SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2025; 42 (3): 16985 DOI: 10.36141/svdld.v42i3.16985

Diagnostic potential of serum ACE and CBC inflammatory markers in silicosis in the ceramic workers

Bilge Akgündüz
Occupational Diseases Clinic, Eskişehir City Hospital, Eskişehir, Turkey

Abstract. This study aims to determine whether serum sACE and CBC inflammatory markers can be used to diagnose silicosis and assess its severity. 231 subjects referred to Eskişehir City Hospital Occupational Diseases Polyclinic between January 2021 and January 2024 were examined. Groups classified into silicosis (n:131), non-silicosis (n:40), and control groups (n:40). sACE and CBC inflammatory markers were analyzed. ROC curve assessed. sACE levels were markedly elevated (85.19±36.06 U/L) in cases with higher profusion scores and disease categories compared to control and non-silicosis groups. sACE has a high diagnostic value for silicosis detection and severity assessment (AUC:0.810,p=0.001). Platelet/Lymphocyte Ratio (PLR) and Monocyte/Lymphocyte Ratio (MLR) were significant predictors of advanced silicosis with large opacities (AUC for PLR: 0.796, p=0.001; AUC for MLR: 0.726, p=0.004). Serum ACE and CBC inflammatory indices serve as valuable biomarkers for diagnosing silicosis and determining disease severity.

Key words: silicosis, angiotensin-converting enzyme, CBC, inflammatory biomarkers

Introduction

Silicosis is an occupational lung disease that causes variable degrees of lung tissue damage as a result of accumulating mineral dust (1). While thoracic computed tomography (CT) is commonly utilized in clinical diagnosis, and chest X-ray (CXR) radiography is often used in screening and compensation contexts, there is no global consensus on the preferred imaging modality for diagnosis and follow-up. To aid in the standardized interpretation of high-resolution CT (HRCT) scans in occupational and environmental lung diseases, the International Classification of HRCT for Occupational and Environmental Respiratory Diseases (ICOERD) was developed. Despite its utility in diagnostic assessment, ICOERD is not yet widely adopted for screening purposes (2). Moreover, there is still no method with both high specificity and sensitivity that does not involve radiation exposure. In recent years, the use of biological markers has gained importance in the scientific literature, particularly in the prevention of occupational diseases associated with mineral dust exposure, in order to evaluate exposure, determine early effects of exposure, and detect pathological changes before silicosis onset (3.4). Serum Osteopontin, Killer Lymphocyte-6, and Syndecan-4 are markers that can be utilized to diagnose coal worker pneumoconiosis (5). Furthermore, peripheral biomarkers could be useful in detecting early changes in workers exposed to silica (6). The lungs are the primary organs responsible for Angiotensin Converting Enzyme(ACE) production. It is known that ACE levels are elevated in both alveolar macrophages and endothelial cells in the pulmonary capillary system. It has been demonstrated that there is a link between alveolar macrophage activity and serum ACE (sACE) levels (7). It is thought that the deposition of mineral dust particles in the lung tissue and their cytotoxic effect on

Received: 17 March 2025
Accepted: 20 May 2025
Correspondence: Bilge Akgündüz,
Eskişehir City Hospital, Occupational Diseases Clinic, 71 Evler Mahallesi, Çavdarlar Sk., 26080 Odunpazarı, Eskisehir, Turkey
E-mail: bilgeuzmezoglu@hotmail.com

macrophages promote structural damage and fibrosis in pneumoconiosis (8-11). In recent years, Complete Blood Count (CBC) inflammatory measures have gained prominence as novel biomarkers that can be evaluated cheaply and easily. Neutrophils, lymphocytes, and monocytes are critical components of the innate immune response; lymphocytes and platelets play important roles in haemostasis, coagulation, and angiogenesis during the inflammatory response (12,13). The immune system's role in the development of silicosis is unclear. Although multiple studies show that the immune response plays a significant role in the pathophysiology of pneumoconiosis, the mechanisms that cause this association remain poorly understood (14,15). According to the understanding of the literature, relatively few studies have shown a link between CBC inflammatory markers and the severity of disease in silicosis (16). These investigations are critical for understanding how the immune response and inflammatory indicators affect pneumoconiosis progression (17). This study aimed to investigate the relationship between sACE levels, CBC inflammatory parameters, silicosis, and International Labour Office (ILO) CXRs pneumoconiosis categories in cases working in the ceramic industry; the purpose was to determine whether they are useful biomarkers in determining the disease and its severity.

Methods

Study population

The study was designed as a retrospective case-control study. The research population was created using data from Eskişehir City Hospital's electronic health record system. Between January 2021 and January 2024, 231 subjects who presented to the Occupational Diseases and Chest Disease outpatient clinic and had their sACE levels and CBC examined included in the study. 151 cases had a history of working in the ceramic industry and diagnosed with silicosis. 40 cases employed in the ceramic industry without silicosis. 40 had no history of working mineral dust exposure sectors. The inclusion criteria for this study required participants to be adults aged 18 years or older. Eligible cases had to have available serum ACE and CBC measurements as part of the study, with these tests being performed at the time of the patient's initial referral to the outpatient clinic, alongside the collection of their clinical and occupational history. Only subjects employed in the ceramic sector were included, ensuring that occupational exposure to ceramic dust was a key factor. Furthermore, to be included, Subjects must have had more than six months of exposure in the ceramic sector. Radiologically, cases had to have underwent a thoracic high-resolution computed tomography (HRCT) with Subjects diagnosed with silicosis being identified using a quality 1 or 2 chest radiograph, classified according to the ILO CXRs Pneumoconiosis Classification. The control group was composed of Subjects without a history of mineral dust exposure and who had a normal HRCT. The exclusion criteria encompassed Subjects with pre-existing systemic diseases, such as sarcoidosis, hypertension, malignancy, cardiovascular disease, chronic kidney disease, or liver disease, particularly those who were using ACE inhibitor treatment. Additionally, Subjects with other interstitial lung diseases, including respiratory bronchiolitis, hypersensitivity pneumonitis, sarcoidosis, idiopathic pulmonary fibrosis, nonspecific interstitial pneumonia, desquamative interstitial pneumonia, or lymphocytic interstitial pneumonia, were excluded from the study. Participants with metabolic or autoimmune conditions, such as uncontrolled diabetes mellitus or any connective tissue disorders, were also excluded. Evidence of active infections at the time of evaluation, as well as the current use of corticosteroids, immunosuppressive drugs, or other medications that might influence inflammatory markers, also disqualified Subjects. Lastly, Subjects who had a history of working in another dust-exposed industry for more than six months, which could confound the effects of ceramic dust exposure, were excluded from the study.

Definition of study groups

Silicosis Group: Subjects with silicosis who had worked in ceramic industry units with mineral dust exposure. HRCT evaluations were conducted at the Eskişehir City Hospital Chest Diseases and Respiratory Occupational Diseases Thoracic Radiology Council. Radiological, laboratory, or interventional procedures were performed as necessary. Silicosis diagnoses were confirmed after differentiation from other interstitial lung diseases (ILD) and classified using the ILO International Classification of CXRs Radiographs of Pneumoconiosis.

