Quantifying Cardiopulmonary Collagen Deposition in a Murine Model of WTC-PM Exposure

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RATIONALE: Particulate matter (PM) exposure can induce an inflammatory response leading to collagen deposition (CD) and permanent scarring and fibrosis. However, collagen deposition is often a subjective description based on histologic staining with few methods of objective quantification. We investigate a murine model of World Trade Center (WTC)-PM exposure and show preliminary results comparing two objective methods to quantify the degree of collagen deposition. METHODS: C57Bl6 mice (n=3/group) had oropharyngeal aspiration of either 200-µg of WTC-PM or PBS control. Heart and lung fixation with 1% paraformaldehyde was performed after one-month. A single 5µm coronal section per exposure was selected based on maximal bioavailable tissue and stained for collagen with Gomori-Trichrome(Thermoscientific). ImageJ(NIH) was used on lung images selected at 40x magnification to maximize visualization of representative alveolar tissue by a single blinded researcher. Images were binarized and area fraction of bioavailable tissue to airspace was calculated. The coronal sections of the whole lung and heart were analyzed in Orbit Image Analysis Software (Version-3.05,http://www.orbit.bio). Annotations of 1000 pixels per image were used to manually classify colors into collagen, background, and other tissue and implemented into the machine learning algorithm. Percent collagen was compared between groups by student's t-test(GraphPad Prism 7). RESULTS PM-exposed mice had significantly increased collagen after 1 month compared to PBS-controls using ImageJ analysis examining alveolar disease, $p<0.01$, Figure-1.1. Representative images of cardiac and lung tissue are shown in Figure-1.A-P delineating the classification process in Orbit. Annotation was performed on 100x sections randomly selected throughout image to isolate collagen. Whole image analysis showed no difference in CD in heart or lungs of PM-exposed mice compared to controls, Figure-1.2-3. CONCLUSIONS Although alveolar tissue analysis through ImageJ show significant difference in CD after exposure, this finding was not replicated in whole lung analysis with Orbit. This may be due to several reasons including being an under-powered study with only n=3 per exposure. Also, whole organ may not demonstrate widespread CD, whereas focal disease was found in alveolar tissue samples that relied more on subjective histologic review by a blinded researcher. Quantifying tissue remodeling by image analysis programs provides potential for an objective alternative to analyzing histological staining. Limitations of this procedure include that manual annotations of pixels give rise to a higher margin of error. Multiple planes of heart and lung sections will be examined in future analysis.

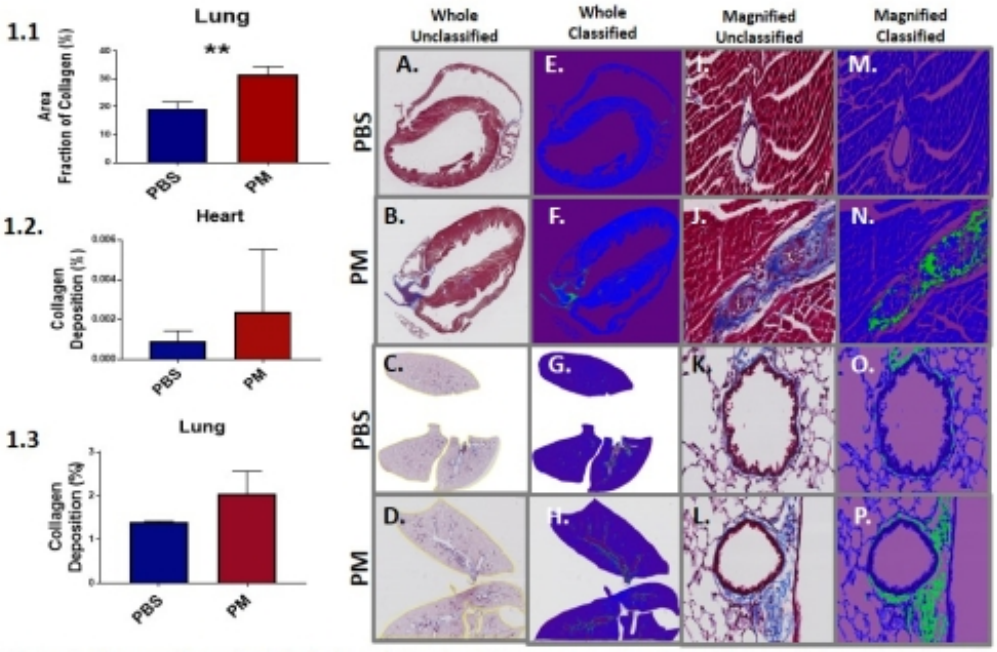

Figure 1. Mean and standard deviation of alveolar Collagen Quantification with **ImageJ** showed significantly greater collagen deposition (% Area Fraction) after PM exposure. **(1.1).** Unclassified whole cardiac and pulmonary specimens of both PM exposed and PBS controls **(A-D)** are shown with their pixel classified counterparts in the adjacent column **(E-H).** Myocardial wall of PM exposed and PBS controls at 100x magnification before **(I-J)** and after pixel classification **(M-N).** Alveolar tissue of exposed and unexposed groups shown at 100x magnification before **(K-L)** and after classification **(O-P).** Images analyzed with Orbit demonstrated trends of greater collagen deposition in PM-exposed group relative to controls **(1.2, 1.3).** **Purple:** Background; **Blue:** Alveolar/Myocardial Wall, **Green:** Collagen. ** P<0.01, **PM**-Particulate Matter; **PBS**-Phosphate Buffered Solution.

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