Virchows Arch (2000) 437:422–428

ORIGINAL ARTICLE

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Centriacinar region inflammatory disease in young individuals: a comparative study of Miami and Los Angeles residents

Received: 6 September 1999 / Accepted: 9 May 2000

Abstract Semiquantitative measurements of chronic inflammation of the centriacinar region (proximal acinus of lung) were compared between 20 Miami and 18 Los Angeles residents (ages 11–30 years) for whom smoking histories were available. Mean extent and severity scores of four lung sites were higher for Los Angeles than Miami residents, with effect of city statistically significant for extent (P=0.02). Also, maximum scores for extent and severity by city were significantly greater for Los Angeles residents (P=0.02, each), but not by smoking history. Smokers did have higher scores for mean extent and severity (by lung site and smoking history), but neither this nor inclusion of smoking and city in the model reached significance. With respect to maximum extent and maximum severity scores, a stratified comparison of cities by smoking history showed a trend (not significant) toward higher scores for Los Angeles residents. Mean extent and severity scores for the lower lobe were higher for basilar sections than for apical sections (each P<0.001). Cumulative data indicate that expanded pathologic studies are essential for efforts to complete a convergence of epidemiological and experimental data implicating exceedences of the Federal ozone standard as a contributor to human lung injury.

Keywords Lung · Ozone · Centriacinar · Human · Autopsy

Disclaimer. Although the research described in this article has been supported by the United States Environmental Protection Agency through Cooperative Agreement CR 821576-01-0 with the University of Southern California, it does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Mention of trade names or commercial products does not constitute endorsement or recommendation for use

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Introduction

Our previous study had shown that severe centriacinar region (CAR) injury was frequently present in the lungs of young Los Angeles residents who died suddenly from vehicular accidents, homicide, or other violence [21]. The pathologic data obtained at that time converged with a large body of epidemiological and experimental data [1, 2, 3, 16] to suggest strongly that ambient levels of ozone caused human lung injury. An opportunity to verify that relationship became available through the cooperation of Medical Examiner Offices in Miami (Dade County) and Los Angeles (Los Angeles County). Those two metropolitan areas were selected for differing levels of ozone in their ambient community atmospheres. Los Angeles was the central consideration in view of frequent exceedences of the National Ambient Air Quality Standard (NAAQS) for ozone, i.e., 0.12 ppm 1-h average (parts per million), as opposed to infrequent exceedences in Miami. It should be noted that the 0.12-ppm NAAQS ozone standard is less stringent than the World Health Organization and California State 1-h mean standards for ozone of 0.056 ppm and 0.09 ppm, respectively. Of further pertinence, the newly introduced 8-h annual mean standard for ozone has been reported to be greater for Los Angeles than for Miami, namely 0.099 ppm and 0.034 ppm, respectively [14]. Complications in the implementation of this project reduced the number of cases available for demographic–pathologic correlations and this obviated definitive conclusions regarding adverse effects of ambient ozone exposure. However, the finding of a greater degree of lung injury in the Los Angeles res-