Non-Silicosis (NS) Group: Subjects with occupational mineral dust exposure but no evidence of silicosis on HRCT. This group included those with normal HRCT findings or unrelated findings with silicosis such as bronchiectasis, pleural thickening, pleural plaque, bulla or emphysema. The International Classification of High-Resolution Computed Tomography (HRCT) for Occupational and Environmental Respiratory Diseases (ICOERD) was used to identify pneumoconiosis. The ICOERD uses 4-point categories to quantify the grades of well-defined rounded opacities (RO), irregular and/or linear opacities (IR), emphysema (EM), ground glass opacities (GGO), and honeycombing (HC) parenchymal opacities (Items) in the upper, middle, and lower zones of each lungs. The summed grade is calculated for each of the Items of the parenchymal abnormalities (RO, IR, EM, GGO, and HC) by adding the scores of each of the 6 zones (2). During the Eskişehir City Hospital Chest Diseases and Respiratory Occupational Diseases Thoracic Radiology Council, the ICOERD criteria were applied. Control (C) Group: Subjects without a history of occupational mineral dust exposure and with normal HRCT findings.

Diagnosis was based on HRCT ICOERD classification findings, and subjects with a profusion score of 0/1 or higher according to the ILO CXRs pneumoconiosis classification were included in the silicosis group. The silicosis cases were further subdivided according to disease severity and compared with the non-silicosis and control groups.

Measurements

Demographic and clinical characteristics, including age, sex, comorbidities, occupation within the ceramic industry, duration of exposure, smoking status (pack-years), pulmonary function test parameters, CBC parameters, and sACE levels, were retrospectively analyzed. Case selection was based on the study objectives and hypotheses.

Classification of dusty departments and/or professions

Dust intensity in various departments or jobs of the ceramics industry was classified on a scale from 1 to 5. The classification was conducted through blind scoring by three independent experts: one occupational health and safety specialist engineer and two occupational health physicians, all with experience in the ceramics industry. In cases of score discrepancies, the researcher's scoring was approved based on the average score.

Dust intensity was rated from 1 (lowest) to 5 (highest). The classifications were as follows: Group 1 (Dust Intensity: 1–3): Ovens, maintenance and repair, packaging, warehouse, quality control, and forklift workers. Group 2 (Dust Intensity: 4): Glazing. Group 3 (Dust Intensity: 4): Press, forming, casting, and forklift workers. Group 4 (Dust Intensity: 5): Retouching. Group 5 (Dust Intensity: 5): Raw material processing, masse and glaze preparation, crushing and grinding, mold breaking, and forklift operators.

ILO International Classification of CXRs Pneumoconiosis Radiographs (18)

Digital radiography was used to obtain Chest X-rays. CXRs were assessed following the ILO International Classification of Pneumoconiosis Radiographs. An occupational disease specialist, certified as an ILO International Pneumoconiosis Radiography Classification reader and responsible for occupational disease notification, conducted the evaluations.

Profusion scores

The term 'profusion of small opacities' describes the density of small opacities in the affected zones of the lung. The classification of profusion is determined by comparison with reference standard radiographs. While written descriptions provide guidance, the standard radiographs are the definitive basis for assessment. The increasing density of small opacities is evaluated accordingly.

Categories: 0, 1, 2, 3,4. Subcategories 0/–; 0/0; 0/1; 1/0; 1/1; ½; 2/1; 2/2; 2/3; 3/2; 3/3; 3/+ Category 0: 0/1; Category 1: 1/0; 1/1; ½; Category 2: 2/1; 2/2; 2/3; Category: 3/2; 3/3; 3/+; Category 4; Large opacity

Size

The shape and size of small opacities are documented, with two types of shapes identified: rounded and irregular. Each shape is further classified into three size categories. For small rounded opacities,

the sizes are labeled as p, q, and r, based on their appearance in the corresponding standard radiographs. These are defined as follows: p = opacities with diameters up to approximately 1.5 mm; q = opacities with diameters between about 1.5 mm and 3 mm; r = opacities with diameters between about 3 mm and 10 mm. Similarly, the three size categories for small irregular opacities are labeled as s, t, and u, also defined by their appearance in the standard radiographs. These are specified as follows: s = opacities with widths up to about 1.5 mm; t = opacities with widths between approximately 1.5 mm and 3 mm; u = opacities with widths between about 3 mm and 10 mm.

Large opacities

A large opacity is defined as an opacity having the longest dimension exceeding 10 mm. Categories of large opacities are defined below. Category A: One large opacity having the longest dimension up to about 50 mm, or several large opacities with the sum of their longest dimensions not exceeding about 50 mm. Category B: One large opacity having the longest dimension exceeding 50 mm but not exceeding the equivalent area of the right upper zone, or several large opacities with the sum of their longest dimensions exceeding 50 mm but not exceeding the equivalent area of the right upper zone. Category C: One large opacity which exceeds the equivalent area of the right upper zone.

Laboratory analyses

Leukocyte subpopulations were analyzed using the automated XN-2000 hematology analyzer (Sysmex, Milton Keynes, UK). Complete Blood Count inflammatory markers were calculated. Neutrophil-to-lymphocyte ratio (NLR), Platelet-to-lymphocyte ratio (PLR), Monocyte-to-lymphocyte ratio (MLR), Systemic immune inflammation index (SII): (Neutrophil × Platelet) / Lymphocyte ratio, Systemic inflammatory response index (SIRI): (Neutrophil × Monocyte) / Lymphocyte ratio, Aggregate index of systemic inflammation (AISI): (Neutrophil × Monocyte × Platelet) / Lymphocyte ratio were calculated. Serum angiotensin-converting enzyme level: sACE levels were measured using the BA-200 analysis system (Biosystem, Spain), with a reference range of 2–52 IU/L.

Pulmonary Function Test (PFT)

Pulmonary function tests were conducted by certified technicians in accordance with the American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines (19). Measurements were obtained using the COSMED Quark PFT device (Italy), which meets ATS/ERS standards and provides high-precision respiratory assessments. Forced Vital Capacity (FVC, FVC%), Forced Expiratory Volume in 1 Second (FEV_1, FEV_1%), FEV_1/FVC (%) parameters were evaluated. Post-bronchodilator values were analyzed, and airway obstruction was defined as an FEV_1/FVC ratio of ≤70%. In the most recent year, diffusion capacity testing (DLCO) was integrated into routine clinical evaluation for patients with pneumoconiosis at our center. Prior to this, logistical challenges related to gas supply limited its consistent application. Consequently, DLCO measurements were not available for all study participants and were therefore not included in the main analysis. However, a subgroup of 53 patients with silicosis underwent complete diffusion testing, including DLCO, total lung capacity (TLC), and DLCO adjusted for alveolar volume (DLCO/VA). These patients were classified according to the International Labour Organization (ILO) chest X-ray profusion score.

Ethical approval

This study was conducted in accordance with the rules of the Declaration of Helsinki and ethical approval was received from Eskişehir City Hospital Scientific Research Ethics Committee with the decision number ESH/BAEK 2024/11 dated 14.03.2024. The cases included in the study had been previously diagnosed or evaluated for differential diagnosis. No specialized examination was conducted for this investigation.

