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Associations of light smoking with serum carcinoembryonic antigen and relative leukocyte telomere length in men with asthma

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Abstract

Smoking and asthma are associated with oxidative stress and chronic inflammation, which may contribute to accelerated cellular aging. Relative leukocyte telomere length (RTL) and serum carcinoembryonic antigen (CEA) are accessible biomarkers linked to these pathways, but their associations with light smoking (1-4 cigarettes/day) in men with asthma remain unclear. This cross-sectional study included 156 men: 73 with asthma and 83 healthy controls, classified as asthmatic light smokers, asthmatic nonsmokers, healthy light smokers, or healthy nonsmokers. Primary analyses used outcome-specific datasets after within-group Tukey 1.5×IQR exclusion, yielding 150 observations for CEA and 148 for RTL; complete-data and age-restricted analyses were sensitivity analyses. CEA differed across groups and was higher in asthmatic light smokers than in healthy nonsmokers (Dunn-Bonferroni p=0.006). In the age-adjusted full-cohort model, light smoking among participants with asthma was associated with higher CEA [adjusted ratio 1.18, 95% confidence interval (CI) 1.01-1.38; p=0.034]. This association became statistically inconclusive after broader adjustment. RTL also differed across groups and was shorter in asthmatic light smokers than in asthmatic nonsmokers (Dunnett T3 p=0.001). In the primary median-regression model, light smoking among participants with asthma was associated with lower RTL (adjusted conditional-median ratio 0.38, 95% bootstrap CI 0.22-0.67; p=0.001), but this association was not consistently retained in severity-adjusted, complete-data, and age-restricted analyses. Asthma-status×smoking-status interactions were nonsignificant for both biomarkers. Light smoking was associated with higher CEA and lower RTL in the primary models, but both findings were sensitive to analytical specification. These exploratory cross-sectional associations do not establish causality and require longitudinal confirmation.

Key words: asthma, smoking, leukocyte telomere length, carcinoembryonic antigen, cross-sectional study.

Introduction

Asthma is a chronic, heterogeneous inflammatory airway disease characterized by variable airflow limitation and airway hyperresponsiveness [1]. In addition to immune-cell recruitment and cytokine signaling, asthma is associated with oxidative stress and airway remodeling, including epithelial injury, airway smooth-muscle hypertrophy and subepithelial fibrosis [2,3]. These processes may contribute to cumulative cellular injury and altered biological ageing.

Two accessible blood biomarkers that may reflect aspects of these pathways are relative leukocyte telomere length (RTL) and carcinoembryonic antigen (CEA) [4,5]. Telomeres are DNA–protein structures that protect chromosome ends and progressively shorten with cell division. Oxidative stress can accelerate telomere attrition, potentially promoting cellular senescence and a pro-inflammatory phenotype [6-8]. CEA, also known as CEACAM5, is widely used as an oncological biomarker but may also be modestly elevated in smokers and in some benign inflammatory conditions. Accordingly, circulating CEA may reflect non-specific epithelial or inflammatory activity rather than malignancy alone [9,10].

Cigarette smoke contains oxidants and reactive chemicals that can amplify airway inflammation and oxidative stress. Among people with asthma, smoking is associated with poorer symptom control and reduced responsiveness to inhaled corticosteroids [11-13]. However, it remains unclear whether light smoking at 1–4 cigarettes per day is associated with measurable differences in RTL or CEA among men with asthma and healthy men. It is also uncertain whether the associations of light smoking with these biomarkers differ according to asthma status. Detecting a biomarker signal at very low cigarette consumption may be relevant to smoking-cessation counselling, particularly for individuals with chronic airway disease. We therefore compared RTL and serum CEA across asthmatic and healthy men stratified by light-smoking status and evaluated whether asthma status modified the associations between light smoking and these biomarkers.

Materials and Methods

Sample collection and study design

Sample collection was conducted after approval by the Medical Ethics Committee of the National Institute for Medical Research Development, Tehran, Iran (IR-NIMAD-REC-1396-032). All participants provided written informed consent. Adult men with physician-diagnosed asthma were recruited from respiratory specialist clinics in Tabriz, Iran. Asthma diagnoses were confirmed

independently by two specialists on the basis of clinical history and spirometry, and asthma severity was classified according to NIH guidelines [14]. Healthy male controls were selected from the Azar Cohort [15] and screened to exclude asthma symptoms, self-reported physician-diagnosed asthma, and use of asthma medications.

For both asthma cases and controls, exclusion criteria included chronic inflammatory diseases other than asthma in the case group, tuberculosis, cancer, immune disorders, chronic obstructive pulmonary disease, and use of hormonal or immunosuppressive medications. Participants with clinically diagnosed metabolic disease were excluded during recruitment. Metabolic syndrome was not used as a recruitment exclusion criterion; it was subsequently derived for the present analysis from measured waist circumference, fasting glucose, triglycerides, HDL-C, and blood pressure.

