Lung Cancer Risk Among Female Textile Workers Exposed to Endotoxin

George Astrakianakis, Noah S. Seixas, Roberta Ray, Janice E. Camp, Dao Li Gao, Ziding Feng, Wenjin Li, Karen J. Wernli, E. Dawn Fitzgibbons, David B. Thomas, Harvey Checkoway

Background

Reduced risk of lung cancer among workers in the cotton textile industry has been observed since the 1970s. Bacterial endotoxin, a contaminant of raw cotton fiber and cotton dust, has been proposed as a protective agent that may act through the innate and acquired immune systems. We examine the association between endotoxin exposure and lung cancer risk in a cohort of female textile workers.

Methods

We conducted a case–cohort study nested within a cohort of 267,400 female textile workers in Shanghai, China. We compared the cumulative exposure histories of 628 case patients diagnosed with incident lung cancer from January 1, 1989, through December 31, 1998, with those of a lung cancer–free reference sub-cohort of 3,184 workers who were frequency matched by 5-year age-groups to all cancer patients in the cohort. Cumulative endotoxin exposure for all participants was based on historic measurements and on additional measurements for this study. Relative risks and 95% confidence intervals (CIs) were estimated by hazard ratios (HRs) from Cox proportional hazards models. We conducted exposure–response trend analyses by use of cumulative exposures with lag times of 0, 5, 10, 15, or 20 years to account for disease latency. All analyses controlled for age and smoking status. All statistical tests were two-sided.

Results

Cumulative exposure to endotoxin was strongly, statistically significantly, and inversely associated with lung cancer risk. The inverse trend was greatest with a 20-year lag time, for which highest endotoxin exposure was associated with a statistically significantly 40% less risk of lung cancer (HR = 0.60, 95% CI = 0.43 to 0.83; \( P_{\text{trend}} \) across quintiles = .002) than nonexposure. From a reported population rate of lung cancer among women in Shanghai of 19.1 per 100,000 for the year 2000 and the estimated reduction in risk of lung cancer observed for 20 years of endotoxin exposure in this population of workers, the incidence of lung cancer in this cohort was reduced by approximately 7.6 per 100,000 (range = 3.2–10.9 per 100,000).

Conclusions

Long-term and high-level exposure to endotoxin, compared with no exposure, appears to be associated with a reduced risk of lung cancer in this cohort.

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Exposure to cotton dust has long been associated with adverse respiratory effects and diminished lung function, which is most evident as byssinosis, a chest tightness experienced by workers on the first day back after a weekend or vacation break (1–3). Most studies indicate that adverse respiratory effects are more closely associated with bacterial endotoxin contamination than with cotton fiber dust (4–6). Endotoxins are complex, heat-stable lipopolysaccharide constituents of the outer membranes of Gram-negative bacterial cell walls. They consist of three main components: the O-specific polysaccharide, the core polysaccharide, and lipid A (which is the least variable but most biologically active component) (7,8). The potentially anticarcinogenic effect of endotoxin appears to be specific to lipid A, as shown by the increased survival of tumor-bearing animals or the reduced growth rate of established tumors inoculated with this component of the lipopolysaccharide (9–11).

