Pathology and Mineralogy Demonstrate Respirable Crystalline Silica is a Major Cause of Severe Pneumoconiosis in US Coal Miners

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Abstract

Rationale: The reasons for resurgent coal workers' pneumoconiosis and its most severe forms, rapidly progressive pneumoconiosis and progressive massive fibrosis (PMF), in the United States (US) are not yet fully understood.

Objective: To compare the pathologic and mineralogic features of contemporary coal miners suffering severe pneumoconiosis to their historical counterparts.

Methods: Lung pathology specimens from 85 coal miners with PMF were included for evaluation and analysis. We compared the proportion of cases with pathologic and mineralogic findings in miners born between 1910 and 1930 (historical) to those born in or after 1930 (contemporary).

Results: We found a significantly higher proportion of silica-type PMF (57% vs. 18%, $p<0.001$) among contemporary miners compared to their historical counterparts. Mineral dust alveolar proteinosis (MDAP) was also more common in contemporary miners compared to their historical counterparts (70% vs. 37%, $p<0.01$). *In situ* mineralogic analysis showed the percentage (26.1% vs. 17.8%, $p<0.01$) and concentration (47.3×10^8 vs. 25.8×10^8 particles/cm^3, $p=0.036$) of silica particles was significantly greater in specimens from contemporary miners compared to their historical counterparts**.** The concentration of silica particles was significantly greater when silica-type PMF, MDAP, silicotic nodules, or immature silicotic nodules were present ($p<0.05$).

Conclusions: Exposure to respirable crystalline silica appears causal in the unexpected surge in severe disease in contemporary miners. Our findings underscore the importance of controlling

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workplace silica exposure in order to prevent the disabling and untreatable adverse health effects afflicting US coal miners.

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The twenty-first century has seen increases in pneumoconiosis globally.(1) In the United States, the prevalence of coal workers' pneumoconiosis (CWP) and its most severe forms, rapidly progressive pneumoconiosis (RPP) (2) and progressive massive fibrosis (PMF), (note: see the Online Supplement for definitions of these terms), has more than doubled since the late twentieth century, from five to more than 10 percent for simple CWP.(3, 4) Cases have been concentrated in the central Appalachian states of Kentucky, Virginia, and West Virginia, where the prevalence of PMF has increased from 0.33% to 3.2% in miners with 25 years or more of tenure.(2, 4–8) While the prevalence of disease declined after the institution of modern dust controls in the 1970s, (9) this trend unexpectedly reversed. This affected active working miners participating in the Coal Workers' Health Surveillance Program where the prevalence of central Appalachian miners with PMF increased tenfold among longer tenured miners, (4, 10) and by 14-fold in former miners applying for Federal Black Lung Program Benefits. (6) Large series of PMF cases have also been reported from individual clinics. (5, 7)

Several lines of evidence point to excessive crystalline silica exposure as the main driving force behind this resurgent epidemic.(11, 12) Improvements in mining equipment and processing technology have enabled profitable recovery of thin coal seams, which involves extraction of large quantities of surrounding rock strata that can contain crystalline silica. In some mines, the rock strata accounts for more than 50% of the total mining height, but it can generate nearly twice as much respirable dust as compared to the coal seam itself.(13, 14) An analysis of Federal dust monitoring data from 1982-2017 showed that respirable quartz mass percent in mines in central Appalachia has been consistently and significantly higher than in most other regions i.e., mean quartz 6.724% in central Appalachia versus 3.886% in other

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regions; and until 2009 more than 15% of all quartz samples in central Appalachia exceeded the permissible exposure limit of 5%. (14, 15) Chest imaging data from medical surveillance of active miners indicated increased prevalence of lesions associated with silicosis in recent years. (11, 16, 17) A case series analyzing the pathologic features of 13 miners with RPP/PMF also provided early evidence supporting this hypothesis, showing accelerated silicosis and mixed dust pneumoconiosis that implicated excessive exposure to respirable silica and silicates. (12) Pathologic features of silicosis were significantly associated with round opacities on chest imaging. Interestingly, pathologic features of focal alveolar proteinosis and interstitial inflammation associated with silicotic nodules pointed toward a more aggressive process than classic coal workers' pneumoconiosis.

We compared miners born between 1910 and 1930 ("historical miners") to those born in or after 1930 ("contemporary miners"). Historical miners worked mainly with conventional mining technology that relied on drilling and blasting, whereas contemporary miners spent at least a substantial portion of their mining tenure working with mechanized equipment which employ high powered cutting heads to shear the coal from the mine face. (18, 19) To date, no study has compared the pathologic and mineralogic features of contemporary miners suffering from this resurgent form of pneumoconiosis to historical counterparts to determine if silica is indeed an important culprit. To address this uncertainty, we used brightfield and scanning electron microscopy with energy dispersive x-ray spectroscopy (SEM/EDS) to analyze lung tissue specimens from materials archived as part of the National Institute for Occupational Safety and Health's (NIOSH) National Coal Workers' Autopsy study (NCWAS)(20) as well as pathologic specimens from contemporary miners with PMF.

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Methods

Study Population and Procedures

Contemporary coal miners attend Black Lung Clinics or approach physicians and attorneys for assistance with medical examinations for the presence and severity of CWP. Records of 1,129 coal miners seeking Black Lung Benefits between 2016 and 2019 were reviewed to identify those with PMF and available lung pathology specimens from biopsy, resection, explantation, or autopsy. NCWAS samples were obtained between 1971-2013. During that time period the program offered families of all coal miners the opportunity to have an autopsy performed to determine the presence and severity of coal workers' pneumoconiosis for use in black lung benefits claims. The NCWAS dataset of 7,762 miners was reviewed to identify cases initially classified as PMF by NIOSH contracted pathologists. Cases were included if they were born after 1910, had $\geq$10 years of underground or surface coal mining, and the archived specimen was adequate for analysis as determined by slide review (FG, AH, and MO). Historical cases were further selected based on the state where they mined to match the geographic distribution of contemporary cases (Figure 1). Non-NCWAS miners met these same inclusion criteria and completed a standardized questionnaire eliciting demographic, smoking, and occupational histories, similar to the one completed by the NCWAS miners' next of kin.

Miners were classified as historical if they were born between 1910 and 1930 and contemporary for those born in or after 1930. The study was approved by the University of Illinois Chicago institutional review board (protocol #2016-0767). Written informed consent was obtained for cases not accessioned through NCWAS.

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Pathology

Brightfield images from hematoxylin and eosin (H&E)-stained lung slides were digitally acquired at 40x magnification using Aperio XT® (Software ImageScope version 8.2; Leica Biosystems, Buffalo Grove, IL). Images were classified and scored by pathologists using a standardized scoring system. Pathologists were blinded to all details of case histories other than prior employment as coal miners and that these miners were thought to have PMF. Three pathologists (JLA, SS, and CC) evaluated and scored specimens individually; two pairs of pathologists (FHYG/AF and JM/NV) evaluated specimens together and submitted joint findings. This approach yielded five separate classifications for all cases.