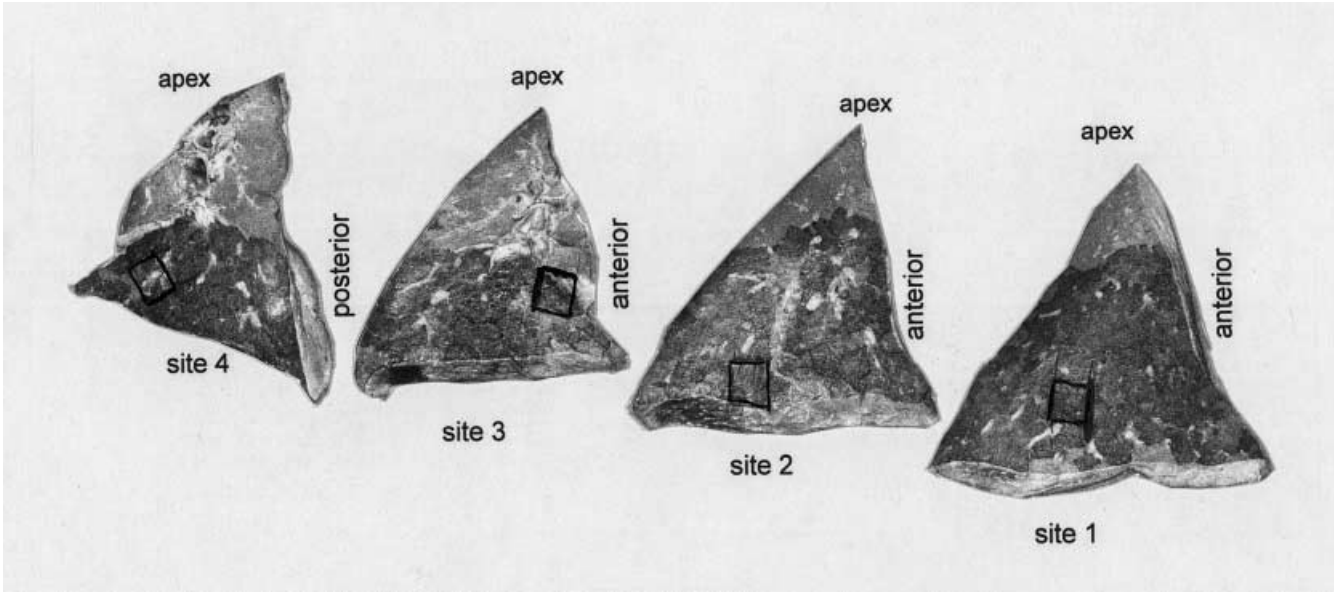


Fig. 1 Lung tissue sample site (sagittal sections). Site 1: a most lateral lung slice, with the section away from the pleura ("central") and in the posterior half of the lower lobe. Site 2: lateral portion of lobe, with section abutting pleura at the base of the lobe. Site 3: medial slice of lobe, with section from anterior aspect and at a level that is below hilum ("central"). Site 4: most medial slice of the lung, with section of lower lobe taken about midway between base and apex (most superoanterior section), and at the level of the hilum (note presence of main stem bronchus). This lung slice is oriented with its posterior margin facing the posterior margin of the adjacent lung slice, and includes a portion of the upper lobe and lingula

idents with and without smoking histories suggests an adverse environmental influence, with ozone highly suspect on the basis of cumulative evidence [1, 3].

Methods

Processing of lungs

Lungs were obtained from autopsies of young residents of Miami (Dade County) and Los Angeles (Los Angeles County) who died suddenly from homicide, vehicular accident, or other violence between 1995 and 1997. Eligibility criteria for lung accessioning were sudden death (at the scene or dead on arrival at the emergency room), residency in Los Angeles or Miami for at least 5 years, age from 12–30 years, no historical record or autopsy evidence of drug use, absence of disease on gross examination at autopsy, and autopsy examination less than 3 days postmortem. The main stem bronchus of a left lung (or right lung when left lung was not suitable due to trauma) was cannulated with a loose-fitting plastic tube and secured at the bronchial margin with an 18-gauge hypodermic needle inserted into a cork. The lung was immersed in a 90-l tank of 10% phosphate-buffered formalin and fixed by perfusion inflation at 25 cm water pressure for 72 h or more. After fixation, lungs at the Miami site were shipped to the principal investigator and accessioned along with lungs from Los Angeles' cases. Accessioning of lungs, gross examination, tissue processing, microscopic study, and semiquantitative measurements were carried out without the principal investigator being aware of the source of the lungs. The gross appearance of each lung was described before and after serial sectioning with the use of an electrical rotary slicer. A peer review of the research protocol [26] agreed with the following: use of the lower lobe of the left lung for the semiquantitative measurements; examination of four tissue sections from each lower lobe in accordance with specific topographical sites as shown in Fig. 1 [specifically, posterolateral central (site 1), lateral basal (site 2), anteromedial central (site 3), and superoanterior (site 4)]; and retention of the upper lobe and lingula for pending quantitative measurements of emphysema by means of diazo print methodology [28]. The sections were processed for paraffin embedding, sectioned at 4 μM, and stained with hematoxylin and eosin (H and E).

