Natural Resources for Human Health

eISSN: 2583-1194

Original Research

Received 11 May 2026
Revised 24 June 2026
Accepted 08 July 2026

Natural Resources for Human Health 2026, Vol. 6 (11s): 1378–1387

KEYWORDS:
Biomarkers; Club Cells; Respiratory Function Tests; Smoking.

Association Between Serum Club Cell Protein 16 (CC16) Levels and $FEF_{25–75}$ in Asymptomatic Active Smokers: A Cross-Sectional Study

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ABSTRACT: Club cell protein 16 (CC16) is a marker of airway epithelial integrity that may reflect early smoking-related injury. Small-airway dysfunction can occur before respiratory symptoms or overt airflow obstruction. This study aimed to determine the association between serum CC16 levels and forced expiratory flow at 25–75% of forced vital capacity ($FEF_{25–75}$) in asymptomatic active smokers and to examine associations with other spirometric parameters. This cross-sectional study included 86 asymptomatic active male smokers aged 20–60 years recruited from hospital security personnel in Makassar, Indonesia. Serum CC16 concentrations were measured using enzyme-linked immunosorbent assay, and pulmonary function was assessed by spirometry. Associations were evaluated using Spearman correlation, group comparisons, and multivariable linear regression adjusted for age, body mass index, and Brinkman Index. The median serum CC16 concentration was 6.27 ng/mL (IQR, 4.45–8.90), while mean $FEF_{25–75}$ was 75.6% predicted (SD, 18.1). Participants with $FEF_{25–75}$ <65% predicted had significantly lower CC16 levels than those with $FEF_{25–75}$ ≥65% predicted (3.87 vs. 7.64 ng/mL; $p<0.001$). Serum CC16 was positively correlated with $FEF_{25–75}$ ($\rho=0.560$, $p<0.001$), FEV_1 ($\rho=0.335$, $p=0.002$), and FEV_1/FVC ($\rho=0.410$, $p<0.001$), but not FVC ($\rho=0.174$, $p=0.109$). After adjustment, each 1-ng/mL increase in CC16 was associated with a 2.62-percentage-point higher $FEF_{25–75}$ ($\beta=2.621$; 95% CI, 1.49–3.75; $p<0.001$). Lower serum CC16 levels are associated with impaired small-airway expiratory flow in asymptomatic active smokers, supporting CC16 as a potential biomarker of subclinical smoking-related airway injury.

1. INTRODUCTION

Club cell secretory protein 16 (CC16), also known as secretoglobin 1A1 (SCGB1A1), is a 15.8-kDa secretory protein predominantly produced by non-ciliated club cells of the distal airway epithelium. Club cells contribute to airway epithelial maintenance, repair, and host defense, while CC16 exerts anti-inflammatory and immunomodulatory effects that support respiratory epithelial homeostasis [1]. Because CC16 can pass from the airway lining fluid into the circulation, its serum concentration has been investigated as a minimally invasive marker of airway epithelial integrity and injury [2]. Chronic exposure

to cigarette smoke is associated with reduced CC16 expression and circulating concentrations, potentially reflecting club-cell dysfunction or depletion and persistent epithelial injury [3]. Importantly, reduced circulating CC16 has been associated with accelerated decline in lung function and an increased risk of airflow limitation, supporting its potential relevance as an early biomarker of smoking-related respiratory injury [4].

Smoking-related airway injury may initially involve the small airways before clinically apparent respiratory symptoms or established airflow obstruction develop. $FEF_{25–75}$, representing the mean forced expiratory flow during the middle 50% of the forced vital capacity, has been used as a spirometric parameter reflecting small-airway function and may provide additional information regarding early peripheral airway abnormalities [5,6]. Experimental and clinical evidence indicates that cigarette smoke exposure can suppress CC16 expression in airway epithelial cells, while lower serum CC16 concentrations are associated with impaired pulmonary function among smokers [4]. Furthermore, circulating CC16 concentrations have been linked to subsequent lung-function decline and the development of airflow limitation, suggesting that alterations in this biomarker may precede clinically significant respiratory disease [7]. Evidence from longitudinal cohorts also indicates that individuals with lower CC16 concentrations have greater subsequent declines in lung function, strengthening the biological rationale for investigating CC16 as an indicator of early smoking-related airway damage [7].