PMF was defined as a dust-related fibrotic lesion measuring greater than one centimeter in longest dimension with irregular or whorled collagen fibers, with or without necrotic areas, and presence of dust consistent with coal mine dust. (21)

To address the question of the role of silica, we developed a classification system characterizing three types of PMF lesions based on the proportion of silicotic nodules in PMF lesions by area in the image(s) reviewed. These were: a “coal”-type of PMF defined as having ≤25% silicotic nodules; a “silica”-type PMF defined as having >75% silicotic nodules; and a “mixed”-type of PMF having >25% and ≤75% silicotic nodules (Figure 2).

Pathologists scored the presence or absence of coal macules, coal nodules, and silicotic nodules when sufficient lung parenchyma surrounding the PMF lesion was present.(12) In addition, pathologists noted the presence or absence of immature silicotic nodules (Figure 3), and mineral dust-related alveolar proteinosis (MDAP), a marker of acute silicosis (Figure 4). Discordant classifications, defined as disagreement on the presence or absence of a finding or

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on the type of PMF, were resolved by consensus conference involving all study pathologists. Previous work demonstrated a substantial level of agreement (kappa=0.62) on type of PMF between the study pathologists in a larger sample of NCWAS cases.(22)

Mineralogy

SEM/EDS was used to characterize size, type and concentration of mineral particles *in situ* using modifications of published morphometric point-counting methods. (23–25) (See Online Supplement for additional detail.)

Statistical Analysis

We used SAS (version 9.4; SAS Institute, Cary, NC) for all analyses. Categorical variables were compared between historical and contemporary groups using Fisher's exact test. Continuous variables were examined across historical and contemporary status as well as PMF type using t-tests with pooled or Satterthwaite results as appropriate. ANOVA with Tukey's pairwise comparison were used to compare mean differences in continuous variables across multiple groups. Levene's test was used to assess homoscedasticity; in cases of unequal distribution we used the Welch's test for ANOVA testing. A p-value ≤ 0.05 was considered significant. Bonferroni corrected p-values were used for multiple comparisons.

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Results

Demographics

Lung tissue from 85 miners was analyzed. We obtained tissue on 16 miners born in or after 1930 who were diagnosed as PMF by their clinical providers. Review by study pathologists confirmed PMF in nine of these cases, the remaining seven cases were excluded from further study since the available specimens did not meet criteria for PMF. An additional 14 contemporary cases of PMF were identified from the NCWAS archive. Sixty-two historical comparison cases were also selected from the NCWAS archive (Figure 1). Contemporary miners were significantly younger at the time their lung tissue was obtained (61 vs 65 years old, p=0.03) and had significantly fewer years of underground mining (30 vs 35 years, p=0.03) as well as a trend toward fewer total years mining (31 vs. 36, p=0.14). There was no difference between groups for work in the central Appalachian states of Kentucky, Virginia, and West Virginia; race; or smoking status and total pack-years (Table 1).

Brightfield microscopy

We found a significantly higher proportion of silica-type PMF (57% vs. 18%, $p < 0.01$) among contemporary miners compared to historical counterparts. In contrast, coal miners born before 1930 had a significantly higher proportion of both coal-type PMF (50% vs. 17%, $p < 0.01$) and mixed-type PMF (33% vs. 26%, $p<0.01$) (Table 2).

Specimens had varying amounts of lung parenchymal tissue surrounding PMF lesions. Five specimens had so little non-PMF parenchyma that neither coal macules and nodules nor

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mature and immature silicotic nodules could be evaluated. Despite differences in the quantity of lung parenchymal tissue surrounding PMF lesions, we found a trend towards an increased proportion of both mature silicotic nodules ($p = 0.17$) and immature silicotic nodules ($p = 0.11$) in contemporary miners. Compared to contemporary miners, miners born before 1930 had a significantly higher proportion of coal macules (93% vs 60%, $p < 0.01$), with a trend towards increased coal nodules in surrounding lung parenchyma (78% vs. 58%, $p = 0.08$) (Table 2).

Findings consistent with MDAP were also more common in contemporary miners compared to historical counterparts (70% vs. 37%, $p < 0.01$) (Table 2). To confirm these findings, 10 specimens with MDAP lesions identified on H&E stains were stained with Periodic Acid-Schiff-Diastase (PAS-D), and all were positive confirming the presence of lipoproteins (Figure 4). MDAP in these specimens was confined to focal involvement of alveolar spaces adjacent to the PMF lesions.

Analysis by mining region showed that the increased proportion of silica-type PMF, MDAP, and the trends towards increased profusion of mature and immature silicotic nodules were largely due to cases of miners who worked in the central Appalachian states of Virginia, West Virginia, and Kentucky compared to those who worked outside of central Appalachia. Similar differences between historical and contemporary miners were also seen outside of central Appalachia, however the numbers were small and were not statistically significant (Table 2).

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Mineralogy

Silica. In situ mineralogic analysis was performed on lung tissues from 17/23 (74%) contemporary miners and 33/62 (53%) historical comparisons. The total concentration of mineral particles in lung specimens did not differ significantly between contemporary and historical miners, (180 x 10^8 vs. 149 X 10^8 particles/cm^3, Table 3A). Most notably, the percentage (26.1% vs. 17.8%, p<0.01) and concentration (47.3 x 10^8 vs. 25.8 X 10^8 particles/cm^3, p=0.036) of silica particles was significantly greater in specimens from contemporary miners compared to their historical counterparts**.**

The concentration of silica particles was more than 50% greater when pathologic features associated with silica exposure were present in the sections analyzed, including MDAP, mature silicotic nodules and immature silicotic nodules (p<0.05, Table 3B). One-way analysis of variance showed nearly double the percentage of silica particles in silica-type PMF compared to mixed- or coal-type PMF (29.6% vs. 16.9% and 16.0%, respectively, p<0.01, Table 3C). Also, the concentration of silica particles appeared to be 70% higher in silica-type PMF compared to mixed or coal-type PMF (42.4 X 10^8 vs. 27.2 x 10^8 and 29.6 X 10^8 particles/cm^3, respectively, p=0.28, Table 3C), however this was not statistically significant in this small sample with high variability.

Other particles. There was a lower percentage of aluminum silicate (SiAl and SiAlK) particles in contemporary miners' lungs compared to historical miners (66.2% vs. 74.6%, p<0.01, Table 3A). The presence of MDAP or mature silicotic nodules was associated with significantly lower percentages, but not lower concentrations, of aluminum silicates compared to silica particles (Table 3B). The percentage of aluminum silicates in miners with silica-type PMF was

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significantly reduced compared to miners with coal- and mixed-type PMF ($p<0.01$, Table 3C). While the concentration of aluminum silicate particles was also reduced in silica-type PMF compared to coal- and mixed-type PMF, it was not statistically significant ($p=0.07$, Table 3C). There were no other significant differences noted for other particles including titanium (Ti) or less commonly found metals.

