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Circulating extracellular vesicles in the context of interstitial lung disease related to systemic sclerosis: A scoping literature review

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ABSTRACT

Background: Interstitial lung disease (ILD) is a significant cause of disability and mortality in systemic sclerosis (SSc), where lung fibrosis stems from the interaction of cells within the epithelial, endothelial, interstitial, and immune cell compartments. Extracellular vesicles (EVs) are particles released by cells capable of transferring functionally active molecules, playing a crucial role in intercellular communication. This scoping review aims to identify and map existing evidence about the role of EVs as biomarkers or pathophysiological actors in SSc-ILD. It also retrospectively assesses the compliance of published articles with the current reporting guidelines established by the International Society of Extracellular Vesicles (ISEV).

Methods: This scoping review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) checklist. The searches were conducted up until 31 May 2023, with no restrictions on the starting year.

Results: Out of 778 publications identified and screened, 9 references were selected. The eligible studies collectively involved a total of 539 SSc patients, with 220 patients presenting with ILD, as demonstrated by high-resolution computed tomography. The studies largely focused on the quantitative assessment of EVs through flow cytometry, primarily concerning larger EVs. The studies primarily focused on the association of EV features with vascular complications, with fibrotic pulmonary involvement typically explored as a secondary finding. The evaluated patients' clinical characteristics were significantly heterogeneous across the studies as well as the association of EV features with the evidence of ILD but none of them longitudinally investigated the relationships with SSc-ILD prognosis. Adherence of these exploratory studies to ISEV reporting guidelines in terms of EV nomenclature, reporting of pre-analytic variables, and qualitative verification of EV separation products was incomplete.

Conclusions: The evidence concerning the clinical association of EV features is limited and conflicting. The interpretation of available data is substantially biased due to patient selection tailored for vascular complications, heterogeneity of separation methodology, and a lack of validation procedures.

1. Introduction

Interstitial lung disease (ILD) is a highly prevalent complication of systemic sclerosis (SSc), accounting for most of the disease-related disability and mortality [1]. Lung fibrosis results from the interaction of cells within the epithelial, endothelial, and interstitial compartments and components of both the innate and adaptive immune system. This ultimately leads to the recruitment and activation of fibroblasts, the differentiation of these fibroblasts into a myofibroblast phenotype, and the subsequent deposition of extracellular matrix [2].

Extracellular vesicles (EVs) can be accurately defined as heterogeneous particles naturally released from almost any type of cell that are encased by a lipid bilayer and cannot replicate due to the absence of a functional nucleus [3]. This collective term indeed encompasses various subtypes of cell-released, membranous structures, including exosomes, microvesicles, microparticles among many other names. EVs can

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functionally transfer between distant cells proteins, lipids, and genetic material, including mRNA transcripts and, such as microRNAs, as well as different types of DNA, such as mitochondrial and genomic DNA. This EV-mediated transfer process is at least partially receptor-mediated and, therefore, cell-specific [4].

The research hypothesis pertaining to the pathophysiological role of circulating EVs in SSc is indeed intriguing. SSc is a complex multi-organ disease, understood to initiate with widespread abnormalities in the endothelial and immune systems, which subsequently evolve into distinct clinical phenotypes and organ damage distributions. Therefore, EVs could potentially serve as a communication network among the endothelium, immune cells, and specific target organs, such as the lungs, acting as effectors of vascular damage and parenchymal fibrosis [5].

The past few decades have seen a significant surge in scientific publications exploring the role of EVs in both immune-mediated and fibrotic lung diseases. However, research in this area has been marked by substantial methodological heterogeneity, which limits the interpretability and reproducibility of the results. Considering the impressive advances made in EV isolation and characterization methodologies, the International Society for Extracellular Vesicles (ISEV) has issued and periodically updated specific guidelines on the minimum information to be included in scientific publications about EVs. These guidelines encompass a broad array of topics, including nomenclature, sample processing, EV separation, characterization of EVs, and their functional characterization [6].

The aim of this scoping review is to identify and map the existing evidence concerning the role of EVs as either biomarkers or pathophysiological actors in SSc-ILD. Furthermore, we retrospectively assessed the compliance of published articles with the current reporting guidelines established by the ISEV.

2. Methods

This scoping review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) checklist [7].

2.1. Eligibility criteria

Eligible references had to involve human subjects diagnosed with SSc according to the ACR/EULAR criteria and provide biological information about EVs derived from these patients' blood samples, regardless of the method of EV separation. Studies involving patients whose EV characteristics were not associated with evidence of ILD on high-resolution computed tomography (HRCT) or its severity according to pulmonary function tests (PFTs) were excluded. Additionally, review articles and conference abstracts where the methods could not be thoroughly reported were also omitted. Lastly, studies not published in English were not taken into consideration.

2.2. Search strategy

Bibliographic database searches were conducted in May 2023, encompassing the Embase and MEDLINE databases. The searches spanned up until 31 May 2023, with no restrictions on the starting year.