Despite growing evidence linking CC16 with smoking exposure and pulmonary function, the relationship between serum CC16 and $FEF_{25–75}$ in asymptomatic active smokers remains insufficiently characterized, particularly in the Indonesian population [4]. Most previous investigations have focused on patients with established chronic respiratory disease, occupationally exposed populations, or broader community cohorts, limiting direct interpretation of CC16 as a marker of subclinical small-airway dysfunction in otherwise asymptomatic smokers. Clarifying this association may help identify early epithelial and functional abnormalities before overt respiratory symptoms or conventional spirometric impairment become apparent [7]. Therefore, this study aimed to determine the association between serum CC16 levels and $FEF_{25–75}$ in asymptomatic active smokers and to further examine the relationships of CC16 with FEV_1, FVC, and FEV_1/FVC.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This analytical observational study employed a cross-sectional design to investigate the association between serum CC16 levels and $FEF_{25–75}$ among asymptomatic active smokers. The study was conducted at a hospital in Makassar, South Sulawesi, Indonesia, from March to May 2026. All participant recruitment, clinical assessment, spirometry, and blood sampling procedures were performed during the study period.

2.2 Participants and Sample Size

The study population comprised asymptomatic active smokers working as hospital security personnel. Eligible participants were aged 20–60 years, currently smoking at least one cigarette per day, and had a smoking history of at least one year. Participants were considered asymptomatic if they reported no chronic cough, chronic sputum production, wheezing, dyspnea, or hemoptysis and had no symptoms of an acute respiratory infection within the preceding four weeks.

Participants were excluded if they had a history of chronic respiratory disease, including asthma, chronic obstructive pulmonary disease, pulmonary tuberculosis, bronchiectasis, or lung cancer, or if they lived in an industrial or factory area. All eligible participants provided written informed consent before enrollment.

The minimum sample size was calculated using the Pearson correlation formula with Fisher's z transformation, assuming a two-sided α of 0.05, 80% statistical power, and an expected correlation coefficient of 0.35. The resulting minimum sample size was 62 participants. Allowing for approximately 10–15% of potentially incomplete or invalid data, including unacceptable spirometry maneuvers or unsuccessful serum analysis, the target sample size was set at approximately 70 participants.

2.3 Sampling and Clinical Assessment

Participants were recruited using consecutive sampling until the required sample size was reached. Potential participants were approached directly at the study site and screened according to the predefined eligibility criteria. Smoking status and smoking history were assessed through structured interviews. Information on the number of cigarettes smoked per day and smoking

duration was recorded, and cumulative smoking exposure was estimated using the Brinkman Index, calculated by multiplying the average number of cigarettes smoked per day by the number of years of smoking.

Asymptomatic status was determined through clinical history and physical examination. Respiratory symptoms, including chronic cough, chronic sputum production, wheezing, dyspnea, and hemoptysis, were assessed. Physical examination included respiratory rate and pulmonary auscultation, with particular attention to wheezing and crackles, as well as the presence of accessory muscle use. Participants with clinical evidence of respiratory disease or recent acute respiratory infection were excluded.

Demographic and anthropometric characteristics were also recorded. Body weight and height were measured using calibrated instruments, and body mass index was calculated as weight in kilograms divided by height in meters squared. Participant data were obtained from the study questionnaire and, when available, medical examination records.