Statistical analyses

Statistical Package for the Social Sciences for Windows 20.0 (SPSS Inc.; Chicago, IL, USA) program was used in the statistical analysis of the data. Frequencies and percentage values of categorical variables, and mean, median and standard deviation values of numerical variables were calculated. Whether the variables showed normal distribution was tested

with Skewness-Kurtosis/Shapiro-Wilk. For normally distributed numerical variables, t-test was applied in the presence of two groups, and ANOVA test was applied in the presence of more than two groups. Categorical variables were tested with the chi-square test. For variables with more than two groups that did not show normal distribution, the Kruskal-Wallis test, one of the non-parametric tests, was applied. Mann-Whitney U test was used to determine the difference within the group. $p < 0.05$ was considered significant.

In this study, all available silicosis cases (n = 151) referred to our occupational diseases clinic were included. However, identifying suitable healthy individuals without occupational exposure or radiological findings was challenging, as our clinic predominantly receives referrals for suspected occupational diseases. Therefore, the control group was limited to 40 individuals who met strict inclusion criteria and showed no signs of silicosis. To allow for balanced group sizes in ROC analysis—an approach known to provide more stable estimates of sensitivity, specificity, and cut-off values—a random sample of 40 silicosis cases was selected using SPSS randomization tools. ROC analyses were then performed between the control group and this randomly selected case subgroup to assess the diagnostic performance of inflammatory indices in detecting silicosis and its radiological features. ROC curves were generated by considering the Control group as the reference group in comparisons such as Control–Silicosis, Control–Non-Silicosis, Control–Profusion scores, and Control–Opacity size. The AUC values were calculated to determine the predictive accuracy, and the Youden Index was used to identify the optimal cut-off points.

Results

Demographic characteristics

A total of 231 participants were included in the study, of whom 151 had a silicosis profusion score of 0/1 or higher. A subset of 40 cases was randomly selected from this group using SPSS software (Figure 1). Based on the International ILO CXRs Pneumoconiosis Classification, 18 cases were categorized as Category 0, while 17 cases had large opacities (Category 4). The remaining cases were distributed as follows: 64 in Category 1, 42 in Category 2, and 10 in Category 3. The control and NS groups each consisted of 40 participants. The mean age of participants was 45.84 ± 8.86 years. The mean duration of occupational exposure was 19.06 ± 8.11 years in the NS group and 18.50 ± 8.89 years in the

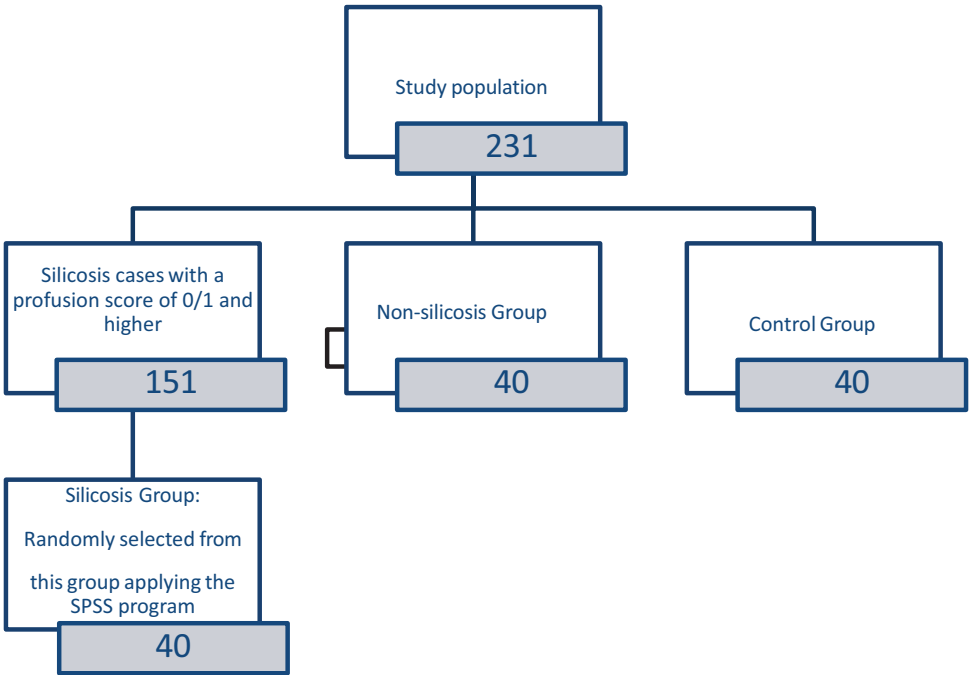

Figure 1. Study population classification.

silicosis group. Notably, patients with large opacities exhibited a significantly lower FEV1/FVC ratio (67.81 ± 16.08%) compared to other profusion categories and the control group (p = 0.012). No significant differences were observed in exposure duration or smoking habits among silicosis subgroups. Table 1 summarizes the demographic and clinical characteristics of the study population. A total of 53 patients diagnosed with silicosis underwent diffusion testing. The mean values in this subgroup were: DLCO 90.71 ± 19.59% predicted, TLC 95.84 ± 16.31% predicted, and DLCO/VA 93.13 ± 16.39% predicted. When categorized by ILO profusion scores, patients with a profusion score of 0/1 exhibited lower DLCO values compared to those with higher profusion scores. These findings suggest that diffusion testing alone may not adequately reflect the extent of radiographic abnormalities or functional impairment in silicosis (Appendix Table).

Serum ACE and CBC inflammatory parameters

No significant differences were observed in sACE levels or CBC-derived systemic inflammatory indices between the NS and control groups. However, when comparing the silicosis group with both the control and NS groups, sACE levels and inflammatory biomarkers were significantly elevated. These findings suggest that these biomarkers may aid in the diagnosis of silicosis and its differentiation from other pulmonary parenchymal diseases.

Importantly, sACE levels were significantly higher in all pneumoconiosis categories except Category 0, indicating that sACE may serve as a biomarker for disease detection beyond early stages. Additionally, PLR and MLR were identified as significant predictors of silicosis severity, particularly in distinguishing large opacity cases from other categories (Table 2).

When sACE levels were analyzed based on workplace departments, workers in high dust exposure environments exhibited significantly elevated levels. The highest serum ACE levels were observed among workers in glazing, pressing, forming, and casting units (Table 3).

ROC analysis results

ROC analysis confirmed that sACE is a reliable biomarker for silicosis diagnosis, with an optimal cut-off value of 40.50 U/L, yielding a sensitivity of 77.5% and specificity of 77.5% (AUC: 0.810, 95% CI: 0.711–0.911; p = 0.001). Notably, sACE demonstrated even higher diagnostic accuracy in assessing silicosis severity, particularly for large opacities, with a sensitivity of 88.2% and specificity of 87.5% (AUC: 0.958, 95% CI: 0.913–1.000; p = 0.001) (Table 4, Figure 2).