Lung cancer mortality among textile workers has received considerable attention since Henderson and Enterline (12) reported an unexpected deficit in such mortality among male cotton workers in the state of Georgia (standardized mortality ratio [SMR] = 55, where an SMR of 100 would have indicated no difference in the observed number of deaths as compared with the population rate). Reduced lung cancer risk was also observed in a study of cotton textile workers in North Carolina (SMR = 40) (13).
In both studies, the prevalence of smoking among the textile workers was reported to be similar to that of the US male population during the study period. In Britain, Hodgson and Jones (14) observed reduced mortality among both male and female textile workers (SMR = 75, 95% CI = 51 to 106 and SMR = 79, 95% CI = 39 to 141, respectively) and statistically significantly reduced lung cancer mortality among nonsmoking textile workers (SMR = 12 and SMR = 13, respectively). They also noted a greater than expected mortality among male heavy smokers (i.e., >15 cigarettes per day; SMR = 176, for <20 years exposed) that declined with the number of years worked (SMR = 118, for 20–40 years worked; and SMR = 110, for >40 years worked). This pattern was not evident among female textile workers who smoked heavily. Among female textile workers in Shanghai, we found a statistically significantly decreased incidence of lung cancer (standardized incidence ratio [SIR] = 0.8, 95% CI = 0.7 to 0.9, where a SIR < 1.0 indicates an observed incidence rate that is lower than the expected rate for the population of Shanghai) and a prevalence of smoking of 4% (15), a value similar to those in previous reports (16). In contrast, a recent study of cancer incidence among a cohort of Lithuanian textile workers found a nonstatistically significant increase in lung cancer risk among men (SIR = 1.4, 95% CI = 1.0 to 1.8) and women (SIR = 1.1, 95% CI = 0.5 to 2.2) (17). The population rate of lung cancer among women in Shanghai has been reported as 19.1 per 100,000 for the year 2000 (18).

Findings from population-based case-control studies of lung cancer are generally supportive of inverse associations with textile industry employment. In a population-based case-control study of cancer incidence in Shanghai, Levin et al. (19) reported reduced risks of lung cancer among male (odds ratio [OR] = 0.7, 95% CI = 0.6 to 0.9) and female (OR = 0.8, 95% CI = 0.6 to 1.0) textile workers. They also observed reduced lung cancer risks among male (OR = 0.7, 95% CI = 0.5 to 1.1) and female (OR = 0.8, 95% CI = 0.4 to 1.6) smokers who worked in the textile industry, compared with smokers employed elsewhere. Wu-Williams et al. (20) observed a reduced risk (OR = 0.5, 95% CI = 0.3 to 1.0) among long-term (>11 years) female textile workers in northern China, after adjustment for age, smoking status, education, and geographic location. However, Wang et al. (21) found no association among women employed in the textile industry and lung cancer risk in Tianjin, China (OR = 1.0, 95% CI = 0.9 to 1.2, after adjustment for age and smoking).

Reduced lung cancer risks have been observed in other occupations that involve endotoxin exposure from sources other than cotton—most notably farming (22). In Italy, Mastrangelo et al. (23,24) observed statistically significantly reduced lung cancer mortality among dairy farmers (SMR = 0.5, 95% CI = 0.3 to 0.7) but not among crop or orchard farmers (SMR = 0.8, 95% CI = 0.5 to 1.3). Reduced mortality has also been observed among workers in the automobile industry exposed to synthetic machining fluids that are contaminated with endotoxin (OR = 0.6, 95% CI = 0.4 to 0.9) (25–27). The objective of this study was to examine the quantitative dose–response relation for endotoxin exposure associated with lung cancer risk among a cohort of female textile workers in Shanghai, China.

Participants and Methods

Case–Cohort Study Design
We conducted a case–cohort study that was nested in a cohort of female textile workers in Shanghai, China. This cohort was originally enrolled in a randomized trial to test the efficacy of breast self-examination on reducing breast cancer mortality, as described previously (28). Briefly, 267,400 women were enrolled from 326 textile factories in Shanghai, China. Women included in this cohort were active and retired female employees of member factories of the Shanghai Textile Industry Bureau and were born between January 1, 1925, and December 31, 1958. At enrollment, all women were administered a questionnaire eliciting basic demographic, lifestyle (including smoking history), and reproductive history information. Subsequently, the cancer incidence patterns of these workers were investigated to determine whether there were associations between numerous site-specific cancers and textile industry exposures (15).

Cohort Enumeration, Follow-up, and Case Ascertainment
Cohort members were followed from their dates of enrollment in the breast self-examination trial (from January 1, 1989, through December 31, 1991) until the date of lung cancer diagnosis, date of death, date that they left the Shanghai Textile Industry Bureau, or the end of follow-up (December 31, 1998). Follow-up was truncated for only 96 subjects when they left the textile industry (28).

