1000 particles typically takes ≥ 30 min for TRPS,

Table 1 Properties of the three most common methods for isolation of extracellular vesicles (EVs)

	Differential centrifugation	Density gradient centrifugation	Size-exclusion chromatograpy
Exposure to high viscosity and hyperosmolarity	No	Yes	No
Recovery	Insufficiently documented	Insufficiently documented	80% [27]
Risk of losing EV subpopulations	Yes	Unknown	No
Change or loss of biological activity	Insufficiently documented	Yes	No
Formation of protein aggregates	Yes	Unknown	No
Contamination by soluble proteins and lipoproteins	Yes	Yes	No*
Time of isolation	~ 2 h	6–48 h	< 30 min

*Contamination will depend on the stationary-phase material of the column. For example, with application of a Sepharose CL-2B column to plasma, the fractions with particles of > 75 nm will contain EVs, chylomicrons, and high molecular weight proteins.

10 min for NTA, and < 1 s for flow cytometry. Second, TRPS and NTA have limited options for identifying EV types and distinguishing EVs from other particles, such as lipoprotein particles. Novel NTA instrumentation has the ability to detect either fluorescence or scatter from a single EV. However, when EVs are stained with common labels, such as green fluorescent protein or antibodies conjugated with fluorescein isothiocyanate, phycoerythrin, or allophycocyanin, then the dye is bleached either before the EVs reach the field-of-view of the microscope or before the EVs can be tracked for a sufficient length of time for accurate size estimation. For comparison, flow cytometry can detect both fluorescence and scatter from a single EV and does not have bleaching problems, because data acquisition is performed within ~ 1 μs, which is shorter than the time required to bleach the dye. Preferably, EV identification should be performed with a label-free technique, which is currently in the research phase [37]. Consequently, fluorescence detection on multiple channels remains the only proven technology, but this technology is not yet supported by TRPS and NTA.

Flow cytometry

Flow cytometry, on the other hand, can provide biochemical information on the cellular origin of single EVs by measuring multiplex fluorescence at a rate of tens of thousands of particles and EVs per minute. The high speed and practicality make flow cytometry suitable for clinical research applications. It is therefore not surprising that most laboratories use flow cytometry to study EVs.

Swarm detection Most of the flow cytometers in use have been developed to measure cells, and not EVs. A major complication was discovered in 2012; swarm detection [38,39]. Because single EVs are small and the concentration of EVs is high as compared with cells, multiple EVs will be present in the laser beam of the flow cytometer. Whereas the scatter or fluorescence signal of a single EV in the laser beam would be too low to exceed the detection threshold, multiple EVs together in the laser beam will generate a detectable signal. Swarm detection can be prevented by diluting the sample, which decreases the EV concentration and thus decreases the probability that multiple EVs in the laser beam will generate a detectable signal.

Standardization Because flow cytometers differ in optical configuration and sensitivity, and because data are expressed in arbitrary units, data interpretation and data comparison between laboratories is not straightforward. Attempts to solve the problems involved in flow cytometry standardization have not succeeded so far. For example, setting size gates with polystyrene beads did not substantially improve standardization [40], because the refractive index of polystyrene beads (1.61) differs from that of EVs (< 1.40). This difference in refractive index leads to a > 10-fold difference in the amount of light scattered from similar-sized particles [4].

Because the Scientific Standardization Committee on Vascular Biology of the ISTH is aware of the above-mentioned problems, a new initiative to standardize EV measurements was started in 2014. Metrology-certified reference materials and biological samples were distributed to 33 laboratories worldwide. To correct for differences in optical configurations of the flow cytometers used, and to correct for differences in light scattering between reference materials and EVs, dedicated software has been developed (available at www.exometry.com). The software is based on Mie theory and numerical optimization algorithms, but is nonetheless easy to use. Although all participating laboratories have a track record for detection of EVs by flow cytometry, one in three instruments was unable to detect EVs of < 1 μm. Furthermore, the flow cytometry performance was strongly user-dependent. The good news, however, is that new flow cytometers have increased sensitivity as compared with the older instruments. Some new flow cytometers are capable of detecting single 100-nm polystyrene beads, which are comparable to EVs of ~ 180 nm after correction for the difference in refractive index. The results of the standardization study will be submitted soon.