Additionally, PLR and MLR were identified as significant biomarkers for both silicosis diagnosis and severity assessment. Figure 3 illustrates the ROC curves for PLR and MLR, highlighting their role in categorizing silicosis severity, particularly in distinguishing large opacity cases from less severe forms of the disease.

Discussion

Silicosis, a chronic lung disease caused by prolonged exposure to silica dust, remains a significant occupational health concern, particularly in industries such as ceramics, mining, and construction. Early diagnosis and monitoring of disease progression are critical for preventing severe complications and improving long-term outcomes for affected workers. While chest radiographs are very useful for diagnosing pneumoconiosis, they are limited in their ability to detect early-stage disease (compered to CT scans) and require repeated radiation exposure. In this study, it was explored the potential of sACE levels and various CBC inflammatory markers as non-invasive, cost-effective biomarkers to diagnose silicosis and assess its severity.

Several biomarkers, including L-selectin, Krebs von den Lungen-6, TNF-alpha, surfactant protein D, and matrix metalloproteinase-2, have been explored as predictors of silicosis, but none have been adopted in clinical practice (20). A study on artificial stone workers found significantly elevated sACE levels in silicosis patients, suggesting its potential as a biomarker for disease progression (21). Consistent with previous studies, it was observed higher PMF rates in affected workers and increased serum ACE levels in foundry workers exposed to silica (22-24).

In this study, ceramic workers with silicosis had significantly elevated ACE levels. However, no difference was found between dust-exposed subjects with normal HRCT findings and those with silicosis, suggesting that sACE may reflect both silica exposure and disease severity. Elevated ACE levels likely

Table 1. Demographic characteristics, pulmonary function test, duration of exposure, and department at workplace

Parameters		Control	Non-silicosis	Silicosis*	Profusion Scores								Total**
					0/1	1/0	1/1	1/2	2/1	2/2 and 2/3	3/2 and 3/3+	Large opacity	
Subjects, n(%)		40(33.3)	40(33.3)	40(33.3)	18(7.8)	20(8.7)	28(12.1)	16(6.9)	21(9.1)	21(9.1)	10(4.3)	17(7.4)	231(100)
Age,year		42.30±12.16	44.05±7.21	51.48±9.07	43.06±5.72	43.50±5.42	43.07±5.97	47.88±7.91	48.38±7.91	50.71±6.21	51.40±7.31	54.35±5.72	45.84±8.86
Male/Female,n		39/1	38/2	40/0	18	20	28	16	21	21	10	17	228/3
Duration of exposure,year		16.03±7.97	19.06±8.11	18.50±8.89	12.72±7.30	16.40±7.32	16.63±7.40	17.34±9.98	20.36±7.43	20.60±6.05	22.30±3.72	17.68±6.72	17.69±7.76
Department at work,n(%)	Group 1	-	15(15.0)	7(5.8)	5(2.2)	6(17.1)	1(0.4)	1(0.4)	6(2.6)	0(0.0)	0(0.0)	1(0.4)	35(15.2)
	Group 2	-	5(4.2)	7(5.8)	1(0.4)	1(3.8)	2(0.9)	3(1.3)	2(0.9)	7(3.3)	2(0.9)	3(1.3)	26(11.3)
	Group 3	-	8(6.7)	12(10.0)	2(0.9)	1(2.1)	10(4.3)	4(1.7)	4(1.7)	7(3.3)	4(1.7)	8(3.5)	48(20.8)
	Group 4	-	9(7.5)	7(5.8)	6(2.6)	9(15.8)	11(4.8)	3(1.3)	5(2.2)	7(3.3)	4(1.7)	3(1.3)	57(24.7)
	Group 5	-	3(2.5)	7(5.8)	4(1.7)	3(12.0)	4(1.7)	5(2.2)	4(1.7)	0(0.0)	0(0.0)	2(0.9)	25(10.8)
Smoking packyear		12.20±10.29	18.10±13.43	22.21±12.20	15.64±9.94	14.93±10.82	15.29±11. 02	27.34±21.92	21.26±12.37	12.38±10.50	12.30±16. 95	13.85±12.04	16.11±13.13
Smoking habit,n(%)	Current smoker	23(19.2)	30(25.0)	21(17.5)	14(6.1)	13(5.6)	20(8.7)	11(4.8)	13(5.6)	13(5.6)	4(1.7)	4(1.7)	145(62.8)
	Ex-smoker	7(5.8)	4(3.3)	12(10.0)	1(0.4)	3(1.3)	5(2.2)	4(1.7)	6(5.6)	2(0.9)	2(0.9)	10(4.3)	44(19.0)
	Non-smoker	10(8.3)	6(5.0)	7(5.8)	3(1.3)	4(1.7)	3(1.3)	1(2.1)	2(0.9)	6(5.6)	4(1.7)	3(1.3)	42(18.2)
Pulmonary function test	FVC,L	4.15±0.93	4.13±0.90	4.21±1.04	4.76±0.88	5.43±1.10	4.48±1.03	4.22±0.86	4.64±0.82	4.27±0.98	4.11±1.03	4.05±1.21	4.40±1.02
	FVC%	91.38±16.71	94.06±16.12	97.40±23.26	105.61±17.89	115.20±19.91	99.36±20.52	97.31±20.68	105.52±22.09	102.76±21.37	99.40±27.08	94.53±27.55	99.29±20.88
	FEV1,L	3.36±0.83	3.23±0.85	3.25±1.05	3.70±0.79	4.35±1.05	3.59±0.95	3.38±0.81	3.73±0.62	3.42±0.90	3.20±0.82	2.80±1.32	3.47±0.95
	FEV1%	87.60±22.24	92.23±16.86	93.65±26.1	100.11±21.35	112.70±22.89	96.25±21.97	95.13±17.98	105.29±21.36	101.05±23.88	96.40±26.90	82.47±35.84	95.95±23.62
	FEV1/ FVC%	81.92±8.48	80.81±7.42	77.35±11.8	77.78±8.99	79.71±5.36	79.70±8.52	81.23±9.30	80.72±5.92	78.94±6.50	79.87±5.10	67.81±16.08	79.39±9.04
	Present FEV1/ FVC<%70, n	4(3.3)	6(5.0)	9(7.5)	4(22.2)	2(0.9)	6(2.6)	2(0.9)	2(0.9)	3(1.3)	1(0.4)	8(3.5)	38(16.5)

*:40 cases were randomly selected from the pneumoconiosis patient group(n:151) using SPSS, the Silicosis group was created. **: Total 231 cases with non-silicosis, silicosis, and control groups. Group 1: Ovens, maintenance and repair, packaging, warehouse, quality control, forklift operators in these units; Group 2: Glazing; Group 3: Presses, forming, casting and forklift operators in this unit; Group 4: Retouching; Group 5: Raw material, masse, glaze preparation, crushing and grinding, mold breaking and forklift operator in these units. FVC: Forced vital capacity; FEV1: Forced expiratory volume exhaled in the first second.