2.4 Serum Collection and CC16 Measurement

Participants were instructed to abstain from smoking for at least 2 h before blood collection to minimize the potential influence of acute cigarette smoke exposure. Venous blood samples were collected in the morning by trained healthcare personnel using standard aseptic procedures. Approximately 3–5 mL of venous blood was collected from the antecubital vein into a plain tube without anticoagulant.

The blood was allowed to clot at room temperature for approximately 30 min and subsequently centrifuged at 3000 rpm for 10–15 min. The separated serum was transferred into sterile microtubes using a micropipette and stored at −80°C until analysis. Serum CC16 concentrations were determined using an enzyme-linked immunosorbent assay with the BioVendor Human Club Cell Protein (CC16) ELISA kit (Catalogue No. RD191022200), according to the manufacturer's instructions. The assay was performed at the HUM-RC laboratory, Hasanuddin University. Results were recorded in ng/mL and analyzed as a continuous variable.

2.5 Spirometry

Spirometry was performed to assess pulmonary function, particularly $FEF_{25–75}$, using a calibrated spirometer in accordance with current American Thoracic Society/European Respiratory Society technical standards. Participants underwent standardized spirometric maneuvers after appropriate instruction and coaching by trained personnel. Measurements included FEV_1, FVC, FEV_1/FVC, and $FEF_{25–75}$. The best value from acceptable and repeatable maneuvers was used for analysis.

$FEF_{25–75}$ was expressed as a percentage of the predicted value. For descriptive classification, a value of ≥65% predicted was considered within the predefined normal range, whereas a value <65% predicted was considered reduced. When available, interpretation relative to the LLN was also considered. Because $FEF_{25–75}$ is influenced by several technical and physiological factors, its interpretation was performed together with the other spirometric parameters rather than as an isolated diagnostic criterion.

2.6 Study Variables

The primary exposure variable was serum CC16 concentration, expressed in ng/mL. The primary outcome was $FEF_{25–75}$ expressed as a percentage of the predicted value. Secondary pulmonary function outcomes included FEV_1, FVC, and FEV_1/FVC. Potential confounding variables included age, sex, body mass index, smoking duration, cigarettes smoked per day, and cumulative smoking exposure as represented by the Brinkman Index.

2.7 Data Management and Statistical Analysis

Data were checked for completeness and consistency before coding and entry into the study database. Data cleaning procedures were performed to identify missing, inconsistent, or implausible values before statistical analysis. Statistical analyses were performed using SPSS software.

Continuous variables were assessed for distributional characteristics and summarized as mean ± standard deviation for normally distributed data or median with interquartile range for non-normally distributed data. Categorical variables were presented as frequencies and percentages. The association between serum CC16 concentration and $FEF_{25–75}$ was assessed using Pearson's

correlation coefficient for normally distributed variables or Spearman's rank correlation coefficient when distributional assumptions were not met.

Differences in CC16 concentrations and $FEF_{25–75}$ between participants classified as having reduced versus non-reduced small-airway function were assessed using an independent-samples t-test for normally distributed data or the Mann–Whitney U test for non-normally distributed data. Multivariable linear regression was performed when $FEF_{25–75}$ was analyzed as a continuous dependent variable to assess the independent association between CC16 and pulmonary function after adjustment for relevant confounders. If reduced $FEF_{25–75}$ was analyzed as a binary outcome, binary logistic regression was performed. Variables with $p < 0.25$ in the bivariate analysis were considered for inclusion in multivariable models. Results were reported as regression coefficients (β), odds ratios (ORs), 95% confidence intervals (CIs), and p values, as appropriate. Statistical significance was defined as a two-sided $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Participant Characteristics

A total of 86 asymptomatic active smokers were included in the analysis. All participants were male, with a median age of 29 years (IQR, 24–36). The median body mass index was 22.76 kg/m² (IQR, 20.76–26.61). The median smoking duration was 13 years (IQR, 8–20.8), with a median consumption of 12 cigarettes/day (IQR, 6–16) and a median Brinkman Index of 142 (IQR, 70–288). Most participants were classified as light smokers according to the Brinkman Index. The median serum CC16 concentration was 6.27 ng/mL (IQR, 4.45–8.90). Baseline characteristics and pulmonary function parameters are summarized in Table 1.

