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REVIEW

Potential of extracellular vesicles for early prediction of severity and potential risk stratification in critical inflammatory diseases

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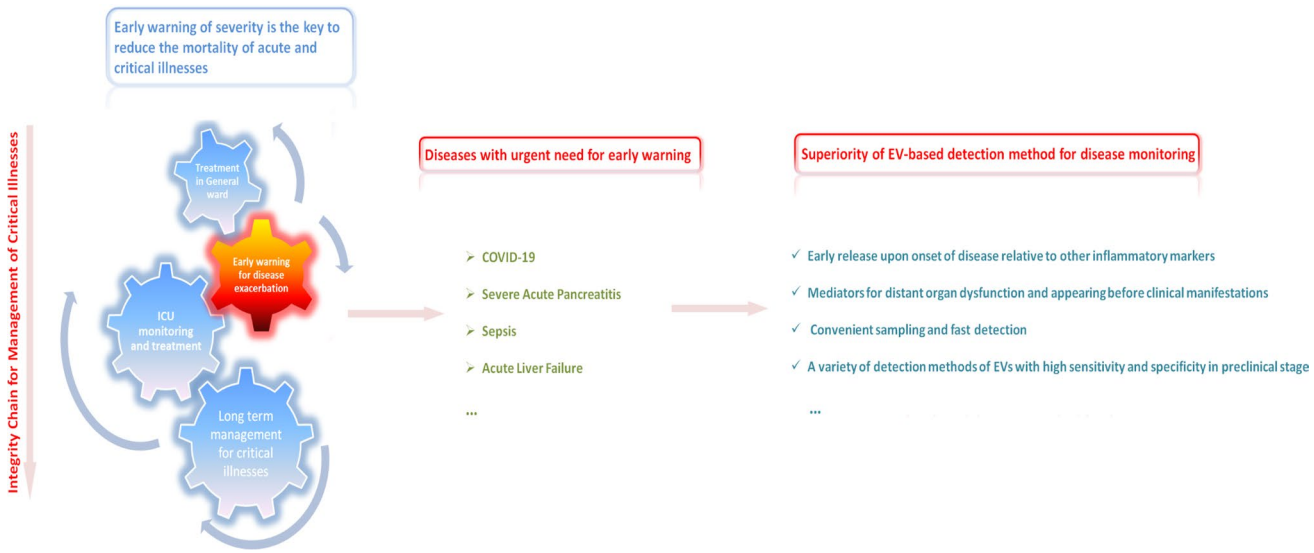
Abstract

Some acute inflammatory diseases are often exacerbated during or after hospitalization, leading to some severe manifestations like systemic inflammatory response syndrome, multiple organ failure, and high mortality. Early clinical predictors of disease severity are urgently needed to optimize patient management for better prognosis. The existing clinical scoring system and laboratory tests cannot circumvent the problems of low sensitivity and limited specificity. Extracellular vesicles (EVs) are heterogeneous nanosecretory vesicles containing various biomolecules related to immune regulation, inflammation activation, and inflammation-related complications. This review provides an overview of EVs as inflammatory mediators, inflammatory signaling pathway regulators, promoters of inflammatory exacerbation, and markers of severity and prognosis. Currently, although relevant biomarkers are clinically available or are in the preclinical research stage, searching for new markers and detection methods is still warranted, as the problems of low sensitivity/specificity, cumbersome laboratory operation and high cost still plague clinicians. In-depth study of EVs might open a door in the search for novel predictors.

Graphical abstract



Keywords Acute pancreatitis · Extracellular vesicle · Inflammation signaling · Early prediction of severity · Critical inflammatory diseases

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Introduction

Inflammation is a physiological or pathological process in which the body responds to stimulators such as infection, tissue stress or dysfunction, and tissue injury and tries to restore the internal homeostasis (Medzhitov 2008). In this process, leukocytes and inflammation-related plasma proteins are recruited to the lesion and perform various effector functions to resist infection or injury. However, when the body's inflammatory response is excessive, the accumulated inflammatory mediators have a severe destructive impact on human tissues. These excessive inflammatory reactions often manifest as systemic inflammatory response syndrome (SIRS) (Singer et al. 2016), cytokine storm (CS) (Ragab et al. 2020) or multiple organ failure (MOF) (Beal and Cerra 1994). Patients with these complications often have a significantly higher mortality rate than those who do not.

The disease course of the patients with the above-mentioned complications often progresses rapidly. Early prediction of disease or stratification of patients by disease severity facilitates more active monitoring and treatment measures and is anticipated to significantly improve the prognosis of patients (Terrasini and Lionetti 2017; Fujita et al. 2021). Inaccurate assessment often leads to treatment delay, increased mortality or poor prognosis. However, the commonly used prediction models and some laboratory tests often have limitations, such as poor sensitivity and specificity, and cannot make early predictions (Mounzer et al. 2012; Germolec et al. 2018). Because organ function is often impaired before obvious clinical manifestations appear, traditional tools have clear deficiencies. Researchers have gradually transitioned from clinical scoring to molecular screening of important markers in disease evolution (Staubli et al. 2015).