Diagnoses were confirmed through computerized matching of records from the Shanghai Textile Industry Bureau for case patients with those from the Shanghai Cancer Registry or, when no computer match was found, from medical record review. A total of 641 case patients with lung cancer were identified by the tumor and death registry of the Shanghai Textile Industry Bureau, and
lung cancer was confirmed in 523 of them. The complete work history records of 13 subjects could not be obtained, and so these subjects were not included in the analysis. Separate analyses were performed for all 628 case patients with work history data and for the subset of 523 case patients with confirmed diagnoses.

A comparison subcohort (n = 3188) was randomly selected from the entire cohort for comparisons with case patients’ exposures. The subcohort was frequency matched in 5-year strata to the age distribution among all cancer patients, including those with lung cancer or cancer at 14 other sites. The size of this subcohort was set to be roughly twice the size of the largest case group, i.e., that with breast cancer. The lung cancer analysis was one of 15 site-specific comparisons with the subcohort in nested case–cohort analyses. In the lung cancer analysis, three members of the randomly selected subcohort were also identified as case patients with lung cancer and, therefore, contributed “time at risk” from entry until the date of their diagnosis. Factory employment records were reviewed to abstract subjects’ job assignments and start and end dates (29). Because work history data could not be obtained for one additional subcohort member, the total number of non–lung cancer subjects included in the subcohort was 3184 workers.

Exposure Assessment

Exposure was assessed and validated as described previously (30). Briefly, quantitative exposures for endotoxin were reconstructed from more than 2400 historic cotton dust measurements made by Chinese factory inspectors and collected from 56 cotton factories between 1975 and 1999. These data were used to estimate job- and period-specific cotton dust exposures. The cotton dust estimates were then converted to endotoxin estimates by use of job-specific endotoxin surveys performed by Christiani et al. (6,31–33) and measurements that we made in three Shanghai factories in 2002 (34). Subjects’ cumulative exposures to cotton dust and endotoxin were based on the sum of estimated exposure for all jobs held by each subject. The correlation coefficient between cumulative exposure estimates of cotton dust and endotoxin is moderate (κ = 0.59) (30).

Ethics Approval

This study was approved by the Institutional Review Boards of the University of Washington, the Fred Hutchinson Cancer Research Center, and the Station for Prevention and Treatment of Cancer of the Shanghai Textile Industry Bureau in accordance with an assurance filed with the Office for Human Research Protections of the US Department of Health and Human Services. Work histories for 95% of study subjects were obtained through review of paper records. For the remainder, subjects were approached directly at home or at work or by telephone, and they provided verbal consent regarding their participation.

Statistical Analysis

The Cox proportional hazards model, adapted for the case–cohort design, was used to estimate dose–response trends for lung cancer in relation to cumulative exposure to cotton dust and endotoxin (35–37). Hazard ratios (HRs) were determined for subjects by use of cumulative cotton dust and endotoxin exposure estimates; however, only analyses related to endotoxin exposure are presented. In the endotoxin analyses, we eliminated workers (26 case patients and 149 subcohort members) who had potential occupational exposures to endotoxin from jobs in noncotton factories (e.g., early-stage wool processing, metal machining, or sanitation) because exposure quantification was possible only for cotton factory jobs. We computed relative risks, estimated as hazard ratios and 95% confidence intervals, with adjustments for age as a continuous variable and smoking status (current, former, or never) as determined at the baseline interview. Categories of endotoxin exposure were based on quintiles of subjects among those whose exposure was assessed as greater than zero. Tests for dose–response trends were assessed by use of the median values of exposure within each category for the subcohort (38), and a two-sided Wald test was used to test the slope of the line. Separate trend tests were computed by alternatively considering the nonexposed group and the lowest exposed group as the reference category to control for potential differences in baseline lung cancer risks between workers from cotton and other factories. There were no substantive differences in the trend results, and therefore only those for which the nonexposed group was the control are presented.

