

Prediction of coronavirus disease severity based on increased levels of microvesicles: A systematic review and meta-analysis

Nastasya Nunki^{1,2}, Yetti Hernaningsih^{3,4,5*}, Puspa Wardhani^{3,4,6}, Citrawati Dyah Kencono Wungu^{6,7}, Agil Saputra⁸, Narazah Mohd Yusoff^{3,9}

¹Laboratory Medicine, Master Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

²Department of Medical Laboratory Technology, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Surabaya, East Java, Indonesia.

³Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

⁴Dr. Soetomo General Academic Hospital, Surabaya, East Java, Indonesia.

⁵Airlangga University Hospital, Surabaya, East Java, Indonesia.

⁶Institute of Tropical Disease, Universitas Airlangga, Surabaya, East Java, Indonesia.

⁷Department of Physiology and Medical Biochemistry, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

⁸Department of Medical Laboratory Technology, Faculty of Medicine, Universitas Palangka Raya, Palangka Raya, Central Borneo, Indonesia.

⁹Genetics Unit, Clinical Diagnostics Lab, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Penang, Malaysia.

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ABSTRACT

Background: Elevated microvesicle (MV) levels are potential biomarkers of coronavirus disease 2019 (COVID-19). We compared different types of microvesicles (total microvesicles, TMVs), platelet-derived microvesicles (PMVs), endothelium-derived microvesicles (EMVs), and tissue factor-bearing microvesicles (TF-MVs).

Objectives: We investigated the correlations between microvesicles and D-dimers, as well as between microvesicles and COVID-19 severity.

Materials and methods: A systematic literature search was conducted in PubMed, Web of Science, Scopus, and ScienceDirect to identify relevant studies comparing microvesicle types between patients with COVID-19 and healthy controls. Pooled estimates of the standardized mean difference (SMD) for each MV type were calculated using RevMan 5.4, along with the corresponding 95% confidence intervals (CIs).

Results: This meta-analysis included six studies comprising 183 COVID-19 patients and 135 healthy controls for quantitative analysis. Compared with controls, COVID-19 patients presented significantly higher levels of circulating TMVs, PMVs, EMVs, and TF-MVs (TMVs: SMD=4.27, 95% CI 2.18-6.36, $p<0.0001$; PMVs: SMD=2.07, 95% CI 0.99-3.15, $p=0.0002$; EMVs: SMD=1.20, 95% CI 0.56-1.84, $p=0.0002$; and TF-MVs: SMD=2.56, 95% CI 1.98-3.15, $p<0.00001$). D-dimer levels were correlated with MV levels ($N=82$, $r=0.430$, 95% CI 0.227-0.592, $p<0.0001$). In general, MVs were correlated with COVID-19 severity ($N=168$; $r=0.942$; 95% CI, 0.558-0.993; $p=0.002$).

Conclusion: Patients with severe COVID-19 had elevated levels of TMVs, PMVs, EMVs, and TF-MVs. Therefore, MVs may contribute to the procoagulant effects associated with prognostic and severity biomarkers of COVID-19.

Corresponding contributor.

Author's Address: Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.

E-mail address: yetti-h@fk.unair.ac.id

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2

(SARS-CoV-2), affects many body systems, including the hematological system, and has garnered global attention since its initial discovery.¹ The invasion of host cells by SARS-CoV-2 can lead to thrombosis,² for which hypercoagulopathy is a contributory factor and potential cause of severe COVID-19.³ Recently, extracellular vesicles have been identified as novel diagnostic biomarkers of triggers of hypercoagulation and subsequent thrombosis in COVID-19 patients⁴ and have been implicated in various human diseases.⁵

Microvesicles (MVs), a type of extracellular vesicle,⁶ are released from various cells following cellular hyperactivation, including platelets, leukocytes, and endothelial cells.⁷ MVs possess intact membrane structures and express membrane glycoproteins specific to their origin cells.⁸ Thus, MVs can be isolated from platelet-free plasma (PFP) by ultracentrifugation and exhibit diverse immunophenotypes.⁸ Upon cellular activation, MVs adhere to the activated endothelium along with neutrophil extracellular traps (NETs), modulate the coagulation cascade, and induce excessive thrombin production, which ultimately results in a hypercoagulable state.⁹