In summary, we identified studies for this review using search terms such as "systemic sclerosis" or "scleroderma" in association with "extracellular vesicles", as well as variations of these terms. Given the expected relatively scarce literature on the subject, we opted not to include ILD-related research terms in our initial search. Instead, we adopted a two-step research strategy. First, we screened all papers related to EVs in SSc, and then homed in on comparisons according to evidence of ILD. This approach was chosen to minimize the risk of overlooking any pertinent literature. The complete electronic search strategy is provided as supplementary material.

2.3. Selection of sources of evidence

The literature retrieved from the database search was imported into the Rayyan platform for deduplication. Studies retrieved using the search terms and parameters were independently screened by two authors (EDL, AR), focusing on the eligibility of the studies' based on abstracts or on full text in case of when the abstract content was not considered decisive for inclusion or exclusion of the reference. The inclusion and exclusion criteria reported above were applied, with any final discrepancies and uncertainties resolved by a third reviewer (FDG). Reasons for the exclusion of sources of evidence were recorded.

2.4. Synthesis of results

The authors collectively developed a data-charting form to identify which variables to extract. The final variables of interest included study design, sample size, patient demographics, reports of comorbidity and treatment, presence of diffuse cutaneous SSc, positivity for anti-centromere antibody (ACA) and anti-Scl70 antibody, disease duration, methods and reporting of EV separation and characterization, and any reported clinical associations with the presence and functional severity of ILD. Two reviewers (EDL, AR) independently charted the data, discussed the results, and continually updated the data-charting form in an iterative process.

3. Results

A total of 778 publications were identified through database searching. After excluding 144 duplicated references, 593 more were eliminated after reviewing their titles and abstracts. An additional 32 were excluded following a full-text review. Ultimately, nine references were included, as they provided information regarding the correlation between circulating EVs and ILD in patients with SSc [8–16] The comprehensive paper selection process, along with detailed reasons for exclusion, is presented in Fig. 1.

The included studies were published between the years 2008 and 2023, with five out of the nine papers being published before and after the 2018 update of the ISEV guidelines on minimal report information. All the studies employed cross-sectional designs, and there were no longitudinal evaluations based on the assessed EV characteristics. Many of the studies were primarily designed to investigate differences in EVs based on the presence of vascular complications such as digital ulcers and pulmonary arterial hypertension, or the severity of skin involvement. The association with fibrotic pulmonary involvement was typically explored as a secondary or incidental finding.

3.1. Report of EV donor characteristics

The eligible studies collectively involved a total of 539 SSc patients. Relevant demographic and clinical information are summarized in Table 1.

Several general factors are known to potentially affect circulating EVs, such as age, gender, body mass index, smoking habits, comorbidities, and medications. The ISEV therefore recommends to comprehensively report these in donor characterization. In the evaluated references, the general characteristics of EV donors were reported with varying levels of detail. While gender and age were more systematically represented, the reporting of the other variables that could potentially affect EV characteristics was inconsistent.

In addition to general characteristics, the reporting of clinical features related to SSc, which are critical for data interpretation and generation, varied across studies. A total of 220 patients presented with ILD, as demonstrated by HRCT, with prevalence ranging from 5.3 to 100% depending on the study. Baseline lung functional impairment, as indicated by reported forced vital capacity (FVC) and alveolar diffusion of carbon monoxide (DLco) values, also varied across studies. Established