Smoking status was determined by participant interview. Current light smoking was defined a priori as consumption of 1–4 cigarettes per day, and only participants meeting this definition were included in the smoker groups. Smoking exposure was not biochemically validated, and information on pack-years was unavailable. The study included 73 men with asthma, comprising 42 light smokers and 31 nonsmokers, and 83 healthy men, comprising 46 light smokers and 37 nonsmokers.

Venous blood was collected from each participant into serum-separation and sodium-citrate tubes. For CEA measurement, blood collected in serum tubes was allowed to clot for approximately 30 minutes and then centrifuged at approximately 1,500–2,000 ×g for 10–15 minutes. Serum aliquots were stored at −40 °C until analysis. Blood collected in sodium-citrate tubes was used for leukocyte DNA isolation and subsequent RTL measurement by quantitative polymerase chain reaction.

DNA extraction for telomere study

Genomic DNA for RTL measurements was extracted from frozen whole-blood leukocytes using a two-step protocol that we have previously validated [16,17]. The extracted DNA was resuspended in TE buffer, quantified, and stored at −20 °C, with freeze–thaw cycles kept to a minimum.

qPCR for RTL

The qPCR was performed using primers specific for telomere repeats and for the single-copy human beta-globin (HBG) gene (Zist Fanavari Pishgam, Iran). Primer sequences and cycling conditions are described in Table 1 [18]. Primer efficiency (E) was evaluated from standard curves. Each reaction had a final volume of 10 µL and was run on a StepOne™ Real-Time PCR System (Applied Biosystems, USA) using SYBR Green for fluorescence detection. Data were analysed with StepOne™ Software v2.0. The RTL was expressed as the ratio of telomere repeat copy number (T) to single-copy HBG copy number (S), i.e., the T/S ratio [19]. RTL was calculated using the ΔΔCt method (Equation 1), with a pooled DNA sample from all participants serving as the reference control [18,20,21].

$$\Delta Ct = Ct_{\text{Telomere sample}} - Ct_{HBG\ \text{sample}}$$

$$\Delta\Delta Ct = (Ct_{\text{Telomere sample}} - Ct_{HBG\ \text{sample}}) - (Ct_{\text{Telomere control}} - Ct_{HBG\text{control}})$$

$$T/S = (1 + E)^{-\Delta\Delta Ct}$$

$$T/S = \frac{(1+E)^{(Ct\ \text{Telomere control})}}{(1+E)^{(Ct\ HBG\ \text{control})}} \times \frac{(1+E)^{(Ct\ HBG\ \text{sample})}}{(1+E)^{(Ct\ \text{Telomere sample})}} \quad \text{(Equation 1)}$$

CEA measurement

Serum CEA was measured by ELISA (Pishtazteb, Iran) according to the manufacturer's protocol. Absorbance was read at 450 nm and concentrations were calculated from kit calibrators (ng/mL).

Statistical analysis

Analyses were performed in R using outcome-specific complete-case datasets. RTL was analyzed as log10(T/S), and CEA was log10-transformed for regression analyses. Continuous variables were summarized as mean +/- SD or median (IQR), as appropriate, and categorical variables as n (%). Metabolic syndrome was defined as the presence of at least three of five NCEP components: waist circumference >=102 cm, fasting glucose >100 mg/dL, triglycerides >150 mg/dL, HDL-C <40 mg/dL, and systolic blood pressure >130 mmHg and/or diastolic blood pressure >85 mmHg.

For primary analyses, CEA and RTL outliers were identified separately within each asthma-smoking group using Tukey's 1.5xIQR criterion and excluded only from analyses of the corresponding outcome. CEA group comparisons used Kruskal-Wallis tests followed by

Bonferroni-adjusted Dunn tests. RTL comparisons used Welch ANOVA followed by Dunnett T3 all-pairs tests.

Primary full-cohort models included age, asthma status, smoking status, and the asthma-by-smoking interaction. Log10(CEA) was analyzed using ordinary least-squares regression with HC3 heteroscedasticity-robust standard errors. RTL was analyzed using median regression. RTL standard errors were estimated using stratified nonparametric bootstrap resampling, with 2,000 replicates for the primary analyses and 1,000 replicates for the complete-data and age-restricted sensitivity analyses. Confidence intervals and two-sided p-values were calculated using the normal approximation based on the bootstrap standard errors.

Among participants with asthma, sequential models adjusted for age, categorical asthma severity, and then alcohol consumption, waist circumference >=102 cm, and elevated blood pressure. Asthma-only RTL bootstraps were stratified by smoking status. Expanded full-cohort models additionally included alcohol consumption and derived metabolic syndrome. Analyses were repeated using complete outcome datasets and among participants aged 35-50 years. Model assumptions, influential observations, heteroscedasticity, and multicollinearity were assessed using standard diagnostics. Coefficients were exponentiated as 10^{β} to obtain adjusted ratios. Two-sided $p<0.05$ was considered statistically significant. R 4.5.0 was used for this analysis.

