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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% 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

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would facilitate the identification of smokers susceptible to tobacco smoke carcinogens and therefore predisposed to either disease.



## 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

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