Fluorescence triggering Recently, several groups have shown that the sensitivity of flow cytometers can be increased by triggering on fluorescence [2,3,23,41,42]. Clearly, fluorescence triggering is a valid approach, although it is hampered by two problems. First, there is no generic label for all types of EV. Although lipid dyes are promising candidates, the unbound dyes form micelles, which have to be removed by sucrose gradient ultracentrifugation before detection [23]. Moreover, lipid dyes are non-specific, because they also stain lipoprotein particles, which are abundant in plasma samples.

Second, detection of fluorescently labeled EVs is difficult to standardize. Preferably, standardization should be based on the number of epitopes, but the relationship between the detected fluorescence signal and the number of epitopes is complex. A possible solution is to trigger on fluorescence and standardize on light scattering, but this requires the light scattering signal to exceed the detection threshold.

Future perspectives on EV detection by flow cytometry It is to be expected that, in the coming years, the sensitivity of flow cytometers will increase further, and that the comparison of measurement results between instruments and institutes will become feasible. Obviously, data comparison is a prerequisite for exploring the full potential of EVs as potentially novel biomarkers of disease. The goal of improving detection of EVs and comparison of measurements results has been recognized not only by the ISTH, but also by the ISEV, the International Society on Advancement of Cytometry (ISAC), and members of the

National Institutes of Health (NIH). Therefore, in April 2015 a Flow Cytometry Work Group was initiated, in which the ISEV (represented by M. Wauben, Utrecht University, Utrecht, The Netherlands), the ISAC represented by J. Nolan, Scintillon Institute, San Diego, CA, USA), the ISTH (represented by R. Nieuwland, Academic Medical Center, Amsterdam, The Netherlands) and the NIH (represented by J. Jones, Center for Cancer Research, National Cancer Institute, Washington, DC, USA) will work together on the improvement of detection and comparison of measurements. During public meetings at the ISEV and ISAC, it was agreed that: (i) standards and references have to be developed for scatter and fluorescence; (ii) instruments have to be characterized in an objective manner; (iii) other measurement techniques, such as NTA and TRPS, have to be used alongside flow cytometry; (iv) fluorescence has to be standardized; (v) education is essential – good practices such as the ISAC-promoted standards and protocols for instrument characterization, fluorescence calibration and data reporting are available, and can be implemented by other societies; (vi) when previous requirements have all been met, it will be possible to perform interlaboratory measurements of EVs; (vii) inter-society communication has to be continued; and (viii) a list of the top 10 antibodies used for flow cytometry of EVs will be prepared. Additional information is available at www.evflowcytometry.org.

Combining techniques

In recent publications, various isolation and detection methods have been combined to obtain complementary information from an EV population [3,29,35]. As EVs have many physical and biological properties that can be assessed with different techniques, it is evident that EV analysis is not limited to a specific technique. Therefore, combining techniques in projects is recommended. For example, in 2015 the project Cancer-ID was started in The Netherlands (www.utwente.nl/tnw/cancer-id). Together with 22 companies, six universities are collaborating to analyze EVs in the same (patient) samples, with the final goal of identifying and fully characterizing tumor-derived EVs for clinical applications, such as diagnosis or monitoring of therapy. Within this project, data on flow cytometry, surface plasmon resonance, different electron microscopy methods, multiple types of Raman spectroscopy, atomic force microscopy and RNA sequencing will be combined to obtain the maximum amount of information from EVs.

Clinical implications

The increased interest in EVs, and the appearance of improved isolation and detection methods, have led to novel insights into and clinical applications of EVs, which will be illustrated in the next three sections.