Semi-quantitative measurements

Semiquantitative measurements of CAR alterations were made in accord with the definition of CAR as a microscopic topographical unit involving the transition zone between the terminal membranous bronchioles and ductal-alveolar respiratory units [10]. A search for CAR alterations in tissue sections at each topographical site (Fig. 1) under 80× magnification was done by sequential field examination with the use of a stage micrometer. Severity of CAR alteration was based on one or more of a complex of findings, with at least a mild respiratory bronchiolitis (some degree of macrophage desquamation) as an invariable component. Additional alterations of the proximal acinus that may or may not have been present included lymphocytic-plasmacellular infiltration of bronchioloalveolar walls, interstitial bronchiolar and/or alveolar infiltration by macrophages, bronchiolar and/or airspace dilation (with or without acinic wall derangement), peribronchiolar fibrosis, and/or epithelial hyperplasia. Every instance of a CAR alteration was scored for severity and extent on a scale of 1–10. The degree of macrophage desquamation and other alterations, if present, determined an overall scoring. A score of 5 or more was considered to be a severe CAR alteration and this usually was a complex that included macrophage desquamation filling at least half of the lumen of a respiratory bronchiole, and/or one or more of the following alterations that alone was at least a score of 5: interstitial tissues infiltrated by macrophages, peribronchiolar fibrosis, epithelial hypertrophy and/or hyperplasia, and/or bronchioloalveolar dilatation with derangement of air spaces. Every CAR alteration in each lung tissue section (lung sites as shown in Fig. 1) was recorded as to severity (0–10; no CAR alteration at some sites), and a single score was assigned per section as a subjective integration ("best judgment mode") of all scores per section. The extent of CAR alteration was estimated as the relative proportion (0–10) of lung parenchyma per section containing altered CAR structures, with a score of 5 reflecting CAR alterations that involved approximately 50% of lung parenchymal tissue in that one section. Lungs

found to have overt disease on histopathologic examination, e.g., acute pneumonitis, desquamative interstitial pneumonitis, and eosinophilic pneumonitis, were excluded from the study, as were cases with marked congestion and/or hemorrhage related to trauma.

Demographic study

The collection of demographic data by next-of-kin interviews, either telephone or in person, was carried out by transplantation groups in Miami and Los Angeles under a contractual arrangement by NHEERL with SRA Technologies (Durham, N.C.). A questionnaire was prepared by SRA and used by both Miami and Los Angeles transplantation groups to collect data that included age, gender, ethnicity, residence, smoking, education, environment (outdoor and indoor), and occupation. Demographic data obtained were not made available to the principal investigator until after the recording and evaluation of all data from the pathologic study.

Statistical methods

Mean values for extent and severity were calculated on the basis of a single score from each lung section. The highest score per lung designated a maximum score and mean maximum scores were derived for severity and extent. A non-parametric Wilcoxon statistic was used to test for differences in maximum extent and severity scores between cities, between smokers and nonsmokers, and between the nine subjects 25 years of age and over and those under 25 years. A standard parametric repeated-measures analysis of variance (ANOVA) was used to analyze extent and severity scores at all sites, and this allowed tests for differences between sites, differences between groups (e.g., smokers and nonsmokers), and interactions between site and group. Analysis was restricted to those individuals with data from all four tissue sites of the lung, which reduced sample size. Analysis was performed using the SAS system.