Discussion

This is the first study of its kind comparing the pathology and *in situ* mineralogy of contemporary miners with PMF to their historical counterparts. Most prior studies of PMF have relied on chest imaging showing pneumoconiotic lesions > 1cm to diagnose PMF and therefore lack the ability to confirm the diagnosis with an evaluation of tissue responses to mineral dust. We used an innovative multi-method approach to investigate possible contributors to the increase in proportion and severity of pneumoconiosis in US coal miners. Prior to this study, the evidence pointing to respirable crystalline silica was indirect and relied on nonspecific clinical variables such as the prevalence of r-type opacities on chest imaging, (11, 16) which have been associated with silicosis.(26) Our prior case series of lung pathology in miners with RPP implicated silica and silicates, however there was no comparison group.(12) This study compares contemporary miners with historical materials and shows significant differences between historical periods.

Our data clearly shows an increased proportion of pathologic features consistent with substantial exposure to respirable crystalline silica in contemporary miners. These pathologic

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findings have been associated with acute and subacute silicosis.(27) They include the presence of foci of MDAP in association with PMF, a finding that has heretofore received little attention in the published literature.(12, 28) We also developed a classification of PMF lesions into silica-, mixed-, and coal-type PMF that proved to be a useful method for characterizing lesions to better understand causal exposures. The increased proportion of mature and immature silicotic nodules seen in lung tissue adjacent to PMF lesions also points strongly toward a silica-driven etiology of disease among younger contemporary miners with significantly fewer years of mining tenure.

Our findings were further informed by results of *in situ* analysis of mineral particles in a subset of these miners. *In situ* findings showed a significant increase in the percentage and concentration of silica particles in PMF lesions in lungs of contemporary coal miners compared to historical miners. There was a corresponding decrease in the percentage of aluminum silicate particles relative to silica particles in contemporary miners. This may reflect changes in geologic and/or mining conditions, including differences in silica content or dust generation from rock strata within or surrounding contemporary coal mining seams. Of note, we found a significant correlation between the concentration and percentage of silica particles and the presence of lung pathologic lesions including PMF, MDAP, and both mature and immature silicotic nodules.

The mineralogy findings in these cases demonstrate some of the highest concentrations of silica particles reported with this *in situ* method.(24) For comparison, the total concentration of inorganic particles in the lungs of persons with no known dust exposures is in the range of $0.1 – 0.2 \times 10^8$ total particles/cm^3 tissue. The concentration of silica particles in PMF lesions reported in sandblasters was 1000 times higher — up to 146×10^8 silica particles/cm^3 tissue.

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(29, 30) Thus, not only do our reported findings confirm the role of exceedingly high silica particles in the development of PMF, but they also provide evidence of the intense exposure to silica experienced by these coal miners, nearly one-third of the concentration seen in sandblasters.(29)

The finding of a higher ratio of silicate to silica particles in the lungs of historical coal miners compared to contemporary miners with PMF may add to our understanding of the pathogenesis of PMF and RPP. Several studies have shown that surface coating of silica particles by silicate (clay) minerals is able to suppress silica toxicity. (31, 32) The mechanism is thought to involve integration of cations of aluminum, magnesium or iron into the surface of the silica (quartz) particle. The coating effectively renders the silica particle into a silicate one associated with reduced toxicity.(33–35) In addition to significant increases in exposure to respirable crystalline silica, it is possible that relative depletion of silicate minerals may also be a contributing factor to disease. In addition to surface occlusion, the toxicity of crystalline silica is dependent on other important factors, including particle size,(36) presence of highly reactive silicon free radicals,(37, 38) and formation of 'nearly free' surface silanols when freshly fractured.(39) Fresh fracturing upsets the long-range ordering of silica's crystal lattice and imparts surface disorder. Future studies could help to determine, which if any, of these factors are associated with the recent upsurge in RPP type PMF as there are potential engineering solutions to mitigate some of these effects. (39)

We divided our subjects based on birth year before and after 1930 in order to segregate miners likely to have worked mainly with historical mining methods from those who worked mainly with modern methods. Mechanized coal extraction devices such as continuous miner

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machines, longwall shears, and other advanced engineering technologies were introduced in the US in the 1950's, the age when a miner born in or after 1930 would have begun their career. These more efficient coal cutting devices,(18, 19) along with improved and more cost-effective methods for separating silica-rich overburden rock from the coal being mined, may be driving increased exposure to respirable crystalline silica(13, 14) and therefore account for the later surge in severe forms of CWP in contemporary miners. Historically, silica has been known to be an important contributor to pneumoconiosis in coal miners. In the early to mid-twentieth century, coal dust was thought to be benign, and coal workers pneumoconiosis could not be diagnosed unless exposure to silica was proven.(40) One study from the UK showed excessive exposure to silica in a group of 21 miners with rapidly progressive CWP, although they did not have pathologic evidence to support this.(41) The concept of rapidly progressive disease and the role of silica in this disease received less attention until the current resurgence of disease in central Appalachia was identified beginning in 2005.(2)

Our study has several strengths. One is the independent blinded review of pathologic materials by seven pathologists with discrepancies addressed through rigorous consensus determination. Study pathologists had no access to clinical and historical information associated with the subjects, therefore minimizing information bias in interpretations. The same was true of the *in situ* mineralogic analyses. Other strengths include the unprecedented number of specimens available for analysis, supplemented by relevant demographic and occupational history data that enabled comparison of contemporary to historical miners. We also developed a standardized scoring system for brightfield microscopy findings. This system assured detailed attention to previously overlooked findings such as immature silicotic nodules

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and MDAP. We utilized digital microscopy platforms, which enabled scoring and consensus review of materials by pathologists separated by long distances. We also used two different microscopy methods to analyze materials and compare findings.

Our study also had several limitations. One limitation was the relatively small (n=23) number of contemporary miner specimens. This is likely related to the small number of miners with advanced disease who underwent surgical lung biopsies, resections, transplants or autopsies. Despite these small numbers, we were able to show significant changes in the pathology and mineralogy of PMF over time. Our small sample size may also reflect participation bias. Subjects who participated in NCWAS, the source of our historical cases, were likely to have had less severe disease and therefore needed the additional pathologic evidence for their claims compared to non-participating miners who may have had stronger claims based on imaging and physiology alone. However, this would not likely have affected our results since our focus was on comparing the most severe disease, PMF, in contemporary miners with historical counterparts. Also, there were limitations in clinical and questionnaire data available for NCWAS specimens such as chest imaging results and details on mining job duties. This is unlikely to have affected our findings since we had the most relevant demographic and work history information including age, smoking status, mining tenure, and geographic location, and pathologic findings that supersede chest imaging for the diagnosis of PMF. The SEM/EDS analysis was constrained to analyzing inorganic particles greater than 0.2 μm in diameter, precluding sizing and counting of smaller particles as well as coal dust particles that may have important roles in disease pathogenesis. Finally, the SEM/EDS analysis provides information about elemental content but not about crystalline structure.