The effect of cumulative endotoxin exposure on lung cancer risk among subjects stratified by smoking history was also examined. Categories of cumulative endotoxin exposure as defined above (i.e., quintiles) were collapsed into low (exposure categories 1 and 2), medium (exposure categories 3 and 4), and high (exposure category 5), and subjects were stratified according to “never smokers” (n = 3637) and “ever smokers” (n = 208), as identified at baseline. Finally, we repeated each analysis by lagging cumulative exposures by 5, 10, 15, or 20 years to take into account disease latency. All statistical tests were two-sided and were performed with Stata version 8.2 (Stata Corp LP, College Station, TX).

Results

Demographic Characteristics

The median number of years worked by the study subjects was 26 years (range = 0.3–46.6 years). On average, the women worked at 1.9 jobs (range = 1–16 jobs) and in 1.2 factories (range = 1–5 factories), although 80% of the subjects had worked in at most two jobs and approximately the same proportion had worked in only one factory. Case patients were older, held slightly more jobs, and worked about the same number of years as the subcohort members. The proportion of subjects who were classified as ever smokers was statistically significantly higher among case patients (12%) than among subcohort members (6%) (Table 1). The risk of lung cancer was statistically significantly higher among current smokers (age-adjusted HR = 2.33, 95% CI = 1.69 to 3.22) than among never smokers and increased with duration of smoking (for smoking of ≥30 years, HR = 2.82, 95% CI = 1.82 to 4.38, compared with never smoking).

Dose–Response Trends for Endotoxin

At the end of follow-up, 1099 subjects were classified as unexposed to endotoxin because we determined that they worked in non-cotton factories or in cotton factories but at nonproduction jobs with no endotoxin exposure. Among the remaining 2538 subjects, the cumulative exposures to endotoxin ranged from 52.2 to 109,030...
endotoxin units (EU)/m$^3$ × years among case patients and from 6.2 to 144.360 EU/m$^3$ × years among noncase subcohort members.

Strong inverse dose–response trends for the risk of lung cancer with cumulative exposure to endotoxin were observed for all lag times analyzed (Table 2) when the group with the highest cumulative endotoxin exposure was compared with the group with no exposure (e.g., for no lag time, HR = 0.70, 95% CI = 0.52 to 0.95, $P_{\text{trend}} = .02$; for a 20-year lag time, HR = 0.60, 95% CI = 0.43 to 0.83, $P_{\text{trend}} = .002$). The dose–response trends were not materially different when the analysis was restricted to the 523 patients with a confirmed lung cancer diagnosis (for 20-year lag, HR = 0.54, 95% CI = 0.37 to 0.79; $P_{\text{trend}} = .02$). Moreover, similar inverse dose–response trends were observed among women who ever smoked and women who never smoked (Table 3), although results for ever smokers were statistically imprecise because of the small number of smokers in the cohort.

Discussion

We found that, among women employed in the Shanghai textile industry, exposure to endotoxin was associated with a decreased risk of lung cancer. The magnitude of the decrease in risk increased with level of cumulative exposure and with increasing time of exposure. The inverse association became increasingly strong as recent exposures were lagged (i.e., discounted), indicating that the observed protective effect of exposure was acting at an early stage during lung carcinogenesis. Although generally consistent patterns of reduced lung cancer incidence and mortality among workers in cotton factories have been reported since the 1970s, our study is the first, to our knowledge, to quantify the associations with endotoxin levels. The inverse dose–response trends for cotton dust exposure (data not shown) were similar to those observed for endotoxin, as expected, given that the endotoxin estimates were scaled conversions of the cotton dust estimates. Other components of cotton dust exposure may also be responsible for reduced risk; however, experimental and epidemiologic evidence indicates that endotoxin is the most biologically active component and, thus, a plausible candidate for a protective factor (5, 23, 25).