Paracrine-mediated complex intercellular communication is very important for maintaining the physiological environment required by different distant organs to respond to stressors. Dysregulation may occur in the early stage of reaching the organ tolerance threshold and lead to the extension of single organ dysfunction to other distant organs (Husain-Syed et al. 2016). The understanding of mechanisms underlying critical illness and the screening of related important molecules may be expected to reveal corresponding indicators for predicting the early evolution of organ failure or individual treatment responses. Studies have shown that extracellular vesicles (EVs) are functionally involved in the progression and aggravation of many diseases (Jia et al. 2021; Hassanpour et al. 2020; Lanyu and Feilong 2019; Su et al. 2020). The identity and quantity of EVs are closely related to disease severity (Fujita et al. 2021; Krishnamachary et al. 2021; Letsiou et al. 2015; Campbell et al. 2021). EVs can indicate not only aggravation of a disease but also the types of complications (Takei et al. 2019; Panich et al. 2017; Delabranche et al. 2016), and EVs are released earlier than other inflammatory markers in the blood (Jansen et al. 2016). Previous reviews have addressed related aspects, including the role of EVs in the personalized management of injury and repair in critical diseases (Husain-Syed et al. 2016), EVs-mediated regulation of sepsis and immune system/blood coagulation disorders (Qiu et al. 2021; Iba and Ogura 2018), etc. To avoid repetition, we do not discuss these aspects in detail. This review introduces the role of EVs in progression of severe acute clinical inflammatory disease, analyzes the potential of EVs as predictors of severity, which is expected to promote the clinical application of EVs as predictive markers.

Brief introduction to EVs

EVs are vesicles composed of a lipid bilayer originating from endosomes or plasma membranes. In 1983, the secretion of EVs from sheet reticulocytes was first discovered by Pan and Johnstone (Pan and Johnstone 1983), but at that time, EVs were considered only a part of the mechanism of cell waste disposal. Currently, it has been shown that almost all types of mammalian cells secrete EVs (Camussi et al. 2011; Timmers et al. 2007; Wang et al. 1950); in addition, EVs are found in most body fluids (Lee et al. 2012; Chaput and Théry 2011; Adeoye and Thomson 2020; Ela et al. 2013; Raeven et al. 2018; Rossaint et al. 2019; Chan et al. 2019). According to the biogenesis pathway, EVs can be classified as exosomes, microvesicles and apoptotic bodies (Hassanpour et al. 2020; Lanyu and Feilong 2019). Since no specific markers for distinguishing EVs subtypes have been identified, determining the biogenesis pathway of EVs remains very difficult (Théry et al. 2018). EVs have numerous sources and diverse functions. How do they play a role in intercellular regulation? The roles of EVs include transfer of membrane components, inhibition of translation via RNA cargos, activation of coagulation cascades, direct signal transduction by receptor recognition, endocytosis of vesicles, regulation of transcription factors and antigen presentation, etc. (Raeven et al. 2018). As the identity and quantity of cargos carried by EVs can reflect the condition of their origin cells and are closely related to the pathological disease state, they have the potential use as biomarkers for clinical diagnosis or prognosis (Urabe et al. 2020; Min et al. 2021).

Exacerbation of acute inflammatory processes by EVs

The pathophysiological manifestations of critical inflammatory diseases usually include severe acute respiratory syndrome (ARDS), shock, systemic inflammatory response syndrome (SIRS), multiple organ dysfunction (MODS), multiple organ failure (MOF) etc.. It has been reported that EVs cause secondary organ failure (SOF) after trauma, invasive treatment or organ transplantation through proinflammatory and prothrombotic effects etc. (Eppensteiner et al. 2018). The pathological factors related to the deterioration process with the involvement of EVs will be discussed in the following.

Direct dissemination of microbe virulence factors by EVs

The pandemic of some infectious diseases can be a global health crisis. During the course of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, host cells would release EVs carrying viral components after virus endocytosis. The presence of viral RNA in the EVs can be identified by the transcription-droplet digital polymerase chain reaction (RT-ddPCR), while no viral material was detected in healthy subjects (Barberis et al. 2021). With the circulation of EVs in body fluid, they would probably contribute to the infection spread of proximal or distal tissues.

Outer membrane vesicles (OMVs) were observed budding from the bacterial cell surface in the course of infection (Gurung et al. 2011). The presence of OMVs has been determined in patient samples and tissue biopsies by electron microscopy (Namork and Brandtzaeg 2002; Stephens et al. 1982). And one of the main purposes for OMVs is thought to be transported of bacterial toxins (MacDonald and Kuehn 2012). With the help of proteomics, many virulence factors of OMVs has been discovered including phospholipase C, proteases, alkaline phosphatase, aminopeptidase, murein hydrolases, hemolysins etc (Choi et al. 2011).

Somatic cell injury mediated by EVs through specific signaling pathway

Oxidative stress signaling

Acute pancreatititis (AP) is a common acute clinical abdominal disease. In most people, the disease is mild, but approximately 20% of patients experience moderate or severe pancreatitis (Dijk et al. 2017) with persistent single or multiple organ failure. The mortality rate of severe cases is approximately 30% (Lankisch et al. 2015; Schepers et al. 2019). Some studies compared inflammatory activation of macrophages by plasma-derived EVs from patients with mild AP and patients with severe AP and found that EVs from patients with severe disease carried more S100A8/S100A9, which can activate NADPH oxidase and promote the production of free radicals, thus promoting inflammatory responses (Carrascal et al. 2021).

