Title

Comparing Human Dust Retention and Fibrotic Severity Between the Upper and Lower Lung Lobes in Residents of the San Joaquin Valley

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Publication Date

2023

Comparing Human Dust Retention and Fibrotic Severity Between the Upper and Lower Lung Lobes in Residents of the San Joaquin Valley

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Forensic Science

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2024

Abstract

The air pollution of the California Central Valley is composed of a complex mixture of gases and particles arising from a diverse number of sources. Exposure to air pollution is especially prevalent in the agricultural professions in Fresno County, California with the exposure of workers to dust and other airborne particles, which can cause pneumoconiosis and fibrosis. This thesis includes a literature review predominantly from publications coming from the Pinkerton laboratory, as well as an observational research project that expands on the research previously completed on coroner cases from the Fresno Medical Examiner's office and in collaboration with students in the Forensic Science Graduate Program at the University of California, Davis. The literature review follows four articles specifically related to the Fresno, CA coroner cases that explore the relationship between particles and the evolution of lung pathology. Furthermore, a fifth article shows how particle size and density affect deposition in different airway generations with an emphasis on first generation respiratory bronchioles using a mathematical model. The research shows a strong correlation between fibrotic severity and the relative score of particles present. All other correlations examined demonstrated no statistically significant differences. The research for this study was limited due to a relatively small sample size of 30 cases for histological assessment, thus reducing statistical power. Nevertheless, prior studies demonstrated strong implications for the effects of black and birefringent particles on the severity of fibrosis. The objective of the present study was to explore the potential differences in particle retention and fibrotic severity between the upper and lower lung lobes to better determine the importance of particle retention and lung tissue remodeling and fibrosis.

The literature review of the Fresno coroner cases studied formed the basis for this thesis research, which explores the pathological differences between the upper and lower lung lobes and its relationship to tissue particle retention. A pulmonary pathologist with extensive expertise graded the lung tissue samples using a semi-quantitative scale between 0 and 3. The pathologist graded 107 cases based on black particle retention, birefringent particle retention, and fibrotic severity. A student t-test revealed that the upper lung

lobe had a significantly higher average for severity in all three variables, compared to the lower lung lobe. After comparing the upper and lower lung lobes, the cases with the highest severity were selected for analysis in the same fashion to determine if the trend was still present for high severity cases (n=30). The results of this analysis demonstrated the trend to also be present for these samples of greater particle retention and fibrosis. These findings demonstrate a statistically significant difference in particle retention and the degree of fibrosis between the upper and lower lobes of the human lung following chronic occupational and environmental particulate exposure.

Background

People working in the agricultural industry in Fresno County, California are exposed to dust and other inhalable particles. Exposure to particulate matter be categorized as black (carbonaceous or carbon-based), birefringent (mineral), and metal particles. These particles easily enter the airways due to their small size (i.e., 2.5 μm) where they can deposit and be retained in lung tissues. These particles can cause pneumoconiosis, oftentimes referred to as occupational lung disease. The literature review follows four previous studies from the Pinkerton laboratory that explored the relationship behind particle deposition/retention and the development of pneumoconiosis in a population of forty to one-hundred and eight medical coroner cases from Fresno County. In addition to the four studies, the literature review conducted also covers one study that shows how particles of varying sizes and densities deposit in different regions of the respiratory system.

Research

From the following four research studies, lung samples had been collected from cases in the Medical Coroner Office of Fresno County, CA. These samples represent one hundred and eight left lung lobes from Hispanic males living in the Fresno region. The lungs were inflation-fixed at necropsy prior to shipment to the Center for Health and the Environment at UC Davis. Upon reception, the lungs were photographed, the airway of the upper left lobe were dissected, and the upper and lower lobes were sampled. All tissue samples were embedded in paraffin and subsequently sectioned using a rotary microtome and placement of tissue sections on slides for microscopic evaluation. For each slide, an expert pulmonary pathologist (Professor Francis Green, University of Calgary Foothill Medical Centre, Calgary, Alberta, Canada) gave a qualitative grade from zero to three, on the degree of fibrotic severity, black particle retention, birefringent particle retention, inflammation, and other pathological changes. A score of zero signified that the slide contained lung tissue that was without disease, normal and healthy, while a score of three signified the lung tissue section demonstrated extensive remodeling in the form of inflammation and lung tissue scarring (i.e., fibrosis and cellular changes). The first published study explored where these particulates deposit in the lungs and whether they were associated with lung tissue remodeling (Pinkerton, Green, Saiki, Vallyathan, Plopper, Gopal, Hung, Bahne, Lin, Ménache, & Schenker, 2000). The second publication explored the development of pneumoconiosis due to dust exposure (Schenker, Pinkerton, Mitchell, Vallyathan, Elvine-Kreis, & Green, 2009). The third and fourth studies (not yet published in the scientific literature, but successfully submitted as thesis papers from UC Davis Forensic Science) expanded on the first two publications by analyzing particle size, elemental composition, and occupation in relation to fibrosis (J. Bautista, 2019; K. Edwards, 2020). One shared limitation between the four publications is a potential variability in the semi-qualitative scoring grades of the lungs among the authors of these publications. In contrast, the first two publications (Pinkerton et al., 2000; Schenker et al., 2009) to describe the entire set of cases had the scoring performed by a single observer trained in pathological assessment.

Distribution of Particulate Matter and Tissue Remodeling

After preparing tissue slides from the lung samples, all authors examined the relationship between dust retention and tissue remodeling. A cross-sectional study was performed to examine where dust accumulates and is retained in the lungs and if these regions demonstrated pathological changes. Of note, there was visible pigmentation along lymphatic vessels in the subpleural interlobular septa (Pinkerton et al., 2000). Furthermore, black pigment was present in tracheobronchial lymph nodes; although it was unclear whether these lymph nodes were enlarged due to the black pigments or the influx of cells containing this black pigment. The study further found a highly significant difference between the severity of histological changes and respiratory bronchiole generation (e.g., first, second, and third generations); the p-value for such changes was less than 0.001. The authors noted that first- and second-generation respiratory bronchioles were more susceptible to pathological changes than third-generation respiratory bronchioles. Pigment was also found to be a strong predictor for severity of fibrosis in the first, second, and third generation respiratory bronchioles; they had an R^2-value of 0.55, 0.53, and 0.31, respectively.

The 2000 Pinkerton et al publication showed strong correlations and highly significant statistics that provided a foundation for future research. The correlation was sufficiently strong to assume the difference in pathological changes between respiratory bronchiole generations was present and needed no further exploration in future studies. Rather, future research should account for how airway generational differences serve as a factor that will affect how pathology develops. The limitations for the 2000 publication was the limited number of cases examined (n=40) and no attempt to identify and compare differences between agricultural workers to non-agricultural workers in Fresno County.