3.2 Small-Airway Function and Serum CC16

The mean $FEF_{25–75}$ was 75.6% predicted (SD, 18.1), and 25 participants (29.1%) had values below 65% predicted, indicating reduced mid-expiratory flow, whereas 61 participants (70.9%) had values ≥65% predicted. Participants with $FEF_{25–75}$ <65% predicted had significantly lower serum CC16 concentrations than those with values ≥65% predicted (median, 3.87 vs. 7.64 ng/mL; $p<0.001$; Table 2).

Spearman analysis demonstrated a significant positive correlation between serum CC16 and $FEF_{25–75}$ ($\varrho=0.560$, $p<0.001$), indicating that higher CC16 concentrations were associated with better mid-expiratory flow (Table 2). The corresponding scatter plot showed a consistent positive trend across the observed range of CC16 concentrations (Figure 1).

When participants were stratified into CC16 quartiles, $FEF_{25–75}$ was substantially lower in the lowest CC16 quartile than in the three higher quartiles. The overall difference across quartiles was significant (Kruskal–Wallis, $p<0.001$), with a significant monotonic trend (Jonckheere–Terpstra, $p<0.001$). Post-hoc analysis showed that the lowest quartile differed significantly from each of the other three quartiles, whereas no significant differences were observed among the second, third, and fourth quartiles. These findings are illustrated in Figure 2. The proportion of participants with $FEF_{25–75}$ <65% predicted also decreased progressively across CC16 quartiles, from 95.5% in the lowest quartile to 19.0% in the second quartile and none in the third and fourth quartiles (Cochran–Armitage trend, $p<0.001$).

3.3 Association With Other Spirometric Parameters

Serum CC16 was significantly lower among participants with FEV_1 <80% predicted than among those with FEV_1 ≥80% predicted ($p=0.024$). Spearman analysis demonstrated positive correlations between CC16 and both FEV_1 ($\varrho=0.335$, $p=0.002$) and FEV_1/FVC ($\varrho=0.410$, $p<0.001$). In contrast, the correlation with FVC was weak and not statistically significant ($\varrho=0.174$, $p=0.109$; Table 3). Because all participants had an FEV_1/FVC ratio ≥70%, categorical comparison according to an obstructive ratio was not applicable.

Table 1. Baseline Characteristics and Pulmonary Function of the Study Participants

Variable	Overall (n=86)
Age, years	29 (24–36)
Male sex, n (%)	86 (100.0)
Body mass index, kg/m²	**22.76 (20.76–26.61)**

Underweight, n (%)	6 (7.0)
Normal, n (%)	42 (48.8)
Overweight, n (%)	7 (8.1)
Obesity class I, n (%)	22 (25.6)
Obesity class II, n (%)	9 (10.5)
Smoking duration, years	**13 (8–20.8)**
<10 years, n (%)	30 (34.9)
10–19 years, n (%)	32 (37.2)
≥20 years, n (%)	24 (27.9)
Cigarettes/day	**12 (6–16)**
1–10 cigarettes/day, n (%)	38 (44.2)
11–20 cigarettes/day, n (%)	38 (44.2)
>20 cigarettes/day, n (%)	10 (11.6)
Brinkman Index	**142 (70–288)**
Light, n (%)	55 (64.0)
Moderate, n (%)	25 (29.1)
Heavy, n (%)	6 (7.0)
$FEF_{25–75}$, % predicted	**75.6 ± 18.1**
$FEF_{25–75}$ <65% predicted, n (%)	25 (29.1)
$FEF_{25–75}$ ≥65% predicted, n (%)	61 (70.9)
FEV_1, % predicted	**76 (70–81)**
FEV_1 <80% predicted, n (%)	56 (65.1)
FEV_1 ≥80% predicted, n (%)	30 (34.9)
FVC, % predicted	**73.76 ± 10.46**
FVC <80% predicted, n (%)	62 (72.1)
FVC ≥80% predicted, n (%)	24 (27.9)
FEV_1/FVC, %	**90.5 (87.2–93.7)**
FEV_1/FVC ≥70%, n (%)	86 (100.0)
Serum CC16, ng/mL	**6.27 (4.45–8.90)**