The terminal stage of Non-alcoholic fatty liver disease (NAFLD) known as acute liver failure (ALF) are often life-threatening clinical syndromes characterized by rapid loss of hepatocyte function in patients without previous liver disease (Thawley 2017). Exogenous EVs from mice with acetaminophen-induced liver injury were found to be internalized into the primary mouse hepatocytes and lead to excessive production of reactive oxygen species (Cho et al. 2018). miR-503 encapsulated in endothelial cells derived EVs under the ischemic myocardial scenarios exacerbated cardiac injury by directly binding to peroxisome proliferator-activated receptor gamma coactivator-1β (PGC-1β) and a mitochondrial deacetylase, sirtuin 3 (SIRT3), thereby triggering ROS production and mitochondrial metabolic dysfunction (Sun et al. 2022).

Apoptosis signaling

As we know, the severe acute respiratory syndrome is one of the most serious complications of Corona Virus Disease 2019 (COVID-19) and marked by endothelial dysfunction and dysregulated immune responses, which was positively correlated to disease severity. EVs from COVID-19 patients plasma significantly increased caspase 3/7 activity of pulmonary microvascular endothelial cells and consequently induced cell apoptosis in the order of disease severity. As discussed above, EVs from mice with acetaminophen-induced liver injury not only induced production of reactive oxygen species (Cho et al. 2018), but also caused the increase of phospho-JNK/JNK, Bax and cleaved caspase-3 in mouse liver after receiving the EVs.

Inflammasome

The NOD-like receptors (NLR) are a family of cytosolic proteins that regulate the cysteine protease caspase-1 within a multiprotein complex known as the inflammasome. Activation of caspase-1 leads to the cleavage and activation of pro-inflammatory cytokines such as interleukin-1beta (IL-1beta) and IL-18, which indicates inflammasome activation (Newton and Dixit 2012). NLR has important roles in innate immunity as intracellular sensors of microbial components and cell injury (Mariathasan and Monack 2007). In sepsis, monocyte-derived EVs promote IL-1β and IL-18 secretion from resident cardiac macrophages through inflammasome activation, resulting in myocardial dysfunction (Wang et al.

2021). EVs produced by hepatocytes under heat stress can activate the NLR signaling pathway in other hepatocytes and lead to liver inflammation and injury (Li et al. 2019). Siminarly, NLRP3 inflammasome activation and subsequent pyroptosis in alveolar macrophages (AMs) is responsible for the lung injury secondary to acute pancreatitis (Wu et al. 2020).

Coagulopathy associated with EVs

As infection is commonly associated with activation of the coagulation system, it is reported that COVID-19 patients often present with thrombosis (Campbell et al. 2021). They had significantly higher levels of D-dimer, circulating extracellular vesicle tissue factor (EVTF) activity, and active PAl-1 compared with healthy controls, which may promote thrombosis due to simultaneous activation of coagulation and inhibition of fibrinolysis. Since tissue factor (TF) expression is induced in monocytes and endothelial cells, the EVTF might be derived from these two cell groups (Campbell et al. 2021). And the EVTF activity was also associated with patient severity and mortality (Rosell et al. 2021). In addition, sepsis patients have a high rate of thrombosis, too. The coagulation disorder contributes to the development of disseminated intravascular coagulation (DIC). A recent article (Iba and Ogura 2018) has reviewed that EVs not only exhibit procoagulant properties during sepsis, but also disseminate prothrombotic activities by transferring their procoagulant properties to distant target cells. To avoid repetition, we do not discuss these aspects in detail.

Induction of inflammatory cell recruitment or differentiation by EVs

In one study about sepsis, EV-associated miRNAs were responsible for EV-induced cytokine production via TLR7-MyD88 signaling. The effects of EVs were resistant to polymyxin B (an endotoxin inhibitor) but significantly inhibited by anti-miR inhibitors. In addition, in vivo, peritoneal cecum ligation and puncture mouse model derived EVs induced significant recruitment of neutrophils. And this study proved the hypothesis that plasma EVs in sepsis were pro-inflammatory (Xu et al. 1950). Lung damage is a frequent complication of acute pancreatitis, of which the pathogenic mechanism is still unknown. PKH26-stained EVs obtained under inflammatory conditions reached alveolar compartment and were internalized by alveolar macrophages. And these EVs activated and polarized these macrophages towards a pro-inflammatory phenotype in vitro (Bonjoch et al. 2016). Furthermore, related study from another research group revealed that acute pancreatitis derived EVs-loaded MALAT1 facilitated M1 polarization of macrophages via miR-181a-5p/HMGB1/TLR4 (Liu et al. 2021).

Superiority of EVs as disease severity predictor

Early occurrence of EVs for disease stratification

As discussed above, many studies have suggested that EVs are significantly correlated with the severity of some diseases. Besides, EVs are even the initiating factor of disease progression. The time at which EVs can be used for disease monitoring in patients with different severities can be much earlier than that of other inflammatory biomarkers. Analysis of blood samples from 50 patients with SIRS after transcatheter aortic valve implantation (TAVI) showed that the endogenous microparticle (EMP) concentration peaked at 4 h after TAVI, while other inflammatory biomarkers peaked at 24 h (PCT, IL-6, IL-8) or 48 h (CRP) after surgery. This difference may exist because EMPs are released immediately after endothelial injury (Jansen et al. 2016).