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Conclusions

Based on integrated pathologic and mineralogic findings in lung tissues from two well-defined coal miner case series with progressive massive fibrosis, our study demonstrates that exposure to crystalline silica appears causal in the unexpected surge in severe disease in contemporary miners. Our findings underscore the importance of controlling workplace silica exposure in order to prevent the disabling and untreatable adverse health effects afflicting US coal miners. (42)

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References

1. Shi P, Xing X, Xi S, Jing H, Yuan J, Fu Z, Zhao H. Trends in global, regional and national incidence of pneumoconiosis caused by different aetiologies: an analysis from the Global Burden of Disease Study 2017. *Occup Environ Med* 2020;77:407–414.
2. Antao VC dos S, Petsonk EL, Sokolow LZ, Wolfe AL, Pinheiro GA, Hale JM, Attfield MD. Rapidly progressive coal workers' pneumoconiosis in the United States: geographic clustering and other factors. *Occup Environ Med* 2005;62:670–674.
3. Blackley DJ, Halldin CN, Laney AS. Continued Increase in Prevalence of Coal Workers' Pneumoconiosis in the United States, 1970-2017. *Am J Public Health* 2018;108:1220–1222.
4. Blackley DJ, Halldin CN, Laney AS. Resurgence of a Debilitating and Entirely Preventable Respiratory Disease among Working Coal Miners. *Am J Respir Crit Care Med* 2014;190:708–709.
5. Blackley D, Crum J, Halldin C, Storey E, Laney A. Resurgence of Progressive Massive Fibrosis in Coal Miners — Eastern Kentucky, 2016. *MMWR Morb Mortal Wkly Rep* 2016;65:1385–1389.
6. Almberg KS, Halldin CN, Blackley DJ, Laney AS, Storey E, Rose CS, Go LHT, Cohen RA. Progressive Massive Fibrosis Resurgence Identified in U.S. Coal Miners Filing for Black Lung Benefits, 1970-2016. *Ann Am Thorac Soc* 2018;doi:10.1513/AnnalsATS.201804-261OC.
7. Blackley D, Reynolds L, Short C, Carson R, Storey E, Halldin C, Laney A. Progressive massive fibrosis in coal miners from 3 clinics in Virginia. *JAMA* 2018;319:500–501.
8. Suarthana E, Laney AS, Storey E, Hale JM, Attfield MD. Coal workers' pneumoconiosis in the united states: regional differences 40 years after implementation of the 1969 Federal Coal Mine Health and Safety Act. *Occup Environ Med* 2011;68:908–913.
9. Federal Coal Mine Health and Safety Act, Public Law no. 91-173 [1969].
10. Laney AS, Weissman DN. The classic pneumoconioses: new epidemiological and laboratory observations. *Clin Chest Med* 2012;33:745–758.
11. Hall NB, Blackley DJ, Halldin CN, Laney AS. Continued increase in prevalence of r-type opacities among underground coal miners in the USA. *Occup Environ Med* 2019;76:479–481.
12. Cohen RA, Petsonk EL, Rose C, Young B, Regier M, Najmuddin A, Abraham JL, Churg A, Green FHY. Lung Pathology in U.S. Coal Workers with Rapidly Progressive Pneumoconiosis Implicates Silica and Silicates. *Am J Respir Crit Care Med* 2016;193:673–680.
13. Sarver E, Keleş Ç, Afrouz SG. Particle size and mineralogy distributions in respirable dust samples from 25 US underground coal mines. *Int J Coal Geol* 2021;247:103851.

ANNALSATS Articles in Press. Published March 30, 2022 as 10.1513/AnnalsATS.202109-1064OC

14. Johann-Essex V, Keles C, Rezaee M, Scaggs-Witte M, Sarver E. Respirable coal mine dust characteristics in samples collected in central and northern Appalachia. *Int J Coal Geol* 2017;182:85–93.

15. Doney BC, Blackley D, Hale JM, Halldin C, Kurth L, Syamlal G, Laney AS. Respirable coal mine dust in underground mines, United States, 1982-2017. *Am J Ind Med* 2019;62:478–485.

16. Laney AS, Petsonk EL, Attfield MD. Pneumoconiosis among underground bituminous coal miners in the United States: is silicosis becoming more frequent? *Occup Environ Med* 2010;67:652–656.

17. Cohen RAC. Is the increasing prevalence and severity of coal workers' pneumoconiosis in the United States due to increasing silica exposure? *Occup Environ Med* 2010;67:649–650.

18. Gardner HH. Mechanization in Alberta Coal Mines. *Can Min Metall Bull* 1951;54:161–166.

19. Shields JJ, Magnuson MO, Haley WA, Dowd JJ. Mechanical Mining in Some Bituminous-Coal Mines; Progress Report 7. Methods of Mining With Continuous-Mining Machines. *Bur Mines Inf Circ* 1954;7696:1–32.

20. Coal Workers' Autopsy Study. *JAMA* 1971;217:1384–1384.

21. Vallyathan V, Landsittel DP, Petsonk EL, Kahn J, Parker JE, Osiowy KT, Green FHY. The influence of dust standards on the prevalence and severity of coal worker's pneumoconiosis at autopsy in the United States of America. *Arch Pathol Lab Med* 2011;135:1550–1556.

22. Almberg K s., Zell-Baran L, Abraham J l., Cool C, Franko A, Green F h. y., Murray J, Sanyal S, Vorajee N, Go L, Rose C s., Petsonk E l., Hubbs A, Orandle M, Clingerman S, Liguori D, Kennedy K, Zulfikar R, Cohen R a. Intra- and Inter-Rater Reliability of Pathologic Classification of Type of Progressive Massive Fibrosis Among Deceased U.S. Coal Miners. *A105 SILICA Inorg DUST Min* American Thoracic Society; 2020. p. A2632–A2632.doi:10.1164/ajrccm-conference.2020.201.1_MeetingAbstracts.A2632.

23. Abraham JL, Burnett BR. Quantitative analysis of inorganic particulate burden in situ in tissue sections. *Scan Electron Microsc* 1983;681–696.

24. Abraham JL, Hunt A, Burnett BR. Quantification of Non-Fibrous and Fibrous Particulates in Human Lungs: Twenty Year Update on Pneumoconiosis Database. *Ann Occup Hyg* 2002;46:397–401.

25. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particulate burden in over 400 lungs. *Scanning Microsc* 1991;5:95–104; discussion 105-108.