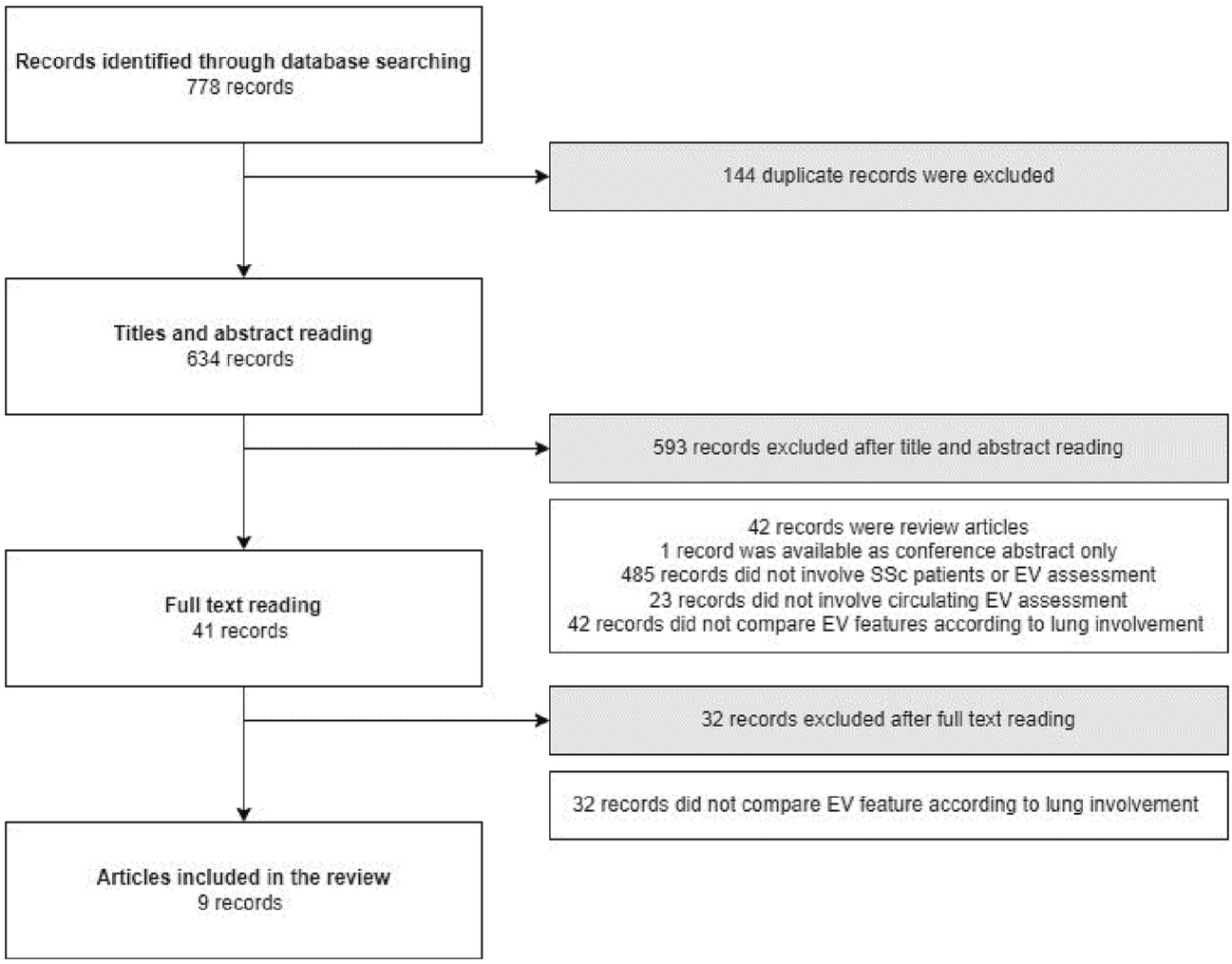

Fig. 1. Selection process for references.
Abbreviations: EV (Extracellular Vesicles), SSc (Systemic Sclerosis).

risk factors for ILD occurrence and progression, such as the Le Roy disease subset, anti-Scl70 antibody positivity, anti-centromere antibody (ACA) negativity, and disease duration, were also represented inconsistently across the studies.

Based on the available information, a substantial heterogeneity in terms of demographic and SSc characteristics, comorbidities, and treatments was observed. This heterogeneity might be the consequence of the inclusion criteria or result from consecutive patient enrolment.

3.2. EV nomenclature

The general term 'extracellular vesicles' is currently endorsed by the ISEV, given the lack of consensus about biomarkers that would unequivocally identify particles of specific biogenesis pathways, such as those of endosomal (exosomes) or plasma membrane origin (ectosomes, microparticles, microvesicles). Instead, the ISEV supports transparent operational terms based on separation or characterization methods, such as size, density, molecular content, or sample derivation.

As indicated in Table 2, only one reference adopted the term 'extracellular vesicles', while 'microparticles' was the preferred term in others. Two studies used the term 'exosomes', but as detailed below, this classification was based on the characteristics of the utilized isolation kit, not on a demonstrated endosomal origin of the particles. Most references identified a specific cellular origin for EVs based on surface markers detected by flow cytometry. However, no studies provided density or size specifications to support their nomenclature. Given the resolution limitations of standard flow cytometry, we can infer that most reported data likely pertains to larger EVs.

3.3. Sample collection, sample processing and EV separation

Due to their potential impact on EV characteristics, it is recommended by ISEV to report donor-related pre-analytical variables such as the time of blood collection, recent food intake, and physical activity. Similarly, pre-analytic variables related to sample collection and processing, such as manipulation, storage, and anticoagulant contamination, should be provided. Among the studies considered, collection-related variables are comprehensively reported in all the papers, while patient-related ones are largely overlooked.

Separation methods are similarly thoroughly reported or referenced in all the studies. Plasma was the most used matrix for the assessment of circulating EVs, and differential centrifugation was the most frequently employed isolation technique. A significant heterogeneity was observed in the centrifugation protocol, and a proper high-speed ultracentrifugation step was included in only one paper. Given the prominent use of relatively low-speed centrifugation steps (<12,000 G), it can be hypothesized that large EVs were the primary object of characterization. Two studies isolated EVs from serum using a commercially available kit based on polyethylene glycol-induced precipitation. The combination of more than one isolation technique was used only in one case, which employed centrifugation and mechanical filtration sequentially. High-specificity separation techniques, including chromatography and

Table 1
Clinical characteristics of the assessed patients.