As MVs are released under pathophysiological conditions, their clinical relevance as disease markers has increasingly generated interest.¹⁰ Furthermore, MVs may influence COVID-19 severity. MVs contribute to blood clotting by exposing negatively charged phospholipids and tissue factors (TFs), which initiate the coagulation pathway *in vivo*.¹¹ MV formation disrupts cellular systems and leads to asymmetry of the phospholipid bilayer in the cell membrane, resulting in the externalization of phosphatidylserine (PS). A notable feature of MVs is their involvement in blood coagulation as a source of TF and as a negatively charged surface on which clotting factor complexes can accumulate.¹¹ Thus, MVs are known for their procoagulant properties.^{12,13} Moreover, D-dimer is a part of the routine screening protocol for determining the severity of COVID-19 and is associated with MV-related tissue factor-dependent activation of blood cells. Thus, the presence of MVs supports fibrin formation, with consequent D-dimer formation and fibrin degradation by plasmin.

This review compared platelet-derived, endothelium-derived, and TF-bearing MV levels in COVID-19 patients with those in healthy controls. Moreover, the correlations between MVs and D-dimer, an essential marker of thrombus formation, and between MVs and COVID-19 severity were assessed. To strengthen these findings, a meta-analysis explored the potential application of MVs as novel biomarkers of thrombus occurrence and disease severity in COVID-19 patients.

Materials and methods

Ethical consideration

The protocol for this systematic review has been registered in the International Prospective Registry of

Systematic Reviews (PROSPERO CRD42022362375). This review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. Only publicly available data from published studies were analyzed, and no direct patient interaction or identifiable data were used.

Type of sampling and reasons for selection

Purposive sampling of studies was used for a systematic review and meta-analysis. The selected studies were chosen based on specific inclusion criteria (human studies on COVID-19 and microvesicles, comparison with healthy controls, quantification of MV levels, and correlation with D-dimer levels and disease severity) to ensure the relevance and robustness of the findings. A systematic literature search was conducted in PubMed, Web of Science, Scopus, and ScienceDirect.

Search strategy

A systematic search was conducted using PubMed, Web of Science, Scopus, and ScienceDirect for articles published through May 10, 2023. The search was not limited by region or release type, and a focused strategy was employed to identify human studies that examined MVs from COVID-19 patients. The search strategy involved a combination of improvisation keywords, MeSH terms (COVID-19, coronavirus, SARS-CoV-2, microvesicles, microparticles, procoagulant), and abbreviations (MVs and MPs).

Inclusion criteria

The eligibility criteria for study inclusion were based on five parameters: population, intervention, comparator, outcome, and study design (PICOS). This meta-research methodology ensures an unbiased search, inclusion, and assessment of articles.

Population

Only studies involving human participants were included.

Intervention

The included studies followed MV isolation protocols for blood samples obtained from human participants; patients who were diagnosed using RT-PCR, patients who were in the post-COVID-19 convalescent stage after up to four weeks of being PCR negative for the virus; patients who were subjected to D-dimer examination; and patients with comorbidities, such as diabetes, hypertension, coronary heart disease, hyperlipidemia, and chronic renal diseases.

Comparator

Blood samples from healthy controls were used as comparator groups. The severity of COVID-19 patients and patients whose D-dimer levels were also measured were used as the assessment groups.

Outcome

MVs were quantified in patients who were clinically evaluated for COVID-19, and disease severity was graded as mild to severe according to World Health Organization guidelines. Compared with those in the control group, the specific MVs of interest in COVID-19 patients included total microvesicles (TMVs), platelet-derived microvesicles (PMVs), endothelium-derived microparticles (EMVs), and tissue factor-bearing microvesicles (TF-MVs). The correlations of MVs with disease severity and D-dimer levels were investigated.

Study design

This systematic review included observational, analytical, cohort, and cross-sectional studies that measured MV levels in cohorts of COVID-19 patients. The studies were published in 2020.