Data are presented as median (IQR), mean ± SD, or n (%), as appropriate.

Table 2. Association Between Serum CC16 and $FEF_{25–75}$

Variable	Result	p-value
Serum CC16 according to $FEF_{25–75}$ category		
$FEF_{25–75}$ <65% predicted, median (IQR), ng/mL	3.87 (2.89–4.12)	<0.001
$FEF_{25–75}$ ≥65% predicted, median (IQR), ng/mL	7.64 (6.07–9.79)	
Spearman correlation	ρ=0.560	<0.001

Mann–Whitney U test for group comparison; Spearman rank correlation for continuous association.

3.4 Multivariable Analysis

In multivariable linear regression, serum CC16 remained independently associated with $FEF_{25–75}$ after adjustment for age, body mass index, and Brinkman Index (Table 4). The overall model was statistically significant ($F(4,81)=5.895$, $p<0.001$) and explained 18.7% of the variance in $FEF_{25–75}$. Each 1-ng/mL increase in serum CC16 was associated with a 2.62-percentage-point higher $FEF_{25–75}$ (% predicted) ($\beta=2.621$, 95% CI 1.49–3.75, $p<0.001$). None of the adjusted covariates showed a significant independent association with $FEF_{25–75}$. Variance inflation factors ranged from 1.01 to 1.73, indicating no evidence of problematic multicollinearity.

Natural Resources for Human Health
eISSN: 2583-1194

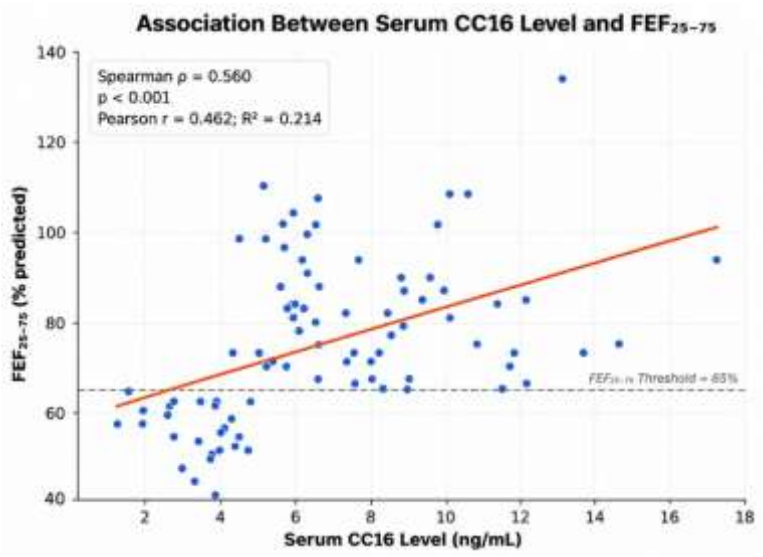

Figure 1. Scatter plot of the association between serum CC16 concentration and $FEF_{25–75}$