Results

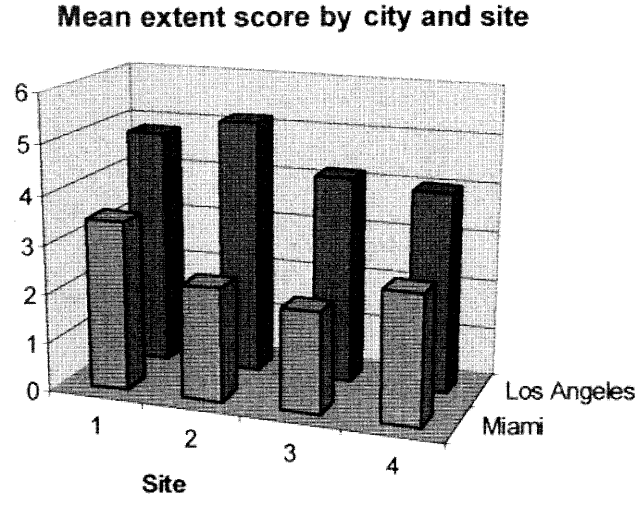
With respect to the Miami facility at the Dade County Medical Examiners Office, semiquantitative measurements were done for 30 (109 lung sections) of the 41 lungs; the remaining 11 were excluded due to technical difficulties. We correlated demographic data with semiquantitative measurements of alterations observed in 20 of the 30 lungs. The remaining 10 of the 30 were excluded from correlation since data were not available for one subject and the other 9 subjects were outside the 11–30 years age range (inclusive) of the protocol. For Los Angeles, 60 lungs were accessioned, and semiquantitative measurements were carried out for 39 (140 lung sections). A correlation of the measurements with demographic data was possible for 18 of the 39, with the remaining 21 excluded from correlation due to a lack of next-of-kin data, age outside of the protocol range, and/or technical difficulty in measurement. In some instances, less than four sections were selected for measurement due to excessive congestion or otherwise poor preservation.

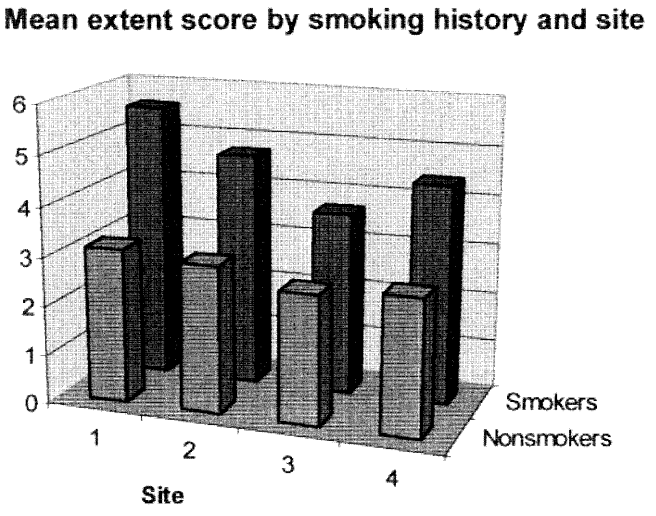
Ages for the two metropolitan groups in the correlation of demographic data with measurements ranged from 15 years to 26 years, with the exceptions of one Los Angeles resident 11 years of age and two Miami residents 28 years of age. The Miami sample (n=20) included significantly older subjects than the Los Angeles sample (n=18) and also significantly fewer smokers (P=0.02 and P=0.01, respectively; Table 1). Neither the distribution of ethnicity nor gender differed significantly by city. Since both age and smoking might be associated with the extent and severity of CAR alterations, both were treated as possible confounding factors (cf. results below).

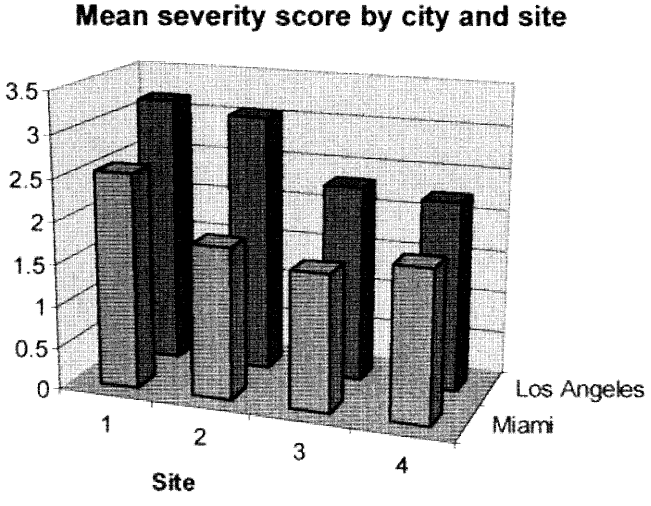
Semiquantitative measurements of CAR alterations for Miami and Los Angeles cases with accompanying demographic data were completed for 20 Miami cases (71 lung sections) and 18 Los Angeles cases (68 lung sections), for a combined total of 38 lungs and 139 lung sections. Mean extent and severity scores for the lungs of the Los Angeles residents were higher than those for Miami residents (Fig. 2), with the effect of city statistically significant for extent but not severity (P=0.03 vs P=0.1, respectively; Table 2). Figure 3 presents mean extent and mean severity scores by lung tissue site and smoking history. Smokers scored higher than nonsmokers at every lung tissue site, but the effect of smoking history was not significant for either mean extent or mean severity (Table 2). Further, when both city and smoking history were included in the model, neither effect was significant (results not shown). Both maximum extent and maximum severity with respect to city effect were significantly higher for Los Angeles subjects than those from Miami (P=0.02 for each; Table 3). There was a tendency (not significant) toward a greater maximum extent (P=0.09) but not maximum severity for smokers versus nonsmokers (Table 3). In order to correct for a probable confounding effect due to smoking, the comparison of Los Angeles and Miami residents was stratified by smoking history (Table 4). The trend of higher maximum extent and maximum severity scores in Los Angeles remained regardless of the smoking history, although it was not statistically significant. Age did not appear to affect maximum extent or severity. As noted, mean extent and severity scores differed significantly between the lower lateral portions of the lower lobe (lung