Table 2. Comparison of serum ACE levels and CBC inflammatory parameters by silicosis and profusion score category with control group

Risk Factor	Groups	N	X	Sum of Square	Rank average	df	Kruskall-Walls H		LSD
							X^2	p	
ACE, U/L	Category 0	18	45.92	37.17	102.81	6	66.12	0.001	2,3,4
	Category 1	64	51.08	29.65	123.22	6			2,3,4,NS,C
	Category 2	42	65.04	35.84	153.05	6			0,1,4,NS,C
	Category 3	10	57.07	29.39	142.20	6			0,4,NS,C
	Category 4	17	85.19	36.06	188.24	6			0,1,2,3,NS,C
	NS*	40	35.14	16.54	79.91	6			1,2,3,4
	C**	40	32.18	15.71	70.33	6			1,2,3,4
PLO	Category 0	18	109.09	34.26	107.56	6	16.76	0.010	3,4
	Category 1	64	110.98	35.27	111.55	6			4
	Category 2	42	111.20	30.44	114.65	6			4
	Category 3	10	147.39	96.18	133.10	6			0
	Category 4	17	175.05	93.14	176.35	6			0,1,2,NS,C
	NS*	40	116.43	51.75	111.11	6			C
	C**	40	106.14	31.24	103.30	6			4,NS
MLO	Category 0	18	0.24	0.09		6	14.09	0.029	3,4
	Category 1	64	0.25	0.09		6			4
	Category 2	42	0.24	0.09		6			4
	Category 3	10	0.35	0.24		6			0
	Category 4	17	0.42	0.33		6			0,1,2,NS,C
	NS*	40	0.25	0.07		6			4
	C**	40	0.27	0.09		6			4
SIRI	Category 0	18	1.17	0.79	101.56	6	14.07	0.029	4
	Category 1	64	1.34	1.04	113.89	6			
	Category 2	42	1.09	0.68	98.98	6			4,NS,C
	Category 3	10	1.34	0.75	100.10	6			
	Category 4	17	2.03	1.80	151.71	6			0,2
	NS*	40	1.36	0.71	126.15	6			2
	C**	40	1.45	0.75	122.40	6			2
AISI	Category 0	18	297.95	235.86	100.22	6	13.07	0.042	4
	Category 1	64	337.19	296.17	113.45	6			
	Category 2	42	258.39	170.30	93.45	6			4,NS,C
	Category 3	10	311.69	268.46	104.00	6			
	Category 4	17	412.81	247.51	146.65	6			0,2
	NS*	40	324.32	196.59	122.90	6			2
	C**	40	356.53	208.17	133.93	6			2
ACE/ Neutrophil	Category 0	18	12.01	14.32	106.47	6	58.22	0.001	2,3,4
	Category 1	64	11.74	7.89	121.78	6			2,4,NS,C
	Category 2	42	17.09	11.01	157.21	6			0,NS,C
	Category 3	10	18.55	16.68	140.70	6			NS,C
	Category 4	17	21.63	13.79	172.35	6			NS,C
	NS*	40	7.96	6.27	81.85	6			1,2,3
	C**	40	6.66	3.63	71.79	6			1,2,3,4

Risk Factor	Groups	N	X	Sum of Square	Rank average	df	Kruskall-Walls H		LSD
							X^2	p	
ACE/ Lymphocyte	Category 0	18	20.85	16.11	101.11	6	58.85	0.001	2,3,4
	Category 1	64	23.56	15.30	118.91	6			2,4,NS,C
	Category 2	42	32.58	23.18	144.24	6			0,4,NS,C
	Category 3	10	36.99	25.30	151.30	6			NS,C
	Category 4	17	74.94	67.80	195.12	6			NS,C
	NS*	40	17.16	1.42	90.85	6			1,2,3,4
	C**	40	13.93	6.27	71.10	6			1,2,3,4
ACE/Monocyte	Category 0	18	90.57	86.71	105.31	6	53.62	0.001	2,3,4
	Category 1	64	104.28	90.25	120.47	6			2,4,NS,C
	Category 2	42	139.96	101.52	154.35	6			0,C
	Category 3	10	113.73	68.30	135.00	6			C
	Category 4	17	202.01	178.78	174.41	6			C,NS
	NS*	40	77.87	78.87	90.28	6			1,4
	C**	40							

Abbreviations: *NS: Non-silicosis group; **C: Control group; LSD: Least Significant Difference; ACE: Angiotensin Converting Enzyme; PLR: Platelet/lymphocyte ratio; MLR: Monocyte/lymphocyte ratio; SIRI: Systemic inflammatory response index: (neutrophil count×monocyte count)/lymphocyte count; AISI: Systemic inflammation aggregate index: (neutrophil x monocyte x platelet)/lymphocyte ratio.

Table 3. Comparison of serum ACE levels by department at workplace

Risk Factor	Departments/ Units	N	X(Mean ACE, U/L)	S.S.	Rank average	df	Kruskall-Walls H		LSD Post-hoc Comparisons*
							X^2	P	
ACE,U/L, mean	NS	40	35.14	16.54	57.95	5	32.65	0.001	2,3,4,5
	Group 1	20	39.69	14.18	73.90	5			2,3,4,5
	Group 2	21	69.60	44.02	120.64	5			NS,1
	Group 3	40	59.43	28.49	110.64	5			NS,1
	Group 4	48	56.20	27.58	107.24	5			NS,1
	Group 5	22	68.71	52.75	110.61	5			NS,1

Group 1: Ovens, maintenance and repair, packaging, warehouse, quality control, forklift operators in these units; Group 2: Glazing; Group 3: Presses, forming, casting and forklift operators in this unit; Group 4: Retouching; Group 5: Raw material, masse, glaze preparation, crushing and grinding, mold breaking and forklift operator in these units. *Mann-Whitney U Test results.

stem from inflammatory processes, as mononuclear phagocytes and alveolar macrophages are primary sources (25-28). While Nordman et al. (24) found no correlation between ACE levels and pneumoconiosis severity, Yano et al. (29) reported a relationship with profusion scores. This study also found significantly higher sACE levels in workers with a profusion score of 0/1.

Workers with 0/1 profusion scores are classified as suspected cases but often remain exposed, lack insurance coverage, and are excluded from research (30). Thorax CT is the preferred diagnostic tool, yet biomarkers like sACE could enhance early detection. However, in this study, sACE was insufficient to identify 0/1 profusion cases, possibly due to small sample size. Still, its 70% sensitivity and specificity in detecting 1/0 profusion cases support its diagnostic utility.

Thoracic HRCT remains the most effective imaging tool for diagnosing pneumoconiosis (31-33).