Reference	Enrolled SSc patients	Age	Males	Diffuse variant	ACA positive	Anti-Scl70 positive	Disease duration	ILD on HRCT	Baseline FVC	Baseline DLco	Vasoactive treatment	Immunosuppressive treatment	Smoke habits and comorbidities
Guiducci 2008	37	63 ± 12 years	10.8%	30.3%	43.2%	35.1%	13 ± 10 years	48.6%	98.5 ± 20.7%	66.0 ± 23.0%	Not Reported	Not Reported	Smoke (ever) 100%, SAH 10.8% Diabetes 2.7%, Dyslipidaemia 0%
Nomura 2008	42	48 ± 11 years	16.7%	Not Reported	Not Reported	Not Reported	Not Reported	59.5%	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported
Iversen 2015	121	57 ± 12 years	15.7%	13.2%	40.5%	12.4%	12 ± 9 years	>5.8%	95.7 ± 21.1%	64.6 ± 19.9%	Not Reported	DMARDs 6.6%	Smoke (ever) 68.6%, SAH 10.8%, Cancer 0%
Nakamura 2016	44	60 years	Not reported	43.2%	22.7%	25.0%	5 years	43.2%	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported
Michalska-Jakubus 2017	47	56 ± 11 years	0.0%	14.9%	36.2%	55.3%	10 ± 7 years	80.9%	Not Reported	Not Reported	ERAi 0%	*CYC* 12.8%	Diabetes 0%, Dyslipidaemia 0% CV disease 0% Cancer 0%
2020	40	52 ± 13 years	30.0%	Not Reported	Not Reported	Not Reported	Not Reported	100%	73.9 ± 18.4%	77.6 ± 15.2%	Not Reported	Not Reported	Smoke (ever) 10.0%
Leleu 2020	96	59 ± 13 years	30.2%	Not reported	42.7%	20.8%	8 ± 7 years	34.4%	1.13 ± 0.21 l	Not Reported	Not Reported	MTX 33.3%, Targeted DMARD 10.5%	Smoke (current) 27.7%
Jud 2021	38	80 ± 9 years	5.3%	0.0%	Not Reported	Not Reported	Not Reported	5.3%	106 ± 18%	89 ± 15%	ACEi/ARB 18.4%, CCB 15.8%, Anti-platelet 15.8%, Anticoagulant 7.9%, Statins 7.9%, Diuretics 5.3%	CS 7.9%, MMF 5.3% HCQ 5.3%, MTX 2.6%, RTX 2.6%, ABA 2.6%	Smoke (ever) 31.6%, Dyslipidaemia 52.6%, SAH 36.8%, Cancer 10.5%, CV disease 0%
De Oliviera 2023	70	49 ± 13 years	10.0%	37.1%	18.6%	18.6%	6 ± 4 years	54.3%	80.9 ± 17.4%	Not Reported	CCB 70.0%, PDEi 12.9%, Statin 11.4%	MMF 21.4%, MTX 17.1%, CS 11.4%, LEF 5.7%, CFS 4.3%, AZT 4.3%, RTX 1.4%	Smoke(ever) 0%, Any comorbidity 52.9%

Abbreviations: ABA (Abatacepts), ACA (Anti-Centromere Antibody), ACEi (Angiotensin-Converting-Enzyme inhibitors), ARB (Angiotensin II Receptor Blockers), AZT (Azathioprine), CCB (Calcium Channel Blockers), CYC (Cyclophosphamide), CS (Corticosteroids), CV (Cardiovascular), DLco (Alveolar Diffusion of Carbon Monoxide), DMARDs (Disease-modifying antirheumatic drugs), ERA (Endothelin receptor antagonists), FVC (Forced Vital Capacity), HCQ (Hydroxychloroquine), HRCT (High-Resolution Computed Tomography), ILD (Interstitial Lung Disease), LEF (Leflunomide), MMF (Mycophenolate mofetile), MTX (Methotrexate), PDEi (Phosphodiesterase type 5 inhibitor), RTX (Rituximab), SAH (Systemic Arteria Hypertension), SSc (Systemic Sclerosis).

Table 2
EV isolation and assessment.

Reference	EV nomenclature	Biologic sample	Sample processing conditions	EV separation or enrichment	EV quantification	Global EV characterization	Single EV characterization
Guiducci 2008	Microparticles	Plasma	Reported	Differential centrifugation (Ultracentrifugation included)	Flow cytometry (CD42, CD235, CD66b, CD14, CD3, CD19, CD144)	Not performed	Not performed
Nomura 2008	Microparticles	Plasma	Reported	Differential centrifugation	Flow cytometry (CD42a, CD40, Annexin V)	Not performed	Not performed
Iversen 2015	Microparticles	Plasma	Reported	Differential centrifugation, Filtration	Flow cytometry (CD42a, CD45, CD146)	Not performed	Not performed
Nakamura 2016	Exosomes	Serum	Reported	PEG precipitation	BCA	ELISA (CD63)	Not performed
Michalska-Jakubus 2017	Microparticles	Plasma	Reported	Differential centrifugation	Flow cytometry (CD51, CD31, CD42b, Annexin V)	Not performed	Not performed
Ryu 2020	Extracellular Vesicles	Serum	Reported	PEG precipitation	Not performed	LC-MS, RT-PCR	TEM
Leleu 2020	Microparticles	Plasma	Reported	Differential centrifugation	Flow cytometry (CD235a, CD41, CD31, CD45, CD66b, CD3, CD19)	Not performed	Not performed
Jud 2021	Microparticles	Plasma	Reported	Differential centrifugation	Flow cytometry (CD31, CD42a, CD51, CD54, CD62E, CD105, CD144)	Not performed	Not performed
De Oliveiera 2023	Microparticles	Plasma	Reported	Differential centrifugation	Flow cytometry (CD42, CD3, CD105, CD14)	Not performed	Not performed