Potential anticarcinogenic effects of endotoxin are probably mediated by the innate and acquired immune systems, although specific mechanisms have yet to be elucidated. Endotoxin exposure results in Th-1 (neutrophil-induced) inflammation that can inhibit tumor growth (39), although there is also evidence that such prolonged inflammation can also accelerate tumor growth (40). Toll-like receptors (TLRs) are signaling proteins located on the surface of various immune cells such as macrophages; TLR4 is the main lipopolysaccharide receptor and is essential for endotoxin recognition (41). The immune response, which is enhanced by lipopolysaccharide-binding protein, includes the induction of cytokines, such as interleukins 1, 6, and 8 and tumor necrosis factor-α (7). The activation of these cytokines results in the mobilization of the defensive B and T cells that are associated with the Th-2 (acquired immune) response, which then suppresses the Th-1 response. Thus, the protective effect of endotoxin may be

### Table 1. Demographic characteristics of the cohort*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case patients, No. (%)</th>
<th>Subcohort members†, No. (%)</th>
<th>HR‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>628</td>
<td>3184</td>
<td>1.11 (1.10 to 1.13)</td>
</tr>
<tr>
<td>Age at baseline, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>35 (6.0)</td>
<td>478 (15.0)</td>
<td>1 (referent)</td>
</tr>
<tr>
<td>40–49</td>
<td>46 (7.2)</td>
<td>463 (14.5)</td>
<td>2.51 (1.59 to 3.98)</td>
</tr>
<tr>
<td>50–59</td>
<td>267 (42.5)</td>
<td>1180 (37.1)</td>
<td>11.49 (7.93 to 16.64)</td>
</tr>
<tr>
<td>60–69</td>
<td>280 (44.6)</td>
<td>1063 (33.4)</td>
<td>18.73 (12.94 to 27.11)</td>
</tr>
<tr>
<td>Years of work</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>22 (3.5)</td>
<td>118 (3.7)</td>
<td>1 (referent)</td>
</tr>
<tr>
<td>≥10 to &lt;20</td>
<td>139 (20.4)</td>
<td>710 (22.3)</td>
<td>0.77 (0.46 to 1.31)</td>
</tr>
<tr>
<td>≥20 to &lt;30</td>
<td>281 (44.7)</td>
<td>1371 (43.1)</td>
<td>1.01 (0.61 to 1.67)</td>
</tr>
<tr>
<td>≥30</td>
<td>186 (29.6)</td>
<td>985 (30.9)</td>
<td>1.41 (0.85 to 2.35)</td>
</tr>
<tr>
<td>Never smoked</td>
<td>556</td>
<td>3039</td>
<td>1.0 (referent)</td>
</tr>
<tr>
<td>Ever smoked (&gt;6 mo)¶</td>
<td>72 (11.5)</td>
<td>145 (4.6)</td>
<td>2.11 (1.56 to 2.86)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>7 (1.1)</td>
<td>26 (0.8)</td>
<td>1.12 (0.48 to 2.61)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>65 (10.4)</td>
<td>119 (3.7)</td>
<td>2.33 (1.69 to 3.22)</td>
</tr>
<tr>
<td>Duration of smoking¶, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>7 (1.1)</td>
<td>29 (0.9)</td>
<td>1.01 (0.44 to 2.30)</td>
</tr>
<tr>
<td>10 to &lt;20</td>
<td>7 (1.1)</td>
<td>32 (0.1)</td>
<td>2.05 (1.08 to 3.92)</td>
</tr>
<tr>
<td>20 to &lt;30</td>
<td>14 (2.2)</td>
<td>32 (0.1)</td>
<td>1.93 (1.01 to 3.68)</td>
</tr>
<tr>
<td>≥30</td>
<td>37 (5.9)</td>
<td>52 (1.6)</td>
<td>2.82 (1.82 to 4.38)</td>
</tr>
</tbody>
</table>

* Proportion of members of the subcohort to case patients was 2:1 and was based on the frequency of the most common cancer site examined, i.e., breast. HR = hazard ratio; CI = confidence interval. Thirteen case patients and one member of the subcohort were not included because information on work history was unavailable.

† Three case patients with lung cancer were randomly selected for the subcohort and, therefore, contributed “time at risk” from entry until their date of diagnosis.

‡ Boldface indicates a statistically significant result.

§ The hazard ratio was evaluated for age as a continuous variable.

|| Hazard ratio was evaluated for age as a categorical variable.