Exclusion criteria

Studies involving nonhuman subjects were excluded. We excluded studies of patients who followed a home isolation protocol, had bacterial inflammation (non-COVID-19) or malignancies, or preclinical animal

models. Review articles, case reports, case series, meta-analyses, duplicate studies, and non-English articles were excluded.

Study and data collection

Titles and abstracts that met the inclusion criteria were screened for full-text review, and all reviewers participated. Any discrepancies were resolved through discussions among the reviewers. Figure 1 depicts the Preferred Reporting Items for Systematic Reviews and Meta-Analyses flowchart of the study selection process. The extracted data were subjected to a double-blinded process. The studies were assessed based on various characteristics, including specimen type, MV isolation technique, instruments used for detection, and other relevant aspects, all of which were recorded and registered in the PROSPERO protocol. To ensure specificity and consistency, the terms “microvesicles” and “microparticles” were used to identify studies that defined MVs as particles in the 100-1,000 nm size range. The characterization of MVs by researchers includes the quantification of MVs, analysis of MV size, and positive labeling of MV markers.

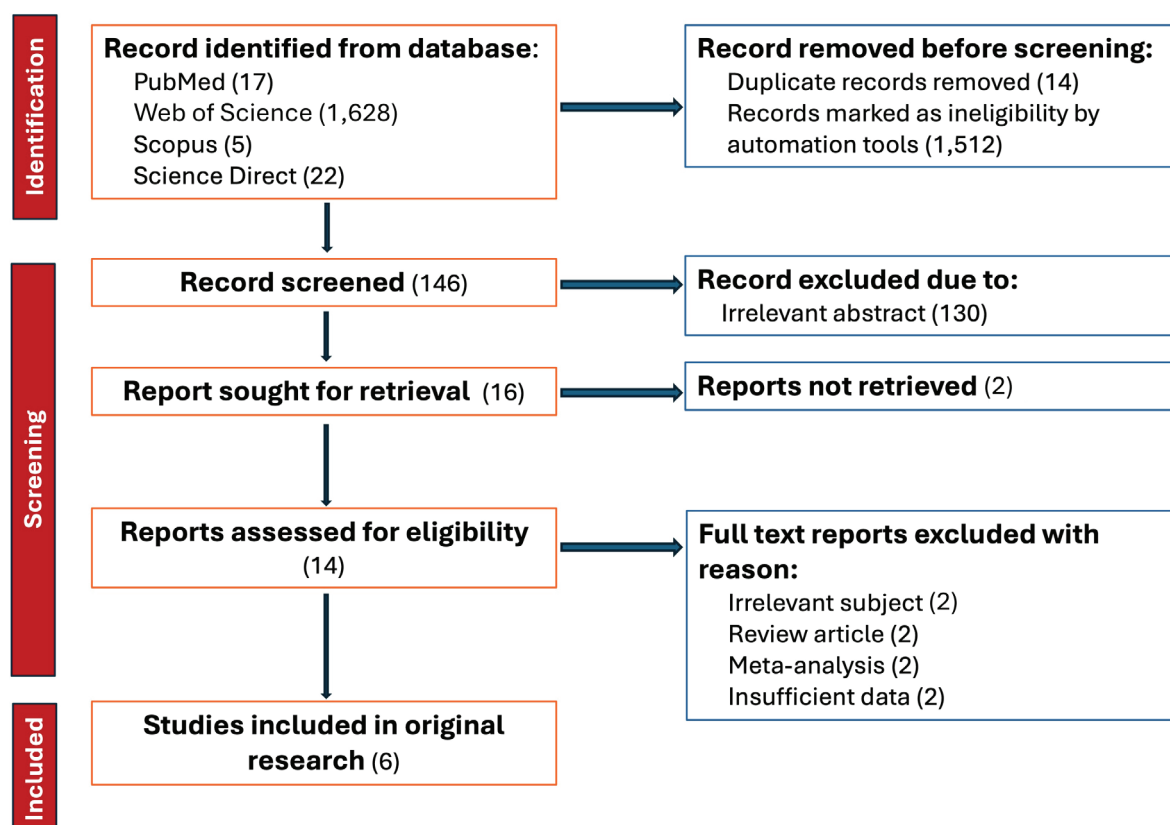


Figure 1. PRISMA flowchart of the literature search and study selection

Quality assessment of studies

The methodological quality of the included studies was evaluated using the Newcastle-Ottawa Quality Scale (NOS), which comprises three components: participant selection, comparability of the study groups, and exposure. Each study was assigned a score from 0 to 10.