Results

The study included 156 men: 42 asthmatic light smokers, 31 asthmatic nonsmokers, 46 healthy light smokers, and 37 healthy nonsmokers. Mean age was slightly higher and more variable in the asthma groups, but the overall difference did not reach statistical significance (Kruskal–Wallis $p = 0.061$). Derived metabolic syndrome was identified in 11 healthy participants—6 light smokers and 5 nonsmokers—but in none of the participants with asthma. Baseline demographic and metabolic characteristics are presented in Table 2, Participant characteristics by asthma and light-smoking status.

After exclusion of six within-group Tukey outliers, 150 participants were included in the primary CEA analysis. Median CEA was highest in asthmatic light smokers at 1.82 ng/mL and lowest in healthy nonsmokers at 1.58 ng/mL. CEA differed significantly across the four groups (Kruskal–Wallis $p = 0.0118$). The clearest difference was observed between asthmatic light smokers and healthy nonsmokers, with asthmatic light smokers showing a median CEA increase

of approximately 0.38 ng/mL (Dunn–Bonferroni $p = 0.0058$, bootstrap 95% CI 0.14–0.65). No other pairwise comparison remained significant after multiplicity adjustment. This pattern suggests a modest elevation in CEA among men who had both asthma and light-smoking exposure. However, because the significant comparison differed simultaneously in asthma and smoking status, the unadjusted contrast alone could not determine whether the higher CEA reflected smoking, asthma, or their combined presence. Group distributions and unadjusted comparisons are shown in Figure 1A, Serum CEA across asthma and light-smoking groups, and Table 3, Primary unadjusted CEA and RTL comparisons after Tukey outlier exclusion.

For RTL, seven Tukey outliers were excluded and one measurement was missing, leaving 148 participants in the primary analysis. RTL differed significantly across groups (Welch ANOVA $p = 0.0010$). Asthmatic light smokers had shorter RTL than asthmatic nonsmokers, with a mean difference of −0.386 $\log_{10}$ (T/S) units (Dunnett T3 $p = 0.0007$, bootstrap 95% CI −0.569 to −0.205). No significant difference was detected between healthy light smokers and healthy nonsmokers. Thus, the principal RTL contrast occurred within the asthma group: men with asthma who smoked lightly had shorter leukocyte telomeres than asthmatic nonsmokers. This finding was consistent with a possible smoking-related cellular-ageing signal in asthma, although the stability of this association was examined further in adjusted and sensitivity analyses. The distributions are shown in Figure 1B, Relative leukocyte telomere length across asthma and light-smoking groups.

In the primary age-adjusted model, light smoking among participants with asthma was associated with higher CEA, corresponding to an adjusted geometric-mean ratio of 1.18 (95% CI 1.01–1.38; $p = 0.0338$). The corresponding estimate among healthy participants was smaller and statistically inconclusive ($p = 0.0703$). The asthma-by-smoking interaction was not significant ($p = 0.5383$, indicating no statistical evidence that asthma altered the smoking–CEA association. A similar pattern was observed for RTL. Among participants with asthma, light smoking was associated with a lower adjusted conditional median RTL (ratio 0.38, 95% bootstrap CI 0.22–0.67; $p = 0.0009$). No association was detected among healthy participants, and the asthma-by-smoking interaction was nonsignificant ($p = 0.3559$). Therefore, although the asthma-stratum estimates were stronger, the interaction tests did not demonstrate that the smoking associations were statistically different between men with and without asthma. Full-cohort results are presented in Table 4, Primary age-adjusted full-cohort models for CEA and RTL, and Figure 2, Adjusted associations of light smoking with CEA and RTL.

Within the asthma group, the CEA association persisted after adjustment for age and asthma severity, with an adjusted ratio of 1.18 ($p = 0.0495$). After additional adjustment for alcohol use, elevated waist circumference, and elevated blood pressure, the estimate decreased to 1.14 and became statistically inconclusive ($p = 0.1334$). This attenuation suggests that the modest CEA elevation was not fully stable under broader adjustment.

For RTL, the age-adjusted association remained strong (adjusted conditional-median ratio 0.40; $p = 0.0009$), but weakened after adjustment for asthma severity (ratio 0.55; $p = 0.0566$). The expanded asthma-only model produced a similar effect estimate but borderline statistical support (ratio 0.50; $p = 0.0487$). These sequential models are reported in Table 5, Primary asthma-only models for CEA and RTL.

Sensitivity analyses supported the CEA pattern more consistently than the RTL pattern. Complete-data analyses continued to show higher CEA among asthmatic light smokers, whereas the complete-data RTL result depended on the statistical method used. In analyses restricted to participants aged 35–50 years, neither biomarker association remained statistically significant. The age-restricted severity-adjusted RTL model was also not reliably estimable because of sparse data.