Table 1 Demographic variables

	Los Angeles n=18	Miami n=20	P value
Gender (male/female)	17/1	15/5	0.18
Ethnic background (white/black/Hispanic)	4/6/8	9/8/3	0.11
Smoking history (yes/no/unknown)	8/6/4	4/16/0	0.01
Age (≥25/<25 years)	1/16	7/10	0.04
Mean age (±SD)	19.1±3.6	22.6±4.3	0.02







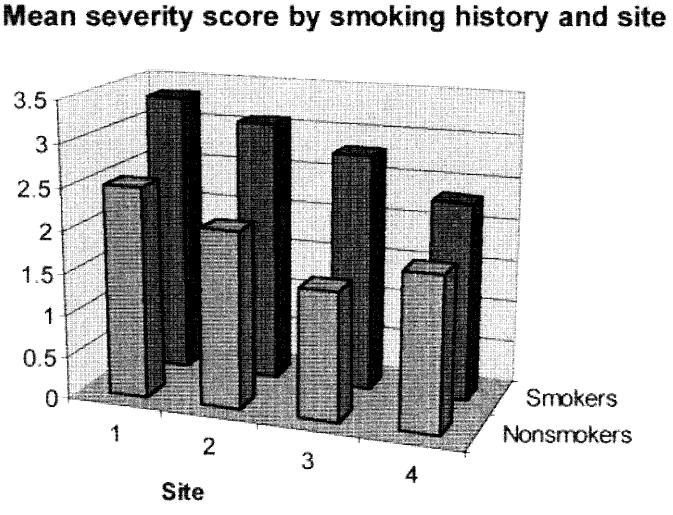


Fig. 2 Mean extent and severity scores by city and site

Fig. 3 Mean extent and severity scores by smoking history

sites 1 and 2) and the upper medial portions of the same lobe (lung sites 3 and 4); P=0.03 and P<0.001, respectively (Fig. 1; Table 2). The lung site differences were also evident when smoking history was included in the model (Table 2).

For Los Angeles residents, CAR alteration was found in all lung sections (4/4) of seven smokers, with 3/3 involvement for the eighth smoker. Of the ten Los Angeles nonsmokers, seven had all lung sections involved (4/4) and involvement for the remaining three was 2/3, 2/2, and 2/4. Overall, every lung from the 18 Los Angeles residents had some degree of CAR involvement (100%). With respect to four Miami smokers, the respective CAR ratios of lung section involvement were 4/4, 3/3, 1/1, and 0/4. For the 16 Miami nonsmokers, CAR alteration of all four lung sections was found in six, 3/3 in one, 3/4 in one, and from 1/3 to 0/4 in four others. Overall, CAR alteration was found in at least one lung section from 15 of the 20 (75%) Miami residents. When we analyzed all semiquantitative data for Los Angeles residents (10 nonsmokers, 8 smokers, and 21 subjects without a smoking history or outside the age range), 37 of the 39 (95%) subjects had some degree of CAR alteration in all of the four lung sections. The remaining two, one a nonsmoker and one of unknown smoking history, each had 2/4 (50%) lung sections involved. In comparison, the 30 Miami residents (16 nonsmokers, 4 smokers, 9 outside age limits, and 1 without demographic data), had 14 (47%) showing all four sections positive, which included 12 nonsmokers, one smoker, and one with smoking history unknown. In addition, 3/3 lung sections were positive for two smokers and one nonsmoker. Positivity for the remaining seven subjects included 1/1, 1/4 (2), 1/3, 2/4 (2), and 2/3. All sections were negative for 5 subjects, 1 smoker and 4 nonsmokers. Overall, some CAR alteration was found in 83% (25/30) of the Miami residents for which we had