Statistical analysis

Heterogeneity between studies was assessed using the Q test and I^2 test. A random or fixed-effects model was used to calculate the pooled standardized mean difference (SMD). When significant heterogeneity ($p < 0.05$) was indicated across studies, a random effects model was used for the analysis. Otherwise, a fixed-effects model was used ($p > 0.05$). The 95% confidence intervals (CIs) of the SMDs were calculated. Begg's funnel plot and Egger's test for publication bias were recommended, with 10 studies included to assess the evidence of publication bias. If publication bias was present, the funnel plot was asymmetric, and Egger's test was significant ($p < 0.05$). Review Manager meta-analysis version 5.4 (The Cochrane Collaboration, UK) was used to calculate the SMD and CI.

Standardized mean difference (SMD) was used to compare microvesicle levels between COVID-19 patients and control groups because the included studies utilized different measurement scales and reporting units. SMD values were interpreted using conventional thresholds: 0.2 (small effect), 0.5 (moderate effect), and ≥ 0.8 (large effect). Positive SMD values indicated higher microvesicle levels in COVID-19 patients than in controls, whereas negative values indicated lower levels.

For studies reporting correlations between microvesicle and D-dimer levels or between

microvesicle levels and COVID-19 severity, correlation coefficients were Fisher-transformed prior to pooled analysis.

Results

Study characteristics

In this systematic review, the initial search retrieved 1,672 studies. After removing duplicate records and records deemed ineligible based on automated tools (e.g., publication year and language), 146 studies remained. The abstracts of these studies were reviewed, and 130 articles were excluded as irrelevant. Two studies were subsequently retrieved for review, whereas two were not, resulting in the eligibility of 14 studies. Finally, based on a thorough examination of the full texts, only six studies met the inclusion criteria and were included in the analysis of MVs/microparticles in COVID-19 patients.

The essential characteristics of the included studies are presented in Table 1. Most of the included studies were conducted in Egypt (N=2), followed by the United States (N=1), Saudi Arabia (N=1), Germany (N=1), and China (N=1). The first study to discuss MVs in COVID-19 patients was published in 2020. Several studies have examined MVs in COVID-19 patients via blood samples. However, only four studies (N=4) conducted original research on MVs in humans. Zahran *et al.* and Guo *et al.* reported data on MVs in patients with varying severities of COVID-19.¹⁴⁻¹⁵ Sun *et al.*, Hamali *et al.*, Rausch *et al.*, and Abdelmaksoud *et al.* reported the presence of MVs in patients with COVID-19.¹⁶⁻¹⁹ Four of the six included studies employed a cross-sectional design, whereas the others used cohort and case-control designs. The quality of the studies was assessed using the NOS, and all studies deemed high quality had scores higher than 7.

Table 1. Characteristics of the systematic studies.