Pneumoconiosis from Agricultural Dust Exposure

The second publication (Schenker et. al, 2009) represented a cross-sectional study that explored the relationship between dust exposure and pneumoconiosis. The authors found that the proximal airways had little dust retention (Schenker, Pinkerton, Mitchell, Vallyathan, Elvine-Kreis, & Green, 2009). Moreover, the bronchioles showed thickened walls, remodeled structures, and inflammation. These pathological changes were associated with dust deposition and were independent from demographics such as occupation, cigarette use, and age (Schenker et al., 2009). They found pneumoconiosis was more dominant in farmworkers than in non-farmworkers. Additionally, farmworkers showed more dust retention than non-farmworkers; that was statistically significant with a p-value less than 0.05. This showed an association with dust and the development of small airway disease and pneumoconiosis (Schenker et al, 2009).

The 2009 publication provided strong correlations and built upon the Pinkerton et. al, 2000 publication to show that pneumoconiosis was independent from other factors such as smoking history and demographic. Even though the data from these two publications are strong, additional questions remains. A primary finding is workers in the agricultural industry are at risk for pneumoconiosis. An important consideration from the Schenker et al. (2000) publication is workers in the agricultural industry should take precautions against inhalation of dust particles.

Metal and Inorganic Particulates in the Lungs

For a decade, little follow-up research was performed. It was not until 2019 when (Bautista, 2019) performed a new study to make associations between occupation, fibrosis, particle size, and elemental composition of the dust particles. It is worth noting the occupations for the cases examined by Bautista were categorized simply as agricultural workers and non-agricultural workers. This classification is different from the earlier article that divided farmworkers and non-farmworkers with several workers in the agricultural field not being actual farmworkers. Bautista explored the relationship between fibrosis and occupation, particle size and occupation, fibrosis and particle size, elemental composition and occupation, elemental composition and fibrosis, and elemental composition. No statistical significance was noted between fibrosis and occupation, particle size and occupation, fibrosis and particle size, elemental composition and fibrosis, and elemental composition and particle size (Bautista, 2019).

Surprisingly, there was statistical significance between elemental composition and occupation. He found that silica dioxide particles were lower in agricultural workers than in non-agricultural workers; the p-value was 0.048 (Bautista, 2019). However, there was no difference for aluminum silicate particles between the two occupations.

The article explored many relationships that created potential factors for future research. Although many relationships demonstrated no statistical significance, this work can be helpful to discount certain factors as contributors to fibrosis. The research demonstrated being in the agricultural industry does not always increase your risk in fibrosis. Moreover, inhalation of particles with varying sizes and composition does not always increase the risk of fibrosis.

The limitation of the Bautista study was quantitative analysis could not be performed. The qualitative analysis of the grading criteria is subjective, even from a pathologist. The small variations that would naturally cancel each other out by averaging multiple qualified opinions was not present. This creates the question on the accuracy and precision of scoring in a limited number of cases. Moreover, one additional limitation between this study and the 2009 study was that the Bautista study analyzed only twenty lung samples from the upper lobe of the lung which did not include samples from the lower lobe. The twenty samples studied by Bautista had the highest fibrotic scores among the 108 necropsy cases.

The Health Effects of Chemical Composition to Particulate Matter-Induced Lung Fibrosis

The fourth study by Edwards (Forensic Science graduate student) was in conjunction with the Bautista study (also a Forensic Science graduate student). Edwards performed a study to see if there was a relationship between fibrosis, black particles, and birefringent particles within identical regions of the lungs where metal and particle recovery had been performed (Edwards, 2020). Additionally, a corollary study analyzed the size of 4000 particles. Using the same grading criteria set by the pathologist in the previous three studies. In this study, all grades were analyzed using linear-regression statistics. Edwards found a significant positive relationship between black pigment and fibrosis with a p-value less than 0.05 and an R-value of 0.77 (Edwards, 2020). Moreover, as the number of birefringent particles increased, so did the severity of fibrosis with a p-value less than 0.05 and an R-value of 0.60. Apart from this, the twenty selected samples from the initial 100 showed no statistical significance between the two variables (Edwards, 2020). The conclusion from the study of Edwards was black pigment and birefringent particles does not always lead to fibrosis.

The corollary study showed that 88% of the particles found were less than 1um in diameter. Of those particles, 41% were silica dioxides and 31% were aluminum silicates (Edwards, 2020). The study found no statistical significance between the severity of fibrosis and particle size.

The study of Edwards has similar limitations as earlier studies. Moreover, individual case information was not available to rule out other variables. Fortunately, the precedent studies minimized this lack of information by being able to rule out variables such as occupation, smoking habits, age, and other demographics.

Particle Deposition in the Human Lung

The final research article to be discussed aimed to find a pattern between particle deposition, size, density, and source. The authors used a mathematical model to predict the deposition of particles in the lung. The article explains the diverse ways that particles deposit in the lungs. These modes are either respired, deposited due to gravity, or through Brownian diffusion (Deng et al., 2019). The main findings were that coarse particles that were more than 1μm in diameter can deposit in the pulmonary region. As particle size decreased, the deposition of particles due to gravity and respiration decreases (Deng et al., 2019). For denser particles, as size increases, the deposition throughout the lungs and tracheobronchial region also increase; this trend does not persist in the tracheobronchial region (Deng et al., 2019). More importantly, both coarse and fine particles from crustal materials show deposition in the pulmonary region, but not in the tracheobronchial region (Deng et al., 2019). Additionally, soil particles deposit through gravity only (Deng et al., 2019).

The mathematical model includes particle dynamics which is both a strength and a limitation. The limitation is it does not simulate real world situations and only simulated average conditions in the airway. The research did not examine the impact of chemical composition and did not consider other dynamics such as particle collision, particle growth, and aggregation. Another limitation is based only on the adult human male airway model. Even though the model has many limitations, it demonstrates coarse particles should also be a major concern for lung pathology (Deng et al., 2019).

Overall Conclusions of Research by Students in Forensic Science – Future Directions

These research articles illuminate the various relationships between pneumoconiosis, particle retention, and particle characteristics. It is well-known occupations in the agricultural industry are at greater risk for pneumoconiosis (Schenker et al, 2009). Moreover, there is a strong relationship between the severity of fibrosis and the amount of black and birefringent particles in the lung (Edwards, 2020). The research completed by (Edwards, 2020) and (Bautista, 2019) only examined the upper lobe of the lung. Based on the findings of (Deng, et al., 2019), soil particles deposit by gravity. It is worth examining whether the lower lung will show greater or less deposition of particles than that found in the upper lung. Because the human lung can accumulate dust without adverse effects (Green, Vallyathan, Hahn, 2007), exploring if there is a pathological difference between the upper and lower lobes of the lung would also be beneficial in determining a relationship.

