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Chronic Obstructive Pulmonary Disease among Lung Cancer-Free Smokers: The Importance of Healthy Controls

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Abstract

Background—The prevalence of chronic obstructive pulmonary disease (COPD) in smokers enrolled as “healthy” controls in studies is 10–50%. The COPD status of ideal smoker populations for lung cancer case-control studies should be checked via spirometry; however, this is often not feasible, because no medical indications exist for asymptomatic smokers to undergo spirometry prior to study enrollment. Therefore, there is an unmet need for robust, cost effective assays for identifying undiagnosed lung disease among asymptomatic smokers. Such assays would help excluding unhealthy smokers from lung cancer case-control studies.

Methods—We used the cytokinesis-blocked micronucleus (CBMN) assay (a measure of genetic instability) to identify undiagnosed lung disease among asymptomatic smokers. We used a convenience population from an on-going lung cancer case-control study including smokers with lung cancer ($n = 454$), smoker controls ($n = 797$), and a self-reported COPD ($n = 200$) contingent within the smoker controls.

Results—Significant differences for all CBMN endpoints were observed when comparing lung cancer to *All* controls (which included COPD) and *Healthy* controls (with no COPD). The risk ratio (RR) was increased in the COPD group vs. *Healthy* controls for nuclear buds (RR 1.28, 95%

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confidence interval 1.01–1.62), and marginally increased for micronuclei (RR 1.06, 0.98–1.89) and nucleoplasmic bridges (RR 1.07, 0.97–1.15).

Conclusion—These findings highlight the importance of using truly healthy controls in studies geared toward assessment of lung cancer risk. Using genetic instability biomarkers would facilitate the identification of smokers susceptible to tobacco smoke carcinogens and therefore predisposed to either disease.

Keywords

COPD; lung cancer; genetic instability; healthy controls

1. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) affects 8–10% of adults and 20–30% of the smoking population. More than 11 million people in the United States have been diagnosed with COPD. If undiagnosed cases are included, the number may rise to 24 million [1]. COPD causes serious long-term disability, and is the third leading cause of death in the United States [2]. As it is for lung cancer, smoking is the most significant risk factor for COPD, accounting for 80–90% of cases [1, 3, 4]. Smokers with mild or moderate COPD have a three-fold risk, increasing to a 10-fold risk with severe COPD, of developing lung cancer within 10 years, as compared to smokers with normal lung function [5, 6].

In a process similar to lung cancer pathogenesis, COPD's inflammatory processes are maintained for an unlimited time. Inflammation is accompanied by a continual cycle of DNA damage and repair, and a higher rate of cell turnover, increasing the likelihood of genetic errors [1]. The risk of developing COPD does not disappear after smoking cessation, thus explaining the persistence or even the progression of the disease in former smokers [7]. With airflow limitation, tobacco carcinogens are not fully cleared from the airway, thus increasing their opportunity to induce DNA damage, mutations, and persistent local inflammation, all of which have been implicated in the pathogenesis of lung cancer [8]. Genome-wide association studies have identified several candidate genes that are associated with host susceptibility for the development of lung cancer and/or COPD, some of which show significant overlap between the two diseases [1, 3, 4]. Moreover, Wang et al., using mediation analysis methods, reported that COPD is a mediating phenotype that explains part of the effect of smoking exposure on lung cancer [9].

Spirometry is the accepted method for diagnosing COPD. Studies have shown that the prevalence of COPD in smokers enrolled as healthy controls is 10–50%, depending on recruitment methods [10–12]. However, spirometry testing of apparently healthy smokers serving as controls may not be feasible for various reasons, including the absence of any medical indication in asymptomatic smokers, recruitment of smokers from non-medical settings (such as nursing homes), lack of insurance coverage, or lack of interest by the participants. Therefore, there is a need to identify smokers with underlying lung disease prior to enrollment in research studies geared at identifying determinants of lung cancer risk.

