STROBE Statement

Section/Topic	Item No	Recommendation	Reported on Page/Relevant Study Content	Reported on Section/Paragraph
TITLE AND ABSTRACT				
Title and abstract	1(a)	Indicate the study's design with a commonly used term in the title or the abstract	Page 1, Title / Abstract	Title indicates differential expression analysis; abstract clarifies the case-control design.
	1(b)	Provide in the abstract an informative and balanced summary of what was done and what was found	Page 1, Full Abstract (Background-Conclusion)	Abstract section: Background, Objective, Methods, Results, and Conclusion are provided
INTRODUCTION				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 1, Whole Introduction Section	Introduction describes clinical significance of acral melanoma, exosomes, and miRNAs
Objectives	3	State specific objectives, including any prespecified hypotheses	Pages 1, Last Sentence of Introduction	Final paragraph of Introduction states objective to explore differential expression and clinical significance of serum exosomal miRNAs
METHODS				
Study design	4	Present key elements of study design early in the paper	Page 1, Materials and Methods (Data and methods/Clinical data)	Case-control study with acral melanoma (cases) and plantar nevus (controls), using pathological diagnosis as gold standard.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 1, Materials and Methods (Data and methods/Clinical data)	Setting: Civil Aviation General Hospital; Recruitment period: 2022.10-2023.10; data collection synchronized with experiment.
Participants	6(a)	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 1, Materials and Methods (Data and methods/Clinical data)	10 participants (5 melanoma, 5 nevus) from the hospital, with clear inclusion/exclusion criteria; no follow-up.
	6(b)	For matched studies, give matching criteria and number of exposed and unexposed	N/A	Not applicable - this is not a matched study
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 2, Materials and Methods	Outcome: miRNA profile; Exposure: melanoma status; Diagnostic criteria and confounders are clearly defined.
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 2, Materials and Methods	Data from serum samples; exosome identification, miRNA detection and validation methods are standardized and comparable.
Bias	9	Describe any efforts to address potential sources of bias	Pages 1-2, Materials and Methods	Strict inclusion/exclusion criteria, standardized experiments and qRT-PCR validation to control selection, information and confounding bias.
Study size	10	Explain how the study size was arrived at	Page 1, Materials and Methods (Data and methods/Clinical data)	5 cases in each group for exploratory research; large-sample validation will be conducted later.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 2, Materials and Methods (Data and methods/Exosome RNA extraction,	miRNA expression normalized; differential expression criteria (≥2-fold change, FDR ≤0.05)

			quality control and miRNA sequencing)	
Statistical methods	12(a)	Describe all statistical methods, including those used to control for confounding	Pages 1-2, Methods	FDR correction, differential screening, qRT-PCR and enrichment analysis methods are described.
	12(b)	Describe any methods used to examine subgroups and interactions	N/A	No subgroup or interaction analyses were reported
	12(c)	Explain how missing data were addressed	N/A	Not reported
	12(d)	Cohort study - If applicable, explain how loss to follow-up was addressed	N/A	This study is a case-control study, with no follow-up or loss to follow-up links.
	12(e)	Describe any sensitivity analyses	Pages 2, Validation of miRNA expression by quantitative real-time PCR (qRT-PCR)	qRT-PCR was used to validate the top 5 most significantly differential miRNAs selected by sequencing as a sensitivity analysis to verify the reliability of the sequencing results.
RESULTS				
Participants	13*(a)	Report numbers of individuals at each stage of study	Page 1, Materials and Methods (Data and methods/Clinical data)	5 melanoma vs. 5 nevus cases
	13*(b)	Give reasons for non-participation at each stage	Page 1, Materials and Methods (Data and methods/Clinical data)	Non-participants were excluded due to unclear diagnosis or comorbidities; no other reasons.
	13*(c)	Consider use of a flow diagram	N/A	No flow diagram provided (described in text only)
Descriptive data	14*(a)	Give characteristics of study participants and information on exposures and potential confounders	Page 1, Materials and Methods (Data and methods/Clinical data)	Participants aged 53-76 years; baseline characteristics of two groups were comparable.
	14*(b)	Indicate number of participants with missing data for each variable of interest	N/A	Not reported
	14*(c)	Cohort study - Summarise follow-up time	N/A	No follow-up in this case-control study.
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A	Study focuses on expression differences, not traditional outcome events
Main results	16(a)	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% CI). Make clear which confounders were adjusted for and why they were included	Page 5, Table 1-2	Table 1 lists top 10 differentially expressed miRNAs with P-values and direction; Table 2 shows qRT-PCR validation results
	16(b)	Report category boundaries when continuous variables were categorized	N/A	Not reported
	16(c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A	Not reported
Other analyses	17	Report other analyses done - e.g., analyses of subgroups and interactions, and sensitivity analyses	Page 5, Figures 3-5	GO and KEGG enrichment analyses reported (Figures 3-5)
DISCUSSION				
Key results	18	Summarise key results with reference to study objectives	Pages 4 and 7, Discussion (sections 2 and 3)	Differential miRNAs were identified; their target genes enriched in tumor pathways; they may be non-invasive biomarkers.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 7, Discussion (sections 5)	Discusses small sample size, lack of functional validation, need for multi-center validation.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and	Pages 4 and 7, Discussion	Interprets findings in context of existing literature on miRNAs and pathways.

		other relevant evidence		
Generalisability	21	Discuss the generalisability (external validity) of the study results	Pages 7, Discussion (sections 5)	Generalizability is limited; multi-center, large-sample studies are needed for verification.
OTHER INFORMATION				
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Pages 8, Acknowledgments	Funded by Civil Aviation Medical Center (Civil Aviation General Hospital).

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.