Current Research

To examine the differences between the upper and lower lung lobes can help to determine the contribution of 1) particle retention and fibrotic severity as well as the importance of 2) where particles deposit and/or are retained in the lungs. If there is a statistically significant difference between the upper and lower lung lobes in particle retention and fibrotic severity, it would imply the retention of inhaled particles may be dependent on gravity, airway path length and/or particle size. If there is a difference in retention, but not in fibrotic severity, then it implies that the retention of particles may not be the driver for lung remodeling, and there could be other factors in play. If there is no statistical significance in particle retention but there is significant fibrotic severity, this could imply that there may be an occult variable that may be contributing to the difference in fibrotic severity that may not be related to particle retention. This occult variable could be particle size. If there is no difference in either, then it implies that retention of particles is not gravity or airway path length dependent.

Experimental Design

A subset of the same cases used by Pinkerton and Schenker will be used for this study. The cases to be examined are from the left lungs of autopsy cases from male Hispanic subjects obtained in the Fresno County coroner's office between June 1994 and June 1995. The lungs are from subjects in the age range of 16 to 73 years. The cause of death was either sudden or unexpected, no deaths were the result of any underlying lung disease. The medical examiners performed autopsies 12-24 hours after discovery. The precise time of death was unknown. As part of the autopsy procedure, the left mainstem bronchus was cannulated and inflation-fixed with 2% glutaraldehyde at a hydrostatic pressure of 30 cm. This procedure provided consistency and uniformity between cases for histopathological analysis and laid out the steps for tissue sampling and preparation described in the Tissue Preparation section.

Each section from the left upper and lower lung lobes was selected for microscopic examination. Figure 1 shows a map of the lungs and shows the location where each section originated.

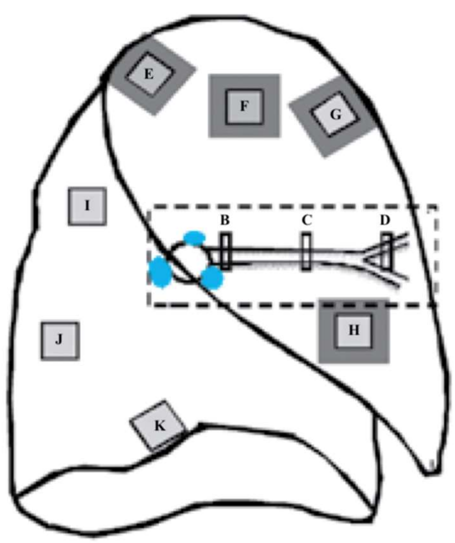

Figure 1 Map of the Lungs. Sections E, F, and G were the primary focus for the study conducted by Edwards, Bautista, Schenker et al, and Pinkerton et al; the sections belong to the left upper lung lobe. Sections I, J, and K are the primary focus for this study; the sections belong to the left lower lung lobe. After preparing tissue blocks from each of the identified regions shown in this map, they were each embedded in paraffin wax. It should be noted the time between embedding and sectioning was 25 years. This does not in any way affect the quality of the prepared sections or the analysis because tissues embedded in paraffin do not degrade in storage as long as the paraffin blocks are maintained in a cool dry environment. They are essentially "immortalized".

After embedding all the tissues in paraffin, each block was sectioned and stained using hematoxylin and eosin. All staining procedures can be found in the appendix. After staining, the tissues were examined under a microscope for black and birefringent particles. Digital pictures were taken of sites demonstrating tissue remodeling and/or for evidence of particle retention as analysis was being completed. Microscopy was done with the Zeiss® Microscope Axio Imager A1 AX10 microscope; the imaging software used was Multi-Image-02 – ZEN 2.3 lite imaging software. The digital pictures were taken by Zeisss® Axiocam 105 color. The photos taken and the pathologist's grade are in the appendix.

Using the pathologist's grades from the previous four studies for each region of the lungs for both the upper and lower lung lobes made, several graphs were made that compared particle retention and fibrotic severity.

The following correlations were made from the data: 1) Mean Black Pigment Score vs Mean Fibrosis Score in the upper lung, 2) Mean Black Pigment Score vs Mean Fibrosis Score in the lower lung, 3) Mean Birefringent Pigment Score vs Mean Fibrosis Score in the upper lung, and 4) Mean Birefringent Pigment Score vs Mean Fibrosis Score in the lower lung. Using parametric statistics including the Student's T-Test was employed to evaluate significant differences in average values between the upper and lower lung scores for black pigments, fibrosis, and polarized pigments. Using statistical analysis methods the goal was to determine whether there is a difference in particle retention or a difference in fibrotic severity between the upper and lower lung.

Results

Along with the graphs, t-tests were conducted to determine if the mean difference between the upper and lower lung was statistically significant. The t-tests analyzed the mean difference in black pigment score, fibrotic score, and birefringent score between the upper and lower lung. The mean score for black pigment in the lower lung is 0.91, and the mean score for black pigment in the upper lung is 1.18; it has a two-tail p-score of 1.59×10^{-5}. The mean fibrotic score in the lower lung is 0.74, and the mean fibrotic score in the upper lung is 0.90; it has a two-tail p-score of 0.003. The mean score for polarized pigment in the lower lung is 0.40, and the mean score for polarized pigment in the upper lung is 0.55; it has a two-tail p-score of 0.002. The difference in all three variables between the upper and lower lung is statistically significant.

Despite the best methods and attempts in reducing variability, some assumptions needed to be made. The assumptions made are the pathologist's grades are 1) a consistent reflection of fibrotic severity, black particle retention, and birefringent particle retention, 2) the average of a region's grades is representative of remodeling of pulmonary tissues in the lungs, and 3) the graded scores of first-generation respiratory bronchioles is consistent with first generation respiratory bronchioles throughout both the upper and lower lobes of the lungs for each case examined.

The scores presented are the average scores of three first-generation respiratory bronchioles for each respective site found in the upper (E, F, G) and lower (I, J, K) lobes of the lung. This score was selected for consistency based on the appearance of the first-generation respiratory bronchiole. This means that the evaluation of the upper and lower lung lobes are made are based for the same anatomical location, i.e., first generation respiratory bronchioles, the primary site for particle deposition in the distal lung as a test of particle retention and tissue remodeling at this anatomical site of the upper and lower left lung lobe.

Figure 2 shows a scatterplot between the three variables in the lower lung. The first graph depicts black pigment score against fibrotic score, and the second graph pitted birefringent score against fibrotic score. A Pearson Correlation is an indication as to how well the data points are correlated in a linear fashion.