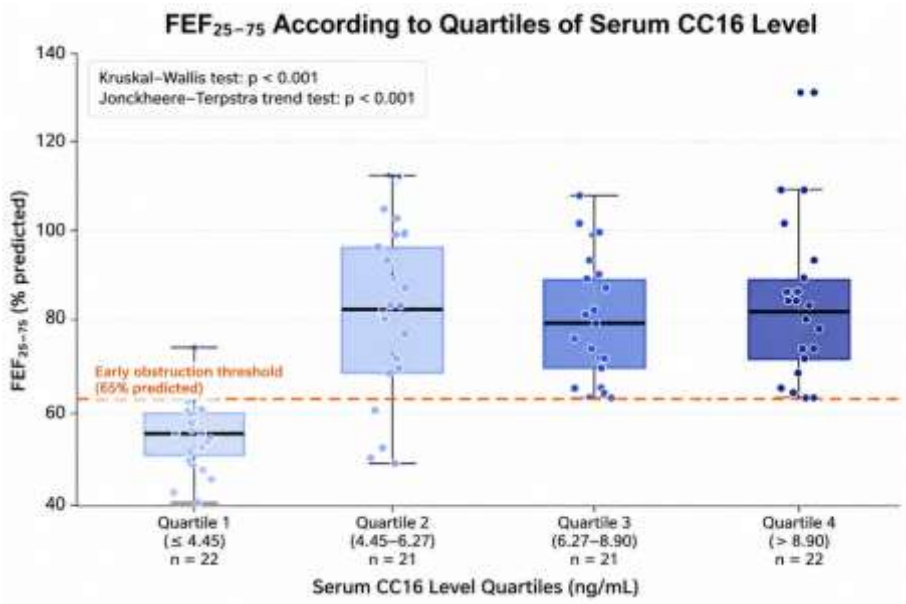

Figure 2. Distribution of $FEF_{25–75}$ According to Serum CC16 Quartiles

Table 3. Association of Serum CC16 Levels with Pulmonary Function Parameters: Group Comparisons and Spearman Correlations

Pulmonary function parameter	CC16, median (IQR), ng/mL	p-value	Spearman ϱ	p-value
FEV_1				
<80% predicted	5.92 (3.99–8.30)	0.024	0.335	0.002
≥80% predicted	6.63 (5.80–9.74)			
FVC				
<80% predicted	6.22 (4.10–8.36)	0.163	0.174	0.109
≥80% predicted	6.44 (5.81–9.64)			
FEV_1/FVC				
≥70%	6.27 (4.45–8.90)	—	0.410	<0.001

Group comparisons were performed using the Mann–Whitney U test, with the corresponding p-value indicating differences in serum CC16 levels between pulmonary function groups. Correlations between serum CC16 levels and pulmonary function parameters were assessed using Spearman's rank correlation, with the corresponding p-value indicating the statistical significance of the correlation. Categorical comparison for FEV_1/FVC was not performed because all participants had values ≥70%.

Table 4. Multivariable Linear Regression for $FEF_{25–75}$

Variable	B	95% CI	p-value	VIF
Serum CC16, ng/mL	2.621	1.49–3.75	<0.001	1.02
Age, years	−0.196	−0.65–0.26	0.395	1.70
Body mass index, kg/m²	−0.196	−0.99–0.60	0.625	1.01
Brinkman Index	0.001	−0.02–0.02	0.897	1.73

R^2=0.225; adjusted R^2=0.187; F(4,81)=5.895; p<0.001; n=86. Outcome: $FEF_{25–75}$ (% predicted). B, unstandardized regression coefficient; CI, confidence interval; VIF, variance inflation factor.

3.5 Discussion

This study demonstrates a clear positive association between serum CC16 concentrations and $FEF_{25–75}$ among asymptomatic active smokers, indicating that lower epithelial biomarker levels are accompanied by poorer small-airway expiratory flow even in the absence of respiratory symptoms. These findings are in line with Oh et al. (2018), who reported a significant positive correlation between serum CC16 and $FEF_{25–75}$ in patients with chronic inflammatory airway diseases. The consistency of the direction of association across clinically heterogeneous populations supports the biological relevance of CC16 to peripheral airway function rather than its association being restricted to established respiratory disease. Similarly, Wei et al. (2024) demonstrated that lower baseline CC16 concentrations were associated with subsequent small-airway dysfunction, further supporting the potential role of CC16 as an early indicator of airway injury [8].