First Author	Study location, Year	MVs sources	MVs isolation, size/ morphology analysis	Surface marker	Sample size		Study design
					COVID-19 patient	Healthy control	
Zahran AM et al. ¹⁴	Egypt, 2021	PFP	Ultracentrifugation, specific monoclonal antibodies with fluorochrome; calibration beads (1.0 µm), flow cytometry	Endothelial MVs (CD146 ⁺ CD45 ⁻ and platelet MVs are CD41 ⁺	42	20	Cross-sectional
Sun Haoyu et al. ¹⁶	USA, 2022	PFP	Ultracentrifugation, specific monoclonal antibodies with fluorochrome, calibration beads (100, 200, 380, 490, and 1,000 nm), TEM, flow cytometry	MVs from white blood cells (CD45 ⁺), platelets (CD61 ⁺), and endothelial cells (CD144 ⁺),	5	5	Cross-sectional
Hamali HA et al. ¹⁷	Saudi Arabia, 2022	PFP	Ultracentrifugation, MV-Activity Kit, ELISA	MVs and TF-bearing MVs	40	37	Case control
Rausch L et al. ¹⁸	Germany, 2021	Peripheral blood mononuclear cells	Ultracentrifugation, TEM, flow cytometry	PS ⁺ EVs and PMVs-marker+ (CD41/CD63/CD274/CD62P) cells.	54	35	Cohort
Guo Da-Zhi et al. ¹⁵	China, 2020	PFP	Ultracentrifugation, flow cytometry	Annexin V ⁺ MVs ⁺	71	30	Cross-sectional
Abdelmaksoud MF et al. ¹⁹	Egypt, 2023	Not described	ELISA Kit	PDMVs (anti-CD42a/GP-IX and anti-CD42b/GPIIb antibodies	25	32	Cross-sectional

Note: PFP: platelet-free plasma; all samples used in all studies were from humans.

Meta-analysis of MVs in COVID-19 patients

This meta-analysis examined the differences in MV levels between patients with COVID-19 and healthy controls. The analysis revealed significantly higher TMV levels in COVID-19 patients than in controls (SMD=4.27, 95% CI=2.18–6.36, $p<0.0001$; Figure 2A). Similar significant differences were observed for PMVs (SMD=2.07, 95% CI=0.99–3.15, $p=0.0002$), EMVs (SMD=1.20, 95% CI=0.56–1.84, $p=0.0002$), and TF-MVs (SMD=2.56, 95% CI=1.98–3.15, $p<0.00001$) between COVID-19 patients and controls (Figure 2B–2D). Notably, PMV levels were higher than those of EMVs;

however, TF-MVs presented the highest levels, followed by PMVs and EMVs (Figure 2B–2D). Furthermore, this study investigated the correlation between MVs and routine COVID-19 test parameters, such as D-dimer levels, which indicate thrombus formation due to increased coagulation activity in COVID-19 patients. The analysis revealed a significant correlation between MVs and D-dimer levels (N=82, $r=0.430$, 95% CI=0.227–0.592, $p<0.0001$) (Figure 2E). Additionally, MVs increased with disease severity (N=168, $r=0.942$, 95% CI=0.558–0.993, $p=0.002$) (Figure 2F).