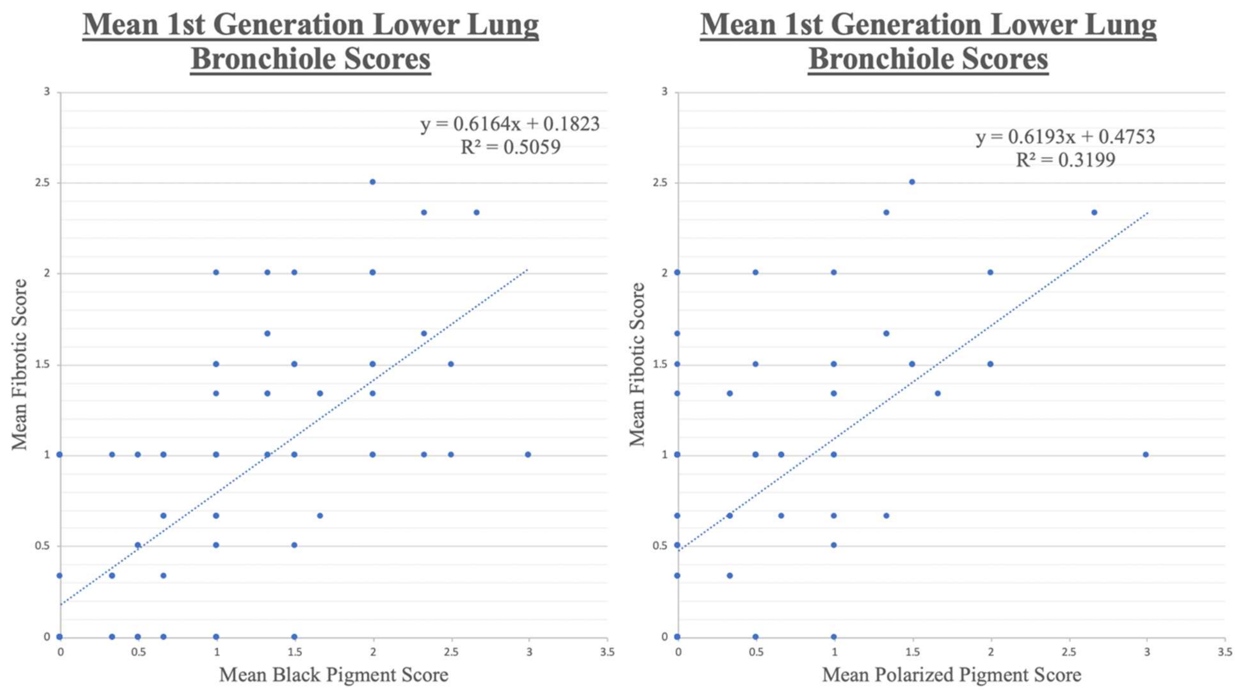

Figure 2. The two graphs display the relationship between black and polarized pigment scores with fibrotic score in the lower lung. The graph on the left has a Pearson Correlation of 0.70, and the graph of the right has a Pearson Correlation of 0.55. Both have a two-tailed P-score of 3.2 x 10^{-3} and 2.21 x 10^{-7} respectively.

Figure 3 shows the same scatterplot with the upper lung. The upper lung has a slightly better Pearson Correlation than the lower lung, but the difference is negligible. This can suggest that the correlation between the variables stays constant in the upper and lower lung. This means that both independent variables have around the same fibrotic effects on the upper lung.

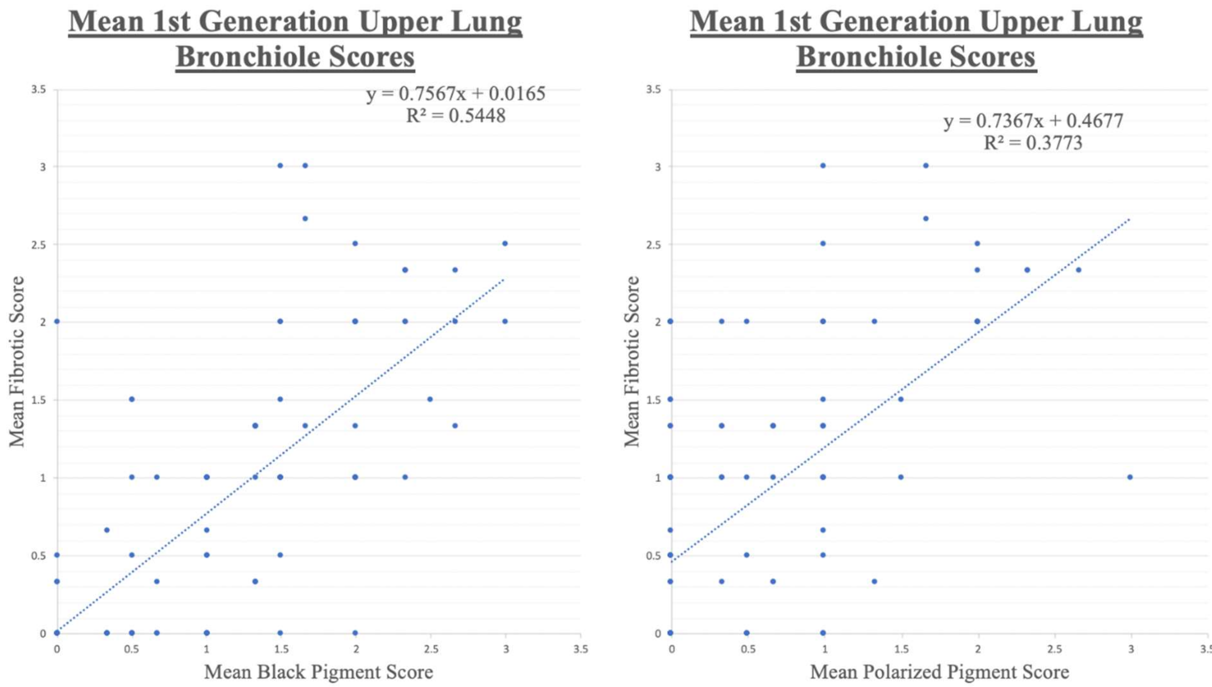

Figure 3. The two graphs display the relationship between black and polarized pigment scores with fibrotic score in the upper lung. The graph on the left has a Pearson Correlation of 0.72, and the graph of the right has a Pearson Correlation of 0.60. Both have a two tailed P-value of 1.84×10^{-5} and 9.84×10^{-7} respectively.

After comparing the scores of all the lungs, the cases with the highest grade in black particle, birefringent, and fibrotic score was compiled to see if the same pattern appears for the cases with the highest pathological significance. The following data shows the cases with the highest pathological grading mixed with ten randomly selected cases to reduce bias in the study.

Figure 4 shows two graphs that placed black pigment and birefringent scores against fibrotic scores in the upper lung.