¶ Hazard ratio for the risk of smoking-related lung cancer was age adjusted.
Table 2. Lung cancer incidence by cumulative exposure to endotoxin*

<table>
<thead>
<tr>
<th>Exposure lag time</th>
<th>Cumulative endotoxin exposure, EU/m² x year†,‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unexposed</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>HR (95% CI) No. of case patients/ No. of subcohort members</td>
</tr>
<tr>
<td></td>
<td>1.0 (referent) 186/916 88/425</td>
</tr>
<tr>
<td>5 y</td>
<td>1.09 (0.83 to 1.45) 94/457 87/422</td>
</tr>
<tr>
<td></td>
<td>1.08 (0.82 to 1.22) 99/509 85/419</td>
</tr>
<tr>
<td>10 y</td>
<td>1.0 (referent) 188/923 94/509 84/399</td>
</tr>
<tr>
<td></td>
<td>1.08 (0.82 to 1.41) 106/557 84/399</td>
</tr>
<tr>
<td>15 y</td>
<td>1.0 (referent) 193/965 106/557 84/399</td>
</tr>
<tr>
<td></td>
<td>1.08 (0.82 to 1.41) 106/557 84/399</td>
</tr>
<tr>
<td>20 y</td>
<td>1.0 (referent) 208/1090 122/543 81/408</td>
</tr>
<tr>
<td></td>
<td>1.19 (0.92 to 1.54) 122/543 81/408</td>
</tr>
</tbody>
</table>

* Data were adjusted for age at baseline and smoking status (never [referent], former, or current). Analysis was based on cumulative exposure to endotoxin, excluding subjects who ever worked as machinists (n = 114), worked with wool (n = 17), or worked in sanitation jobs in production (n = 44). EU = endotoxin units; HR = hazard ratio; CI = confidence interval.
† Exposure categories represent quintiles of exposure among noncase patients. Category labels are equal to median of exposure range for each quintile.
‡ Boldface indicates a statistically significant result.
§ Two-sided Wald test for the hypothesis that slope = 0 of hazard trend treated as a continuous variable, including unexposed category; category scores were based on median of category exposure range. At the end of follow-up, the hazard ratio among former smokers was 0.98 (95% CI = 0.39 to 2.43) and among current smokers was 2.36 (95% CI = 1.6 to 3.11). At a 20-year exposure lag time, the hazard ratio among former smokers was 0.94 (95% CI = 0.38 to 2.35) and among current smokers was 2.23 (95% CI = 1.6 to 3.12).

The net effect of the interplay between Th-1 and Th-2 responses, which is mediated by TLRs (42). Study of the modulation of lung tumor growth in animal models with and without functional TLR4 (10,11) may provide further insight on how the innate and acquired immune responses that are triggered by endotoxin exposures result in the inverse association that we observed in this study.

Our study has several strengths. The findings are based on the observations of a well-characterized cohort of 267,400 women workers in the textile industry. The large size of the cohort and the availability of quantitative endotoxin exposure data permitted statistically robust estimation of the dose–response effect for endotoxin. No previous epidemiologic study, to our knowledge, has provided quantitative assessments of exposure–response relations for endotoxin associated with lung cancer. Confounding by smoking was probably not a bias because of the low smoking prevalence in the cohort. Nonetheless, the availability of smoking data enabled the evaluation of risk estimates by adjustment and by stratification.

Our study also has several potential limitations. A potential source of bias in this study of textile workers is the healthy worker survivor effect (43). Sensitive workers may have transferred from highly exposed jobs to jobs with lower exposure, whereas unaffected workers who are less sensitive to the respiratory effects associated with endotoxin exposure would likely not have transferred jobs. Previous studies of textiles workers in Shanghai have reported some administrative intervention among sensitized workers, primarily from respiratory effects of endotoxin (6). Therefore, long-term workers may represent a pool of survivors resistant to the acute effects of endotoxin exposure. This form of bias is typically of concern when the study goal is to test positive relations between an exposure and disease, but the same behavior of bias toward the null may apply for inverse associations. As such, the removal of more sensitive workers could result in an observed inverse trend for lung cancer that would be attenuated when compared with the true relation between exposure and outcome. More simulation studies are required to properly characterize the bias introduced by the healthy worker survivor effect in the presence of a true protective effect. In this study, we do not have data on respiratory symptoms related to cotton dust and endotoxin in the cohort to examine the healthy worker selection bias. However, job mobility in this cohort was limited. On average, case patients with lung cancer held 2.0 jobs and members of the subcohort held 1.9 jobs. Among those who only worked in the cotton factories, case patients held 1.9 jobs and subcohort workers held 1.8 jobs.