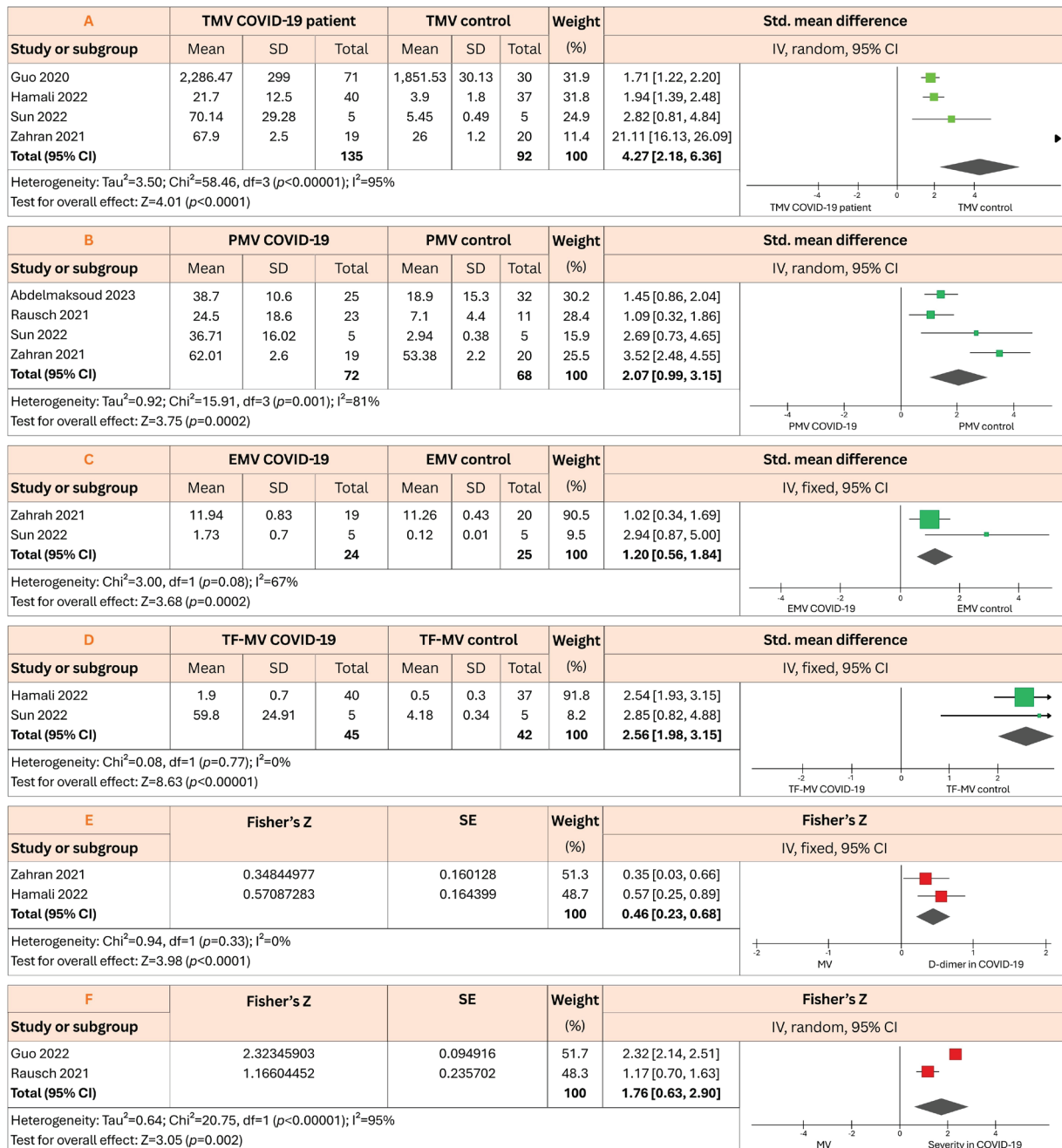


Figure 2. Forest plots and meta-analyses comparing microvesicle types between COVID-19 patients and controls. A: Forest plot and meta-analysis of TMVs, B: Forest plot and meta-analysis of PMVs, C: Forest plot and meta-analysis of EMVs, D: Forest plot and meta-analysis of TF-MVs, E: Forest plot and meta-analysis of MVs correlated with D-dimer levels in COVID-19 patients, F: Forest plot and meta-analysis of MVs correlated with disease severity in COVID-19 patients.

Assessment of studies

Three independent reviewers assessed the quality of the studies to evaluate the risk of bias (Supplementary data). The included cohort studies did not indicate publication bias, and none were deemed of low quality. The NOS assessment tool incorporates assessment parameters specific to each study design. All studies measured TMVs in COVID-19 patients, except the study by Rausch *et al.*, which precisely measured CD41 PMVs.¹⁸ However, the quality assessment results did not differ significantly between the studies (Supplementary data). Specifically, this analysis focused on TMVs, PMVs, EMVs, and TF-MVs expressed in COVID-19 patients and healthy controls. All the results collected within the groups were consistent with the overall analysis. To understand the role of MVs in COVID-19, this study analyzed the association between MVs and COVID-19 severity, as well as the correlation between MVs and D-dimer, a coagulation parameter. All the findings are consistent with the original analysis.

Publication bias and sensitivity analysis

Begg's funnel plot or Egger's test was not performed to assess evidence of publication bias because there were fewer than 10 studies; according to the Cochrane guidelines, funnel plots are recommended for studies exceeding this threshold.