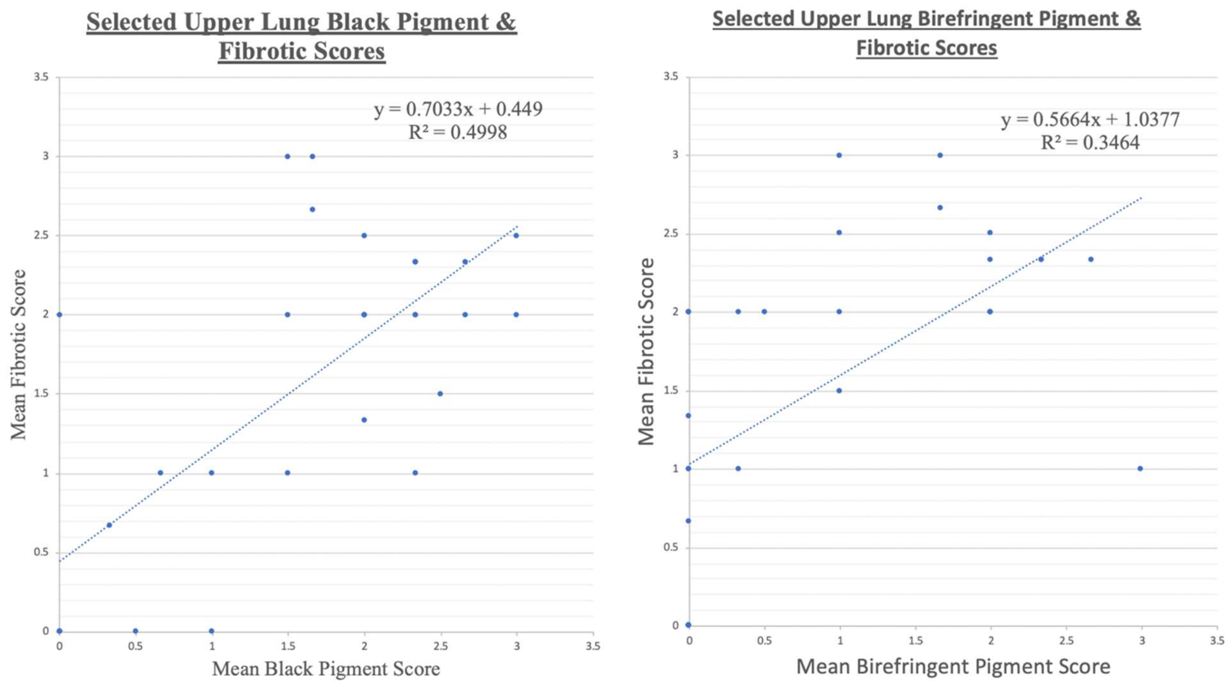

Figure 4. The two graphs showed no correlation between black pigment score and fibrotic scores in the upper lung. The graph on the left has a Pearson correlation of 0.64; the graph on the right has a Pearson correlation of 0.55.

Figure 5 shows two graphs that pitted black pigment and birefringent scores against fibrotic scores in the lower lung.

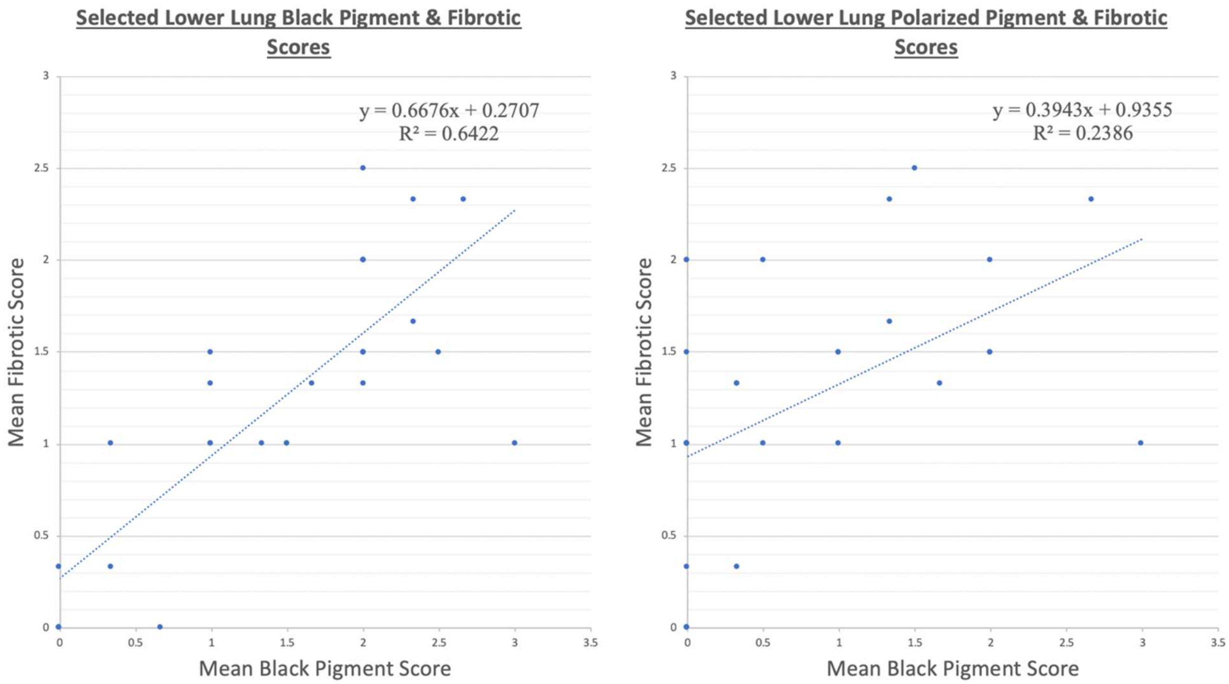

Figure 5. The two graphs showed no correlation between birefringent particles and fibrotic score in the lower lung. However, it shows some support for black pigmented particles having a decent positive relationship with fibrotic score. The graph on the left has a Pearson correlation of 0.80; the graph on the right has a Pearson correlation of 0.49.

Following the determination of the relationship between fibrosis and particle retention in the lungs, the difference in birefringent particle retention, fibrotic severity, and polarized pigment retention between the upper and lower lung was analyzed. In the selected cases, the lower lung has a black particle score of 1.45, and the upper lung has a mean of 1.69; the two-tail t-test score is 0.012. The lower lung mean fibrotic score is 1.24, and the upper lung has a mean of 1.67; the two-tail t-test score is 0.0003. The lower lung mean polarized pigment score is 0.78, and the upper lung has a mean of 0.99; the two-tail t-test score is 0.04. The difference in all three variables between the upper and lower lung is statistically significant.

Discussion

For both the upper and lower lobes of the human lung, the relationship between black particle retention and fibrotic score is stronger than the relationship between polarized pigment retention and fibrotic score. This might suggest that retention of black particulates in the lung could be a better indicator of fibrosis than polarized pigment.

Furthermore, t-tests performed on the observed data show that the differences in fibrotic score, black particle retention, and birefringent particle retention between the upper and lower lung lobes are statistically significant. In all three variables, the lower lung had a lower score than the upper lung. These findings suggest the lower lung is less likely to have pathological changes than the upper lung in humans, perhaps due to differences in particle deposition and/or retention.

For the selected cases with the highest grade of lung remodeling, the data shows the same trends and relationships as depicted in the data that included all cases. The only difference is that the relationship between black particle retention and fibrosis in the lower lung seems to have a stronger relationship than all the others. This difference suggests that black pigment retention could serve as a strong indicator of fibrosis in the lower lung. However, further research is needed to determine if this is by random chance, other unknown variables, or has a relevant of pathological significance for observed differences between the upper and lower lobes of the human lung. The similarities between the complete data base and those selected only for cases with the highest degree of fibrosis provided a strong degree of credibility for differences between the upper and lower lung lobes as being true.