The observation of impaired small-airway function in asymptomatic smokers is clinically relevant because structural and functional abnormalities of the distal airways may develop before overt airflow obstruction or respiratory symptoms. This concept is consistent with the "silent zone" of the lung, in which extensive loss or narrowing of small airways may occur while conventional spirometric indices remain relatively preserved [9]. Ronish et al. (2022) further demonstrated that $FEF_{25–75}$ provides information related to small-airway disease, emphysema, and hyperinflation beyond that captured by FEV_1 or FVC [10]. The present findings therefore suggest that the combination of a circulating epithelial biomarker and a small-airway flow parameter may provide complementary information regarding subclinical smoking-related airway injury.

The markedly lower CC16 concentrations among participants with reduced $FEF_{25–75}$ are biologically plausible because cigarette smoke directly injures the bronchiolar epithelium and alters club-cell integrity. Gribben et al. (2022) reported lower circulating CC16 concentrations among active smokers with chronic obstructive pulmonary disease and demonstrated a relationship between CC16 and smoking-related lung-function impairment [11]. Lam et al. (2018) similarly showed that reduced CC16 was associated with smoking exposure and impaired pulmonary function in community-based participants without substantial respiratory symptoms [12]. These observations support the interpretation that reduced circulating CC16 reflects epithelial injury associated with cigarette smoke exposure and may accompany early impairment of distal airway function.

The relationship between CC16 and $FEF_{25–75}$ may also reflect the biological functions of CC16 beyond its value as a passive marker of epithelial injury. CC16 has anti-inflammatory and antioxidant properties and participates in maintaining airway epithelial homeostasis. Berthiaume Fox et al. (2026) described evidence that CC16 modulates inflammatory signaling and oxidative stress in airway epithelial cells and macrophages, providing a mechanistic basis for its association with preservation of airway integrity [2]. Persistent cigarette-smoke exposure may reduce club-cell abundance and suppress CC16 expression, thereby weakening epithelial protection and facilitating inflammation, mucus-related obstruction, and remodeling of the distal airways. This mechanism provides a plausible explanation for the parallel reduction in CC16 and expiratory flow observed in asymptomatic smokers.

The present findings also extend previous observations by demonstrating that the association between CC16 and small-airway function is detectable in a relatively young, asymptomatic working population. Herath et al. (2025) described a comparable adult male smoking population, whereas Kwon et al. (2020) demonstrated that reduced $FEF_{25–75}$ can be present despite otherwise preserved spirometric findings [13,14]. Bhattarai et al. (2026) likewise reported lower $FEF_{25–75}$ among smokers with otherwise normal spirometry, supporting the concept that small-airway abnormalities may represent an early manifestation of smoking-related respiratory injury [15]. The detection of this pattern before the development of overt symptoms emphasizes the potential value of assessing distal airway function in smokers who are traditionally considered clinically healthy.

The association of CC16 with FEV_1 and FEV_1/FVC, but not with FVC, further supports its closer relationship with airflow characteristics than with static lung volume. These findings are consistent with Oh et al. (2018) [16], who observed associations between CC16 and both FEV_1 and FEV_1/FVC, and with Rojas-Quintero et al. (2026) [17], who reported that lower CC16 was associated with poorer FEV_1. Gribben et al. (2022) similarly found that CC16 was more closely related to airflow-related indices than to FVC [11]. This pattern is biologically coherent because CC16 primarily reflects the condition of the bronchiolar epithelium, whereas FEV_1 and FEV_1/FVC are strongly influenced by airway caliber and expiratory flow.