Table 4. The sensitivity and specificity for serum ACE levels in silicosis and profusion scores compared with control group

Risk Factors		AUC (95%)	ACE Cut-off	P	Sensitivity (%)	Specificity (%)
Silicosis with control		0.810(0.711-0.911)	40.50	0.001	77.5	77.5
Silicosis with non-silicosis		0.782(0.677-0.887)	45.15	0.001	77.5	77.5
Profusion Scores	0/1	0.639(0.468-0.809)	35.00	0.093	72.2	70.0
	1/0	0.796(0.678-0.915)	36.00	0.001	70.0	70.0
	1/1	0.771(0.657-0.885)	34.95	0.001	71.4	70.0
	1/2	0.601(0.405-0.797)	31.50	0.242	56.3	57.5
	2/1	0.830(0.719-0.942)	40.50	0.001	76.2	77.5
	2/2 ve 2/3	0.898(0.817-0.979)	43.95	0.001	85.7	85.0
	3/2 ve 3/3+	0.800(0.616-0.984)	39.50	0.004	70.0	72.5
	Large opacity	0.958(0.913-1.000)	48.50	0.001	88.2	87.5
Non-silicosis with control		0.549(0.422-0.676)	30.50	0.450	55.0	52.5

However, a compensation-independent biomarker with high sensitivity and specificity is needed to aid in early detection, avoiding close radiation exposures. In this study, ACE levels were higher in workers in high-dust-intensity departments (e.g., retouching, pressing, raw material processing) than in lower-exposure areas (e.g., glazing, maintenance, packaging). Unlike prior studies, it was found no association between exposure duration and sACE levels, suggesting dust intensity may be a more critical factor (34).

Inflammatory markers like sedimentation rate, C-reactive protein, NLR, and PLR are used to assess systemic inflammation (35). PLR is prognostic in several diseases, including lung conditions (36-37). A case-control study of ceramic workers found significantly elevated NLR and PLR values in both early-stage and advanced pneumoconiosis (38). In this study, PLR and MLR were useful for early detection, and SII, SIRI, AISI were more sensitive and specific for PMF than simple silicosis.

Importantly, the study also highlighted that these biomarkers not only differentiate between pneumoconiosis and non-silicosis groups, but also provide valuable insight into the severity of the disease. Elevated levels of sACE, as well as inflammatory markers like PLR and MLR, were particularly notable in Subjects with more advanced silicosis (higher profusion scores), suggesting that these biomarkers could serve as reliable indicators of disease progression. The ROC analysis further supported the diagnostic potential of sACE, with a cut-off value of 40.50 U/L demonstrating promising sensitivity and specificity for silicosis diagnosis. These results suggest that sACE, along with CBC-based inflammatory markers, could be utilized as part of a multifaceted approach to monitoring workers exposed to mineral dust and assessing their risk of developing silicosis.

The strengths of this study include its large sample size, the use of multiple biomarkers for a comprehensive assessment of disease, and the inclusion of a control group with no exposure to mineral dust, which strengthens the validity of this study findings. Additionally, the study's inclusion of workers from various departments within the ceramic industry allowed for an evaluation of silicosis across different levels of dust exposure intensity, which is an important topic when studying occupational diseases.

Limitations

However, several limitations and bias must be acknowledged. First, the study was cross-sectional, which limits ability to establish causality or determine the long-term progression of disease. Longitudinal studies are needed to confirm whether these biomarkers can predict future disease development and progression. Second, the study focused solely on workers in the ceramic industry, which may limit the generalizability of the study findings to other industries with different types of dust exposure. This study's dust exposure classification was based on expert judgment due to the lack of standardized workplace measurements. While this approach provided a

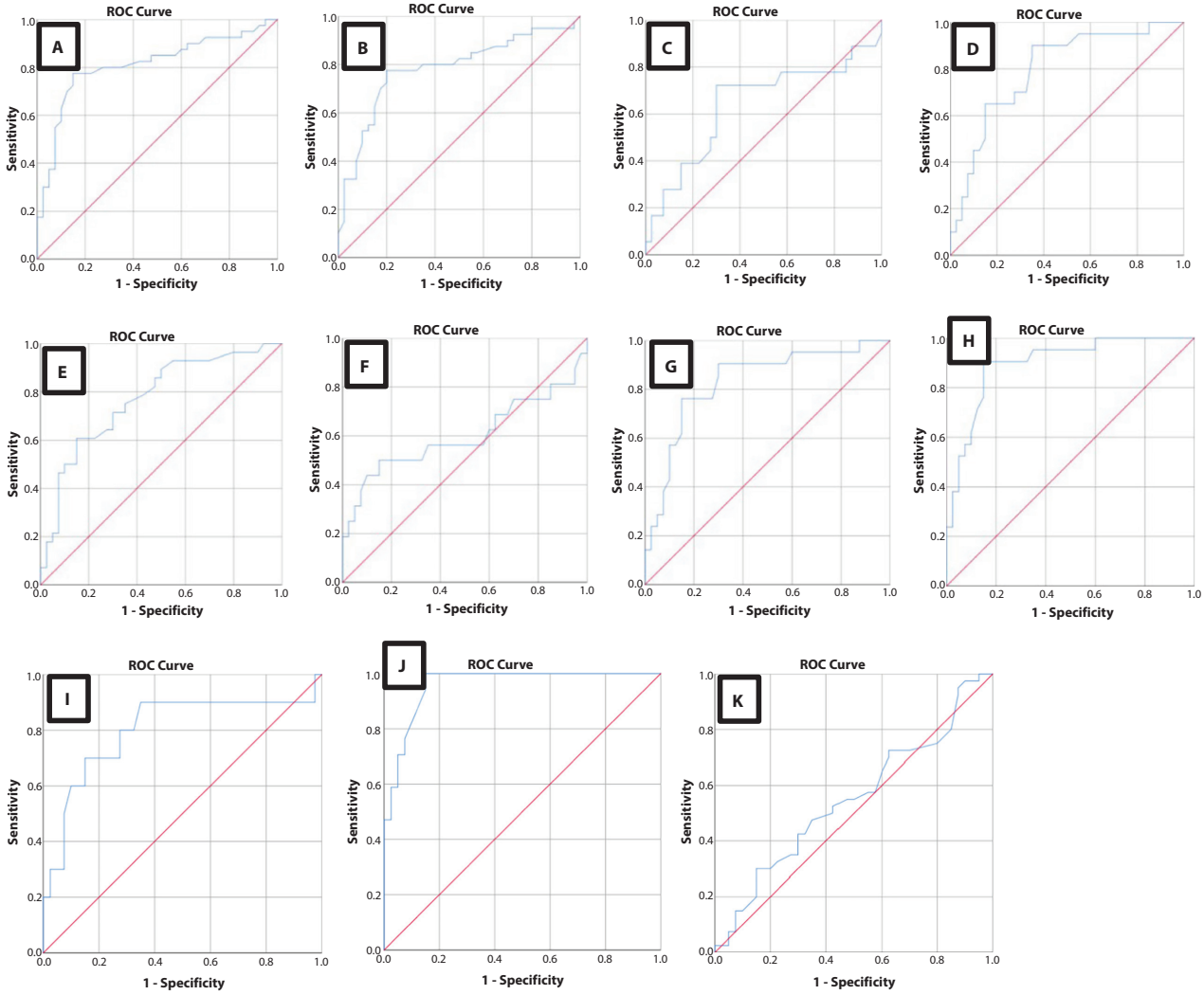

Figure 2. For serum ACE levels ROC curves. Graph A: Silicosis with control group; Graph B: Silicosis with non-silicosis group; Graph C: Profusion score 0/1 vs control group ; Graph D: Profusion score 1/0 vs control group; Graph E: Profusion score 1/1 vs control group; Graph F: Profusion score 1/2 vs control group; Graph G: Profusion score 2/1 vs control group; Graph H: Profusion score 2/2 and 2/3 vs control group; Graph I: Profusion score 3/2 and 3/3+ vs control group; Graph J: Category 4(Large Opacity) vs control group; Graph K: Non-silicosis vs control group.

practical estimation, it may introduce bias, especially given 1) variations in workplace controls and preventive measures, 2) and recall bias, which could result from relying on self-reported data for occupational exposure. Additionally, differences in factory size and layout—particularly in smaller enterprises without compartmentalization—could lead to cross-exposure among workers in different roles. These factors may affect the accuracy of exposure classification and should be considered when interpreting the results. Finally, while sACE and CBC-derived inflammatory markers showed promise in differentiating silicosis from other respiratory conditions, further research is necessary to validate these biomarkers in a broader patient population and in the context of other occupational diseases.