From the unique findings of this study, it is possible to consider black particulate retention in the lungs as a measure of a patient's risk for fibrosis. It also suggests that black particulates may be a large contributor to the development of fibrosis. Occupational conditions that expose workers to black particulates should take greater precaution in limiting exposure to these particulates to reduce fibrosis and increase quality of life.

Since the upper lung lobe possesses a higher retention of black pigment retention and fibrotic score than the lower lung lobe, there could be a rational reason for this trend. One explanation could be that the upper lung lobe is more sensitive to environmental effects than the lower lung lobe. If this is the case, then analyzing the upper lung for pathological significance could be useful for medical applications. Another explanation could be that the upper lung is not more sensitive; however, it could be that more particles and other debris make their way into the upper lobe compared to the lower lobe.

The patterns that were found from the data from all cases were also present in selected cases with the most severe fibrotic and particle retention scores. This implies that the trends found in all cases with varying degree of black pigment retention correlate to the relative degree of fibrosis (lung remodeling as a function of particle exposure and is not due to other confounding factors or outliers).

Interview with the Pathologist

The pathologist (Dr. Francis Green) and the author for the Pinkerton et al., 2000 and Schenker et al., 2009 publications (Dr. Kent Pinkerton) expressed their opinions on the findings in an interview on October 20, 2022. During the interview, the topic of consistency for the association between particle retention and fibrosis, and the possible histopathological findings for these relationships was confirmed.

Consistency Present in the Analysis

In the interview, Pinkerton and Green emphasized on the remarkable consistency that the pattern had in the selected thirty cases. The fact that the selected thirty cases—of which twenty showed the most fibrotic severity, and ten are randomized—still had the same positive relationship with a strong statistical significance means that there is consistency in how the observational experiment was performed (Carillo, Pinkerton, Green, 2022). The subset of the most severe cases could be an indicator of sensitivity and could show how the experiment is not just significant when taking the entire sample population.

Associations Between Particle Retention and Fibrosis

The observational experiment shows that there is a dose response when it comes to an increase in particle deposition and retention. The fibrotic scores show that there is a direct correlation between the two types of particles, and that there is an underlying reason on why the relationship between the variables exist. With an increase in particle deposition and retention, there is an increase in fibrotic response. Moreover, black pigment particles show a stronger association with fibrosis than polarized pigments.

Possible Explanations of The Relationships

Green's histopathological explanation on how these relationships arose was based on the effect of ventilation-perfusion ratios in different regions of the lungs on particle retention. The ventilation-perfusion ratio is poorer in the upper lobe, while there is a better ratio in the lower lobe; the higher VQ ratio in the lower lobe could be better for particle clearance (Carillo, 2022). Green highlighted his prior observations in miners' and smokers' lungs by stating that miners and smokers tend to have more severity in upper lobe tissues remodeling than the lower lobe; upper lung lobe remodeling is more prominent in smokers and coal miners (Carillo, 2022). Green's observations on coal miners and smokers supports the hypothesis that better particle clearance in the lower lobe could explain why the upper lobe shows greater scores for black pigment, polarized pigment, and fibrotic severity than the lower lobe.

A hypothesis that relates to the ventilation-perfusion ratio is an increase in lymphatic vessels in the lower lung due to the ventilation-perfusion ratio. The lymphatic vessels could allow for better leukocyte mobility into areas affected by black and birefringent particles. The increase in leukocyte mobility can enhance the clearance of particles and the reduction in particle retention of the lower lobe.

Study Limitations

One of the clear limitations of this observational experiment is that there is no control human lung lobe samples to compare against cases from the Fresno Medical Coroner Office. The sample cases were obtained only from the Fresno County area of California. The occupation among cases was mixed and, in some cases, unknown. Control cases for this experiment would ideally come from another location in California, although it would be difficult to find any location free of exposure to atmospheric pollutants and airborne particles. Due to the lack of control lung samples, the statistical significance can only be inferred to the public if the hypothetical control shows that there is no significant difference between the upper and lower lobes in healthy and non-fibrotic lungs.

Another limitation is that the data was not discriminatory based on smoking history. There was no data on which cases came from heavy, light, or non-smokers. In contrast, this limitation for the inclusion of smoker is associated with enhanced black pigment deposition and retention in the lungs which suggests these patterns are a direct result of smoking history rather than occupation or place of residency.

Conclusion

The conclusions of this study were drawn from a population of 108 cases. Improvements to this observational study could include a wider population other than Hispanic males in the Fresno region, and encompass more professions other than those in the agricultural industry. This improvement can expand the significance to the general population. Because smoking history was not recorded, it could be possible that most of these lungs came from individuals with a smoking history that could alter the data. Moreover, the cases came from individuals in Fresno county. The selection process was not discriminatory on the length of residency in the county. Both factors could have played a role in how the data showed different trends; however, with over 100 different cases supplying individual data points, and most of the p-scores being beyond the statistical significance of 0.05, it is likely that these factors averaged out. The methods did not allow for proper quantitative analysis and instead relied on qualitative analysis based on the ability from a pathologist; however, it still showed significant trends that is worth expanding on using a different method that allows quantitative analysis.

Appendix

H&E Staining Procedures

Hematoxylin and Eosin Staining protocol for paraformaldehyde fixed tissue.

1.	Deparaffinize (and set up steps 3-12):	
	a. Toluene/Xylene	5 minutes
	b. Toluene/Xylene	5 minutes
	c. Toluene/Xylene	2 minutes
2.	Rehydrate:	
	a. 100% EtOH	2 minutes
	b. 95% EtOH	2 minutes
	c. 70% EtOH	2 minutes
3.	Staining with Harris Hematoxylin	10 minutes
4.	Dip in double distilled water (2-3 dips)	
5.	Soak in double distilled water	1 minute
6.	Differentiating solution* (2-3 dips)	
7.	Dip in double distilled water (2-3 dips)	
8.	Soak in double distilled water	1 minute
9.	Bluing solution*	45 seconds
10.	Dip in double distilled water (2-3 dips)	
11.	Soak in double distilled water	1 minute
12.	Eosin Y*	2-3 minutes
13.	Dehydrate:	
	a. 70% EtOH	2 minutes
	b. 95% EtOH	2 minutes
	c. 100% EtOH	2 minutes
14.	Clear:	
	a. Toluene/Xylene	2 minutes
	b. Toluene/Xylene	2 minutes
	c. Toluene/Xylene	2 minutes
15.	Coverslip slides ASAP with Clear-Mount	

Pathological Features and Grading Criteria

Airway orientation is not considered. Airways and Bronchioles that were not found were not graded. The grading system is based off of the system of *Wright et al., Arch Pathol Lab Med* 109:163-165, 1985).

Fibrosis

This is graded primarily on the elastic trichrome stain.

