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Original Research

Nicotine in E-cigarette aerosol may lead to pulmonary inflammation

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ABSTRACT

Background: Cigarette smoking stands as one of the leading causes of preventable death globally. Alternative tobacco products, such as e-cigarettes, have gained popularity due to the general perception of being less harmful. However, much is still unknown about the health implications of these novel products. In this study, we aimed to investigate if e-cigarettes could induce pulmonary inflammatory responses by measuring lung-related circulating extracellular vesicles (EVs) in the blood of healthy volunteers following brief e-cigarette vaping sessions, with and without nicotine.

Methods: 22 healthy volunteers were included. Employing a randomized, double-blind, cross-over design all participants vaped 30 puffs of e-cigarette aerosol, with and without nicotine, over a 30-min period. Blood samples were collected at baseline, 30- and 105-min following exposure. Lung-related EVs were quantified using flow cytometry. Analyzed markers included angiotensin converting enzyme (ACE), aldehyde dehydrogenase 3B1 (ALDH3B1), palate, lung and epithelial clone (PLUNC), complement component 3 (C3), C-C motif chemokine ligand 3 (CCL3), also known as macrophage inflammatory protein 1 alpha (MIP-1α), and uteroglobin, also known as club cell protein 16 (CC16). All these markers are associated with pulmonary inflammation.

Results: E-cigarette use, with nicotine but not without, resulted in a significant increase in three out of the six lung-related inflammatory markers measured and clear increases though not statistically significant in the remaining three.

Conclusion: The observed increase in levels of circulating lung-related inflammatory EV markers following vaping e-cigarette aerosol containing nicotine suggests that inhaled nicotine plays a central role in triggering pulmonary inflammation.

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1. Introduction

The World Health Organization (WHO) reports that tobacco claims over 8 million lives annually [1]. In response to increasing regulations, the tobacco industry has redirected focus toward alternative products including e-cigarettes, smokeless tobacco, and heated tobacco products. While marketed as "reduced harm" options and potential cessation aids, their health effects remain insufficiently characterized.

Current evidence on e-cigarettes' effectiveness for smoking cessation remains highly inconclusive [2–6], with major health authorities including WHO, FDA, and EU not recommending them for this purpose [7–9].

The harmful effects of conventional cigarette smoking have largely been attributed to combustion by-products [10–13]. E-cigarettes, which operate below combustion temperatures, are therefore promoted on this aspect as safer alternatives. However, most e-cigarettes deliver nicotine, which itself has documented adverse health effects [14–16]. This raises important questions about nicotine's specific role in potential e-cigarette related harm.

Extracellular vesicles (EVs) are membrane-bound particles released

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during cell activation or apoptosis [17,18] and serve as useful biomarkers of cellular damage. In healthy individuals, most circulating EVs originate from platelets, with elevated levels observed in various cardiovascular conditions [19]. Our previous research has demonstrated an increase in endothelial-derived EVs following exposure to both conventional cigarette smoke and nicotine-containing e-cigarettes [20,21], suggesting vascular involvement. However, the relationship between nicotine products and lung-related EVs remains unexplored.

For this study, we selected six lung-related inflammatory markers based on their established roles in pulmonary inflammation and response to oxidative stress (Table 1). These include: (1) Aldehyde dehydrogenase 3B1 (ALDH3B1), which protects against oxidative stress and is highly expressed in lungs [22–24]; (2) Angiotensin Converting Enzyme (ACE), a pro-inflammatory mediator specific to lung tissue and elevated in various pulmonary conditions [25,26]; (3) Club cell protein 16 (CC16/Uteroglobin), which protects lung epithelium and serves as a biomarker for lung injury [27,28]; (4–5) Complement component 3 (C3) and C-C motif chemokine ligand 3 (CCL3/MIP-1α), which play critical roles in immune regulation and pulmonary inflammation [29–31]; and (6) Palate, lung and nasal epithelial clone protein (PLUNC), which functions in airway immune defense and shows altered expression in smokers [24,32,33].

Building on our previous findings of vascular effects, the present study aims to investigate whether brief use of e-cigarettes (with and without nicotine) elicits measurable pulmonary effects by analyzing lung-related EV markers in the circulation of healthy volunteers. This approach enables us to test the hypothesis that inhaled nicotine specifically contributes to pulmonary inflammatory responses.