26. Fernie JM, Ruckley VA. Coal workers' pneumoconiosis: correlation between opacity profusion and number and type of dust lesions with special reference to opacity type. *Br J Ind Med* 1987;44:273–277.

27. Craighead J, Kleinerman, J, Abraham JL, Gibbs AR, Green FHY, Harley RA, Ruettner JR, Vallyathan V, Juliano EB. Diseases associated with exposure to silica and non fibrous silicate minerals. Silicosis and Silicate Disease Committee. *Arch Pathol Lab Med* 1988;112:673–720.

ANNALSATS Articles in Press. Published March 30, 2022 as 10.1513/AnnalsATS.202109-1064OC

28. Honma K, Chiyotani K. Pulmonary alveolar proteinosis as a component of massive fibrosis in cases of chronic pneumoconiosis. An autopsy study of 79 cases. *Zentralbl Pathol* 1991;137:414–417.

29. Abraham JL, Wiesenfeld SL. Two cases of fatal PMF in an ongoing epidemic of accelerated silicosis in oilfield sandblasters: lung pathology and mineralogy. *Ann Occup Hyg* 1997;41:440–447.

30. Abraham JL. *Analysis of Fibrous and Non-Fibrous Particles. Ch 18. in Rom W (ed) Environmental and Occupational Medicine*, 4th ed. Philadelphia : Wolters Kluwer/Lippincott Williams & Wilkins; c2007.

31. Chen W, Hnizdo E, Chen J-Q, Attfield MD, Gao P, Hearl F, Lu J, Wallace WE. Risk of silicosis in cohorts of Chinese tin and tungsten miners, and pottery workers (I): An epidemiological study. *Am J Ind Med* 2005;48:1–9.

32. Harrison J, Chen J-Q, Miller W, Chen W, Hnizdo E, Lu J, Chisholm W, Keane M, Gao P, Wallace W. Risk of silicosis in cohorts of Chinese tin and tungsten miners and pottery workers (II): Workplace-specific silica particle surface composition. *Am J Ind Med* 2005;48:10–15.

33. Wallace WE, Harrison J, Keane MJ, Bolsaitis P, Eppelsheimer D, Poston J, Page SJ. Clay occlusion of respirable quartz particles detected by low voltage scanning electron microscopy-X-ray analysis. *Ann Occup Hyg* 1990;34:195–204.

34. Stephens JW, Harrison JC, Wallace WE. Correlating auger electron spectroscopy with scanning electron microscopy-energy dispersive spectroscopy for the analysis of respirable particles. *Scanning* 1998;20:302–310.

35. Harrison JC, Brower PS, Attfield MD, Doak CB, Keane MJ, Grayson RL, Wallace WE. Surface composition of respirable silica particles in a set of U.S. anthracite and bituminous coal mine dusts. *J Aerosol Sci* 1997;28:689–696.

36. Wirth JJ, Carney WP, Frederick Wheelock E. The effect of particle size on the immunodepressive properties of silica. *J Immunol Methods* 1980;32:357–373.

37. Vallyathan V, Shi XL, Dalal NS, Irr W, Castranova V. Generation of free radicals from freshly fractured silica dust. Potential role in acute silica-induced lung injury. *Am Rev Respir Dis* 1988;138:1213–1219.

38. Vallyathan V, Castranova V, Pack D, Leonard S, Shumaker J, Hubbs AF, Shoemaker DA, Ramsey DM, Pretty JR, McLaurin JL. Freshly fractured quartz inhalation leads to enhanced lung injury and inflammation. Potential role of free radicals. *Am J Respir Crit Care Med* 1995;152:1003–1009.

39. Pavan C, Santalucia R, Leinardi R, Fabbiani M, Yakoub Y, Uwambayinema F, Ugliengo P, Tomatis M, Martra G, Turci F, Lison D, Fubini B. Nearly free surface silanols are the critical molecular moieties that initiate the toxicity of silica particles. *Proc Natl Acad Sci U S A* 2020;117:27836–27846.

40. Derickson A. *Black lung : anatomy of a public health disaster*. Cornell University Press; 1998.

ANNALSATS Articles in Press. Published March 30, 2022 as 10.1513/AnnalsATS.202109-1064OC

41. Seaton A, Dodgson J, Dick JA, Jacobsen M. QUARTZ AND PNEUMOCONIOSIS IN COALMINERS. *The Lancet* 1981;318:1272–1275.

42. *NIOSH hazard review: health effects of occupational exposure to respirable crystalline silica.* U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health; 2002. doi:10.26616/NIOSHPUB2002129.

ANNALSATS Articles in Press. Published March 30, 2022 as 10.1513/AnnalsATS.202109-1064OC

Figure Legends:

Figure 1: Flow chart of cases accessioned into the study

Figure 2: Representative examples of coal-, mixed- and silica-types of PMF, (hematoxylin and eosin stains). A: Coal-type PMF lesion (≤25% silicotic nodules). This lesion consists of one large nodule fused to two smaller nodules below. There is substantial collagen with varying orientation surrounded by a rim of coal dust-laden histiocytes with fibrotic extensions into the adjacent parenchyma. There is prominent central necrosis with large quantities of dust. Mature or immature silicotic nodules are not seen, with the possible exception of the small collagenized nodule at bottom left. B: Mixed-type PMF (>25% and ≤75% silicotic nodules). This PMF lesion is composed of fused nodules, some with features of coal dust nodules, others showing features of mature silicotic nodules (arrows). Some of the nodules show central necrosis, and there is extensive necrosis with cavitation on the left side of the lesion. Black coal dust pigment is prominent in all areas. C: Silica-type PMF (>75% silicotic nodules). This lesion is composed almost entirely of mature silicotic nodules. Silicotic nodules are also seen in the adjacent parenchyma with bridging fibrosis (arrow) to the PMF lesion. Black coal mine dust is markedly less apparent than in the other PMF types.

Figure 3: Immature Silicotic Nodule. Example of an immature silicotic nodule (hematoxylin and eosin stain). The nodule is composed of central collagen bundles lacking the characteristic central whorling of a mature silicotic nodule. The periphery is composed of fibrohistiocytic cells

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with prominent lymphocytes. The latter extend into the adjacent lung interstitium. Note: These nodules should not be confused with granulomas which differ from immature (and mature) silicotic nodules in that they are composed of activated histiocytes and do not have the central collagen bundles.

Figure 4: Example of mineral-dust alveolar proteinosis (MDAP). This feature was characterized by the finding of scattered alveoli containing dark pink, finely granular, lipo-proteinaceous material (A), that stained with PAS (B). Characteristic cracking artefact (arrows) was also seen.