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To address potential selection bias, we conducted endotoxin exposure–response analyses among women who held only one job (272 case patients and 1413 noncase members of the subcohort). We observed statistically significant reduced risks for all categories of endotoxin exposure in analyses with a 20-year lag time, especially among the subjects with the highest endotoxin exposure, compared to those subjects with only one job but with no exposure (HR = 0.5, 95% CI = 0.3 to 0.9). Thus, a healthy worker survivor effect was probably not an important bias in our study.

Another possible limitation is that, through each of the analyses with lag times of 0, 5, 10, 15, or 20 years, a small nonstatistically significant excess risk was observed among subjects in the lowest exposure category as compared with unexposed subjects. This result raises the question of whether the unexposed category or the low-exposed category was best suited as the reference group. When we performed the analyses both ways, we found identical patterns of responses. We therefore have no interpretation of the excess risk in the low-exposure group.

All exposures assigned retrospectively are subject to the effect of misclassification. Another limitation of this study relates to the errors associated with the exposure reconstruction procedures. However, any misclassification bias introduced was nondifferential because the estimates of exposure were assigned irrespective of case status. Nondifferential misclassification typically results in risk estimates that are biased to the null, which applies similarly for factors that increase or decrease risk. Thus, the true positive association between the risk of lung cancer and endotoxin exposure may be stronger than what we have observed.

Still another limitation relates to the inherent uncertainties associated with the reconstruction of historic endotoxin exposure. Workplace concentrations of endotoxin can vary depending on the source of cotton, humidity, and handling procedures. We did not have historic data on those factors, and so we relied on the observed correlations between cotton dust and endotoxin that were derived from studies by Christiani et al. and from our own measurements to estimate past exposures (34). The historic dataset used to estimate cotton dust exposures included measurements made between 1975 and 1999. Exposures occurring before 1975 were assigned estimates that were based on the predicted levels for 1975. This assumption might have underestimated exposures before 1975 and, consequently, amplified the observed exposure effect. An additional sensitivity analysis was performed by assuming a step increase in exposures in 1955, the midway point between the earliest employment and 1975. Of the 3812 subjects, 1320 were employed before 1955. We assumed that pre-1955 exposures were 50% higher than 1975 exposure levels, a reasonable but arbitrary value. The results of the dose–response analysis for endotoxin that were based on this modified exposure scheme were not different from the previous results (for the highest exposure category of cumulative exposure, compared with the lowest category, with a 20-year lag time, HR = 0.5, 95% CI = 0.4 to 0.8; *P* < .001).

### Table 3. Lung cancer incidence by cumulative exposure to endotoxin; stratified by smoking status*

<table>
<thead>
<tr>
<th>Exposure lag time</th>
<th>Smoking history</th>
<th>Categories of cumulative endotoxin, EU/m³ × years†</th>
<th>Unexposed</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never smoked</td>
<td>HR (95% CI)</td>
<td>1.0 (referent)</td>
<td>1.02 (0.80 to 1.30)</td>
<td>0.82 (0.64 to 1.05)</td>
<td>0.71 (0.51 to 0.97)</td>
<td>.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of case patients/No. of subcohort members</td>
<td>164/868</td>
<td>162/819</td>
<td>144/807</td>
<td>65/400</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ever smoker</td>
<td>HR (95% CI)</td>
<td>1.0 (referent)</td>
<td>0.92 (0.41 to 2.08)</td>
<td>1.02 (0.48 to 2.17)</td>
<td>0.71 (0.28 to 1.81)</td>
<td>.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of case patients/No. of subcohort members</td>
<td>22/45</td>
<td>16/30</td>
<td>20/42</td>
<td>9/24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Never smoked</td>
<td>HR (95% CI)</td>
<td>1.0 (referent)</td>
<td>0.99 (0.79 to 1.24)</td>
<td>0.71 (0.54 to 0.92)</td>
<td>0.60 (0.43 to 0.85)</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of case patients/No. of subcohort members</td>
<td>186/1045</td>
<td>183/915</td>
<td>115/619</td>
<td>51/315</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ever smoker</td>
<td>HR (95% CI)</td>
<td>1.0 (referent)</td>
<td>1.05 (0.49 to 2.26)</td>
<td>0.93 (0.43 to 2.02)</td>
<td>0.65 (0.25 to 1.71)</td>
<td>.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of case patients/No. of subcohort members</td>
<td>22/45</td>
<td>20/36</td>
<td>17/37</td>
<td>8/23</td>
<td></td>
</tr>
</tbody>
</table>