2. Materials and method

2.1. Subjects

The study included 22 healthy women and men who were occasional smokers or snus-users, with a maximum consumption of 10 cigarettes or 10 pouches of snus per month. All volunteers were required to abstain from caffeine and alcohol for 12 h, and refrain from heavy exercise for at least 24 h prior to study. Nicotine-containing products (including cigarettes, e-cigarettes or Swedish snus) and anti-inflammatory medications were not allowed within 7 days of the study days. A clinical examination, routine blood tests, dynamic spirometry, ECG, blood pressure measurement and pregnancy test were completed before inclusion in the study. Exclusion criteria included cardiovascular or pulmonary disease, any systemic or chronic disorder such as rheumatologic or metabolic diseases, as well as pregnancy. Furthermore, subjects had to be free of symptoms of infection or inflammation within 4 weeks of the study day. 5 subjects were excluded from analysis due to missing data.

2.2. Electronic cigarette exposure

The study protocol adhered to the 1975 Helsinki declaration and was approved by the Swedish Ethical Review Authority. All included subjects underwent a simple randomization by the study supervisor. Using a randomized cross-over design (Fig. 1), volunteers vaped e-cigarette aerosol with and without nicotine for 30 min, with a frequency of 1 puff per minute, using a third-generation e-cigarette equipped with dual nickel coils and adjustable settings (eVic-VT, Shenzhen Joyetech Co., Ltd., (China). The e-cigarette was set to identical settings (temperature 230 °C, effect 32 W, resistance 0,20 Ω), used in all exposures. The two exposures were conducted in a specially designed room with adequate ventilation, and each session was separated by at least a one-week washout period to avoid carry-over effects. The e-liquid used (Valeo laboratories GmbH, Germany) consisted of a mixture of ethanol (5 %), glycerine (44.4 %) and propylene glycol (49.4 %) without added flavours. The e-liquids were purchased premixed with and without nicotine (19 mg/ml and 0 mg/ml L-nicotine, respectively). The two e-liquids were stored in identical containers separated by differing letters and blinded to both parties. Samples remained blinded until after analysis.

2.3. Blood sampling

Blood samples were drawn into test tubes containing 1/10 volume of 0.129 M sodium citrate, EDTA and serum at baseline, at 30 min, and 105 min after exposure. Platelet-poor plasma was prepared by centrifugation at 2,000*g* for 20 min in room temperature (RT) and then frozen at −80 °C until analysis.

2.4. Flow cytometric analysis of EVs

High speed centrifugation was used to separate and concentrate the EVs. In brief, platelet-poor plasma was thawed in a water bath and centrifuged at 20,160 g for 45 min at 21 °C. After centrifugation, the supernatant was discarded, and the remaining pellet was vortexed to obtain an EV-enriched pellet. Twenty μl of the supernatant was then transferred to a 96-well plate containing 5 μl of conjugated antibodies targeting specific EV markers, as described in the table below. The plate was incubated in the dark for 20 min before adding 120 μl of CytoFLEX sheath fluid. EVs were measured using the CytoFLEX flow cytometer. The EV gate was determined using Spherotech Nano fluorescent Yellow Particles of sizes 0.22 μm, 0.45 μm, 0.88 μm & 1.35 μm. EVs were defined as vesicles that were between 0.1 and 1 μm and positive for antibodies. Through a review of the current literature, we identified six markers of interest [13], each of which has been shown to correlate to lung tissue inflammation. Results are presented as frequencies of EVs (%): the number of marker-positive EVs events (as presented in Table 1) divided by the total number of EV events present in the EV gate.

2.5. Statistical analysis

The power calculation was based on the expectation of observing at least a 30 % difference in endothelial-derived EV markers, as previously demonstrated following brief exposure to nicotine-containing products.

Table 1
Overview of Lung-related inflammatory markers analyzed in the study.