Grade 0: Normal, thin wall.
Grade 1: Expansion of the wall by loose connective tissue.
Grade 2: Further expansion with condensation of collagen.
Grade 3: Dense connective tissue with or without *thinning* of wall.

Pigment

Opaque and birefringent particles within the airway walls are graded separately.

Opaque particles:
0 = none
1 = small quantities
2 = moderate amounts
3 = dense deposits

Birefringent particles:
0 = none
1 = small quantities
2 = moderate amounts
3 = dense deposits

Slide photos

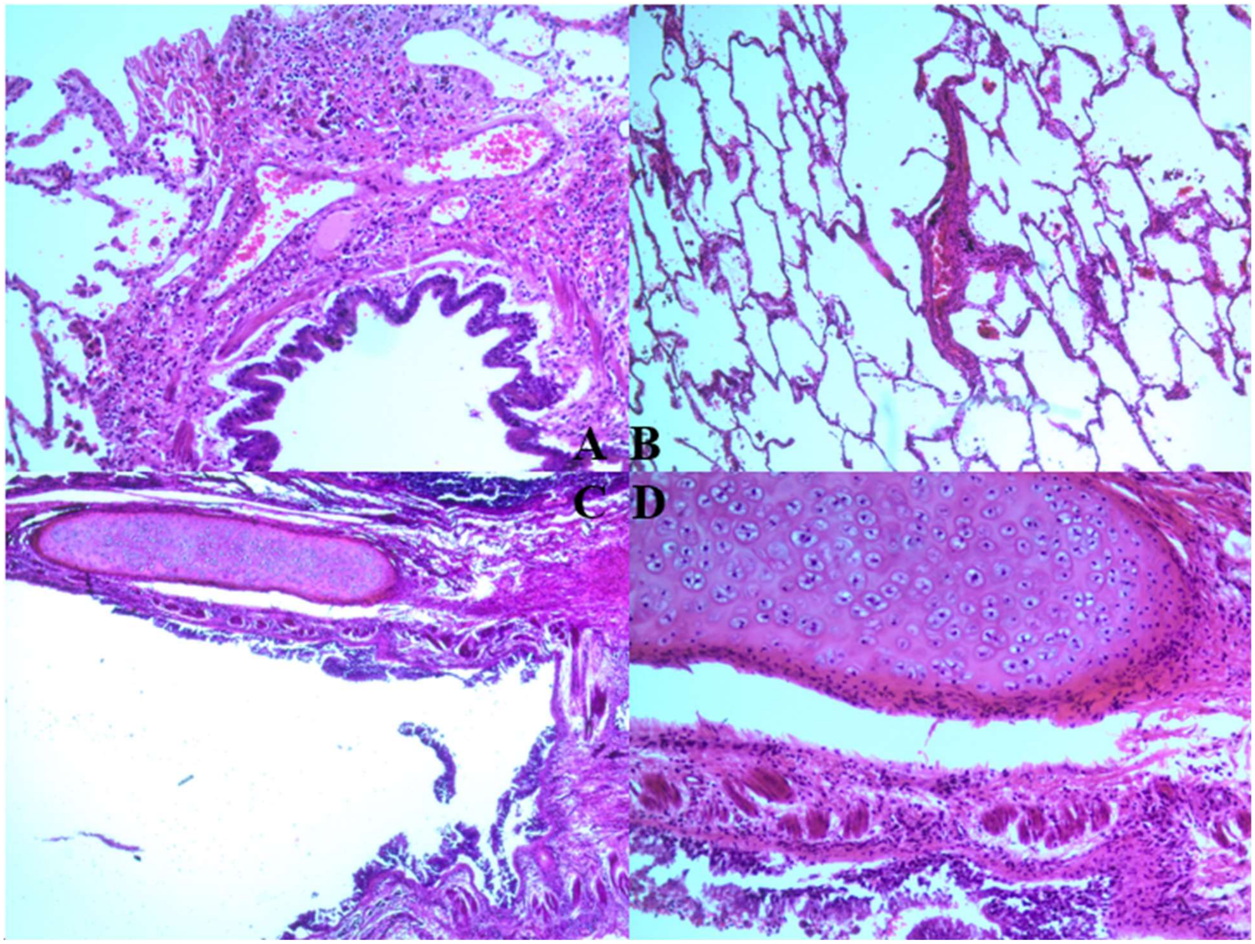

Photo 1. This photo consists of four microscopic images of lung tissue from the same case study. Photo 1A was taken using a 5x magnification. It shows an airway with an intact epithelium. Although black pigments were embedded into the tissue, there was little evidence of fibrosis. Photo 1B was taken using 5x magnification, this photo shows a slightly collapsed alveoli with significant black pigment deposits in the blood vessel. Photo 1C was taken with 2.5x magnification. The photo shows a large bronchiole cutting across the slide with sloughing epithelium. Photo 1D is in 10x magnification and is a close up view of photo C. Black pigments are shown embedded in the tissue.