In a case–control study, the Intensive Care Unit with septic shock (SS) patients and matched healthy volunteers were recruited and various microparticle subtypes (AnnexinV+, E-selectin+, thrombomodulin+ , leukocyte-derived (CD45+ , CD36+) and platelet-derived MP) from plasma were studied to evaluate their possible association with severity of illness and sepsis-related complications including DIC and acute kidney injury (AKI). It turned out that a global response through MP derived from endothelial cells, leukocytes and platelets on day 1 of SS was confirmed and are useful to evaluate SS severity and DIC occurrence, which was earlier than the detection of inflammation related cell activation (Boscolo et al. 2019).

Convenient sampling and detection of EVs-derived markers

In clinic, specially ICU, various scoring systems are often used to evaluate the severity of the disease and the most commonly used include Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), the Rason score etc. And these scoring systems are always cumbersome, time-consuming and subjective (Mounzer et al. 2012). Laboratory examination methods to predict diseases by measuring inflammation-related biomolecules such as cytokines, chemokines and acute phase proteins also have issues with limited sensitivity and specificity (Staubli et al. 2015). And convenient and fast alternatives are urgently needed.

The level of EVs in several body fluids, including serum/plasma, urine (Panich et al. 2017), ascites fluid (Bonjoch et al. 2016; Jiménez-Alesanco et al. 2019), breast

milk (Admyre et al. 1950), saliva (Cheng et al. 2020), and alveolar lavage fluid (Soni et al. 2016; Lee et al. 2017), was found to be increased in inflammatory pathological conditions. Therefore, EVs samples can be obtained by noninvasive method. Although clinical application of EVs detection methods is challenging because of the low concentration, small size and heterogeneity of EVs, various emerging technologies are flourishing and impressive (Garcia-Cordero and Maerkl 2020; Salafi et al. 2016). Above all, EVs-based detection methods are still highly expected.

Association with severe complications of EVs

As discussed above, EVs are related to inflammation-related complications, and patients with these complications often have a significantly higher mortality and rapid disease progression. So, EVs are promising indicators of disease severity. It has been discussed in "Coagulopathy associated with EVs" section that EVs exhibit procoagulant properties during sepsis and contributes to the development of DIC. In another study, increased E-selectin^{+}MPs in subjects who developed sepsis-related AKI were observed (Boscolo et al. 2019). Besides, circulating endothelial microparticles (EMPs) expressing higher levels of angiotensin-converting enzyme (ACE) on admission were significantly increased in septic patients who developed ARDS compared with septic patients who did not. Therefore, circulating ACE EMPs may be a prognostic marker for the development of ARDS in the septic patients (Takei et al. 2019).

Indication of disease severity, prediction of hospitalization duration as well as mortality by EVs

Acute liver failure (ALF) is a prototypical syndrome of systemic inflammatory response syndrome (SIRS) associated with a procoagulant state. And it has been verified that patients with ALF develop increased procoagulant microparticles (MPs) in proportion to the severity of systemic complications and adverse outcome (Takei et al. 2019). Specifically, MP concentrations was correlated with laboratory predictors of death/liver transplant (LT) (higher phosphate and creatinine; lower bicarbonate), and day 1 and 3 MPs were higher in patients who died or underwent LT, compared to survivors. Another study on the role of EVs for COVID-19 suggested that EVs from critically-ill patients with enrichment of proinflammatory and procoagulation protein signatures can delineate disease severity and predict length of hospitalization (Krishnamachary et al. 2021).

Excellent predictive value of EVs for patient stratification

The predictive markers for early characterizing the variability of COVID-19 patients are still lacking, and emerging evidence indicates that EVs components might be key determinants to predict disease severity. The prediction accuracy of an EV-related marker EV COPB2, a subunit of the Golgi coatomer complex, was systemically studied. And EV COPB2 exhibited significantly higher abundance in patients remained mild than developed severe/critical COVID-19 with a predictive value of AUC 0.85 (95% CI 0.73–0.97) for early patient stratification (Fujita et al. 2021). In clinic, 50% of sepsis patients might develop AKI and sepsis patients with AKI had a higher mortality rate. Therefore, early sepsis-AKI biomarkers would be indispensable, however, no specific biomarker for sepsis-AKI is available currently. In a study, urinary exosomal activating transcriptional factor 3 (uATF3) has been compared with the standard AKI biomarker serum creatinine (Scr) and potential sepsis-AKI marker urinary neutrophil gelatinase associated lipocalin (uNGAL). And AUROC of uNGAL and uATF3 were 64% (95% CI 0.54–0.74) and 84% (95% CI 0.77–0.91), respectively, which suggested that uATF3 is an interesting sepsis-AKI biomarker (Panich et al. 2017).

Various cargos on EVs as markers for distinguishment of disease severity

EVs contain various bioactive substances, such as lipids, proteins and RNAs, and all are widely explored.