Conclusion

This study provides evidence that sACE and CBC inflammatory markers could serve as valuable, non-invasive biomarkers for diagnosing silicosis and

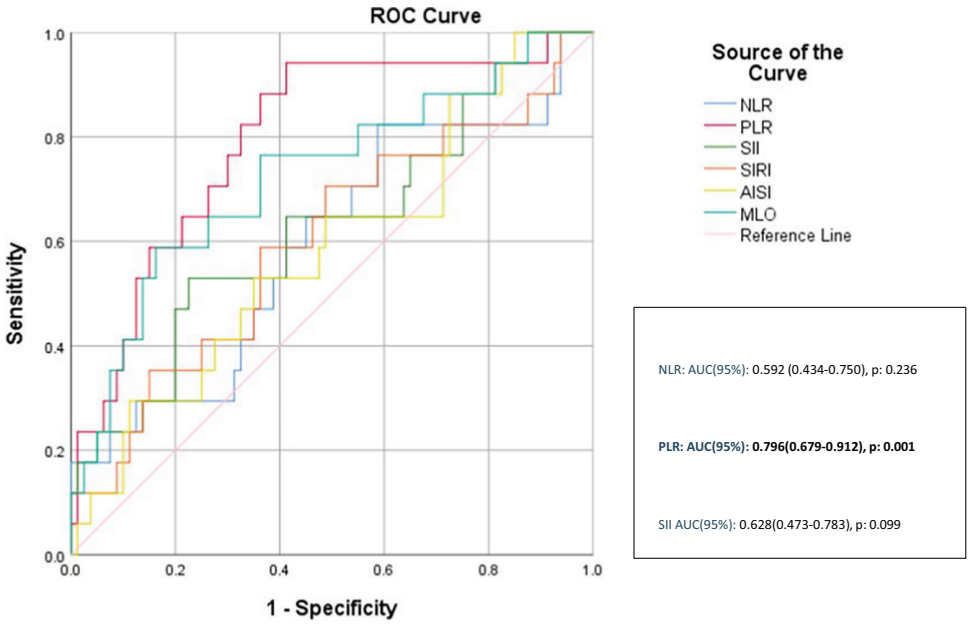

Figure 3. Comparison of sensitivity and specificity of CBC inflammatory parameters of large opacities with control group.

assessing its severity. These markers have the potential to improve early detection and monitoring of silicosis, reducing the need for repeated radiographic imaging and minimizing radiation exposure to workers. These biomarkers are relatively inexpensive, and their monitoring could therefore serve as an effective tool for secondary prevention by occupational physicians overseeing the health surveillance of workers exposed to silica. Further research, including longitudinal studies and larger cohort analyses, is necessary to confirm these findings and refine the use of these biomarkers in clinical practice. Future studies should aim to include also direct measurements/workplace data to improve exposure assessment. As the understanding of the immunological mechanisms behind silicosis evolves, monitoring these biomarkers at dusty workplaces could play a crucial role in preventing disease and improving the health outcomes of workers exposed to mineral dust.

Author Contributions: Substantial contribution to the conception and design of the work: BA. Acquisition, analysis, or interpretation of data for the work: BA. Drafting or revising the article critically for important intellectual content: BA. Agreement to be accountable for all aspects of the work, in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved: BA.

Funding: None.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article..

Ethical Approval: This study was conducted in accordance with the rules of the Declaration of Helsinki and ethical approval was received from Eskişehir City Hospital Scientific Research Ethics Committee with the decision number ESH/BAEK 2024/11 dated 14.03.2024.

References

1. Austin EK, James C, Tessier J. Early Detection Methods for Silicosis in Australia and Internationally: A Review of the Literature. Int J Environ Res Public Health. 2021;18(15):8123.
2. Tamura T, Suganuma N, Hering KG, et al. Relationships (I) of International Classification of High-resolution Computed Tomography for Occupational and Environmental Respiratory Diseases with the ILO International Classification of Radiographs of Pneumoconiosis for parenchymal abnormalities. Ind Health. 2015;53(3):260-70.
3. Borm PJ. Biological markers and occupational lung disease: mineral dust-induced respiratory disorders. Exp Lung Res. 1994;20(5):457-70.
4. Kunpeuk W, Julchoo S, Phaiyarom M, et al. A Scoping Review on Occupational Exposure of Silica and Asbestos among Industrial Workers in Thailand. OSIR. 2021;14(2) :41-51.
5. Hou Z, Zhang X, Gao Y, et al. Serum Osteopontin, KL-6, and Syndecan-4 as Potential Biomarkers in the Diagnosis of Coal