Marker	Full Name	Primary Function	Expression in Lung	Relevance to Inflammation/Disease
ALDH3B1	Aldehyde Dehydrogenase 3B1	Detoxifies aldehydes, protects against oxidative stress	Highly expressed in lung tissue [24]	Increased levels associated with lung cancer [22,23]; upregulated in response to cigarette smoke
ACE	Angiotensin Converting Enzyme	Converts angiotensin I to angiotensin II, regulating inflammatory pathways	Metabolized in lung vascular endothelial cells	Clinical marker for sarcoidosis; increased in ARDS [25]; mediates pro-inflammatory effects [26]
CC16	Club Cell Secretory Protein 16 (Uteroglobin)	Protects lung epithelium from inflammation and oxidative damage	Expressed by club cells in small airways	Biomarker for lung injury; increased levels correlate with worse outcomes in ARDS [27,28]
C3	Complement Component 3	Enhances immune response through opsonization and chemotaxis	Present in lung tissue	Upregulation linked to COPD and asthma; chronic activation causes host cell damage [30]
CCL3	C-C Motif Chemokine Ligand 3 (MIP-1α)	Recruits and activates inflammatory cells	Found in lung tissue during inflammation	Elevated in sarcoidosis patients' bronchoalveolar lavage fluid [31]
PLUNC	Palate, Lung, and Nasal Epithelial Clone	Acts as surfactant; aids innate immunity	Abundant in airway secretions	Regulates neutrophil recruitment during inflammation [32]; decreased in current smokers [24]

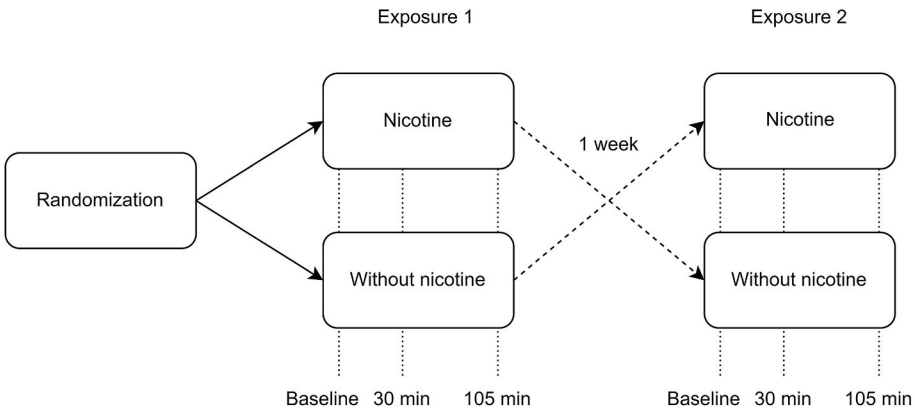

Fig. 1. Study design and bloods sampling timeline. 22 healthy male and female subjects were randomized for e-cigarette vape exposure with and without nicotine. Blood samples were collected at baseline and at 30 and 105 min after exposure.

To achieve a power of 80 % at a two-sided significance level of $p < 0.05$, a sample size of 12 subjects would be sufficient. Statistical analysis was performed using JMP 18 (SAS Institute, Cary, NC, USA) for multivariate analysis and GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA) for figure generation. Data was assessed for normality visually and using the Shapiro-Wilk test. A repeated measures multivariate analysis of variance (MANOVA) was employed to analyze the interaction between time and exposure (nicotine vs. nicotine-free). Only subjects with data from both exposures were included in the analysis ($n = 17$). A p-value of <0.05 was considered statistically significant.

3. Results

Of the 22 initially included, data from 17 participants, 7 males (41,2 %) and 10 females (58,8 %) with a mean age of 25.8 (18–49) years were included in the analysis. Subject characteristics are shown in Table 2. Data was collected between September 2019 and January 2020.

3.1. Extracellular vesicles

The analysis revealed significant increases in ACE, ALDH3B1, and PLUNC levels following vaping e-cigarette aerosol containing nicotine, as demonstrated by the MANOVA results see (Fig. 2, 3 and 4). These significant differences were driven by the interaction between time and nicotine exposure, highlighting an effect of nicotine over time. Other proteins, including C3, MIP-1α and Uteroglobin, did not exhibit statistically significant changes and are summarized in Table 3 for reference.

This table presents the p-values from a repeated measures multivariate analysis of variance (MANOVA) used to analyze the interaction effect of time and nicotine exposure (nicotine vs. nicotine-free conditions) on the expression levels of various proteins. Only subjects with samples available at all time points were included in the analysis. Statistically significant interactions between time and exposure are indicated by p-values ($p < 0.05$), which are highlighted in orange.