Discussion

Regardless of the type of control or comorbidity, our systematic review revealed a significant increase in annexin V-labeled TMVs, PMVs, and EMVs in patients with COVID-19. This is the first systematic review to evaluate MVs in COVID-19 patients and compare them with MV levels in healthy controls. Despite the potential heterogeneity among studies, the results of the subgroup and sensitivity analyses were robust and indicated a significant increase in MVs in COVID-19 patients. Many severe cases of COVID-19 are associated with hemostatic markers such as D-dimer.²⁰ The increased coagulation and thrombosis observed in COVID-19 are triggered by cellular hyperactivation, which is initiated when the spike surface receptor of SARS-CoV-2 binds to ACE-2 receptors on host cells through the transmembrane protease serine-type 2 (TMPRSS2), facilitating fusion with host cells.²¹

SARS-CoV-2 infection involves the endothelium, and entry of the virus into ACE-2-expressing endothelial cells leads to endothelial dysfunction and loss of endothelial integrity.²² Consequently, uncontrolled viral invasion triggers endothelial dysfunction, activating the immune system and hemostatic responses. Endothelial dysfunction, in turn, contributes to the release of MVs from the endothelium, further increasing the production of proinflammatory cytokines, such as IL-6, IL-8, IL-10, and TNF- α , and ultimately exacerbates the severity of COVID-19.²³ Additionally, during acute

inflammation, various cells, including neutrophils, monocytes, lymphocytes, and platelets, undergo activation and release MVs.²⁴

According to Balbi *et al.*, many EVs carrying surface antigens found in COVID-19 patients originate from platelets and the endothelium.⁸ Consistent with this, the studies included in our meta-analysis predominantly employed markers derived from platelets and the endothelium, with more recent studies investigating tissue factor expression. Kaur *et al.* reported the upregulation of IL-6, IL-8, IL-10, and TNF- α , along with increased prothrombotic and coagulation factors, in patients with severe COVID-19.²⁵ MVs can form coagulation complexes and support thrombin formation, contributing to the prothrombotic changes observed in COVID-19 patients and playing a significant role in the development of a hypercoagulable state.¹⁷ MVs of COVID-19 patients with comorbidities are more procoagulant. In addition to adhering to the endothelium, it enables the assembly of enzyme complexes and thrombin formation, thus contributing to hypercoagulation.¹⁶ In line with these findings, Guo *et al.* confirmed that increased production of circulating MVs is associated with COVID-19 progression and that the number of MVs may indicate disease severity.¹⁵

Previous studies have consistently demonstrated an increase in the number of microvesicles, and our meta-analysis further confirmed that MV levels are elevated not only in COVID-19 patients but also in healthy controls and are positively correlated with D-dimer levels. Furthermore, the expression of TFs on MV surfaces was significantly higher in COVID-19 patients than in healthy controls. Notably, TF-bearing MVs appeared to be the most abundant, followed by MVs originating from platelets and the endothelium.

TFs are expressed on the surface of MVs when cells are activated, causing cell membrane translocation and facilitating TF-initiated coagulation.²⁶ Several tissue factors are released in response to inflammation.²² Generally, TF is found in the subendothelial matrix. In previous studies, TF-MVs were reported to play a role in thrombosis, indicating their severity and death, and these TF-bearing MVs are procoagulants that trigger the start of the coagulation cascade.^{27,28,12}

The presence of hyperactive platelets in patients with COVID-19 increases the number of PMVs in circulation.¹⁸ MV derivatives, such as PMVs, contain cellular receptors that may trigger an inflammatory storm. The ability of PMVs to mediate inflammatory cytokines can exacerbate the inflammatory response. In COVID-19, there is an increase in PMVs due to secondary inflammatory conditions when the virus is active.¹⁹ PMVs can change the function of cells, including neutrophils, endothelial cells, and megakaryocytes, as platelet precursors, thereby enabling communication between cells during infection.¹¹

PMVs and EMVs influence the expression of cell

adhesion molecules, enabling interactions with the endothelium and the recruitment of inflammatory cells.²⁹ Garnier *et al.* reported that the concentration of EMVs was higher in intubated COVID-19 patients than in uninfected controls.³⁰ Subgroup analysis of molecular cluster of differentiation (CD) markers was not performed because of insufficient data. Increased D-dimer levels in COVID-19 patients are associated with cell activation and coagulation due to the release of MVs.^{30,31,14} D-dimer is often found in patients with COVID-19, is a risk factor that correlates with disease severity and can predict hypercoagulability status due to the risk of thrombosis and mortality.³²⁻³⁴ Thus, when D-dimer levels correlate with MV release, MV may indirectly trigger the presence of cross-linked fibrin breakdown products.