Cellular origin of EVs in human plasma

There is consensus in the literature that most EVs in human plasma originate from either platelets or megakaryocytes [43,44]. Most evidence comes from studies in which EVs were isolated from plasma by centrifugation, followed by detection and establishment of their cellular origin by electron microscopy and flow cytometry. With conventional flow cytometers, however, we have learned that most single EVs in human body fluids are too small to be detected [2,36]. Furthermore, when plasma is subjected to centrifugation, even at relatively low centrifugation speeds of 20 000 $\times$ *g*, EVs of megakaryocytic and/or platelet origin will aggregate (personal communication with Y. Yuana) [18]. This aggregation increases the risk that the concentration of megakaryocytic and/or platelet EVs will be overestimated when a non-sensitive detection method is used (Fig. 2). Thus, despite numerous studies on the cellular origin of single EVs in human plasma, the true cellular origin of most EVs remains elusive, and in fact may have to be reconsidered.

Because most EVs in plasma and other body fluids are so small and thus below the detection limit of flow cytometry, we recently performed a pilot experiment to study the protein composition of circulating EVs from an overnight fasting healthy individual by proteomics. The plasma was centrifuged three times to prepare platelet-depleted plasma. Subsequently, EVs were purified by SEC [27,29] and concentrated by ultracentrifugation. The 20 most abundant membrane-associated proteins are characteristic for leukocytes and erythrocytes rather than platelets or megakaryocytes. Although these data are preliminary and need further confirmation and validation, the cellular origin of EVs in human plasma and blood will need to be reinvestigated with improved isolation and detection methods.

Functional transfer of mRNA in vitro *and* in vivo

One of the most exciting findings regarding EVs is their ability to transfer functional mRNA between cells. EVs of eukaryotic cells contain different types of RNA, and this RNA can be transferred and translated by recipient cells *in vitro* [45]. The uptake of this RNA may change the phenotype of recipient cells. For example, angiogenesis is promoted by uptake of EVs from glioblastoma cells containing mRNA encoding a truncated epidermal growth factor receptor [46,47]. In turn, angiogenesis promotes tumour growth [47]. At present, there is compelling evidence that mRNA can be transferred by tumor-derived EVs *in vivo*. Ridder *et al*. demonstrated that EVs from peripheral blood of transgenic mice transfer Cre-encoding mRNA into the liver, lung, small intestine and brain of non-Cre-encoding mice, leading to reporter gene expression in recipient cells [48–50]. With the same model system, transfer of mRNA present in EVs from malignant

tumor cells to less malignant tumor cells was shown to enhance the migration and metastatic capacity of the recipient cells [51]. Together, these findings show that EVs are capable of exchanging functional genetic information between cells *in vivo*, and understanding this intercellular communication mechanism is likely to be of the utmost biological and clinical relevance.

Clinical applications of EVs

At the ISTH, EVs are best known for their coagulant properties, owing to their exposure of TF and negatively charged phosphatidylserine. Plasma of cancer patients contains TF-exposing EVs. The role of TF-exposing EVs in the development of venous thromboembolism (VTE), a frequent complication in cancer patients, was discussed during ISEV 2014 [52]. Therefore, the Academic Medical Center (Amsterdam) has started a multicenter trial to study whether the ability of TF-exposing EVs present in plasma of cancer patients can be used to identify patients at high risk for VTE. The role of EVs in the pathogenesis of cancer has been excellently summarized elsewhere [53], and glypican-1, a protein exposed on tumor-derived EVs, was recently shown to be useful for the early diagnosis of pancreatic cancer [54].

To conclude this review, we propose use of the term 'EV' instead of the now common terms 'microparticles' and 'exosomes' within the ISTH, until there is consensus regarding nomenclature. This consensus may follow from recent progress regarding the isolation and detection of EVs, as well as from new insights into their distribution and function. To reveal all aspects of EVs, interdisciplinary collaboration is required, which can be pursued by merging research from different disciplines and societies. Together, these developments have ensured that EV research has gained noticeable biological and clinical momentum.

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Disclosure of Conflict of Interests

E. van der Pol is a cofounder and shareholder of Exometry B.V. All other authors state that they have no conflict of interest.

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