Table 2 Repeated measures analysis of variance tests of city and smoking history (SHx) effects

	P value[a]
Mean extent	
Model: site[b] and city (*n*=29)	
City effect	0.03
Site effect	0.03
Site and city interaction	0.26
Model: site[b] and SHX (*n*=26)	
SHx effect	0.07
Site effect	0.02
Site and SHx interaction	0.47
Mean severity	
Model: site[b] and city (*n*=29)	
City effect	0.10
Site effect	<0.001
Site and city interaction	0.07
Model: site[b] and SHX (*n*=26)	
SHx effect	0.18
Site effect	0.004
Site and SHx interaction	0.26

[a]*P* value for testing the null hypothesis of no effect
[b]Topographocal site of lung tissue. Data limited to lungs with all four sites represented

Table 3 Maximum extent and maximum severity by city and smoking history

	Mean (±SD)
Maximum extent	
By city	
Los Angeles (*n*=18)	6.06±1.70
Miami (*n*=20)	3.80±2.88
Wilcoxon *P* value	0.02
By smoking history	
Smokers (*n*=12)	6.16±2.37
Nonsmokers (*n*=22)	4.15±2.63
Wilcoxon *P* value	0.09
Maximum severity	
By city	
Los Angeles (*n*=18)	3.89±1.08
Miami (*n*=20)	2.80±2.09
Wilcoxon *P* value	0.02
By smoking history	
Smokers (*n*=12)	3.75±1.60
Nonsmokers (*n*=22)	3.00±1.93
Wilcoxon *P* value	0.20

Table 4 Maximum extent and maximum severity by city, stratified by smoking history

	Smokers	Nonsmokers
Maximum extent		
Los Angeles (*n*=8 and 6, respectively)	5.33±1.87	6.88±3.20
Miami (*n*=4 and 16, respectively)	3.56±2.85	4.75±1.64
Wilcoxon *P* value	0.25	0.37
Maximum severity		
Los Angeles (*n*=8 and 6, respectively)	3.67±1.37	4.13±0.99
Miami (*n*=4 and 16, respectively)	3.00±2.45	3.00±2.45
Wilcoxon *P* value	0.17	0.66

semiquantitative data, which compares with 75% for the 20 lungs of the correlative study, as noted above.

Discussion

The main goal of the project was to determine whether young Los Angeles residents had a greater amount of CAR alteration than a comparable population in Miami and, if so, did it relate to Los Angeles County but not Miami frequently exceeding the federal ozone standard? That goal could not be achieved due to the relatively small number of subjects for which demographic data were available, but it was possible to complete semiquantitative measurements of CAR alterations involving 68 residents from two metropolitan areas and to correlate measurements with smoking histories for a combined 38 residents. The comparison studies point to a trend toward a greater degree of CAR alterations in Los Angeles residents that cannot be attributed solely to smoking since mean extent differed significantly with respect to city but not smoking effect (*P*=0.03; Table 2), as did maximum severity and maximum extent (*P*=0.02 each; Table 3). In the latter respect, analysis by maximum scores (single highest score per lung), as opposed to mean scores per lung section per lung, avoids diluting out severe but infrequent instances of CAR injury. Smoking did appear to have some influence on CAR alteration as evident by higher mean scores (not significant) for each of the four lung sites of Miami and Los Angeles smokers combined (Fig. 3; Table 2). The lack of a smoking significance is likely to be explained by the reduction in sample sizes when analysis was stratified for smoking history (Table 4).