Overall, light smoking was associated with modestly higher CEA and shorter RTL among men with asthma in the primary analyses. However, the strength and statistical certainty of both associations—particularly RTL—varied with covariate adjustment, outlier handling, and age restriction. Sensitivity analyses and model diagnostics are reported in *Supplementary Tables 1-5*.

Discussion

Asthma is characterized by chronic airway inflammation and oxidative stress, which drive epithelial injury, airway remodeling, and may contribute to accelerated biological ageing [2,3]. Cigarette smoke adds oxidants and inflammatory signaling that can extend beyond the lungs [22,23]. In the present study, we evaluated whether two accessible blood biomarkers—RTL and serum CEA—differed according to asthma and light-smoking status in men reporting very low-intensity smoking (1–4 cigarettes/day). We restricted analyses to men to reduce heterogeneity related to sex-linked biology and gender-related exposures [24-26]. In the primary unadjusted analyses, asthmatic light smokers had shorter RTL than asthmatic nonsmokers, while serum CEA was higher in asthmatic light smokers than in healthy nonsmokers. In age-adjusted full-cohort

models, smoking status was associated with higher CEA and shorter RTL among participants with asthma; however, the asthma-status×smoking-status interaction was not statistically significant for either biomarker. Therefore, the present data do not establish that the associations of smoking status with CEA or RTL differ between participants with and without asthma.

Among participants with asthma, the association between light-smoking status and CEA remained statistically significant after simultaneous adjustment for age and asthma severity. In contrast, the association with shorter RTL was statistically significant in the age-adjusted model but was attenuated by approximately one third and became statistically inconclusive after additional adjustment for asthma severity. These findings indicate that the CEA association was more consistent than the RTL association across the principal models, although neither estimate can be considered independent of residual confounding.

Telomeres are vulnerable to oxidative damage, and repeated ROS exposure can accelerate telomere attrition and promote leukocyte senescence with a pro-inflammatory phenotype [27,28]. Leukocyte telomere length has been widely discussed as a biomarker of ageing and age-related disease processes [29]. Asthma-related inflammatory activation and impaired antioxidant defenses can increase oxidative stress and DNA damage [30]. The shorter RTL observed among asthmatic light smokers is consistent with, but does not demonstrate, a possible contribution of the combined biological context of asthma-related inflammation and cigarette-smoke exposure [31]. However, the attenuation of the smoking coefficient after adjustment for asthma severity suggests that the unadjusted and age-adjusted RTL findings may have been influenced partly by differences in disease severity. Accordingly, the RTL result should be interpreted as suggestive rather than as a robust association. This finding is biologically compatible with established relationships among oxidative stress, chronic inflammation, and telomere biology, although the specific biological processes underlying the observed association remain to be clarified. Because exposure and RTL were measured concurrently, the present study cannot determine whether smoking preceded or contributed to the observed difference in RTL.

CEA (CEACAM5), although best known as an oncological marker, can be modestly elevated in smokers and benign inflammatory conditions and has been discussed as a biomarker of airway inflammation [32,33]. In the primary unadjusted comparison, CEA was higher in asthmatic light smokers than in healthy nonsmokers, yet remained within typical reference ranges. Although this unadjusted comparison differed simultaneously in asthma and smoking status, the asthma-only

multivariable analysis provided additional evidence that light-smoking status remained associated with higher CEA after adjustment for age and asthma severity. In the primary Tukey-filtered age- and severity-adjusted analysis, light smoking was associated with approximately 18% higher CEA among men with asthma. The complete-data sensitivity analysis yielded a larger estimate of approximately 26%. This difference may reflect subtle epithelial or inflammatory variation, but these processes were not directly measured in the present study. The adjusted association remains observational and cannot establish that light smoking caused the higher CEA values. Moreover, the magnitude of the difference was modest, and values remained within the established clinical reference range. Therefore, the finding should be interpreted as a subtle biomarker association rather than evidence of clinically meaningful disease progression.

Overall, our findings are consistent with the established associations of oxidative stress and chronic inflammation with both asthma and cigarette smoking and provide a biologically plausible context for the observed biomarker patterns [30-32,34,35]. These mechanisms were not directly evaluated and should therefore be regarded as possible interpretations rather than demonstrated pathways linking light smoking to RTL or CEA. The nonsignificant asthma-status×smoking-status interactions indicate that the present sample does not provide statistical evidence that asthma status modifies the association between smoking and either biomarker. At the same time, adjustment for asthma severity had different consequences for the two outcomes: it had little influence on the CEA estimate but materially attenuated the RTL estimate. Thus, asthma severity should be considered a potential confounder of the RTL association rather than dismissed on the basis of a nonsignificant severity coefficient.