Table 2
Mean values and standard deviation (SD) of age, waist circumference, BMI and routine blood samples at inclusion.

Subject characteristics	Mean	SD
Age [years]	25.82	7.22
Waist [cm]	79.71	9.76
BMI [kg/m^2]	23.88	3.28
Hb [g/L]	136.12	15.47
Creatinine [μmol/L]	71.35	12.50
WBC [x 10^9/L]	5.49	1.13

4. Discussion

In this study, brief exposure to e-cigarette aerosol containing nicotine, but not nicotine-free aerosol, caused significant increases in extracellular vesicles positive for ACE, PLUNC, and ALDH3B1. Additionally, we observed increases in CCL3, C3, and CC16-positive EVs, though these did not reach statistical significance. These findings suggest that inhaled nicotine triggers an acute inflammatory reaction in the lungs.

Historically, the harmful effects of conventional cigarettes have been predominantly attributed to combustion by-products rather than to nicotine itself. Our results challenge this perspective by isolating nicotine's specific contribution to pulmonary inflammation. This aligns with a recent meta-analysis by Glantz et al. [34], which found significant associations between e-cigarette aerosol exposure and respiratory conditions including asthma and COPD, particularly in never--smokers—supporting the role of non-combustion components like nicotine in lung pathology.

The inflammatory markers we investigated function within a complex balance of protective and potentially harmful effects. Under normal conditions, these proteins help defend against oxidative stress and regulate immune responses. However, their chronic activation or dysregulation—as might occur with regular nicotine exposure—can lead to tissue damage and disease progression. This dual nature explains why they serve both protective and potentially harmful roles in respiratory health.

ALDH3B1, ACE, and PLUNC showed the most pronounced responses to nicotine exposure. ALDH3B1 normally protects against oxidative stress by detoxifying aldehydes, but its persistent upregulation has been linked to cancer development [35,36]. ACE, through its conversion of angiotensin I to angiotensin II, promotes inflammation that contributes to various pulmonary disorders [37–43]. Our finding of increased ACE-positive EVs after nicotine exposure provides the first human evidence of nicotine's direct effect on this system. PLUNC, which typically protects airways through innate immune functions, shows altered expression patterns in smokers [44–47], suggesting disruption of normal protective mechanisms.

The potential pathological effects of nicotine extend beyond acute inflammation. Grando's review [48] demonstrates nicotine's ability to affect cellular processes through nicotinic acetylcholine receptors (nAChRs) on non-neuronal cells, potentially linking chronic exposure to long-term disease. Particularly relevant is emerging evidence that nicotine-induced inflammation may represent an early stage in pathological processes including tissue remodelling. While our study focuses on acute effects, our observations align with growing evidence suggesting direct tissue effects of nicotine independent of combustion products.