An important finding is that the association between CC16 and $FEF_{25–75}$ remained evident after adjustment for relevant demographic and smoking-related factors. This is consistent with Dy et al. (2025) [18], who demonstrated persistent associations between CC16 and lung function across multiple population cohorts after adjustment for major demographic and smoking-related variables. Rojas-Quintero et al. (2026) likewise reported an independent relationship between CC16 and pulmonary function after adjustment for smoking exposure and other relevant covariates [17]. Together, these findings suggest that the observed relationship is unlikely to be explained solely by differences in smoking intensity or demographic characteristics.

The distributional pattern across CC16 levels, in which the lowest biomarker group showed the greatest impairment in small-airway flow, may indicate a clinically relevant threshold effect rather than a strictly linear biological response. This interpretation is compatible with the concept that small-airway disease can remain functionally compensated until a substantial proportion of distal airways is affected. Li et al. (2026) described progressive bronchiolar narrowing, mucus plugging, and loss of terminal bronchioles as key features of early small-airway disease, with substantial structural injury occurring before major increases in total airway resistance become clinically apparent [19]. Thus, very low CC16 concentrations may identify individuals in whom epithelial injury has progressed sufficiently to coincide with measurable impairment of distal expiratory flow.

Overall, these findings support CC16 as a promising biomarker of early smoking-related small-airway injury in asymptomatic active smokers. Its association with $FEF_{25–75}$, together with relationships with airflow-related spirometric indices, suggests that CC16 may provide complementary biological information to conventional pulmonary function testing. Rather than serving as a stand-alone diagnostic marker, CC16 may be particularly useful as part of an early-risk assessment strategy aimed at identifying smokers with subclinical airway injury before clinically overt respiratory disease develops. The findings also reinforce the importance of smoking cessation at an early stage, when structural and functional abnormalities may still be potentially modifiable.

This study has several limitations. The relatively small sample was recruited from a single occupational setting and consisted exclusively of male participants, predominantly young adults, which may limit generalizability to women, older populations, and other occupational groups. The predominance of light smokers may also have reduced the ability to detect dose–response relationships between smoking exposure and the study outcomes. In addition, the absence of a non-smoking control group precluded direct comparison of CC16 concentrations between smokers and non-smokers. $FEF_{25–75}$ is also relatively sensitive to expiratory effort and therefore may have greater measurement variability than other spirometric parameters. Finally, because internationally standardized serum CC16 reference thresholds have not yet been established and absolute concentrations may vary according to assay reagents, direct comparison of CC16 concentrations across studies using different assay platforms should be interpreted cautiously.

4. CONCLUSION

Serum CC16 is associated with pulmonary function in asymptomatic active smokers, particularly with small-airway expiratory flow and airflow-related spirometric parameters. Lower CC16 concentrations are accompanied by impaired $FEF_{25–75}$ and reduced FEV_1, while FVC does not show a significant association and the FEV_1/FVC ratio remains preserved. These findings

indicate that epithelial injury reflected by reduced CC16 may be detectable alongside early functional abnormalities before the emergence of respiratory symptoms or overt airflow obstruction. The persistence of this association after adjustment for demographic characteristics, body mass index, and smoking exposure further supports the potential role of CC16 as a biomarker of subclinical smoking-related airway injury and early small-airway dysfunction.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the participants and the laboratory staff who assisted with specimen processing and spirometry measurements.

AUTHOR CONTRIBUTIONS

MIK: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft.

SN: Methodology, Supervision, Validation, Writing – review & editing.

EA: Methodology, Supervision, Validation, Writing – review & editing.

MI: Investigation, Resources, Data curation, Writing – review & editing.

ID: Supervision, Project administration, Validation, Writing – review & editing.

BN: Conceptualization, Supervision, Project administration, Writing – review & editing.

All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants received an explanation of the study objectives, procedures, potential benefits, and their rights as research participants before providing written informed consent. The study protocol received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (No. 1150/UN4.6.4.5.31/PP36/2025).

DATA AVAILABILITY STATEMENT

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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