Transfusion and Apheresis Science 55 (2016) 23–34

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Review

Measurement of extracellular vesicles as biomarkers of consequences or cause complications of pathological states, and prognosis of both evolution and therapeutic safety/efficacy

Jean Amiral [a,*], Jerard Seghatchian [b]

[a] Hyphen BioMed, Neuville sur Oise, Paris, France
[b] International Consultancy in Blood Components Quality/Safety Improvement, Audit/Inspection and DDR Strategies, London, UK

ARTICLE INFO

Keywords:
Extracellular vesicles
Biomarkers
Cell activity
Disease state
Intercellular cross-talk

ABSTRACT

Utility of EVs, as biomarkers of cause or consequence of various pathological complications, and prognosis of blood components' therapy in terms of safety/efficacy and their potential associated hazards, primed by EVs involvements in pro-inflammatory, immunomodulatory and activations of both pro/anti-coagulatory and others associated pathways, as well as various cellular cross talks, are highlighted as the fundamental. Today EVs are becoming the "buzz" words of the current diagnosis, development and research [DDR] strategies, with the aim of ensuring safer therapeutic approaches in the current clinical practices, also incorporating their potential in long term cost effectiveness in health care systems. The main focus of this manuscript is to review the current opinions in some fundamental areas of EVs involvements in health and diseases. Firstly, our goal is highlighting what are EVs/MVs/MPs and how are they generated in physiology, pathology or blood products; classification and significance of EVs generated in vivo; followed by consequences and physiological/pathological induced effects of EVs generation in vivo. Secondly, specific cell origin EVs and association with malignancy; focus on EVs carrying TF and annexin V as a protective protein for harmful effects of EVs, and associations with LA; and incidence of anti-annexin V antibodies are also discussed. Thirdly, utility of EVs is presented: as diagnostic tools of disease markers; prognosis and follow-up of clinical states; evaluation of therapy efficacy; quality and risk assessment of blood products; followed by the laboratory tools for exploring, characterizing and measuring EVs, and/or their associated activity, using our own experiences of capture based assays.

Finally, in perspective, the upcoming low volume sampling, fast, reliable and reproducibility and friendly use laboratory tools and the standardization of measurement methods are highlighted with the beneficial effects that we are witnessing in both wound healing and tissue remodeling, with an expected blockbuster status EVs as future therapeutic directions.

Contents

* Corresponding author. Hyphen BioMed, Neuville sur Oise, Paris, France. Fax: +33134487236.
E-mail address: jamiral@hyphen-biomed.com (J. Amiral).

http://dx.doi.org/10.1016/j.transci.2016.07.009
1473-0502/

1. Introduction

This manuscript focuses on the diagnostic aspects of all circulating blood cells derived EVs/MVs currently used as biomarkers of health/disease states [i.e. using circulating proteins and lipid markers, blood cells and tumor cells, circulating cell-free DNA (cfDNA) and extracellular vesicles, including exosomes, microvesicles, micro-particles, ectosomes and blood cell apoptotic bodies] [1–3].

Extracellular vesicles (EVs) are gaining increasing interest in many biomedical fields for their multiple applications, as major indicators of cell life, activity and disease aggressions. Many scientific and biochemical articles are being published on the different aspects of EVs [4–7]. These EVs are released from a large variety of cells in response to cell stimulation (side effects of blood activation, inflammation, antibody binding, etc…), pathogenic activation, or programmed cell death (apoptosis) [2,8,9]. These "cell dusts" (as they were formerly called) are present in many different sizes (from <100 nM to >1 μM), and they can expose procoagulant phospholipids such as phosphatidyl serine (P-Ser), membrane antigens, and transmembrane proteins anchored onto the EV phospholipid surface, which allow to identify the cell origin of these EVs [8,10–12]. They can also contain soluble proteins. When originated from platelets (the most abundant EVs in blood circulation), EVs surface is more than that of activated platelets, exposing the phospholipids surface, phosphatidyl-serine (P-Ser) rich, and it can then focus the coagulation cascade [13,14]. However, EVs are present in all body fluids and organs, including saliva, brain, etc… [2]. According to EVs' size and composition, we can distinguish nanoparticles (NPs), exosomes, ectosomes, microparticles (MPs) and apoptotic bodies present in highly variable concentrations in health and disease [1,8,15–17]. Basically there are 3 major groups of EVs: nano-particles/exosomes; ectosomes and MPs; apoptotic bodies. In addition, these "cell dusts" can carry specific activities, which induce usually bad activities, as they can worsen a disease state through amplification of inflammation and blood activation, disseminate pathologic information from cancer or infectious cells (metastasis, inflammation) and increase thrombosis risk, favoring cell to cell cross-talk and cell adhesion to some physiological surfaces [18–23]. Nevertheless, EVs can be good in a few cases, when their beneficial effects supersede the harmful ones, especially for their contribution to tissue remodeling and wound healing, and for permitting remote exchanges of cell information, and some biological activities' modulation (as immunological or inflammatory responses) [1,12,19,24,25]. They also allow intercell transfer of nucleic acids/DNA/RNA and can contribute to reprogram some cell functions [10,19,25].

Scientific studies on EVs are first aimed to document their heterogeneity and their associated activities or their possible pathogenic effect, and also for tracking in-vivo cell activity, which is better characterized through these released particles. Many other studies are focused on the diagnostic value of these EVs, or only on some of them, duly characterized, for evaluating disease states [16–18]. They can be useful as prognosis tools of disease evolution and complications, or for assessing the efficacy of some therapies, such as those implemented in patients with heart diseases [26–30], or for treating hemophiliacs A with inhibitors using Factor VIIa [31], and can be promising for some cancers in association with progenitor cells [32]. Their characteristics open a new therapeutic avenue, as they can be used as cargos for specific drugs, targeted to well characterized target cells [33].

Lastly, EVs can be generated ex-vivo, in blood cell concentrates (RBC), especially red blood cell concentrates or platelet concentrates (PC), during storage, and can then contribute to the adverse effects of these therapeutic fractions [24,34–37]. They are usually reported as indicators of "cell storage lesion". Their measurement can have a major interest for testing possible health risks associated to concentrates with storage damages, which could induce adverse effects in patients receiving these pouches. An increased morbidity and mortality can be associated to the use of these blood derived products required for treating hemorrhagic complications and anemia [38], while diminution of EVs in stored plasma reduces its hemostatic potential [39].

Present technologies have highly improved the preparation of cell concentrates, and their storage conditions, and complications directly associated to presence or ex-vivo generation of EVs are very rare. Nevertheless, perspectives to specifically remove EVs from blood cell concentrates are foreseen, in order to still reduce the side effects of these transfusion products.

Concerning the laboratory tools available for measuring EVs, on patients' plasmas, or blood cell concentrates or plasma derived products, different technologies can be considered. According to their detection mode, different types of EVs are measured. Flow cytometry (FACS: fluorescence-activated cell sorting) is one of the most popular tools, but is usually performing only for the largest EVs, with sizes ranging from 0.2/0.4 μM to >1.0 μM. New flow cytometers are able to detect smaller particles, especially when they are homogeneous in purified milieus, but are not useable for the heterogeneous presentation of EVs in blood or anticoagulated plasma from patients [8,39]. Functional/capture based assays [40] and emerging technologies offer large investigation possibilities, and will be discussed in this article, which is focused mainly on EVs present in blood circulation [41–43].