Abbreviations: BCA (Bicinchoninic acid assay), CD (Clusters of differentiation), ELISA (Enzyme-Linked Immunosorbent Assay), EV (Extracellular Vesicle), LC-MS (Liquid Chromatography - Mass Spectrometry), PEG (Polyethylene glycol), RT-PCR (Real Time Polymerase Chain Reaction), TEM (Transmission electron microscopy).

antibody-mediated selection, were not used in any of the studies.

Given the details provided, most of the reported isolation methods exhibited low EV-specificity and a high recovery rate. Non-EV lipidic structures, such as lipoproteins, and cellular fragments were possibly included in most of the final samples.

Notably, ISEV does not endorse specific isolation methods, as the separation process should be tailored according to the experimental question, provided that the technique is comprehensively described, and the quality of the separation process's product is subsequently verified.

3.4. EV quantification and characterization

The definition of EV features can potentially encompass various aspects of their nature, as informed by several techniques. This critical process represents both the verification of the separation process results and the exploration of the specific nature of the isolated EVs. Generally, these methods could be categorized into four primary types: global quantification, global characterization, single EV characterization, and functional studies.

Global quantification involves indirect methods, such as reporting the baseline volume of the sample used for EV separation, and direct methods like assessing the total protein amount or total particle numbers. The latter can be done using nanoparticle tracking analysis (NTA), standard flow cytometry for larger EVs, high-resolution flow cytometry for smaller EVs, or other methods.

In the studies considered, flow cytometry was the most frequently used tool for EV quantification. However, the antibodies used did not typically target endorsed general surface or cytosolic EV markers; instead, they occasionally assessed endorsed cell-specific surface molecules such as CD14 or CD42a. One single study used total protein content as a proxy for EV total content. None of the studies employed the currently recommended practice of performing global quantification by at least two methods. Notably, different studies were only partially consistent in terms of markers used to label EVs from different cellular origins.

Global characterization for EV isolation quality assessment is based on the evaluation of at least one each of transmembrane or membrane-anchored protein localized in EVs, cytosolic proteins with membrane-binding abilities, and proteins associated with compartments supposed to be absent or poorly represented in EV-enriched samples.

The studies reviewed were quite deficient in this regard, as only one included a CD63 evaluation as an EV marker. The singular study in which a comprehensive proteomic evaluation based on mass spectrometry was performed focused on comparing ILD and non-ILD SSc patients and did not provide available information about the representation of EV markers in the assessed samples. Notably, this was also the only study that assessed mitochondrial DNA among potentially evaluable nucleic acid content of the EV-enriched samples.

Single EV characterization includes both the direct visualization of single EVs, typically based on electron microscopy, and single-particle analysis techniques that can calculate biophysical parameters such as NTA and light scattering, or fluorescence detection in high-resolution flow cytometry. Only one of the considered studies included the electron microscopy assessment of separated EVs, while the flow cytometry techniques used did not provide substantial information about EV physical characteristics for any of the studies.

Finally, functional studies on lung-derived cell lines, such as pulmonary fibroblasts, epithelial cells, endothelial cells, or bronchoalveolar lavage fluid-derived macrophages, were not provided in any of the selected references.

3.5. Clinical associations of EV characteristics

The reported association between EV characteristics and ILD in SSc patients are summarized in Table 3. The total number of EVs was the most used as a measure to compare EV characteristics with the presence or severity of ILD. As the only possible alternative, Nakamura et al. specifically used CD63 as a surrogate measure of EV abundance, while Ryu provided a comprehensive proteomic characterization and a quantification of mitochondrial DNA content within EVs. The clinical correlations explored were based on the presence of ILD for all the studies except one, with some studies also providing a correlation of EV characteristics with the severity of pulmonary function as assessed by PFTs.

Table 3
Association of circulating EV characteristics with ILD presence and severity.