There is no prior report comparing semiquantitative measurements of CAR alterations in young residents of two metropolitan areas, but there are related studies. Kleinerman and Rice [13] presented a preliminary report on emphysema, chronic bronchitis, and pigment deposition (parenchymal and pleural) based on an autopsy study of 330 males 16–65 years of age who died suddenly from natural, accidental, or violent causes. Their preliminary results indicated that “emphysema and chronic bronchitis do occur in young populations and that several environmental factors acting together may be related to the presence of the disease entities”. A subsequent study of the same material by Niewoehner et al. [15] was based on a selection of 39 subjects who did not have emphysema, of which 19 were smokers and 20 nonsmokers (mean age 25 years). They recorded the frequency of respiratory bronchiolitis, which they defined as “clusters of brown pigmented macrophages in the first-order and second-order respiratory bronchioles distal to the terminal membranous bronchiole (and) frequently associated with edema, fibrosis, and epithelial hyperplasia in the adjacent bronchiolar and alveolar walls”. All 19 smokers (100%) had respiratory bronchiolitis in at least three of five lung sections, and all five lung sections were involved in 84%, versus 25% and 10%, respectively, for the nonsmokers. The respiratory bronchiolitis of smokers and nonsmokers was

considered to be "still largely reversible", but with a proviso that the lesion may be a precursor of centriacinar emphysema. They also observed a smoking relationship to inflammation of membranous bronchioles.

The present study of CAR alterations differs in two main respects, the first being a definition of CAR alteration that includes the basic lesion of respiratory bronchiolitis (macrophage clusters within the lumina of the proximal acinus) and with or without one or more of the following: macrophage and/or chronic inflammatory cell infiltration of bronchiolar and/or alveolar walls, respiratory bronchiolitis associated with perivascular chronic inflammation, associated focal interstitial and/or peribronchiolar fibrosis, epithelial hyperplasia, and architectural derangement of acini. Second, our data, based on four lung sections versus five in the study by Niewoehner et al. [15], are not strictly comparable but the results suggest a somewhat greater extent of CAR alteration in our study of nonsmokers – specifically 100% for Los Angeles (10/10) and 75% for Miami (12/16), versus 25% (5/20) in the study by Niewoehner et al. [15]. Los Angeles' smoker data were comparable with those from the study by Niewoehner et al. [15], both having 100% of lung sections involved. However, extent for Miami smokers was 75% (3/4), reflecting one individual with no CAR alteration in any of the four lung sections.

The pathogenesis of CAR alteration is undoubtedly multifactorial, with respiratory infection and adverse environmental influences as two of the major considerations. In the latter respect, finding 100%, 75%, and 25% respiratory bronchiolitis with or without other CAR alterations in nonsmokers from Los Angeles, Miami, and the Niewoehner et al. study [15], respectively, raises the question of a more adverse health effect of environment for Los Angeles residents. However, we recognize that the 25% involvement reported by Niewoehner et al. [15], presumably for the Cleveland, Ohio, area, is not strictly comparable since that population was studied at least 20 years earlier (1974 report). Respiratory infection is highly suspect as a frequent underlying factor since it is virtually ubiquitous in the lungs of the general population. Childhood respiratory trouble [9] is an important example of poorly defined lung injury where CAR alteration and respiratory infection are likely to be playing some underlying role, with the immediate adverse effect of increased susceptibility to disease and the long-term potential of chronic lung disease in adult life [4]. It is noteworthy that respiratory bronchiolitis is known to have an adverse effect on lung function [27], and Thurlbeck has pointed out [25] that the inflammation may be "more than just a predecessor to centrilobular emphysema". Further, the limitation of CAR alteration primarily to the proximal portion of the acinus has the disproportionate consequence of adversely affecting airflow to the entire acinus. Another topographical consideration is the implication from the present findings that the basilar-lateral portion of the lower lobe is more susceptible than the apical-medial portion of that lobe to injury, i.e., lung sites 1 and 2 versus sites 3 and 4 (Fig. 1).