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Table 1: Demographic and mine work characteristics of study participants

	Historical			Contemporary		
	n=62	**SD**	**Range**	**n=23**	**SD**	**Range**
Age (Mean Years)	65.4	5.7	55 - 82	61.1	3.7	48 - 79
Birth Year (Mean Year)	1919	4.9	1910 – 1928	1942	11	1930 - 1961
White Race Yes n (%)	53 (87)			21 (91)		
Smoker Yes; n (%)	50 (81)			17 (74)		
Mean Pack-Years Smoking	20.1	19.0	0 – 96	18.6	18.4	0 – 60
[Work in] Central Appalachia* (Yes)	39 (63)			17 (74)		
Mean Years of Coal Mining	35.8	10.0	10-50	31.4	2.2	10 – 42
Mean Years Worked Underground	34.9	8.9	3 – 50	30.2	8.7	10 – 42
Mean Years Worked at the Surface	2.6	10	0 – 45	0.8	1.3	0 – 3

*Central Appalachia refers to the states of Kentucky, Virginia, and West Virginia

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Table 2: Pathologic Findings in Historical compared to Contemporary Coal Miners with PMF, including all cases and findings by US geographic region

	All Regions			Central Appalachia[§]			Rest of the US		
Finding	**Historical n=62**	**Contemporary n=23**	**P value**	**Historical n=39**	**Contemporary n=17**	**P value**	**Historical n=23**	**Contemporary n=6**	**P value***
PMF Type									
Silica	11 (18)	13 (57)	**0.0015****	5 (13)	10 (59)	**0.001****	6 (26)	3 (50)	0.60
Mixed	20 (33)	6 (26)	**0.0015****	10 (26)	4 (24)	**0.001****	10 (44)	2 (33)	0.60
Coal	31 (50)	4 (17)	**0.0015****	24 (66)	3 (18)	**0.001****	7 (30)	1 (17)	0.60
Surrounding lung parenchyma[†]									
Silicotic Nodules	20 (33)	10 (52)	0.17	12 (32)	7 (50)	0.33	8 (35)	3 (60)	0.35
Immature Silicotic Nodules	11 (18)	7 (37)	0.11	6 (16)	4 (29)	0.43	5 (22)	3 (60)	0.12
Coal Macules	56 (93)	12 (60)	**0.0011****	34 (92)	10 (67)	0.035	22 (96)	2 (40)	0.012
Coal Nodules	47 (78)	11 (58)	**0.08**	33 (89)	8 (57)	**0.018**	14 (61)	3 (60)	1.00
MDAP[†]	22 (37)	16 (70)	**0.007**	14 (36)	12 (71)	**0.021**	8 (35)	4 (67)	0.198

All values are presented as n (%).

* P < 0.05 in bold.

** P < Bonferroni correction for testing 24 comparisons value of 0.0021.

[†]Five cases had only PMF lesions without evaluable surrounding parenchyma. Total evaluated = 80.

[‡] MDAP: presence of mineral dust-related alveolar proteinosis

[§]Central Appalachia refers to the states of Virginia, West Virginia and Kentucky.

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Table 3A: In situ Lung Mineralogy Findings in Historical vs. Contemporary Coal Miners with PMF: Percentages and Concentrations by Particle Type

	Historical Mean (SD) n=33	**Contemporary Mean (SD)** n=17	**P value***
Total particle concentration†	149 (77)	180 (156)	0.43
Particle Type:			
Silica (Si)			
% of Particles	**17.8 (10.0)**	**26.1 (10.0)**	**0.007****
Particle concentration†	**25.8 (19.7)**	**47.3 (37.0)**	**0.036**
Aluminum Silicates (SiAl and SiAlK)			
% of Particles	**74.6 (9.9)**	**66.2 (9.9)**	**0.006****
Particle concentration†	112 (60.5)	120 (118)	0.78
Titanium (Ti)			
% of Particles	5.8 (3.0)	6.2 (2.3)	0.68
Particle concentration†	8.8 (7.4)	11.4 (10.7(	0.32

* P < 0.05 in bold.

** P < Bonferroni correction for testing 6 comparisons value of 0.0085.

†Particle concentrations are particles X 10^8 per cm^3 of tissue.

Note: Data for particles comprising < 5% not shown, therefore percentages in Table 3 do not total 100%.

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Table 3B – In Situ Lung Mineralogy-Pathology Correlations in Coal Miners with PMF:
Percentages and Concentrations by Particle Type

Pathology Finding	**MDAP Absent** **Mean (SD)**	**MDAP Present** **Mean (SD)**	**P value**	**Sil Nod Absent** **Mean (SD)**	**Sil Nod Present** **Mean (SD)**	**P value**	**Immature Sil Nod Absent** **Mean (SD)**	**Immature Sil Nod Present** **Mean (SD)**	**P value***
Type of Particle	n=26	n=24		n=30	n=16		n=34	n=12	
Silica (Si)									
% of particles (SD)	**17.1 (7.3)**	**24.5 (12.4)**	**0.016**	**16.9 (8.0)**	**25.8 (11.6)**	**0.004**	17.8 (7.9)	26.0 (13.8)	0.071
Particle concentration (SD) †	**24.6 (20.3)**	**42.3 (3.3)**	**0.030**	**24.4 (22.4)**	**44.2 (31.6)**	**0.017**	**26.2 (23.8)**	**45.8 (32.4)**	**0.031**
Aluminum Silicates (SiAl and SiAlK)									
% of particles (SD)	**75.4 (7.9)**	**67.8 (11.9)**	**0.011**	**74.8 (8.4)**	**67.5 (12.4)**	**0.022**	74.2 (8.0)	66.7 (14.5)	0.11
Particle concentration (SD) †	116.8 (96.5)	112.0 (67.9)	0.84	110.6 (68.3)	105.7 (60.5)	0.81	108.0 (68.7)	112.0 (56.4)	0.85
Titanium (Ti)									
% of particles (SD)	5.70 (3.1)	6.16 (2.80)	0.58	6.2 (3.2)	5.8 (2.9)	0.69	6.1 (3.4)	5.89 (1.8)	0.76
Particle concentration (SD) †	9.3 (9.6)	10.2 (7.7)	0.72	9.4 (7.8)	9.7 (8.6)	0.89	9.3 (8.6)	10.1 (6.1)	0.75

* P < 0.05 in bold.

** P < Bonferroni correction for testing 18 comparisons value of 0.0028.

†Particle concentrations are particles X 10^8 per cm^3 of tissue.

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Table 3C: Lung Mineralogy-Pathology Correlations by PMF Type:
Percentages and Concentrations by Particle Type

Pathology Finding	**Type of PMF**			
	Silica-type Mean (SD)	**Mixed-type Mean (SD)**	**Coal-type Mean (SD)**	**P value**
Type of Particle	n=16	n=13	n=21	
Silica (Si)				
% of particles	**29.6 (12.5)**	**16.9 (5.5)**	**16.0 (6.8)**	**<0.001****
Particle concentration†	42.4 (33.6)	27.2 (20.0)	29.6 (28.0)	0.28
Aluminum Silicates (SiAl and SiAlK)				
% of particles	**62.3 (11.2)**	**76.8 (6.7)**	**75.8 (7.1)**	**<0.001****
Particle concentration†	75.6 (39.7)	137.6 (118.5)	129.8 (74.5)	0.07
Titanium (Ti)				
% of particles	6.2 (2.9)	5.0 (2.2)	6.2 (3.4)	0.44
Particle concentration†	7.7 (5.6)	8.4 (8.7)	12.1 (10.2)	0.25

* P < 0.05 in bold.