Protein

The protein markers are the most widely studied EV-based markers. Till now, dozens of research studies have reported human EV-derived protein markers to differentiate critical inflammatory diseases. Proteomic approach is usually used to map the circulating EVs from the patients and healthy controls. And the marker molecules reported are involved in the immune response, inflammation, activation of the coagulation and complement pathways including FIBA, C1R (Barberis et al. 2021), TF (Rosell et al. 2021), COPB2 (Fujita et al. 2021) etc..

microRNA

An overall mortality for patients with community-acquired pneumonia (CAP) is 17.3%, and frequent secondary complications of CAP include sepsis, SS or acute pulmonary failure etc. Novel biomarkers for CAP will help to identify patients at risk for progress to sepsis and facilitate early intervention

and treatment. And expression levels of miR-1246 showed significant changes with an increase in overall disease severity, which is a promising marker for CAP (Hermann et al. 2020).

As the mortality of SS is approaching 50%, novel biomarkers served as prompt indicators are urgently needed for early diagnosis and treatment as well as patient survival. EVs-derived miRNA (miR-125b-5p) contributes significantly to sepsis diagnosis and survival prediction and could be used as newly identified targets for the development of novel sepsis biomarkers (Reithmair et al. 2017).

Lipids

Hitherto evidence has proved that multivesicular bodies (MVBs)/late endosomes were implicated in extracellular release of viruses, which contributed to virus infection for distant cells. Specifically, mature viral particles are released by infected cells in the form of transport vesicles from the trans-Golgi network (TGN). As the trafficking routes are present between TGN and late endosomes, the virus particle release might be affected by the homeostasis of some phosphate receptor or lipids. One study suggests that COVID-19 patients with elevating disease severity have increased enrichment in monosialodihexosyl gangliosides (GM3)-containing EVs, and may partake in pathological processes of COVID-19 pathogenesis (Song et al. 2020).

Total EVs content

As discussed in "Indication of disease severity, prediction of hospitalization duration as well as mortality by EVs" section, one research study investigated the role of microparticles in mediating complications and outcome of ALF. MPs with size range of 0.1–1.0 μm were enumerated. Total MPs (0.15–1.0 μm) were present in nearly 19-fold higher concentrations in ALI/ALF patients compared to healthy controls, and MPs (0.36–0.64 μm) increased in direct proportion to SIRS severity and grade of hepatic encephalopathy (HE) (Stravitz et al. 2013).

Discussion

Other than the academic publications mentioned above, nearly 10 clinical studies have been initiated for evaluating EVs as sensitive criteria of early evaluation and supervision of therapeutic effect for acute disease including sepsis, acute lung injury, acute myocardial infarction, immune reconstitution syndrome in HIV/TB co-infection, traumatic brain injury etc. (Table 1). The study purpose and design of these clinical trials may offer a glimpse of the potential of EVs derived biomarker for predicting disease severity and prognosis.

Over the past decade, research on the function of EVs in inflammation has increased significantly. Given that EVs and inflammation are two complex fields, the existing research is insufficient to fully reveal their interrelationship. However, two points are certain. First, regarding inflammation, EVs play a strong regulatory role in disease as inflammatory mediators. Second, the cargos carried by inflammatory EVs can reflect the pathological state of patients.Concerning the second aspect, EVs can be used as diagnostic/prognostic markers of disease and promote more accurate and personalized treatment.

Distinction of EVs subtypes is also a development direction worthy of attention (Shao et al. 2018). EVs themselves are highly heterogeneous lipid containers with diverse sources, sizes, and contents; indeed, they perform different functions and even play opposite roles in the same disease. For example, epithelial cell-derived EVs isolated from intestinal lavage fluid in septic mice inhibited inflammation; in contrast, EVs isolated from plasma promoted inflammation (Appiah et al. 2020; Gao et al. 2019). If these EVs subtypes are not distinguished, the conclusions may be puzzling. Therefore, numerous novel technologies including single EV analysis (SEA), surface-enhanced Raman spectroscopy (SERS), cryo-transmission electron microscopy (cryo-TEM) and total internal reflection fluorescence miscroscopy etc. are on their way to development to unveil different components and functions of various EV subgroups (Krishnamachary et al. 2021; Hosseinkhani et al. 2020). However, due to the limitations of separation and characterization technology, research on EVs subtypes isolated from a specific body fluid (such as plasma) or a specific cell type (such as T cells) in the inflammatory state is lacking. It is conceivable that if a specific subtype is selected, the prediction ability of EVs-based markers may be increased. Therefore, assessing these patterns is a meaningful research direction.

Table 1 Clinical study of EVs as an early assessment of acute diseases and supervision of treatment effects