Workers' Pneumoconiosis: A Case-Control Study. Pharmgenomics Pers Med. 2023;16:537-49. doi: 10.2147/PGPM.S409644.
6. Peruzzi CP, Brucker N, Bubols G, et al. Occupational exposure to crystalline silica and peripheral biomarkers: An update. J Appl Toxicol. 2022;42(1):87-102.
7. Yasar Z, Özgül MA, Cetinkaya E, et al. Angiotensin-converting Enzyme as a Predictor of Extrathoracic Involvement of Sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2016 Jan 18;32(4):318-24.
8. Vanka KS, Shukla S, Gomez HM, et al. Understanding the pathogenesis of occupational coal and silica dust-associated lung disease. Eur Respir Rev. 2022;31(165):210250. doi: 10.1183/16000617.0250-2021.
9. Marrocco A, Ortiz LA. Role of metabolic reprogramming in pro-inflammatory cytokine secretion from LPS or silica-activated macrophages. Front Immunol. 2022;13:936167.
10. Yin H, Fang L, Wang L, et al. Acute Silica Exposure Triggers Pulmonary Inflammation Through Macrophage Pyroptosis: An Experimental Simulation. Front Immunol. 2022;13:874459.
11. Zhang Z, Zhang XR, Wang J. [Research progress on immune pathogenesis of pneumoconiosis]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2022;40(6):471-6.
12. Kovalchuk TA, Rubtsov RV. Diagnostic value of inflammatory biomarkers among mining and metallurgical workers with pneumoconiosis in combination with chronic obstructive pulmonary disease. Ukrainian journal of occupational health 2022;18(4).
13. Hu XX, Liu SP, Zhou RS, Hu MN, Wen J, Shen T. [Correlation analysis between blood routine-derived inflammatory markers and respiratory function in pneumoconiosis patients]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 2022;40(7):508-14.
14. Üzmezoğlu B, imşek C, Gülgösteren S, Gebeşoğlu B, Sarı G, Çelik D. Sarcoidosis in iron-steel industry: mini case series. Sarcoidosis Vasc Diffuse Lung Dis. 2017;34(4):36572.
15. Mayeux JM, Escalante GM, Christy JM, Pawar RD, Kono DH, Pollard KM. Silicosis and Silica-Induced Autoimmunity in the Diversity Outbred Mouse. Front Immunol. 2018;9:874.
16. Kang HY, Cao SY, Shao S, Liang LR, Tong ZH. The systemic immune-inflammation index is significantly associated with the severity of silicosis: a 9-year retrospective study in Beijing. Front Med (Lausanne). 2024;11:1351589.
17. Li ZG, Li BC, Li ZW, et al. The Potential Diagnostic Biomarkers for the IgG Subclass in Coal Workers' Pneumoconiosis. J Immunol Res. 2023;2023:9233386.
18. International Labour Organization. Guidelines for the use of the ILO International Classification of Radiographs of Pneumoconioses – Revised edition. ILO.2022. https://www.ilo.org/resource/ilo-international-classification-radiographs-pneumoconioses-1
19. Ponce MC, Sankari A, Sharma S. Pulmonary Function Tests. [Updated 2023 Aug 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482339/
20. Oț elea MR, Andrei N, Moise LG. The significance of the neutrophil to lymphocyte ratio in silicosis. J Contemp Clin Pract. 2018;4(2):53-9.
21. García-Núñez A, Jiménez-Gómez G, Hidalgo-Molina A, Córdoba-Doña JA, León-Jiménez A, Campos-Caro A. Inflammatory indices obtained from routine blood tests show an inflammatory state associated with disease progression in engineered stone silicosis patients. Sci Rep. 2022;12(1):8211.
22. Beshir S, Aziz H, Shaheen W, Eltahlawy E. Serum Levels of Copper, Ceruloplasmin and Angiotensin Converting Enzyme among Silicotic and Non-Silicotic Workers. Open Access Maced J Med Sci. 2015;3(3):467-73.
23. Marčetić D, Samaržija M, Vukić Dugac A, Knežević J. Angiotensin-Converting Enzyme 2 (ACE2) as a Potential Diagnostic and Prognostic Biomarker for Chronic Inflammatory Lung Diseases. Genes (Basel). 2021;12(7):1054.
24. Nordman H, Koskinen H, Fröseth B. Increased activity of serum angiotensin-converting enzyme in progressive silicosis. Chest. 1984;86(2):203-7. doi: 10.1378/chest.86.2.203.
25. Orfanos SE, Armaganidis A, Glynos C, et al. Pulmonary capillary endothelium-bound angiotensin-converting enzyme activity in humans. Circulation. 1999;99(12):1593-9.
26. Liu TT, Sun HF, Han YX, Zhan Y, Jiang JD. The role of inflammation in silicosis. Front Pharmacol. 2024;15:1362509.
27. Romano C, Sulotto F, Peruccio G, et al. Serum angiotensin-converting enzyme level in silicosis. Med Lav. 1985;76:366-70.
28. Romano C, Sulotto F, Peruccio G, Pavan I, Parola S. Pulmonary capillary endothelium-bound angiotensinconverting enzyme activity in acute lung injury. Circulation. 2000;102:2011–8.
29. Yano E, Takeuchi K, Sato M. Serum angiotensin converting enzyme activity in silicosis. Ind Health. 1987;25(1):11-8.
30. Tamura T, Suganuma N, Hering KG, et al. Relationships (I) of International Classification of High-resolution Computed Tomography for Occupational and Environmental Respiratory Diseases with the ILO International Classification of Radiographs of Pneumoconioses for parenchymal abnormalities. Ind Health. 2015;53(3):260-70.
31. Hayashi H, Ashizawa K, Takahashi M, Kato K, Arakawa H, Kishimoto T, et al. The diagnosis of early pneumoconiosis in dust-exposed workers: comparison of chest radiography and computed tomography. Acta Radiologica. 2022;63(7):909-13.
32. Satija B, Kumar S, Ojha UC, Gothi D. Spectrum of high-resolution computed tomography imaging in occupational lung disease. Indian J Radiol Imaging. 2013;23(4):287-96.
33. Takahashi M, Nitta N, Kishimoto T, Ohtsuka Y, Honda S, Ashizawa K. Computed tomography findings of arc-welders' pneumoconiosis: Comparison with silicosis. Eur J Radiol. 2018;107:98-104.
34. Hoy RF, Hansen J, Glass DC, Dimitriadis C, Hore-Lacy F, Sim MR. Serum angiotensin converting enzyme elevation in association with artificial stone silicosis. Respir Med. 2021;177:106289.
35. Jin Z, Cai G, Zhang P, et al. The value of the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio as complementary diagnostic tools in the diagnosis of rheumatoid arthritis: A multicenter retrospective study. J Clin Lab Anal. 2021;35(1):e23569.
36. Üçsular FD, Polat G, Kardeniz G, et al. Diagnostic value of platelet-to-lymphocyte and neutrophil-to-lymphocyte ratios in hypersensitivity pneumonia. Sarcoidosis, Vasculitis and Diffuse Lung Diseases. 2012; 38(2):164–9
37. Cai C, Zeng W, Wang H, Ren S. Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Monocyte-to-Lymphocyte Ratio (MLR) as Biomarkers in Diagnosis Evaluation of Acute Exacerbation of Chronic Obstructive Pulmonary Disease: A Retrospective, Observational Study. Int J Chron Obstruct Pulmon Dis. 2024;19:933-43.
38. Karataş M, Gündüzöz M, Öziş TN, Özakıncı OG, Ergün D. Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio as haematological indices of inflammatory response in ceramic workers' silicosis. Clin Respir J. 2019;13(3):159-65.

Appendix

Table S1. Distribution of DLCO, DLCO/VA, and TLC among 53 silicosis cases, categorized by ILO chest X-ray profusion scores and assessed via pulmonary diffusion testing.

		N	Mean	Std. Deviation
DLCO	Category 0	5	67.40	17.27
	Category 1	26	95.58	17.032
	Category 2	13	92.54	16.01
	Category 3	2	99.50	38.89
	PMF	7	83.43	22.45
	Total	53	90.71	19.59
DLCO/VA	Category 0	5	77.00	5.83
	Category 1	26	94.77	16.67
	Category 2	13	89.92	13.84
	Category 3	2	99.00	39.60
	PMF	7	102.86	11.50
	Total	53	93.13	16.39
TLC	Category 0	5	79.60	18.47
	Category 1	26	98.46	12.52
	Category 2	13	102.38	11.41
	Category 3	2	109.00	19.80
	PMF	7	81.86	21.71
	Total	53	95.84	16.31

Abbreviations: DLCO: The carbon monoxide diffusion lung capacity; DLCO/VA: Diffusing capacity of the lung for carbon monoxide divided by alveolar volume; TLC: Total lung capacity; PMF: Progressive Massive Fibrosis.