Microvesicles have been shown to increase in number and correlate with D-dimer, which predicts hypercoagulability. MVs can be identified early in the diagnosis of thrombosis and have potential as diagnostic biomarkers. In addition, as part of the EV subtype, microvesicles play an essential role in cellular communication, making MVs promising candidates for therapeutic agents and drug delivery systems. MV may be a promising noninvasive biomarker for monitoring biological processes in the diagnosis and treatment of thrombosis.^{35,36}

Microvesicles may also serve as promising therapeutic candidates due to their natural role in intercellular communication, ability to carry bioactive cargo (including proteins, lipids, and nucleic acids), biocompatibility, and low immunogenicity. These characteristics make them potential targeted drug-delivery systems for improving therapeutic efficacy while minimizing adverse effects.³⁵

The results of this meta-analysis were consistent with those of previous studies. This meta-analysis used the standard deviation of the mean to validate studies that presented only numerical data. This study was conducted at the end of the COVID-19 pandemic. For this reason, studies have discussed potential markers that are not widely known for the treatment of COVID-19, such as PMV, EMV, and TF-bearing MVs, which can be used as new reliable markers of the response to pathophysiological severity in patients with COVID-19.

The potential role of microvesicles in predicting COVID-19 severity may be reflected by the magnitude of the pooled SMD, where larger SMD values indicate greater differences in microvesicle levels between severe COVID-19 patients and comparison groups.³⁷ Hypercoagulation and thrombosis in COVID-19 patients are attributable to MVs from platelets and endothelium derivatives or TF-bearing MVs, which increase over time, along with the severity of the effects. However, there is no consensus regarding the association of MVs with COVID-19. MV quantification in COVID-19 patients should be developed to initiate management

to minimize disease worsening and high mortality.

These studies are limited in that few studies discuss microvesicles in COVID-19 patients, and not all have examined the associations between MVs and other laboratory parameters. Investigating this association in future studies could provide insights into the complex pathophysiology resulting from increased MVs in COVID-19 patients, particularly hypercoagulation and thrombosis. Another limitation of this study is the heterogeneity of microvesicle isolation, characterization, and detection methods across included studies, which may influence measurement consistency and comparability of reported microvesicle levels. This meta-analysis included a limited number of studies with relatively small sample sizes, which may affect the generalizability of the findings. Therefore, the results should be interpreted cautiously, and larger multicenter studies are needed to validate the role of microvesicles as predictive biomarkers in COVID-19.

Conclusion

In conclusion, the number of MVs detected in COVID-19 patients is significantly higher than that detected in healthy controls. The presence of MVs in COVID-19 patients is positively associated with disease severity. Specifically, TF-MVs are present at higher levels in patients with COVID-19 and are more abundant than PMVs and EMVs. MVs also demonstrate a positive correlation with D-dimer, a crucial parameter in hemostasis. The predictive value of MVs in assessing COVID-19 severity has been shown consistently across numerous studies, with high statistical significance ($p < 0.05$). Therefore, MVs hold promise as valuable markers for predicting the prognosis of patients with COVID-19.

Ethical approval

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Conflict of Interest

The Authors declared no conflict of interest.

CRediT authorship contribution statement

Nastasya Nunki: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing: review and edit, project administration; **Yetti Hernaningsih:** conceptualization, validation, writing: original draft, supervision, funding acquisition; **Puspa Wardhani:** conceptualization, supervision; **Citrawati Dyah Kencono Wungu:** investigation, data curation, visualization; **Agil Saputra:** methodology, formal analysis, resources; **Narazah Mohd Yusoff:** supervision. All authors have read and agreed to the published version of the manuscript.

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