We hypothesized that mild or moderate COPD among apparently healthy smokers would correlate with genetic instability that could potentially modify the data from the overall control group. We used the multi-endpoint cytokinesis-blocked micronucleus (CBMN) genetic instability biomarker assay to measure the extent of genetic instability associated with cigarette smoke exposure in a convenience sample of clinic-based controls. The CBMN assay in human lymphocytes is one of the most commonly used methods for measuring DNA damage, an established risk factor for lung cancer. The CBMN biomarker assay measures exposure to DNA breakage in binucleated cells in terms of binucleated-micronuclei (BN-MN, originating from chromosome fragments or whole chromosomes that lag behind when the cell divides), binucleated-nucleoplasmic bridges (BN-NPB, formed when centromeres of dicentric chromosomes or chromatids are pulled to opposite poles of the cell), and binucleated-nuclear buds (BN-NBUDS, which are markers of gene amplification) [13–15].

The mechanisms relating COPD to lung cancer remain uncertain. We have previously reported that the CBMN endpoints are strong predictors of lung cancer risk [16–20]. Because COPD is thought to be a mediator between smoking and lung cancer [1, 3, 4, 9], we investigated whether the same endpoints could be used to predict COPD. The purposes of our investigation were to determine the extent of genetic instability observed in smoking controls with and without self-reported COPD, and to determine the extent of change in lung cancer risk when COPD is included in a smoking control population.

2. PATIENTS AND METHODS

2.1 Study Populations

The parent study, from which the study participants were drawn, is a lung cancer case-control study at the University of Texas MD Anderson Cancer Center. The demographic and clinical characteristics of the study population are detailed elsewhere [19, 20]. Briefly, patients with lung cancer were consecutively recruited, with newly diagnosed, previously untreated, and histologically confirmed lung cancer. Smoking controls were frequency matched to cases with respect to age (± 5 years) and sex. The controls were recruited from the Kelsey-Seybold Clinics, a multispecialty physician group. A risk-factor questionnaire was used to obtain medical history, family history of cancer, smoking habits, and occupational history. The questionnaire included a section dedicated to lung diseases, and self-reported COPD status was determined by asking the subjects if a doctor had ever informed them that they had chronic bronchitis, emphysema, or COPD. After giving informed consent, all participants donated 10 ml of blood. The study was approved by the institutional review boards of MD Anderson Cancer Center and Kelsey-Seybold Clinics.

The current study was conducted using a convenience subset of participants, and included only smokers (former or current) with lung cancer ($n = 454$) and smoker controls ($n = 797$) without lung cancer. Within the control sample was a smaller sample with positive history of COPD ($n = 200$). No spirometry information was available on the smokers with self-reported COPD, since these participants were asymptomatic, with no medical indication for performing the test prior to enrollment in the research study.

2.2 Lymphocyte Cultures for CBMN Assay

The CBMN assay was performed using the cytochalasin B technique described by Fenech and Morley [8], following the scoring procedures of the International Collaborative Project on Micronucleus Frequency in Human Populations (HUMN Project) [21]. Details of the technique and scoring are described elsewhere [16–20].

2.3 Statistical Analyses

“*All* controls” defines the overall control group of smokers with and without COPD. “*Healthy* controls” defines the control group of smokers without COPD. The Pearson Chi-squared test was used to test for differences between lung cancer cases and controls in categorical variables (e. g., sex, smoking intensity: light, moderate or heavy). For differences between means of continuous variables (e. g., age and CBMN endpoints), we used t-tests and one-way ANOVA. Post-ANOVA comparisons by Tukey’s Honest Significant Difference test were used to evaluate differences between lung cancer cases and *All* controls, lung cancer cases and COPD cases, and COPD cases and *Healthy* controls. Two-way ANOVA was used for differences among the lung cancer, *All* controls, COPD, and *Healthy* controls categories within smoking intensity groups. Tests for trend were conducted by modeling the lung cancer, COPD, and *Healthy* controls groups as a continuous variable in a standard regression model. We estimated the relative risk for lung cancer cases versus *All* controls and COPD participants versus *Healthy* controls, adjusted for age, sex, and smoking intensity, using Poisson regression. P values less than 0.05 were considered statistically significant. All analyses were conducted using R version 3.2.3 [22].

3. RESULTS

3.1 Study population

The demographic characteristics of our study population (lung cancer cases and *All* controls) are shown in Table 1. Unlike the parent study, only smokers were included, and we collapsed race/ethnicity into three categories (Caucasian, African American and other). The *All* controls group averaged about 5 years younger than the group with lung cancer (mean $\pm$ SE = 57.5 $\pm$ 0.48 years vs. 62.7 $\pm$ 0.48 years, respectively). Most participants were Caucasian, but 17% of the lung cancer group were African American, compared to 10% of the *All* controls group. Former smokers comprised 51% of the lung cancer group, as compared to 43% of the *All* controls group. More heavy smokers were in the lung cancer group (55%) than in the *All* controls group (31%).