Reference	Associations with ILD presence on HRCT	Associations with PFT
Guiducci 2008	• No difference in total EV number evaluated though flow-cytometry between SSc patients with and without ILD. • No difference in platelet-derived (CD42+), erythrocyte-derived (CD235+), neutrophile-derived (CD66b+), monocyte-derived (CD14+), T cell-derived (CD3+), B cell-derived (CD19+), endothelium-derived (CD144+) EV number evaluated though flow-cytometry between SSc patients with and without ILD.	• No correlation of total EV number evaluated though flow-cytometry with FVC and DLco. • No correlation of platelet-derived (CD42+), erythrocyte-derived (CD235+), neutrophile-derived (CD66b+), monocyte-derived (CD14+), T cell-derived (CD3+), B cell-derived (CD19+), endothelium-derived (CD144+) EV numbers evaluated though flow-cytometry with FVC and DLco.
Nomura 2008	• The number of platelet-derived (CD42a+) or procoagulant monocyte-derived (CD14+ Annexin V+) EVs evaluated though flow-cytometry was higher in SSc patients with ILD compared to those without ILD.	• Not assessed or not reported.
Iversen 2015	• Not assessed or not reported.	• Platelet-derived (CD42a+) and endothelium-derived (CD146+) EV number evaluated though flow-cytometry had a weak inverse correlation with both FVC and DLco. • No correlation of leukocyte-derived (CD45+) EV number evaluated though flow-cytometry with FVC or DLco.
Nakamura 2016	• No difference in EV levels based on ELISA CD63 assessment between SSc patients with and without ILD.	• No correlation of EV levels based on ELISA CD63 assessment with FVC or DLco.
Michalska-Jakubus 2017	• No difference in total endothelium-derived (CD31+/CD42b-), activated endothelium-derived (CD62e+/Annexin V-), apoptotic endothelium-derived (CD62e+/Annexin V+ or CD51+) EV number evaluated though flow-cytometry between SSc patients with and without ILD.	• No correlation of total endothelium-derived (CD31+/CD42b-), activated endothelium-derived (CD62e+/Annexin V-), apoptotic endothelium-derived (CD62e+/Annexin V+ or CD51+) EV number evaluated though flow-cytometry with FVC or DLco.
Ryu 2020	• A total of 38 proteins, mainly related to platelet activation, cell adhesion, and immune responses assessed through mass spectrometry were differently represented in EV-enriched samples of SSc-ILD patients compared to healthy controls. • Mitochondrial DNA was overrepresented in EV-enriched samples of SSc-ILD patients compared to healthy controls.	• Not assessed or not reported.
Leleu 2020	• The total EV number evaluated though flow-cytometry was higher in SSc patients with ILD compared to those without ILD. • No difference in platelet-derived (CD41+, CD235-) and endothelium-derived (CD31+, CD235-, CD41-) EV number evaluated though flow-cytometry between SSc patients with and without ILD.	• The total EV number evaluated though flow-cytometry had a moderate inverse correlation with FVC and a weak inverse correlation with TLC, and Kco. • Platelet-derived (CD41+, CD235-) EV number had a weak inverse correlation with FVC. • No correlation of endothelium-derived (CD31+, CD235-, CD41-) EV number evaluated though flow-cytometry with FVC or DLco.
Jud 2020	• No difference in platelet-derived (CD31+/CD42b-) EV number evaluated though flow-cytometry between SSc patients with and without ILD.	• Not assessed or not reported.
De Oliveiera 2023	• No difference in platelet-derived (CD42+/CD31+), neutrophile-derived (CD66b+), monocyte-derived (CD14+), or endothelium-derived (CD105+) EV number evaluated though flow-cytometry between SSc patients with and without ILD.	• Not assessed or not reported.

Abbreviations: CD (Clusters of differentiation), DLco (Alveolar Diffusion of Carbon Monoxide), ELISA (Enzyme-Linked Immunosorbent Assay), EV (Extracellular Vesicle), FVC (Forced Vital Capacity), ILD (Interstitial Lung Disease), Kco (Carbon monoxide transfer coefficient), TLC (Total Lung Capacity).

Regarding EV levels, the data provided across the studies present conflicting results, possibly also due to significant methodological heterogeneity. A similar number of studies either support or refute the association between the presence and severity of functional impairment in ILD and the number of total and platelet-derived, endothelium-derived, or leukocyte-derived EVs. Importantly, there is no discernible pattern linking the conclusions of these studies to the reported methodologies of separation or characterization.

Interestingly, the sole stdy that provided a comprehensive exploration of differences in protein composition and mitochondrial DNA content found substantial discrepancies between ILD and non-ILD patients.

4. Discussion

This scoping review aimed to provide a preliminary assessment of the scope, nature, and extent of the available literature on circulating EVs in ILD related to SSc using a rigorous methodology in terms of reference selection and evaluation of adherence to current ISEV guidelines about the minimal information to be provided in these studies.

The available evidence on the topic is still limited. The available studies were primarily pioneering and exploratory in nature, largely focusing on the association with skin fibrosis and microvascular impairment, rather than on the comparison of patients according to lung fibrotic involvement. Notably, data about the EV profiles of SSc-ILD are considerably more scarce compared to other forms of ILD such as idiopathic pulmonary fibrosis, sarcoidosis, and hypersensitivity pneumonia, where deregulation of protein and RNA content of circulating EVs have been extensively reported [17,18]

A further observation is that the available studies are remarkably heterogeneous in terms of patient sample characteristics and EV separation methodology. Key characteristics related to SSc, such as disease duration, Le Roy cutaneous variant, autoantibody specificity, and proportion of patients, are variably represented across the samples, and some studies did not even comprehensively describe all the crucial clinical features. Moreover, even though a standard in the EV process does not exist, the final product of the different EV separation processes was likely to produce different EVs in terms of purity, release modality,

size, and cellular derivation across the studies. Both these aspects significantly limit the comparison of findings and their generalization to the SSc-ILD population.