Related diseases	ClinicalTrials.gov Identifier	Study description	EVs-related outcome measures
Sepsis	NCT03267160	A study of exosome proteomics and hemodynamics in sepsis	To purify exosomes in blood and urine from septic patients who had multiple organ failures and to study exosome proteomics in these specimens. To analyze autophage and apoptosis related biomarkers of exosomes by bioinformatics. To find the correlations between exosomes biomarkers and hemodynamic parameters
	NCT05229328	Study on the establishment of a system for early warning and prognostic evaluation of patient with sepsis	To collect peripheral blood to separate and extract plasma, PBMC, and plasma exosomal miRNA, and sequence to find indicators related to disease deterioration and prognosis
	NCT05061212	To study the mechanism of extracellular vesicles containing mitochondrial DNA in ARDS lung injury caused by extrapulmonary sepsis	To collect peripheral blood samples on 24 h and 48 h after admission to the ICU. To isolate EVs from the plasma, and evaluate mtDNA concentration of plasma DNA by RT-qPCR
Acute rejection (AR) after heart transplantation	NCT04921774	The purpose of this clinical research is to achieve early diagnose of acute rejection in patients with heart transplantation, thus it may be helpful for timely intervention to improve the patient's prognosis	Combining multimodality MR imaging with circulating exosomal miRNA expression to evaluate acute rejection in patients with heart transplantation
Acute respiratory distress syndrome (ARDS)	NCT05451342	Explore potential plasma and BALF biomarkers for identifying ARDS endotypes	1, Comparing the PBMC/alveolar macrophage derived exosome levels; 2, Exosome extraction from plasma and BLAF supernatant and related omics research
Heat stroke	NCT05155358	Study on the establishment of a system for early warning and prognostic evaluation of patients with heat stroke	To collect the peripheral blood of the normal population, the 24 h after the onset of heat stroke and the patients in the recovery period, to separate and extract plasma, PBMC, and plasma exosomal miRNA, and sequence to search for suitable indicators
COVID-19	NCT04367662	To study early and more specific markers of hypercoagulability and markers of routine endothelial dysfunction, as soon as the patient is hospitalized, in order to predict the risk of hospitalization in intensive care	The plasma level of many hypercoagulability related factors including D-dimers, fibrin, antithrombin, prothrombin, microvesicles etc. were determined

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Declarations

Competing interests The authors declare that they have no competing interests. (Miyake et al. 2020)

Ethics approval Not applicable.

Consent for publication Not applicable.

References

Adeoye J, Thomson P (2020) 'The Double-Edged Sword'—an hypothesis for Covid-19-induced salivary biomarkers. Med Hypotheses 143:110124

Admyre C, Johansson SM, Qazi KR et al (2007) Exosomes with immune modulatory features are present in human breast milk. J Immunol (baltimore, Md: 1950) 179(3):1969–1978

Appiah MG, Park EJ, Darkwah S et al (2020) Intestinal epithelium-derived luminally released extracellular vesicles in sepsis exhibit the ability to suppress TNF-a and IL-17A expression in mucosal inflammation. Int J Mol Sci 21(22):8445

Barberis E, Vanella VV, Falasca M et al (2021) Circulating exosomes are strongly involved in SARS-CoV-2 infection. Front Mol Biosci 8:632290

Beal AL, Cerra FB (1994) Multiple organ failure syndrome in the 1990s: systemic inflammatory response and organ dysfunction. JAMA 271(3):226–233

Bonjoch L, Casas V, Carrascal M, Closa D (2016) Involvement of exosomes in lung inflammation associated with experimental acute pancreatitis. J Pathol 240(2):235–245

Boscolo A, Campello E, Bertini D et al (2019) Levels of circulating microparticles in septic shock and sepsis-related complications: a case-control study. Minerva Anestesiol 85(6):625–634

Campbell RA, Hisada Y, Denorme F et al (2021) Comparison of the coagulopathies associated with COVID-19 and sepsis. Res Pract Thromb Haemost 5(4):e12525

Camussi G, Deregibus MC, Bruno S, Grange C, Fonsato V, Tetta C (2011) Exosome/microvesicle-mediated epigenetic reprogramming of cells. Am J Cancer Res 1(1):98–110

Carrascal M, Areny-Balagueró A, de-Madaria E, et al (2022) Inflammatory capacity of exosomes released in the early stages of acute pancreatitis predicts the severity of the disease. J Pathol 256(1):83–92

Chan BD, Wong W-Y, Lee MM-L et al (2019) Exosomes in inflammation and inflammatory disease. Proteomics 19(8):1800149

Chaput N, Théry C (2011) Exosomes: immune properties and potential clinical implementations. Semin Immunopathol 33(5):419–440

Cheng Y, Pereira M, Raukar NP et al (2020) Inflammation-related gene expression profiles of salivary extracellular vesicles in patients with head trauma. Neural Regen Res 15(4):676–681

Cho YE, Seo W, Kim DK et al (2018) Exogenous exosomes from mice with acetaminophen-induced liver injury promote toxicity in the recipient hepatocytes and mice. Sci Rep 8(1):16070

Choi DS, Kim DK, Choi SJ et al (2011) Proteomic analysis of outer membrane vesicles derived from *Pseudomonas aeruginosa*. Proteomics 11(16):3424–3429

Delabranche X, Quenot JP, Lavigne T et al (2016) Early detection of disseminated intravascular coagulation during septic shock: a multicenter prospective study. Crit Care Med 44(10):e930-939

El Andaloussi S, Mäger I, Breakefield XO, Wood MJ (2013) Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 12(5):347–357

Eppensteiner J, Davis RP, Barbas AS, Kwun J, Lee J (2018) Immunothrombotic activity of damage-associated molecular patterns and extracellular vesicles in secondary organ failure induced by trauma and sterile insults. Front Immunol 9:190

Fujita Y, Hoshina T, Matsuzaki J et al (2021) Early prediction of COVID-19 severity using extracellular vesicle COPB2. J Extracell Vesicles 10(8):e12092