* Data were adjusted for age at baseline and stratified by smoking status (never or ever). Analysis was based on cumulative exposure to endotoxin, excluding subjects who ever worked as machinists (n = 114), worked with wool (n = 17), or worked in sanitation jobs in production (n = 44). Exposure categories were from Table 2, collapsed as follows: low = 89 and 1627 EU/m³ × years; medium = 2402 and 3883 EU/m³ × years; and high = 24350 EU/m³ × years. EU = endotoxin units; HR = hazard ratio; CI = confidence interval.

† Boldface indicates a statistically significant result.

‡ Two-sided Wald test for the hypothesis that slope = 0 of hazard trend was treated as a continuous variable, including unexposed category. Category scores were based on the median of the category exposure range.
The likelihood of incomplete case ascertainment, a possible limitation, was small. All women who worked in the textile industry, including retirees, obtained all medical services through the textile industry until 2002. Medical records were maintained at the factory clinics of the Shanghai Textile Industry Bureau and were regularly updated by factory clinic personnel. The cases of lung cancer identified in the Shanghai Textile Industry Bureau tumour and death registry were verified as such by use of data from the Shanghai Cancer Registry and by review of medical records. Although it is possible that a woman could retire, return to her village, subsequently be diagnosed with lung cancer, but not notify the Shanghai Textile Industry Bureau of her status and therefore not be included in the Shanghai Cancer Registry, it is unlikely. Only 96 women in the cohort severed all ties to the Shanghai Textile Industry Bureau and were lost to follow-up before the end of the study. There was no evidence of case misclassification. The analyses of data from all identified case patients with lung cancer and all case patients with confirmed lung cancer resulted in identical patterns of reduced risk.

A final possible limitation is confounding by unaccounted endotoxin exposure from employment in other industries (e.g., animal farming). However, this possibility is an unlikely source of confounding. The textile industry was the sole or predominant employer of women in the cohort. Moreover, if other such employment were to have occurred, there is no reason to suspect that it would have been associated with endotoxin exposure levels in the cotton factories. There is no reason to suspect that exposures to other lung carcinogens, such as indoor coal burning or environmental tobacco smoke, both of which are ubiquitous exposures, would be related to textile industry endotoxin exposure levels. Exposure to high doses of endotoxin, especially those of at least 20 years, were associated with an approximately 40% reduction of lung cancer risk among women textile workers. On the basis of a reported population rate of lung cancer among women in Shanghai of 19.1 per 100 000 for the year 2000 (18) and the estimated reduction in risk of lung cancer observed for 20 years of endotoxin exposure in this population of workers, the incidence of lung cancer in this endotoxin-exposed cohort was reduced by approximately 7.6 per 100 000 (range = 3.2–10.9 per 100 000). These findings indicate that endotoxin exposure is positively associated with early-stage anticarcinogenic activity in humans. Biologic plausibility for this association is supported by experimental research and corroborative evidence for reduced lung cancer among endotoxin-exposed workers in other industries (23–25). Further research into the mechanisms of this activity of endotoxin could provide useful information for lung cancer prevention and treatment strategies.

References

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Notes
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