A major limitation of all the studies is the absence of an exhaustive verification of the EV separation process. EV-biomarkers were incompletely assessed or not assessed at all, limiting the comprehension of the translational impact of the reported findings. Moreover, negative EV-markers, i.e., proteins that indicate the degree of contamination with non-EV components after the separation process, were not assessed in any of the studies. This is particularly crucial in nucleic acid and proteomic characterization, given the possibility of extra-EV components affecting the results. However, this lack of comprehensive verification is not uncommon in earlier and even some more recent EV studies. Indeed, it was this observed praxis that led to the development and implementation of the ISEV guidelines [19].

The provided information is also still limited from both biological and clinical perspectives. From a biological perspective, all the studies, except one, are focused only on the abundance of EVs. A quantitative-based approach completely ignores the heterogeneity in terms of quality of protein and nucleic acid content of EVs that have been assessed in a single study on SSc-ILD patients so far. Notably, lipid characterization and transcriptomic characterization of circulating EVs in SSc patients have not been provided yet. Furthermore, small EVs were unlikely to be separated by most of the reported methods.

From a clinical perspective, none of the studies employed a longitudinal design that could discern baseline EV characteristics according to the clinical prognosis of SSc-ILD. This is of specific interest considering the identification of a subset of SSc-ILD patients with a rapidly progressive course, a more profound impact on survival, and distinct therapeutic needs [20].

Finally, none of the studies assessed the potential in vitro biological effects of EVs on pulmonary pathophysiology, nor the correlation between patients' clinical characteristics and the EV molecular profile. It is conceivable that EVs could serve not merely as a biomarker, but also as a therapeutic target or tool. This hypothesis aligns with increasing evidence of biological activity in EVs separated from the supernatants of SSc patient cell cultures [21] and the effect of EVs on fibrosis in animal models [22,23] In line with a potential pathogenic role for EVs in SSc-ILD, these particles have been shown to traverse biological barriers [24] or fibrotic tissues [25] and lungs are recognized as major sites of circulating EV localization in animal models [26,27]

5. Conclusions

In conclusion, the evidence regarding the clinical association of EV features provided by the available pioneering studies is limited and conflicting. The interpretation of the available data could be biased due to patient selection tailored for vascular complications, heterogeneity of separation methodology, and a lack of validation procedures. Most of the studies focused on larger circulating EVs. Future research should strictly adhere to the available guidelines for minimal information to draw robust inferences about the role these particles play in SSc-ILD.

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Ethical approval information

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Pubblic data

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autrev.2023.103401.