Gao K, Jin J, Huang C et al (2019) Exosomes derived from septic mouse serum modulate immune responses via exosome-associated cytokines. Front Immunol 10:1560

Garcia-Cordero JL, Maerkl SJ (2020) Microfluidic systems for cancer diagnostics. Curr Opin Biotechnol 65:37–44

Germolec DR, Shipkowski KA, Frawley RP, Evans E (2018) Markers of inflammation. In: DeWitt JC, Rockwell CE, Bowman CC (eds) Immunotoxicity testing: methods and protocols. Springer, New York, pp 57–79

Gurung M, Moon DC, Choi CW et al (2011) Staphylococcus aureus produces membrane-derived vesicles that induce host cell death. PLoS ONE 6(11):e27958

Hassanpour M, Rezaie J, Nouri M, Panahi Y (2020) The role of extracellular vesicles in COVID-19 virus infection. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 85:104422

Hermann S, Brandes F, Kirchner B et al (2020) Diagnostic potential of circulating cell-free microRNAs for community-acquired pneumonia and pneumonia-related sepsis. J Cell Mol Med 24(20):12054–12064

Hosseinkhani B, van den Akker NMS, Molin DGM, Michiels L (2020) (Sub)populations of extracellular vesicles released by TNF-α –triggered human endothelial cells promote vascular inflammation and monocyte migration. J Extracell Vesicles 9(1):1801153

Husain-Syed F, Slutsky AS, Ronco C (2016) Lung-kidney cross-talk in the critically Ill patient. Am J Respir Crit Care Med 194(4):402–414

Iba T, Ogura H (2018) Role of extracellular vesicles in the development of sepsis-induced coagulopathy. J Intensive Care 6:68

Jansen F, Rohwer K, Vasa-Nicotera M et al (2016) CD-144 positive endothelial microparticles are increased in patients with systemic inflammatory response syndrome after TAVI. Int J Cardiol 204:172–174

Jia YC, Ding YX, Mei WT et al (2021) Extracellular vesicles and pancreatitis: mechanisms, status and perspectives. Int J Biol Sci 17(2):549–561

Jiménez-Alesanco A, Marcuello M, Pastor-Jiménez M et al (2019) Acute pancreatitis promotes the generation of two different exosome populations. Sci Rep 9(1):19887

Krishnamachary B, Cook C, Kumar A, Spikes L, Chalise P, Dhillon NK (2021) Extracellular vesicle-mediated endothelial apoptosis and EV-associated proteins correlate with COVID-19 disease severity. J Extracell Vesicles 10(9):e12117

Lankisch PG, Apte M, Banks PA (2015) Acute pancreatitis. The Lancet 386(9988):85–96

Lanyu Z, Feilong H (2019) Emerging role of extracellular vesicles in lung injury and inflammation. Biomed Pharmacother 113:108748

Lee Y, El Andaloussi S, Wood MJ (2012) Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 21(R1):R125-134

Lee H, Zhang D, Wu J, Otterbein LE, Jin Y (2017) Lung epithelial cell-derived microvesicles regulate macrophage migration via MicroRNA-17/221-induced integrin β1 recycling. J Immunol 199(4):1453–1464

Letsiou E, Sammani S, Zhang W et al (2015) Pathologic mechanical stress and endotoxin exposure increases lung endothelial microparticle shedding. Am J Respir Cell Mol Biol 52(2):193–204

Li Y, Zhu X, Zhang M, Tong H, Su L (2019) Heatstroke-induced hepatocyte exosomes promote liver injury by activating the NOD-like receptor signaling pathway in mice. PeerJ 7:e8216

Liu J, Niu Z, Zhang R et al (2021) MALAT1 shuttled by extracellular vesicles promotes M1 polarization of macrophages to induce acute pancreatitis via miR-181a-5p/HMGB1 axis. J Cell Mol Med 25(19):9241–9254

MacDonald IA, Kuehn MJ (2012) Offense and defense: microbial membrane vesicles play both ways. Res Microbiol 163(9–10):607–618

Mariathasan S, Monack DM (2007) Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nat Rev Immunol 7(1):31–40

Medzhitov R (2008) Origin and physiological roles of inflammation. Nature 454(7203):428–435

Min L, Wang B, Bao H et al (2021) Advanced nanotechnologies for extracellular vesicle-based liquid biopsy. Adv Sci (weinheim, Baden-Wurttemberg, Germany). 8(20):e2102789

Miyake H, Lee C, Chusilp S et al (2020) Human breast milk exosomes attenuate intestinal damage. Pediatr Surg Int 36(2):155–163

Mounzer R, Langmead CJ, Wu BU et al (2012) Comparison of existing clinical scoring systems to predict persistent organ failure in patients with acute pancreatitis. Gastroenterology 142(7):1476–1482

Namork E, Brandtzaeg P (2002) Fatal meningococcal septicaemia with "blebbing" meningococcus. Lancet (london, England) 360(9347):1741

Newton K, Dixit VM (2012) Signaling in innate immunity and inflammation. Cold Spring Harbor Perspect Biol 4(3):a006049

Pan BT, Johnstone RM (1983) Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell 33(3):967–978