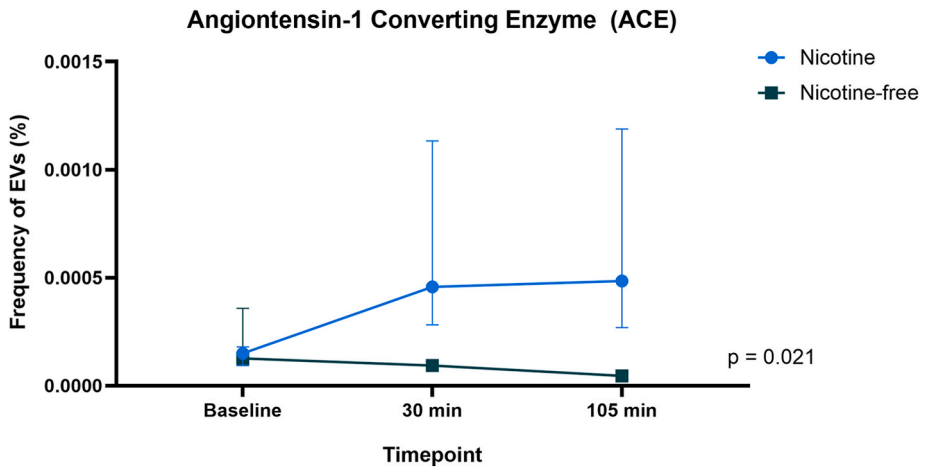

Fig. 2. Frequency (%) of EVs expressing angiotensin-1 converting enzyme (ACE) after nicotine and nicotine-free e- cigarette vape exposure. The figure illustrates the frequency of EVs expressing Angiotensin-1 Converting Enzyme (ACE), as measured by flow cytometry, at baseline, 30 minutes, and 105 minutes post-exposure to nicotine-containing and nicotine-free e-cigarette vapor. Data points represent the median values with interquartile range (IQR) between the 25th and 75th percentiles. p- values were derived from repeated measures multivariate analysis of variance (MANOVA), assessing the interaction between time and nicotine exposure (n=17).

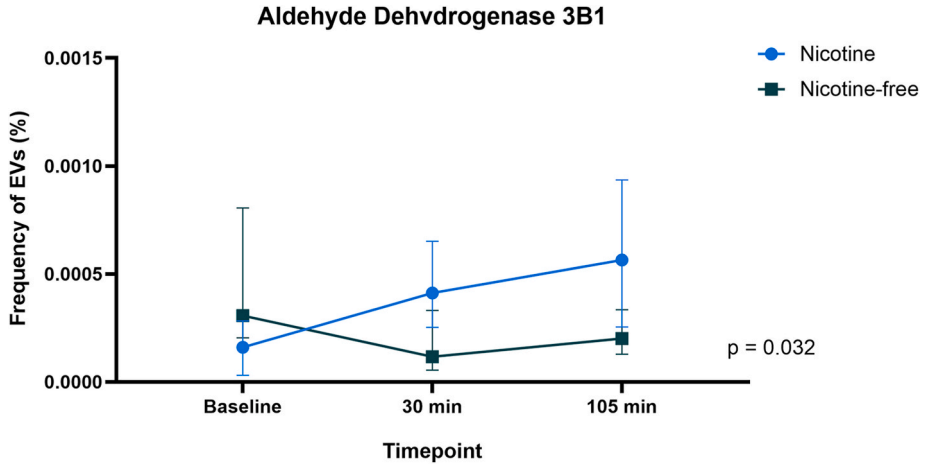

Fig. 3. Frequency (%) of EVs expressing Aldehyde Dehvdrogenase 3B1 after nicotine and nicotine-free e-cigarette vape exposure. The figure illustrates the frequency of EVs expressing Aldehyde Dehvdrogenase 3B1, as measured by flow cytometry, at baseline, 30 minutes, and 105 minutes post-exposure to nicotine-containing and nicotine-free e-cigarette vapor. Data points represent the median values with interquartile range (IQR) between the 25th and 75th percentiles. p-values were derived from repeated measures multivariate analysis of variance (MANOVA), assessing the interaction between time and nicotine exposure (n=17).

The increases, albeit non-significant, in CC16, C3, and CCL3 still merit attention as part of a broader inflammatory pattern. Previous research has shown that CC16 levels increase with lung injury [49,50], while C3 and CCL3 upregulation contributes to pulmonary inflammation in smoking-related conditions [51–58]. The consistency of directional change across all markers strengthens our conclusion regarding nicotine's inflammatory effects.

These findings align with our previous research demonstrating vascular endothelial effects of vaping nicotine-containing e-cigarette [21]. Together, these studies suggest nicotine plays a more significant role in tobacco-related health effects than previously recognized. Given that conventional smoking's most serious consequences often take decades to manifest, early markers of inflammation from nicotine-containing products may serve as important warning signs.

It is notable that these inflammatory responses occurred after just 30 puffs—substantially less than typical daily e-cigarette use (150–250 puffs) [59,60]. This raises concerns about potential cumulative effects with regular use. The inflammatory response appears specific to nicotine, as the identical aerosol without nicotine produced no significant changes.

Our results have implications for both current and future nicotine delivery systems. While transdermal or oral nicotine replacement therapy products provide slower, more gradual absorption and are generally considered safer [61], the rapid absorption from inhalation may present unique risks. This should inform ongoing debates about e-cigarettes as smoking cessation tools.