3.2 CBMN endpoints among the study groups

The frequency distribution of the CBMN endpoints among the lung cancer cases and *All* controls is also shown in Table 1. The extent of genetic damage was significantly higher among the lung cancer cases, for all the endpoints tested. Mean values of the CBMN endpoints among the three groups are shown in Table 2. The lung cancer cases had significantly higher mean counts of CBMN endpoints than the COPD participants and *Healthy* controls for BN-MN (mean $\pm$ SEM = 3.53 $\pm$ 0.045; 1.91 $\pm$ 0.060, and 1.78 $\pm$ 0.035, respectively) (P_{trend} 0.001). Higher mean values were also found for BN-NPB in the lung

cancer cases (mean $\pm$ SEM = 4.26 ± 0.035) when compared to COPD (1.03 ± 0.039) and *Healthy* controls (0.97 ± 0.025) ($P_{\text{trend}} = 0.001$). The same pattern was seen for BN-NBUDS: lung cancer cases (mean $\pm$ SEM = 0.59 ± 0.043); COPD (0.38 ± 0.034); *Healthy* controls (0.30 ± 0.019); ($P_{\text{trend}} = 0.001$). When stratified by smoking intensity, lung cancer cases had higher mean counts for all CBMN endpoints compared to COPD and *Healthy* controls (Table 2).

Results from Poisson regression models created to estimate the relative risk for lung cancer, adjusted for age, sex, race, and smoking intensity, are shown in Table 3. The strongest association was found for BN-NPB, with lung cancer cases having over 4 times greater risk than the control groups. Comparing lung cancer cases to *All* controls showed a relative risk (RR) and 95% confidence interval (CI) of 4.36, 4.17–4.57. The risk was slightly elevated when lung cancer cases were compared to the *Healthy* controls: RR and 95% CI = 4.46, 4.24–4.71. Similarly, lung cancer cases displayed nearly 2 times the risk for BN-MN (RR and 95% CI = 1.98, 1.88–2.08) and BN-NBUDS (RR and 95% CI = 1.95, 1.64–2.32), compared to *Healthy* controls. The relative risk for BN-MN and BN-NBUDS were slightly higher when comparing lung cancer cases to *All* controls (BN-MN: RR and 95% CI = 1.95, 1.86–2.04; BN-NBUDS: RR and 95% CI = 1.79, 1.51–2.09, respectively). Comparing the risks for COPD and *Healthy* controls, only BN-NBUDS (RR and 95% CI = 1.29, 1.02–1.62) showed a significantly increased risk, while BN-MN and BN-NPB were borderline (RR and 95% CI = 1.05, 0.97–1.13 and 1.05, 0.95–1.16, respectively).

4. DISCUSSION

Lung cancer and COPD are complex, phenotypically variable diseases, with a potential for mechanistic overlap that has led researchers to suggest that COPD be considered a sub-phenotype of lung cancer [1, 3, 4]. COPD is similar to lung cancer in that both are characterized by an interaction between host genetic susceptibility and environmental exposures. Prospective studies have shown that smokers with spirometry-diagnosed COPD have a 4- to 6-fold increased risk of lung cancer compared to smokers with normal lung function or smokers randomly selected from the community [1, 3, 4]. Furthermore, genetic, molecular, and epidemiologic findings suggest that the susceptibility of smokers to COPD and lung cancer may involve overlapping molecular and pathogenetic pathways [4, 23, 24].

We have previously reported that the CBMN endpoints are predictors of lung cancer risk among smokers [16–20]. Here we tested whether the CBMN endpoints are predictive of COPD status among smoker controls. Our results showed that smokers with self-reported COPD had a higher frequency of CBMN endpoints, reflecting an increase in genetic instability, as compared to *Healthy* controls. However, only the BN-BUDS endpoint was associated with a significantly elevated relative risk of COPD. The BN-MN and BN-NPBs were associated with a non-significant increase in risk, most likely due to the limited sample size of the COPD population in our study. More importantly, our results showed a higher risk of lung cancer associated with the CBMN endpoints compared to the *Healthy* control group (the smoker control group with no COPD), highlighting the importance of excluding smokers with undiagnosed lung disease such as COPD from being recruited as controls.