Several limitations should be noted. The cross-sectional design precludes causal inference, and smoking exposure was self-reported without information on duration, pack-years, passive exposure, or cotinine verification. Although models adjusted for age and asthma severity, data on inhaled corticosteroid use, disease duration, BMI, socioeconomic and environmental factors, and lung function, including FEV1% predicted, were unavailable, leaving potential residual confounding. The modest sample size limited multivariable and interaction precision. qPCR measurement error and Tukey-based outlier exclusion may also have influenced estimates, although complete-data sensitivity analyses were performed. Findings may not generalize beyond men from this region.

Conclusions

Among men with asthma, light-smoking status was associated with higher serum CEA after adjustment for age and asthma severity, although the magnitude of the association was modest and CEA values remained within typical clinical reference ranges. Unadjusted and age-adjusted analyses also indicated shorter RTL among asthmatic light smokers; however, this association was attenuated and was no longer statistically significant after additional adjustment for asthma severity. Evidence for a severity-adjusted association with RTL was less consistent and remained susceptible to residual confounding. The asthma-status×smoking-status interactions were not statistically significant for either biomarker, so the present study does not establish that smoking–biomarker associations differ between men with and without asthma. These cross-sectional findings cannot establish temporal or causal relationships. Larger longitudinal studies incorporating objective tobacco-exposure measures and more comprehensive adjustment for treatment, disease duration, BMI, passive smoking, and environmental exposures are needed to confirm the findings and determine their clinical significance.

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Online supplementary material

Table 1. Primer sequences and qPCR program.

Target	Primer Sequence			Ref.
HBG (F)	5`GCTTCTGACACAACTGTTCACTAGC 3`			
HBG (R)	5` CACCAACTTCATCCACGTTCACC 3`			
Telomere (F)	5`CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT 3`			
Telomere (R)	5`GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT 3`			
qPCR program	Step	Time (seconds)	Temperature (^{0}C)	
HBG	Denaturation	30	95	
	annealing	30	60	[18]
	Extension	30	72	
Telomere	Denaturation	15	95	
	annealing	15	65	
	Extension	15	72	
	Holding stage	600 (10 min)	95	
	Melting curve	15	95	

Table 2. Participant characteristics by asthma and light-smoking status

Characteristic	Asthmatic light smoker	Asthmatic nonsmoker	Healthy light smoker	Healthy nonsmoker	Overall p-value
Age, years, mean +/- SD (range)	47.93+/-15.75 (19-89)	45.84+/-15.97 (19-92)	41.07+/-4.24 (35-50)	40.65+/-4.19 (35-50)	0.061
Sex, male, N (%)	42 (100.0%)	31 (100.0%)	46 (100.0%)	37 (100.0%)	
Smoking intensity, cigarettes/day	1–4	-	1–4	-	
Asthma severity: Intermittent, N (%)	4 (9.5%)	4 (12.9%)	-	-	0.933
Asthma severity: Mild persistent, N (%)	17 (40.5%)	13 (41.9%)	-	-	
Asthma severity: Moderate persistent, N (%)	12 (28.6%)	9 (29.0%)	-	-	
Asthma severity: Severe persistent, N (%)	9 (21.4%)	5 (16.1%)	-	-	
Alcohol use, N (%)	13 (31.0%)	3 (9.7%)	1 (2.2%)	0 (0.0%)	<0.001
Waist circumference >=102 cm, N (%)	17 (40.5%)	7 (22.6%)	3 (6.5%)	2 (5.4%)	<0.001
Fasting glucose >100 mg/dL, N (%)	0 (0.0%)	2 (6.5%)	21 (45.7%)	15 (40.5%)	<0.001
Triglycerides >150 mg/dL, N (%)	0 (0.0%)	1 (3.2%)	15 (32.6%)	13 (35.1%)	<0.001
HDL-C <40 mg/dL, N (%)	0 (0.0%)	0 (0.0%)	25 (54.3%)	13 (35.1%)	<0.001
Elevated blood pressure, N (%)	7 (16.7%)	2 (6.5%)	4 (8.7%)	5 (13.5%)	0.527
Metabolic syndrome, N (%)	0 (0.0%)	0 (0.0%)	6 (13.0%)	5 (13.5%)	0.005
CEA, ng/mL, median (IQR); N	1.82 (1.47-2.34); n=39	1.74 (1.14-1.99); n=31	1.62 (1.36-1.97); n=43	1.58 (1.26-1.76); n=37	0.012
RTL, $\log_{10}$(T/S), mean +/- SD; N	-0.58 +/- 0.48; n=39	-0.20 +/- 0.29; n=28	-0.41 +/- 0.38; n=45	-0.28 +/- 1.05; n=36	0.001

Values are presented as mean ± SD (range), median (IQR), or N (%), as indicated. Light smoking was defined as 1–4 cigarettes/day. CEA and RTL summaries use the primary outcome-specific datasets after within-group Tukey 1.5×IQR outlier exclusion. Metabolic syndrome was derived from the measured metabolic components. Overall p values compare the four study groups. CEA, carcinoembryonic antigen; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; RTL, relative leukocyte telomere length; SD, standard deviation; T/S, telomere-to-single-copy-gene ratio.