** P < Bonferroni correction for testing 6 comparisons value of 0.0085.

†Particle concentrations are particles X 10^8 per cm^3 of tissue.

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Figure 1: Flow chart of cases accessioned into the study

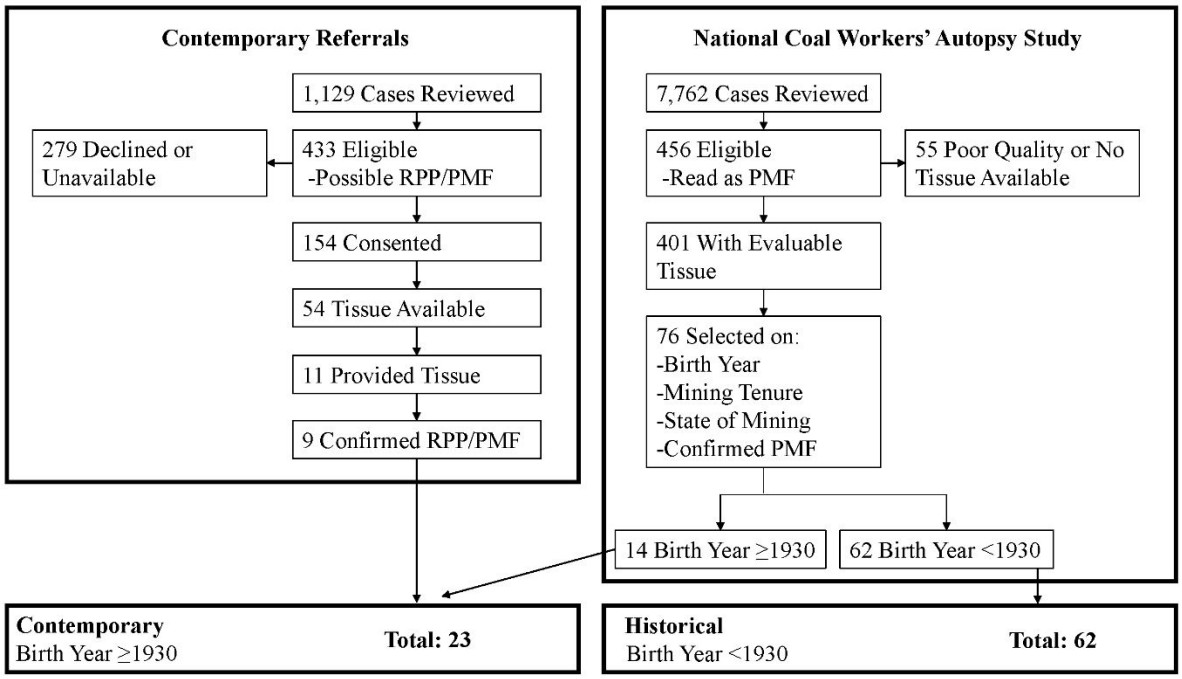

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Figure 2A
Coal-type PMF

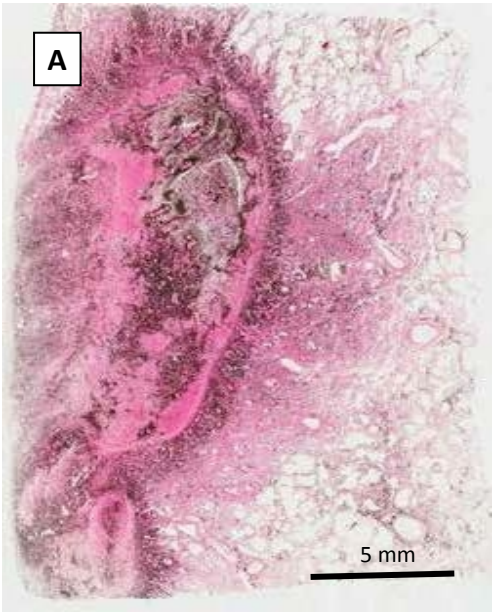

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Figure 2B
Mixed-type PMF

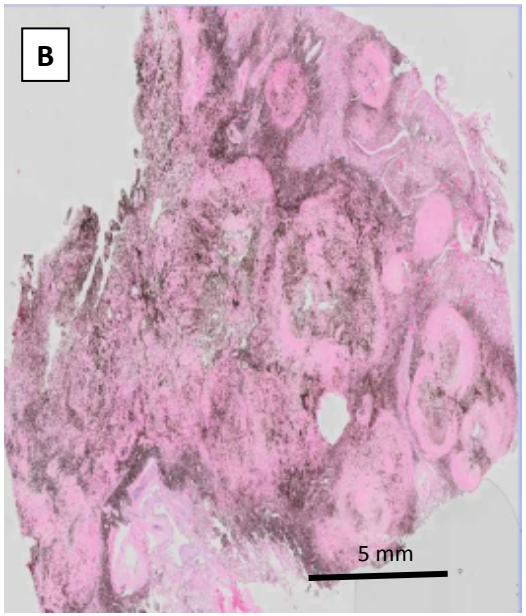

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Figure 2C
Silica-Type PMF

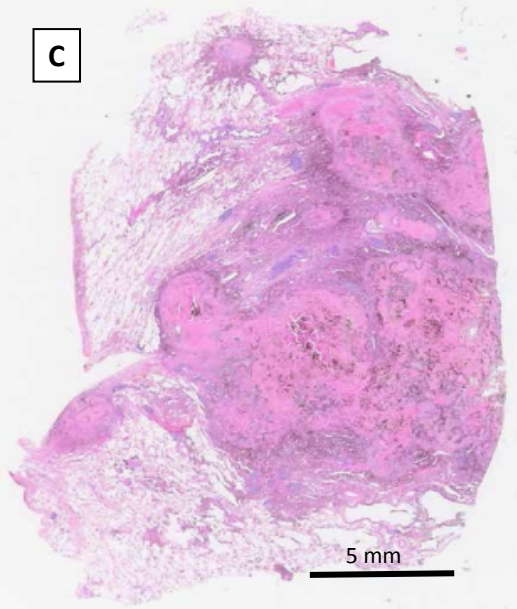

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Figure 3

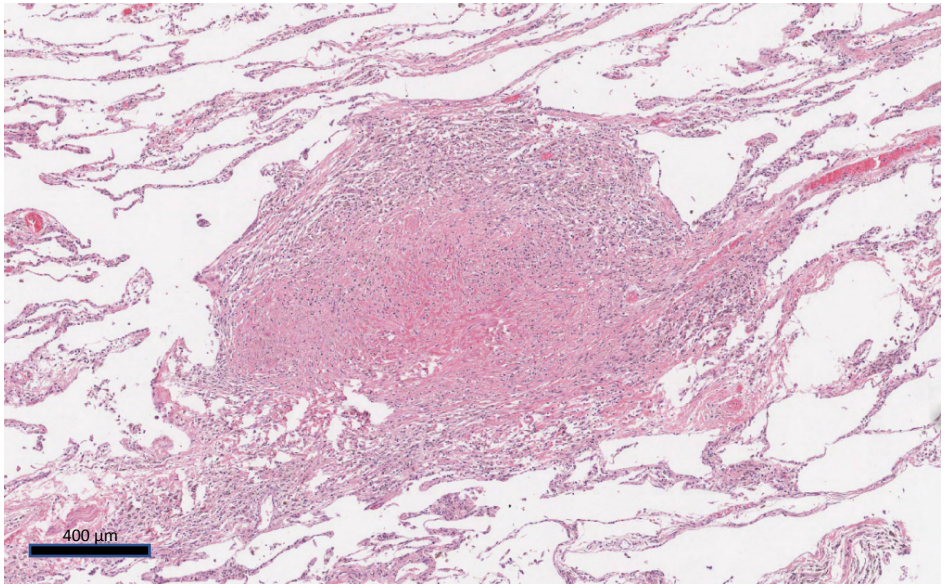

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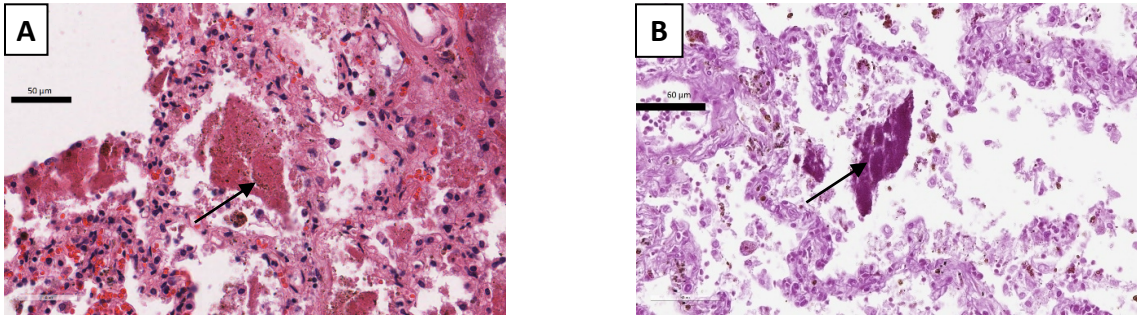
Figure 4

MDAP on H&E Stain

MDAP on PAS Stain



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Online Supplement

Manuscript Title:

Pathology and Mineralogy Demonstrate Respirable Crystalline Silica is a Major Cause of Severe Pneumoconiosis in US Coal Miners

Authors: Robert A. Cohen, Cecile S. Rose, Leonard H. T. Go, Lauren M. Zell-Baran, Kirsten S. Almberg, Emily A. Sarver, Heather A. Lowers, Cayla Iwaniuk, Sidney M. Clingerman, Diana L. Richardson, Jerrold L. Abraham, Carlyne D. Cool, Angela D. Franko, Ann F. Hubbs, Jill Murray, Marlene S. Orandle, Soma Sanyal, Naseema I. Vorajee, Edward L. Petsonk, Rafia Zulfikar, Francis H. Y. Green

Online Supplement

Definitions and Abbreviations of Occupational Pulmonary Terms

1) RPP - Rapidly Progressive Pneumoconiosis is defined as pneumoconiotic opacities increasing by more than one subcategory of the ILO classification system over five years after 1985, or the development or progression of PMF over 5 years after 1985.(1)
2) PMF - Progressive Massive Fibrosis is defined as a pneumoconiotic lesion > 10 mm in long-axis diameter as seen on chest imaging or in pathologic specimens.
3) NIOSH – National Institute for Occupational Safety and Health – This agency is part of the US Centers for Disease Control and Prevention and is the federal agency responsible for conducting research and making recommendations for the prevention of work-related injury and illness.
4) NCWAS – National Coal Workers Autopsy Study – This NIOSH program was actively recruiting cases from 1971-2013. Very few cases were accessioned after that date. Rule

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changes that became effective in 2021 now allow compensation of pathologists at contemporary rates for the public health needs of the program, although it is no longer an entitlement program.

