Background: Smoking status is often not known in autopsy studies. Potential surrogates for this information include severity of centriacinar emphysema (CE) and autofluorescent macrophages. Cigarette smoke contains many gaseous and particulate components, and a strong intracellular fluorescence of these components is present in unfixed alveolar macrophages from smokers and ex-smokers, but has not been studied in paraffin-embedded tissue.

Design: Using lung tissue from 100 random cases in the National Coal Workers' Autopsy Study, we reviewed the intensity (qualitative, 0-2+) and alveolar area fraction of autofluorescent macrophages (AAFAM) in unstained, deparaffinized sections at three different wavelengths (blue, ultraviolet (DAPI)) at 360-370 nm excitation, green (FITC) at 460-500 nm excitation, and red (Texas RED) at 532.5-587.5 nm excitation. Quantitative studies utilized a commercial image analysis system (Metamorph® Imaging System, Universal Imaging Corporation). In addition, the severity of CE (0-2+) was evaluated by 2 blinded observers, but focal emphysema (associated with coal workers'
pneumoconiosis) was not scored as part of this. These results were compared to the next-of-kin reported smoking status using SAS statistical software.

**Results:** CE and smoking status were not significantly associated (p=0.2658) in these miners. Qualitative assessment of autofluorescence intensity correlated with smoking status, as did AAFAM at 532.5-587.5 nm excitation (p<0.0001, Kruskal-Wallis test). The results at the other wavelengths were not significant. In addition, there was a linear decrease of AAFAM in relation to the number of years quit for ex-smokers (r=0.331, p<0.01).

**Conclusion:** While not usually observed in H&E stained sections, red autofluorescent pigments reported in unfixed macrophages from smokers are retained in paraffin-embedded tissues. Their presence in large numbers in unstained sections may be used as a surrogate for smoking status, but in our study of coal miners, severity of CE was not associated with smoking status.