Table 3. Primary unadjusted comparisons of serum CEA and relative leukocyte telomere length across study groups.

Outcome	Primary N	Omnibus comparison	Key pairwise comparison	Effect estimate (95% CI)	Adjusted pairwise p-value
CEA	150	Kruskal–Wallis H(3)=10.99; p=0.012	Asthmatic light smokers vs healthy nonsmokers; Dunn–Bonferroni test	Hodges–Lehmann difference: 0.380 ng/mL (0.140 to 0.650)	0.006
RTL	148	Welch ANOVA F(3,75.80)=5.97; p=0.001	Asthmatic light smokers vs asthmatic nonsmokers; Dunnett T3 test	Mean difference: −0.386 $\log_{10}$(T/S) units (−0.569 to −0.205)	0.001

Primary analyses used outcome-specific datasets after within-group Tukey 1.5×IQR outlier exclusion. Positive CEA differences indicate higher concentrations in asthmatic light smokers, whereas negative RTL differences indicate shorter RTL in asthmatic light smokers. Effect estimates are presented with 95% confidence intervals. All p-values are two-sided. ANOVA, analysis of variance; CEA, carcinoembryonic antigen; CI, confidence interval; RTL, relative leukocyte telomere length; T/S, telomere-to-single-copy-gene ratio.

Table 4. Primary age-adjusted full-cohort models for the associations of light smoking with serum CEA and relative leukocyte telomere length.

Outcome	Term or model contrast	N	β (95% CI)	Adjusted ratio (95% CI)	p-value
CEA	Age, centered	150	0.001 (−0.001 to 0.003)	1.002 (0.998 to 1.007)	0.330
CEA	Asthma status	150	0.030 (−0.035 to 0.094)	1.070 (0.923 to 1.242)	0.367
CEA	Smoking association among healthy participants	150	0.047 (−0.004 to 0.097)	1.114 (0.991 to 1.252)	0.070
CEA	Asthma×smoking interaction	150	0.026 (−0.058 to 0.111)	1.062 (0.875 to 1.290)	0.538
CEA	Smoking association among participants with asthma	150	0.073 (0.006 to 0.140)	1.183 (1.013 to 1.382)	0.034
RTL	Age, per year	148	0.003 (−0.005 to 0.010)	1.006 (0.989 to 1.023)	0.502
RTL	Asthma status	148	0.145 (−0.489 to 0.778)	1.395 (0.325 to 5.999)	0.654
RTL	Smoking association among healthy participants	148	−0.105 (−0.729 to 0.519)	0.786 (0.187 to 3.303)	0.742
RTL	Asthma×smoking interaction	148	−0.315 (−0.983 to 0.354)	0.484 (0.104 to 2.257)	0.356
RTL	Smoking association among participants with asthma	148	−0.420 (−0.666 to −0.173)	0.381 (0.216 to 0.671)	0.001

Full-cohort models included age, asthma status, light-smoking status, and the asthma×smoking interaction. Healthy nonsmokers were the reference group. CEA was analysed after $\log_{10}$ transformation using ordinary least-squares regression with HC3 robust standard errors. RTL was analysed using median regression with 2,000 bootstrap replicates stratified by asthma–smoking group. Adjusted ratios were calculated as 10^{β}; they represent geometric-mean ratios for CEA and conditional-median ratios for RTL. The smoking association among participants with asthma was estimated by combining the smoking and asthma×smoking coefficients. Confidence intervals are 95%, and all p-values are two-sided. β, regression coefficient; CEA, carcinoembryonic antigen; CI, confidence interval; HC3, heteroscedasticity-consistent covariance estimator type 3; RTL, relative leukocyte telomere length.

Table 5. Asthma-only sequential models for the associations of light smoking with serum CEA and RTL.

Outcome	Adjustment model	N	β (95% CI)	Adjusted ratio (95% CI)	p-value	Attenuation vs age-only model
CEA	Age adjusted	70	0.073 (0.005 to 0.141)	1.183 (1.011 to 1.384)	0.036	—
CEA	Age + asthma severity	70	0.071 (0.000 to 0.142)	1.178 (1.0003 to 1.388)	0.0495	2.40%
CEA	Age + severity + alcohol + waist circumference + blood pressure	70	0.056 (−0.018 to 0.129)	1.137 (0.961 to 1.345)	0.133	23.80%
RTL	Age adjusted	67	−0.403 (−0.641 to −0.164)	0.395 (0.228 to 0.685)	0.001	—
RTL	Age + asthma severity	67	−0.262 (−0.532 to 0.007)	0.547 (0.294 to 1.017)	0.057	34.90%
RTL	Age + severity + alcohol + waist circumference + blood pressure	67	−0.298 (−0.594 to −0.002)	0.504 (0.255 to 0.996)	0.049	26.10%