Our findings are supported by observations reported by Maluf et al., [25] who found that COPD patients had higher levels of DNA damage measured by two different assays, the Comet and micronucleus assays. These reported findings, unlike ours, were highly significant for the COPD patients, since their study was designed specifically to recruit COPD patients with different stages of the disease (moderate to very severe COPD), who were diagnosed via clinical history, physical examination, and spirometry. In a recent study on buccal cells, da Silva et al. [26] reported the presence of apoptosis and necrosis among clinically diagnosed COPD patients. In our study, we used a convenience sample of self-reported COPD participants, recruited through an on-going lung cancer study, as part of the smoker control group. Recruiting smokers without clinical evaluation for underlying lung disease is a common practice in many epidemiological case control studies, which may in turn affect the estimated cancer risk.

Although cigarette smoke is a well-documented environmental exposure factor that accounts for 90% of COPD and lung cancer cases, only 15% of smokers develop lung cancer, and 30% develop clinically significant COPD [3, 4]. Since only a minority of cigarette smokers develop either disease, the argument for host genetic susceptibility to either disease is robust, as evidenced by genome-wide association studies that have identified several candidate genes that are associated with either lung cancer, COPD, or both [1, 3, 4]. When classification of COPD is based on symptoms and medical history alone, approximately 50%–70% of COPD cases are missed due to the lack of lung function testing [1, 10, 27]. To accurately distinguish healthy and resistant smoker controls for COPD or lung cancer studies, smokers should have their COPD status confirmed either by spirometry or, as we did here, by robust DNA damage assays that are reflective of sensitivity to tobacco carcinogens as well as host susceptibility to the disease. Both lung cancer and COPD are considered “dynamic” phenotypes that occur almost exclusively after exposure to cigarette smoke and other air pollutants [1, 3, 4]; therefore, having an untainted smoker control group is crucial, because the expression of genes conferring “susceptibility” or “resistance” to lung cancer and/or COPD is dependent on the effects of smoking exposure.

Our study is not without limitations. Although we used a matched case-control design, our changes in stratification for this sub-study (e.g., omitting ‘never smokers’ and combining ‘Hispanics’ with ‘others’) led to differences across the groups, and in some analyses, small sample sizes may have led to attenuation in power. A proportion of our “healthy” controls may have had some form of COPD; however, this is more likely to produce a bias towards the null and is unlikely to have led to false positive results. The COPD participants were self-reported, not spirometry-diagnosed or staged according to the GOLD diagnosis, since the participants were recruited under the premise of being healthy smoker controls and were asymptomatic, with no medical indication for spirometry evaluation.

4.1. Conclusion

Our present study highlights the importance of not drawing from a convenience sample, and selecting smoke-resistant controls as the healthy control population when designing lung cancer and/or COPD case-control studies. We show that undiagnosed COPD smokers can harbor genetic damage, due to susceptibility to tobacco smoke carcinogens that may be a

mediator in lung carcinogenesis [1, 3, 4, 9]. Furthermore, using affordable, robust genetic instability biomarkers, such as the CBMN endpoints, would facilitate the identification of high-risk smokers who are susceptible to tobacco smoke carcinogens. Identifying this high-risk sub-group would have value for developing interventions and disease prevention. Consequently, understanding of the underlying mechanisms associated with lung cancer and COPD could potentially improve clinical and economic outcomes of both diseases.