Panich T, Chancharoenthana W, Somparn P, Issara-Amphorn J, Hirankarn N, Leelahavanichkul A (2017) Urinary exosomal activating transcriptional factor 3 as the early diagnostic biomarker for sepsis-induced acute kidney injury. BMC Nephrol 18(1):10

Qiu P, Zhou J, Zhang J, Dong Y, Liu Y (2021) Exosome: the regulator of the immune system in sepsis. Front Pharmacol 12:671164

Raeven P, Zipperle J, Drechsler S (2018) Extracellular vesicles as markers and mediators in sepsis. Theranostics 8(12):3348–3365

Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R (2020) The COVID-19 cytokine storm; what we know so far. Front Immunol 11:1446

Reithmair M, Buschmann D, Märte M et al (2017) Cellular and extracellular miRNAs are blood-compartment-specific diagnostic targets in sepsis. J Cell Mol Med 21(10):2403–2411

Rosell A, Havervall S, von Meijenfeldt F et al (2021) Patients with COVID-19 have elevated levels of circulating extracellular vesicle tissue factor activity that is associated with severity and mortality-brief report. Arterioscler Thromb Vasc Biol 41(2):878–882

Rossaint J, Margraf A, Zarbock A (2019) Perioperative inflammation. Anaesthesist 68(7):421–427

Salafi T, Zeming KK, Zhang Y (2016) Advancements in microfluidics for nanoparticle separation. Lab Chip 17(1):11–33

Schepers NJ, Bakker OJ, Besselink MG et al (2019) Impact of characteristics of organ failure and infected necrosis on mortality in necrotising pancreatitis. Gut 68(6):1044

Shao H, Im H, Castro C, Breakefield X, Weissleder R, Lee H (2018) New technologies for analysis of extracellular vesicles. Chem Rev 118(4):1917–1950

Singer M, Deutschman CS, Seymour CW et al (2016) The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA 315(8):801–810

Song JW, Lam SM, Fan X et al (2020) Omics-driven systems interrogation of metabolic dysregulation in COVID-19 pathogenesis. Cell Metab 32(2):188-202.e185

Soni S, Wilson MR, O'Dea KP et al (2016) Alveolar macrophage-derived microvesicles mediate acute lung injury. Thorax 71(11):1020–1029

Staubli SM, Oertli D, Nebiker CA (2015) Laboratory markers predicting severity of acute pancreatitis. Crit Rev Clin Lab Sci 52(6):273–283

Stephens DS, Edwards KM, Morris F, McGee ZA (1982) Pili and outer membrane appendages on *Neisseria meningitidis* in the cerebrospinal fluid of an infant. J Infect Dis 146(4):568

Stravitz RT, Bowling R, Bradford RL et al (2013) Role of procoagulant microparticles in mediating complications and outcome of acute liver injury/acute liver failure. Hepatology (baltimore, MD) 58(1):304–313

Su G, Ma X, Wei H (2020) Multiple biological roles of extracellular vesicles in lung injury and inflammation microenvironment. Biomed Res Int 2020:5608382

Sun P, Wang C, Mang G et al (2022) Extracellular vesicle-packaged mitochondrial disturbing miRNA exacerbates cardiac injury during acute myocardial infarction. Clin Transl Med 12(4):e779

Takei Y, Yamada M, Saito K et al (2019) Increase in circulating ACE-positive endothelial microparticles during acute lung injury. Eur Respir J. 54(4):1801188

Terrasini N, Lionetti V (2017) Exosomes in critical illness. Crit Care Med 45(6):1054–1060

Thawley V (2017) Acute liver injury and failure. Vet Clin North Am Small Anim Pract 47(3):617–630

Théry C, Witwer KW, Aikawa E et al (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7(1):1535750

Timmers L, Lim SK, Arslan F et al (2007) Reduction of myocardial infarct size by human mesenchymal stem cell conditioned medium. Stem Cell Res 1(2):129–137

Urabe F, Kosaka N, Ito K, Kimura T, Egawa S, Ochiya T (2020) Extracellular vesicles as biomarkers and therapeutic targets for cancer. Am J Physiol Cell Physiol 318(1):C29-c39

van Dijk SM, Hallensleben NDL, van Santvoort HC et al (2017) Acute pancreatitis: recent advances through randomised trials. Gut 66(11):2024–2032

Wang L, Zhao H, Xu H et al (2021) Targeting the TXNIP-NLRP3 interaction with PSSM1443 to suppress inflammation in sepsis-induced myocardial dysfunction. J Cell Physiol 236(6):4625–4639

Wang G, Huang W, Wang S et al (2021) Macrophagic extracellular vesicle CXCL2 recruits and activates the neutrophil CXCR2/PKC/NOX4 axis in sepsis. J Immunol (baltimore, Md: 1950) 207(8):2118–2128

Wu XB, Sun HY, Luo ZL, Cheng L, Duan XM, Ren JD (2020) Plasma-derived exosomes contribute to pancreatitis-associated lung injury by triggering NLRP3-dependent pyroptosis in alveolar macrophages. Biochim Biophys Acta 1866(5):165685

Xu J, Feng Y, Jeyaram A, Jay SM, Zou L, Chao W (2018) Circulating plasma extracellular vesicles from septic mice induce inflammation via MicroRNA- and TLR7-dependent mechanisms. J Immunol (baltimore, Md: 1950). 201(11):3392–3400

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