Analyses were restricted to participants with asthma and used the primary outcome-specific Tukey-filtered datasets. CEA was analysed after $\log_{10}$ transformation using ordinary least-squares regression with HC3 robust standard errors. RTL was analysed using median regression with 2,000 bootstrap replicates stratified by smoking status. Adjusted ratios were calculated as $10^{\beta}$and compare light smokers with nonsmokers. Attenuation represents the percentage reduction in the absolute smoking coefficient relative to the age-adjusted model. Confidence intervals are 95%, and all p-values are two-sided. β, regression coefficient; CEA, carcinoembryonic antigen; CI, confidence interval; HC3, heteroscedasticity-consistent covariance estimator type 3; RTL, relative leukocyte telomere length.

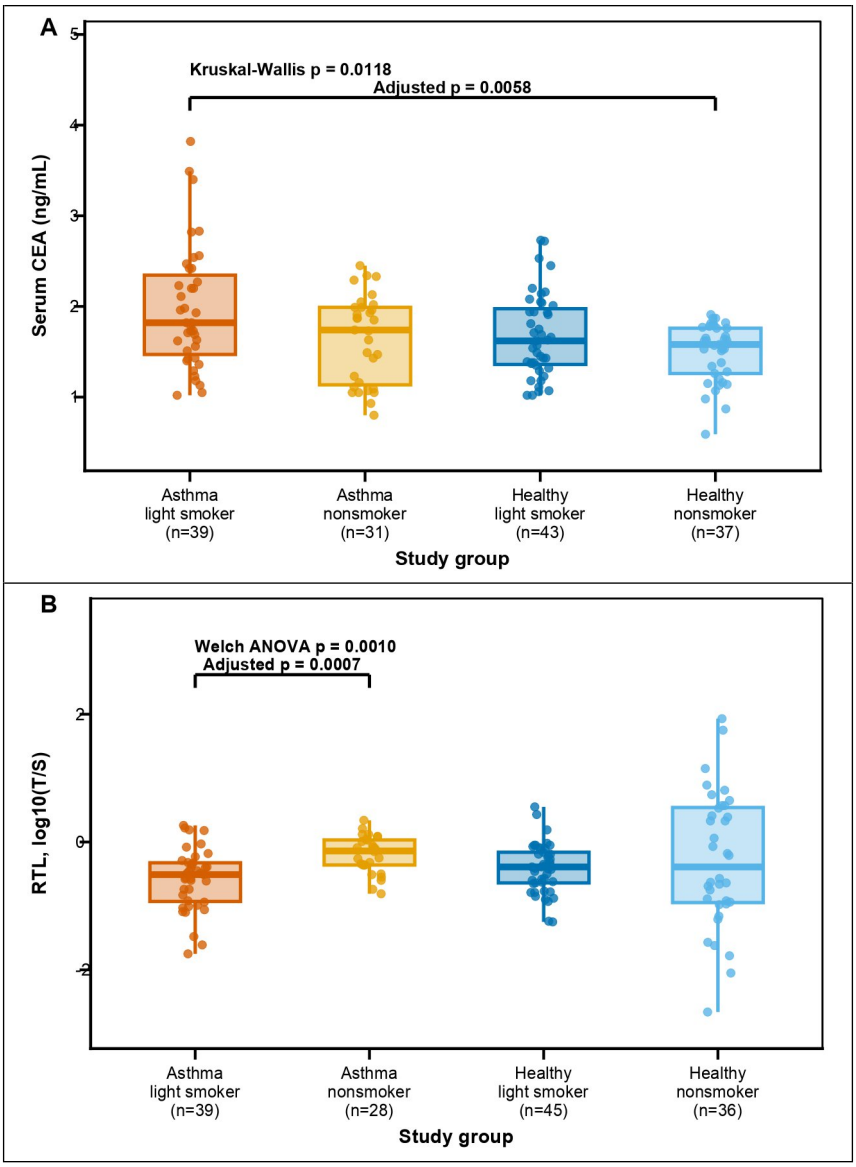

Figure 1. Primary group comparisons of CEA (A) and RTL (B) after Tukey outlier exclusion. (A) Serum carcinoembryonic antigen (CEA), a marker of epithelial and inflammatory activity, was highest in asthmatic light smokers and was significantly greater in this group than in healthy nonsmokers. (B) Relative leukocyte telomere length (RTL), expressed as $\log_{10}$ (T/S), was lower in asthmatic light smokers than in asthmatic nonsmokers, consistent with a possible cellular-ageing signal associated with light smoking in asthma. Boxes represent the median and interquartile range, whiskers extend to 1.5×IQR, and points represent individual participants. Analyses used outcome-specific datasets after within-group Tukey 1.5×IQR outlier exclusion. Adjusted p-values for the principal between-group comparisons are shown in the panels.