References

[1] Tyndall AJ, Bannert B, Vonk M, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR scleroderma trials and research (EUSTAR) database. Ann Rheum Dis 2010;69(10):1809–15. https://doi.org/10.1136/ard.2009.114264.
[2] Wei J, Bhattacharyya S, Tourtellotte WG, Varga J. Fibrosis in systemic sclerosis: emerging concepts and implications for targeted therapy. Autoimmun Rev 2011;10(5):267–75. https://doi.org/10.1016/j.autrev.2010.09.015.
[3] Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014;30:255–89. https://doi.org/10.1146/annurev-cellbio-101512-122326.
[4] Yáñez-Mó M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles 2015;4:27066. Published 2015 May 14, https://doi.org/10.3402/jev.v4.27066. Published 2015 May 14.
[5] Čolić J, Matucci Cerinic M, Guiducci S, Damjanov N. Microparticles in systemic sclerosis, targets or tools to control fibrosis: this is the question! J Scleroderma Relat Disord 2020 Feb;5(1):6–20. https://doi.org/10.1177/2397198319857356. Epub 2019 Jun 28. PMID: 35382401; PMCID: PMC8922594.
[6] Théry C, Witwer KW, Aikawa E, et al. 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 2018;7(1). https://doi.org/10.1080/20013078.2018.1535750. 1535750. Published 2018 Nov 23.
[7] Tricco AC, Lillie E, Zarin W, et al. PRISMA extension for scoping reviews (PRISMA-ScR): checklist and explanation. Ann Intern Med 2018;169(7):467–73. https://doi.org/10.7326/M18-0850.
[8] Guiducci S, Distler JH, Jüngel A, et al. The relationship between plasma microparticles and disease manifestations in patients with systemic sclerosis. Arthritis Rheum 2008;58(9):2845–53. https://doi.org/10.1002/art.23735.
[9] Nomura S, Inami N, Ozaki Y, Kagawa H, Fukuhara S. Significance of microparticles in progressive systemic sclerosis with interstitial pneumonia. Platelets. 2008;19(3):192–8. https://doi.org/10.1080/09537100701882038.
[10] Iversen LV, Ullman S, Østergaard O, et al. Cross-sectional study of soluble selectins, fractions of circulating microparticles and their relationship to lung and skin involvement in systemic sclerosis. BMC Musculoskelet Disord 2015;16:191. Published 2015 Aug 12, https://doi.org/10.1186/s12891-015-0653-8. Published 2015 Aug 12.
[11] Nakamura K, Jinnin M, Harada M, et al. Altered expression of CD63 and exosomes in scleroderma dermal fibroblasts. J Dermatol Sci 2016;84(1):30–9. https://doi.org/10.1016/j.jdermsci.2016.06.013.
[12] Michalska-Jakubus M, Kowal-Bielecka O, Smith V, Cutolo M, Krasowska D. Plasma endothelial microparticles reflect the extent of capillaroscopic alterations and correlate with the severity of skin involvement in systemic sclerosis. Microvasc Res 2017;110:24–31. https://doi.org/10.1016/j.mvr.2016.11.006.
[13] Ryu C, Walia A, Ortiz V, et al. Bioactive plasma mitochondrial DNA is associated with disease progression in scleroderma-associated interstitial lung disease. Arthritis Rheumatol 2020;72(11):1905–15. https://doi.org/10.1002/art.41418.
[14] Leleu D, Levionnois E, Laurent P, et al. Elevated circulatory levels of microparticles are associated to lung fibrosis and vasculopathy during systemic sclerosis. Front Immunol 2020;11:532177. Published 2020 Oct 23, https://doi.org/10.3389/fimmu.2020.532177. Published 2020 Oct 23.
[15] Jud P, Meinitzer A, Strohmaier H, et al. Evaluation of endothelial dysfunction and clinical events in patients with early-stage vasculopathy in limited systemic sclerosis. Clin Exp Rheumatol 2021;39(Suppl. 131):57–65. https://doi.org/10.55563/clinexprheumatol/243mpp (4).
[16] de Oliveira SM, de Azevedo Teixeira IL, França CN, de Oliveira Izar MC, Kayser C. Microparticles: potential new contributors to the pathogenesis of systemic sclerosis? Adv Rheumatol 2023;63(1):19. Published 2023 Apr 25, https://doi.org/10.1186/s42358-023-00299-y. Published 2023 Apr 25.
[17] D'Alessandro M, Bergantini L, Bargagli E, Vidal S. Extracellular vesicles in pulmonary fibrosis models and biological fluids of interstitial lung disease patients: a scoping review. Life (Basel) 2021;11(12):1401. Published 2021 Dec 15, https://doi.org/10.3390/life11121401. Published 2021 Dec 15.
[18] Trappe A, Donnelly SC, McNally P, Coppinger JA. Role of extracellular vesicles in chronic lung disease. Thorax. 2021;76(10):1047–56. https://doi.org/10.1136/thoraxjnl-2020-216370.
[19] EV-TRACK Consortium, Van Deun J, Mestdagh P, et al. EV-TRACK: transparent reporting and centralizing knowledge in extracellular vesicle research. Nat Methods 2017;14(3):228–32. https://doi.org/10.1038/nmeth.4185.
[20] De Lorenzis E, Natalello G, Di Donato S, et al. AB0841 concordance and prognostic relevance of different definition of systemic sclerosis interstitial lung disease progression. Ann Rheum Dis 2023;82:1633–4.

[21] Bryon J, Wasson C, Ross R, Zeqiraj E, Del Galdo F. Systemic sclerosis dermal fibroblast-derived exosomes trigger a type 1 interferon Rresponse in keratinocytes through TBK1 [abstract]. Arthritis Rheumatol 2022;74(Suppl. 9).
[22] Rozier P, Maumus M, Maria ATJ, et al. Mesenchymal stromal cells-derived extracellular vesicles alleviate systemic sclerosis via miR-29a-3p. J Autoimmun 2021;121:102660. https://doi.org/10.1016/j.jaut.2021.102660.
[23] Rozier P, Maumus M, Maria ATJ, et al. Lung fibrosis is improved by extracellular vesicles from IFNγ-primed mesenchymal stromal cells in murine systemic sclerosis. Cells 2021;10(10):2727. Published 2021 Oct 13, https://doi.org/10.3390/cells10102727. Published 2021 Oct 13.
[24] Banks WA, Sharma P, Bullock KM, Hansen KM, Ludwig N, Whiteside TL. Transport of extracellular vesicles across the blood-brain barrier: brain pharmacokinetics and effects of inflammation. Int J Mol Sci 2020;21(12):4407. Published 2020 Jun 21, https://doi.org/10.3390/ijms21124407. Published 2020 Jun 21.
[25] Lenzini S, Bargi R, Chung G, Shin JW. Matrix mechanics and water permeation regulate extracellular vesicle transport. Nat Nanotechnol 2020;15(3):217–23. https://doi.org/10.1038/s41565-020-0636-2.
[26] Wiklander OP, Nordin JZ, O'Loughlin A, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. J Extracell Vesicles 2015;4:26316. Published 2015 Apr 20, https://doi.org/10.3402/jev.v4.26316. Published 2015 Apr 20.
[27] Morishita M, Takahashi Y, Nishikawa M, Takakura Y. Pharmacokinetics of exosomes-an important factor for elucidating the biological roles of exosomes and for the development of exosome-based therapeutics. J Pharm Sci 2017;106(9):2265–9. https://doi.org/10.1016/j.xphs.2017.02.030.