As noted above, the planned correlation of CAR alterations with ozone levels could not be carried out due to the relatively small number of cases available for study. Presumably, the limited number of cases in the study by Niewoehner et al. [15] also obviated the planned correlation of respiratory bronchiolitis with air pollution, specifically sulfur dioxide and particulates. However, our preliminary results are in accord with cumulative data suggesting that frequent exceedences of the federal ozone standard in Los Angeles has had an adverse effect on the human lung [1, 2, 3, 16]. Our finding of a significant city but not smoking effect has the support of a recent study of lungs showing injury to airways and membranous bronchioles that was apparently related to air pollution [22]. Specifically, the study compared 50 residents of Guarullos (a suburb of Sao Paulo, Brazil) with 34 residents of two agricultural communities. Although only particulates were measured, Guarullos is well known for exceptionally high levels of ozone in the community atmosphere. Additional support comes from an experimental study using bronchoalveolar lavage and bronchial biopsy showing a relationship between acute airway inflammation and exposure to ambient levels of ozone [3]. Of related importance, injury to the human nasal mucosa has been linked to high levels of ozone present in the ambient air of Mexico City [5]. With respect to Los Angeles and surrounding areas, a recent report on pulmonary function tests of children has shown a statistically significant relationship between exposure to ozone in community air and altered lung function in girls with asthma and in boys who spent more time outdoors [19]. Another study of school children suggests that "long term ambient ozone exposure might negatively influence lung function growth" [8]. In the latter respect, an exclusive role for ozone has been questioned by Tager [24] who believes that the more important contribution of that report [8] is providing "prospective evidence for an association between air pollution and lung function alteration in children". Numerous experimental animal studies add to the evidence linking ambient levels of ozone in community air to CAR alteration [3, 6, 7, 17, 20]. Of particular importance is the production of a mild form of respiratory bronchiolitis in primates exposed to 0.15 ppm ozone [11], a level essentially at the 0.12 ppm 1-h average of the federal standard for ozone.

The results strongly support the potential of human autopsy data, as part of a comprehensive multi-city study, to establish definitively that ambient levels of ozone in community air can injure human respiratory tissue. While there are formidable obstacles to be overcome with autopsy studies [17], we believe, as Tager [23] has stated, that the use of human postmortem specimens "is one of the essential elements in future epidemiologic studies of the health effects of O_3 exposure". Attention to CAR alteration is especially meaningful since the injury is positioned at the gateway to the distal acinus and is thus especially suspect for a disproportionately important role in accelerating the lung functional decline experienced by all adults. Further, a decline of lung structure, which CAR alteration

in part reflects, warrants special attention since it is a priori a far more sensitive measure of lung injury than is lung function testing. Thus, an inventory of human CAR alteration at autopsy can fulfill a critical yet largely unaddressed need, namely the acquisition of secular data on the rate of lung structural reserve loss in the general population. The loss of lung structural reserve, in large part a clinically silent disappearance of lung tissue, is the hallmark of emphysematous diseases and an important component of chronic obstructive pulmonary disease (COPD) in general. With COPD now the fourth leading cause of death in the United States [12] and rising in incidence, notably for women and males over 85 years in the United States [18] as well as worldwide [12], the magnitude of CAR alteration, overt and covert, should be taken into consideration in establishing ozone and other air pollutant standards.

Acknowledgements This study was supported by a Cooperative Agreement (CR 821576) between the National Health and Environmental Effects Research Laboratory (NHEERL), U.S. Environmental Protection Agency, and the University of Southern California. Assistance was provided by R. B. Everson, PhD, formerly of the Human Studies Division of the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, Raleigh, NC 27711; Lakshmanan Sathyavagiswaran, MD, Chief Medical Examiner of the Los Angeles County Department of Coroner, Joseph H. Davis, MD, and Roger E. Mittleman, MD, Scott Hanks, and Ann C. Hughes of the Dade County Medical Examiner's Office; Jeffrey A. Thomas and Margaret Acosta of the Lions-Doheny Eye and Tissue Bank. The project was approved by the Institutional Review Board of the Los Angeles County-University of Southern California Medical Center and the Research Committee of the Department of Coroner of Los Angeles County.

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