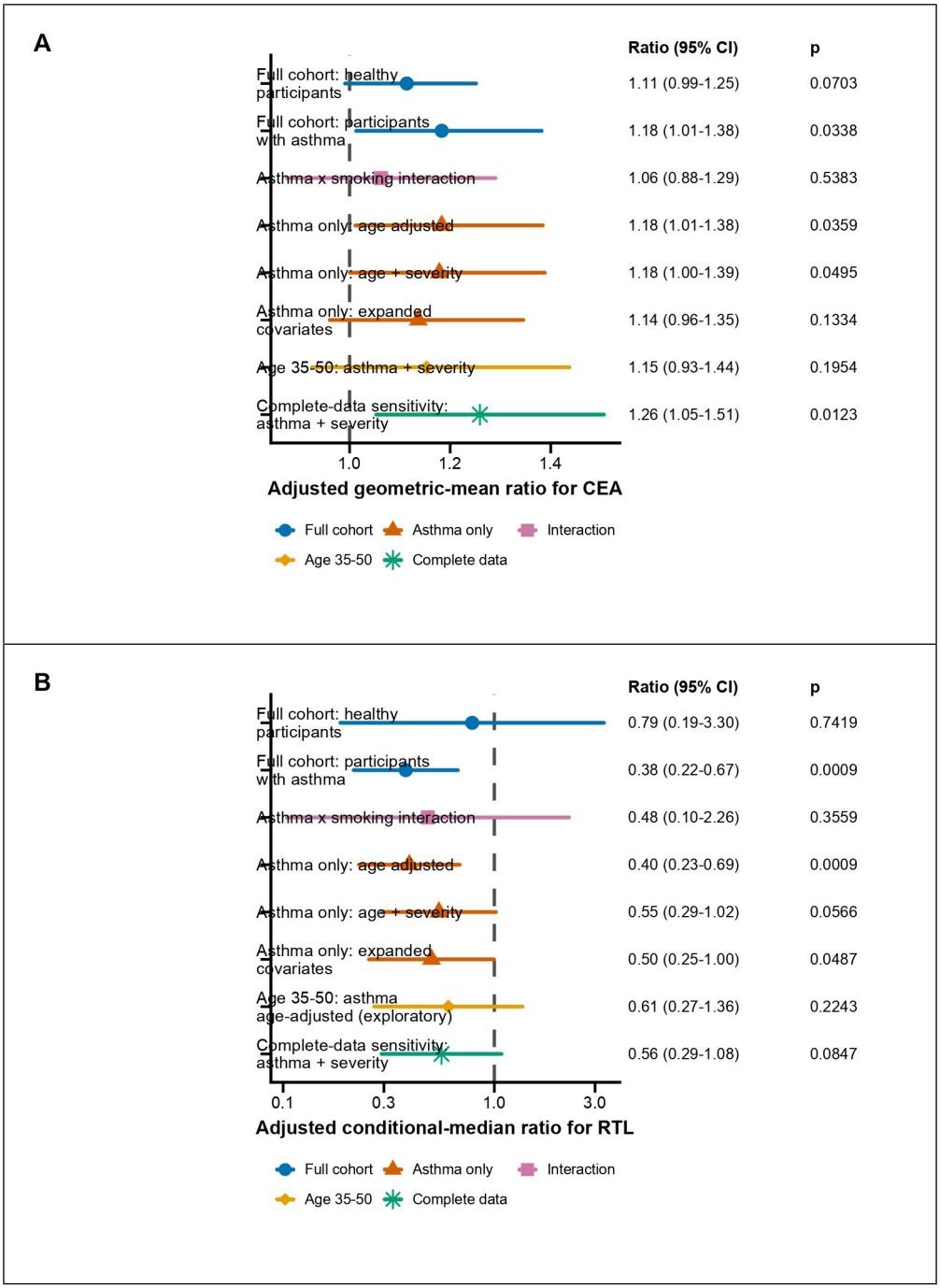

Figure 2. CEA and RTL adjusted forests. Adjusted associations of light smoking with circulating CEA (A) and leukocyte RTL (B). Light smoking was associated with modestly higher CEA among men with asthma in the primary models, consistent with increased epithelial or inflammatory activity, although the association weakened after broader covariate adjustment (A). Light smoking was associated with lower RTL among men with asthma in the primary models, suggesting a possible cellular-ageing signal, but this association was less consistent across severity-adjusted, complete-data, and age-restricted analyses (B). Asthma-by-smoking interaction estimates were nonsignificant for both biomarkers, providing no evidence that asthma significantly modified either association. Points represent adjusted ratios and horizontal lines represent 95% confidence intervals. Ratios above 1 indicate higher CEA, whereas ratios below 1 indicate shorter RTL. The vertical dashed line indicates the null value of 1.