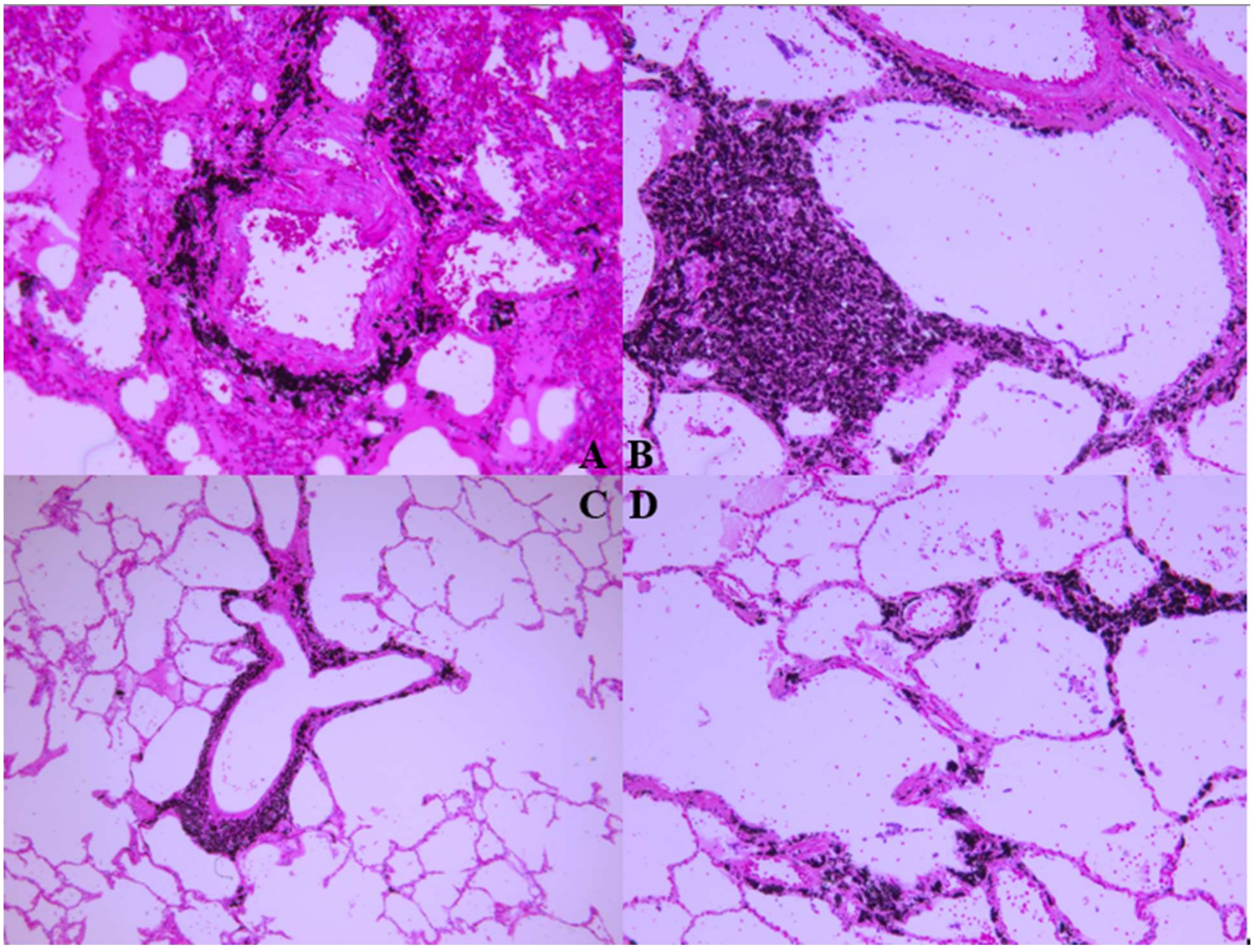

Photo 2. The photo consists of four microscopy photos from the same case study. The lungs in this study were graded high in both fibrotic score and black pigment retention. Photo 2A shows black pigments aggregating around an airway and its associated blood vessel. The photo shows thickened walls. Photo 2B shows heavy deposits of black pigments around a bronchiole. The slide showed moderate degradation of the alveoli. Photo 2C was taken under 2.5x magnification. The bronchiole shown in the photo has black pigments surrounding it, and the alveoli shows heavy degradation as only a few remain intact. Photo 2D shows a 10x magnification of degraded alveoli with black pigments surrounding the alveolar walls.

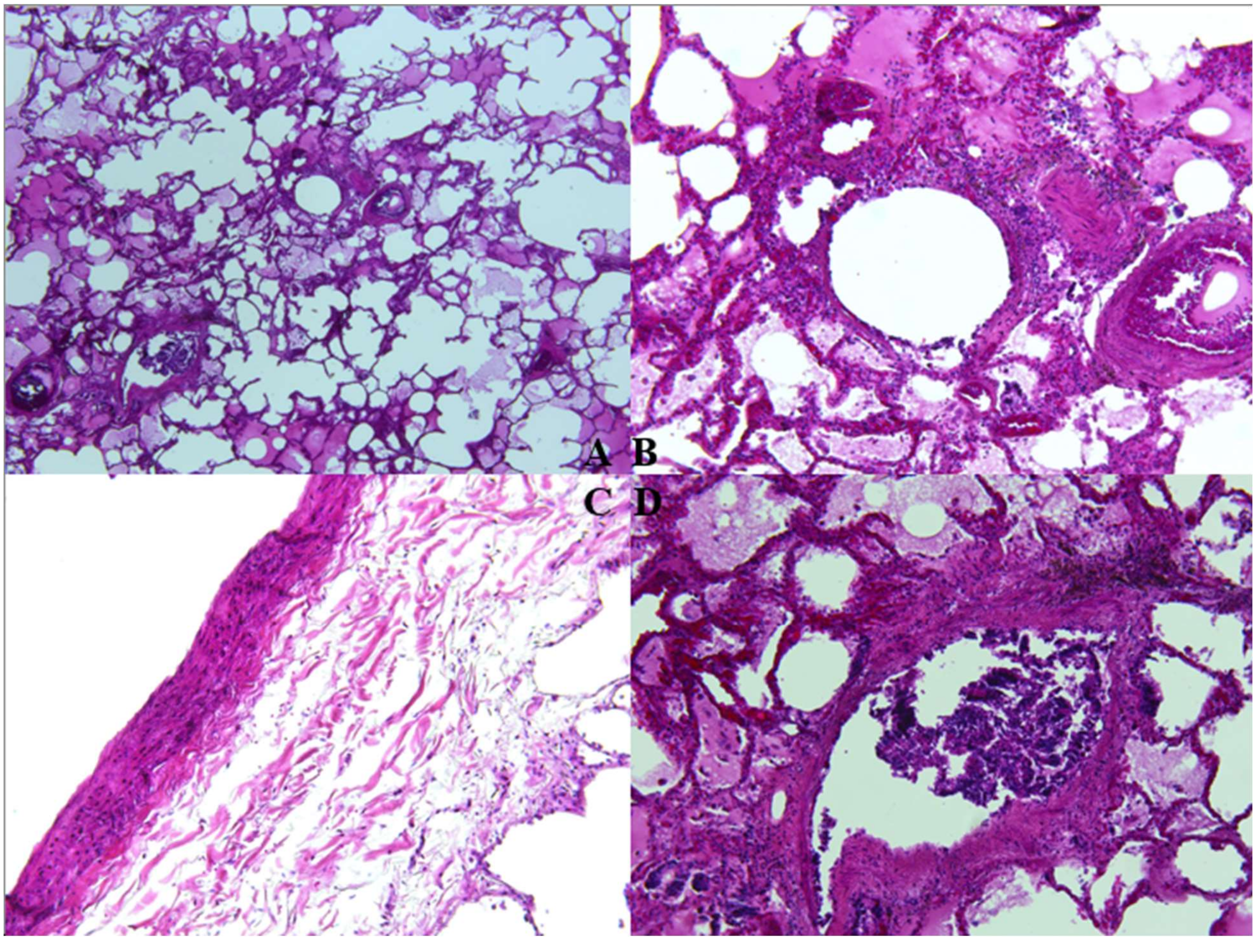

Photo 3. The photo consists of four microscopy photos from the same case study. The lungs in this study were graded low in fibrotic score and in black pigment retention. Photo 3A is a 2.5x magnification of alveoli and airways. The photo shows moderately intact airways and alveoli. Photo 3B was taken under 10x magnification. It depicts a bronchiole and blood vessel fully intact with little presence of black pigments. Photo 3C shows part of a blood vessel, and the tissue remains mostly intact with no evidence of black particles. Photo 3D shows a 10x magnification of an intact bronchiole. Despite being too pigmented, the structures were clear, and little black pigment deposition was found. The entire photo supports the notion that fibrotic severity tends to follow a positive relationship alongside black particle retention.

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