4.1. Limitations

The novel methodology used limits our ability to fully quantify the clinical relevance of observed EV increases. Future studies comparing these biomarkers across various inhalants would establish better context for interpreting their significance. Additionally, our sample size was originally based on endothelial-derived EVs from earlier studies, which may not adequately reflect the variability or effect size of the newly explored lung-associated markers, potentially limiting our ability to detect statistically significant differences. Finally, investigating these markers in current smokers and those with established lung disease would provide valuable insights for smoking cessation applications.

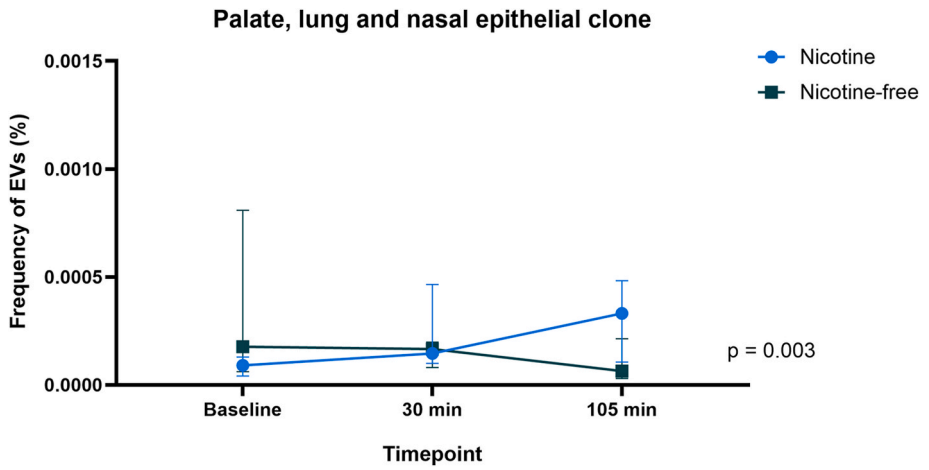

Fig. 4. Frequency (%) of EVs expressing Palate, lung and nasal epithelial clone, after nicotine and nicotine-free e-cigarette vape exposure. The figure illustrates the frequency of EVs expressing Palate, lung and nasal epithelial clone, as measured by flow cytometry, at baseline, 30 minutes, and 105 minutes post-exposure to nicotine-containing and nicotine-free e-cigarette vapor. Data points represent the median values with interquartile range (IQR) between the 25th and 75th percentiles. p- values were derived from repeated measures multivariate analysis of variance (MANOVA), assessing the interaction between time and nicotine exposure (n=17).

Table 3
Results of repeated measures MANOVA assessing the interaction between time and nicotine exposure on biomarker expression.

Repeated Measures Analysis (MANOVA) Factor: Time vs Exposure	
Marker	*p* value
ACE	0.021
ALDH3B1	0.032
C3	0.060
MIP-1α (CCL3)	0.060
PLUNC	0.003
Uteroglobin (CC16)	0.130

4.2. Conclusion

This study provides evidence that even brief vaping of nicotine-containing e-cigarette aerosol induces measurable changes in lung-related inflammatory markers expressed on extracellular vesicles. These findings suggest caution regarding e-cigarettes as smoking cessation tools, as even at low doses, they appear capable of triggering pulmonary inflammatory responses that could potentially contribute to respiratory disease with chronic exposure.

CRediT authorship contribution statement

Mikael Kabéle: Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Gustaf Lyytinen:** Writing – review & editing, Investigation, Formal analysis. **Jenny A. Bosson:** Writing – review & editing, Supervision, Conceptualization. **Linnea Hedman:** Writing – review & editing, Supervision, Conceptualization. **Lukasz Antoniewicz:** Writing – review & editing, Formal analysis, Conceptualization. **Magnus Lundbäck:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization. **Fariborz Mobarrez:** Writing – review & editing, Visualization, Validation, Supervision, Investigation, Formal analysis, Conceptualization.

Data availability

The data and study protocol is available upon request and after a confidentiality evaluation.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Magnus Lundback reports financial support was provided by Swedish Heart and Lung foundation. Magnus Lundback reports financial support was provided by Swedish Heart and Lung Association. Magnus Lundback reports financial support was provided by Stockholm County Council. Jenny A. Bosson reports financial support was provided by County Council of Västerbotten. Magnus Lundback reports financial support was provided by Swedish Society of Medicine. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2025.108101.

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