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Table 1

Distribution of study participants by selected characteristics

Variables	Lung Cancer	^a All Controls	p-value
	N = 454	N = 797	
Age (y), mean $\pm$ SE	62.7 $\pm$ 0.48	57.5 $\pm$ 0.48	<0.01
Sex, n (%)			
Male	224 (49.3)	384 (48.2)	0.737
Female	230 (50.7)	413 (51.8)	
Race, n (%)			
Caucasian	365 (80.4)	673 (84.4)	<0.01
African American	78 (17.2)	83 (10.4)	
Other	11 (2.4)	41 (5.1)	
Smoking in Pack Years ^b , mean $\pm$ SE	48.5 $\pm$ 1.390	31.3 $\pm$ 1.404	<0.01
Smoking, n (%)			
Former	231 (50.9)	340 (42.7)	<0.01
Current	223 (49.1)	457 (57.3)	
Smoking Intensity, n (%)			
Light ($\leq$ 20 pack years)	99 (22.1)	352 (44.6)	<0.01
Moderate (< 20 to < 40 pack years)	106 (23.7)	193 (24.4)	
Heavy ($\geq$ 40 pack years)	243 (54.2)	245 (31.0)	
CBMN Endpoints, mean $\pm$ SE			
Micronuclei (BN-MN)	3.53 $\pm$ 0.045	1.81 $\pm$ 0.030	<0.01
Nucleoplasmic Bridges (BN-NPBs)	4.26 $\pm$ 0.035	0.99 $\pm$ 0.021	
Nuclear Buds (BN-NBUDS)	0.59 $\pm$ 0.032	0.32 $\pm$ 0.017	

^a All controls include COPD participants^b Missing data on 13 study participants: 6 patients with lung cancer and 7 controls

Table 2

Multiple comparisons of CBMN endpoints, split by smoking intensity

	Lung Cancer			COPD			Healthy Controls			
	N	Mean	Std Error	N	Mean	Std Error	N	Mean	Std Error	
Micronuclei (BN- MN)										
<i>Overall</i>	448	3.53	(0.045)	200	1.91	(0.060)	590	1.78	(0.035)	$P_{trend} < 0.001$
<i>Light</i>	99	3.52	(0.093)	63	1.95	(0.112)	289	1.72	(0.051)	
<i>Moderate</i>	106	3.55	(0.100)	50	1.88	(0.133)	143	1.83	(0.069)	
<i>Heavy</i>	243	3.53	(0.059)	87	1.92	(0.086)	158	1.81	(0.072)	
	P = 0.969			P = 0.904			P = 0.371			
Bridges (BN- NPB)										
<i>Overall</i>	448	4.26	(0.035)	200	1.03	(0.039)	590	0.97	(0.025)	$P_{trend} < 0.001$
<i>Light</i>	99	4.36	(0.752)	63	1.05	(0.694)	289	0.99	(0.035)	
<i>Moderate</i>	106	4.22	(0.746)	50	1.00	(0.857)	143	1.01	(0.053)	
<i>Heavy</i>	243	4.24	(0.049)	87	1.06	(0.061)	158	0.90	(0.048)	
	P = 0.315			P = 0.819			P = 0.255			
Buds (BN- NBU DS)										
<i>Overall</i>	448	0.59	(0.043)	200	0.38	(0.034)	590	0.30	(0.019)	$P_{trend} < 0.001$
<i>Light</i>	99	0.55	(0.071)	63	0.35	(0.061)	289	0.34	(0.028)	
<i>Moderate</i>	106	0.68	(0.069)	50	0.36	(0.069)	143	0.29	(0.038)	
<i>Heavy</i>	243	0.56	(0.044)	87	0.42	(0.056)	158	0.26	(0.036)	
	P = 0.251			P = 0.669			P = 0.216			

Table 3

Poisson regression of CBMN endpoints, lung cancer, and COPD, adjusted for age, sex, race, and smoking intensity

Lung Cancer vs. ^aAll Controls	Relative Risk (95% Confidence Interval)
Micronuclei (BN-MN)	1.95 (1.86–2.04)
Bridges (BN-NPB)	4.36 (4.17–4.57)
Buds (BN-BUDS)	1.79 (1.51–2.09)
Lung Cancer vs. ^bHealthy Controls	Relative Risk (95% Confidence Interval)
Micronuclei (BN-MN)	1.98 (1.88–2.08)
Bridges (BN-NPB)	4.44 (4.22–4.68)
Buds (BN-BUDS)	1.95 (1.64–2.32)
COPD vs. ^bHealthy Controls	Relative Risk (95% Confidence Interval)
Micronuclei (BN-MN)	1.05 (0.97–1.13)
Bridges (BN-NPB)	1.05 (0.95–1.16)
Buds (BN-BUDS)	1.29 (1.02–1.62)

^a All controls include COPD participants

^b Healthy controls do not include COPD participants