5) CWHSP – Coal Workers Health Surveillance Program – The NIOSH Coal Workers' Health Surveillance Program (CWHSP) studies the causes and effects of respiratory diseases related to coal mine dust exposure and provides vital health information to coal miners through health screenings and surveillance.

Supplemental Information for Mineralogy Methods:

SEM/EDS was used to characterize size, type and concentration of mineral particles *in situ* using modifications of published morphometric point-counting methods. (2–4) Briefly, a 5-µm-thick tissue section including the most severely affected area seen in brightfield microscopy was mounted on a carbon disc, and a transect was drawn through the section. The number of particles in the backscattered electron image of 50 consecutive fields (each 691.7 μm^2) separated by 0.5 mm steps across this transect were counted. Up to 10 particles per field were analyzed by EDS for type (silica, aluminum silicates, and metals). To ensure sampling of at least one full transect of the tissue when high numbers of particles were present, the EDS analysis was done in every third field. Counting and characterization across one or more transects was continued until a minimum of 50 fields were counted and a minimum of 100 particles per section were analyzed. The results of the EDS classification were extrapolated (weighted) for each field when the number of particles analyzed was less than the total number counted. This allowed calculation of the concentration of particles per volume of tissue by particle type.

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Supplemental References

E1. Antao VC dos S, Petsonk EL, Sokolow LZ, Wolfe AL, Pinheiro GA, Hale JM, Attfield MD. Rapidly progressive coal workers' pneumoconiosis in the United States: geographic clustering and other factors. *Occup Environ Med* 2005;62:670–674.

2. Abraham JL, Burnett BR. Quantitative analysis of inorganic particulate burden in situ in tissue sections. *Scan Electron Microsc* 1983;681–696.

3. Abraham JL, Hunt A, Burnett BR. Quantification of Non-Fibrous and Fibrous Particulates in Human Lungs: Twenty Year Update on Pneumoconiosis Database. *Ann Occup Hyg* 2002;46:397–401.

4. Abraham JL, Burnett BR, Hunt A. Development and use of a pneumoconiosis database of human pulmonary inorganic particulate burden in over 400 lungs. *Scanning Microsc* 1991;5:95–104; discussion 105-108.

ANNALSATS Articles in Press. Published March 30, 2022 as 10.1513/AnnalsATS.202109-1064OC