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Possible pro-carcinogenic association of endotoxin on lung cancer among Shanghai women textile workers

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Background: Endotoxin (lipopolysaccharide) is a widespread contaminant in many environmental settings. Since the 1970s, there has been generally consistent evidence indicating reduced risks for lung cancer associated with occupational endotoxin exposure.

Methods: We updated a case–cohort study nested within a cohort of 267 400 female textile workers in Shanghai, China. We compared exposure histories of 1456 incident lung cancers cases diagnosed during 1989–2006 with those of a reference subcohort of 3022 workers who were free of lung cancer at the end of follow-up. We applied Cox proportional hazards modelling to estimate exposure–response trends, adjusted for age and smoking, for cumulative exposures lagged by 0, 10, and 20 years, and separately for time windows of $\leqslant 15$ and >15 years since first exposure.

Results: We observed no associations between cumulative exposure and lung cancer, irrespective of lag interval. In contrast, analyses by exposure time windows revealed modestly elevated, but not statistically significant relative risks (~ 1.27) at the highest three exposure quintiles for exposures that occurred >15 years since first exposure.

Conclusions: The findings do not support a protective effect of endotoxin, but are suggestive of possible lung cancer promotion with increasing time since first exposure.

Endotoxin (lipopolysaccharide), produced by Gram-negative bacteria, is a widespread environmental contaminant in numerous industrial and agricultural settings. Especially high concentrations are found in cotton textile factories, sewage treatment, and poultry and livestock farming (Rylander, 2002). The majority of epidemiologic studies of occupational cohorts exposed to endotoxin conducted since the 1970s have shown reduced risks for lung cancer. Summary relative risks of 0.72 and 0.62, respectively, among cotton textile worker cohorts, which included data from our previously reported study of women workers in Shanghai (Astrakianakis *et al*, 2007, 2010) and agricultural cohorts were reported in a 2011 meta-analysis (Lenters *et al*, 2010). Endotoxin-induced inflammation leading to immune system upregulation has been proposed as a likely anti-carcinogenic mechanism (Lundin and Checkoway, 2009). However, some subsequently published literature provides conflicting evidence regarding the effect of endotoxin exposure on lung cancer. Reduced lung cancer risks continue to be observed in studies of UK cotton textile (McElvenny *et al*, 2011) and US agricultural cohorts (Beane Freeman *et al*, 2012), whereas moderately elevated risks associated with endotoxin

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were recently reported from the large multi-country population-based SYNERGY case–control study (Peters *et al*, 2012). Here, we report findings for endotoxin exposure and lung cancer from an extended follow-up of the Shanghai cotton textile worker cohort (Astrakianakis *et al*, 2007, 2010). Our objectives were to assess the temporal stability of the previously observed inverse relation, and to identify exposure time windows where exposure-related effects may be most prominent.

MATERIALS AND METHODS

Study subjects, follow-up, and case ascertainment. As described in detail previously (Astrakianakis *et al*, 2007), the base cohort was composed of 267 400 women born between 1 January 1925 and 31 December 1958 who had been employed in factories included in the Shanghai Textile Industry Bureau (STIB). The cohort was originally enumerated from 526 Shanghai textile factories during 1989–1991, and enroled in an intervention trial to test the efficacy of breast self-exam on reducing breast cancer incidence and mortality (Thomas *et al*, 1997, 2002). At enrolment, questionnaires eliciting information on basic demographics, reproductive history, and smoking were administered to participants in the trial (Thomas *et al*, 1997).

Computerised matching of the cohort with the STIB Tumor and Death Registry (1989–1998) and subsequently with the Shanghai Cancer Registry (1999–2006) identified a total of 1456 incident lung cancer cases. Medical record reviews were conducted in instances where computerised matching with the registries could not be confirmed. Diagnoses were based on histology (564, 39%), clinical investigation, including X-ray, ultrasounds, MRI, and CT scan (495, 34%), cytology (244, 17%), physical exam (27, 2%), autopsy, exploratory surgery, or immunological testing (21, 1%). Diagnostic methods were unknown for 105 (7%) cases.

A comparison subcohort composed of 3199 workers was randomly selected from the entire cohort at baseline. The subcohort was frequency-matched in 5-year age strata to the age distribution of all cancer cases in the cohort identified during the initial follow-up, 1989–1998 (Astrakianakis *et al*, 2007). The subcohort size was originally set to have the same age distribution of all cancer cases and to be roughly twice the size of the largest case group, breast cancer. There were 20 lung cancer cases who also were in the subcohort.

Exposure assessment. Quantitative endotoxin exposures were estimated for cases and subcohort members by applying a validated job–exposure matrix to factory and job assignment data spanning complete work history experience (Astrakianakis *et al*, 2006b). The job/exposure matrix was based on data from >2400 historical cotton dust measurements made between 1975 and 1999 in 56 textile factories during government inspections that were converted to endotoxin estimates from job-specific surveys conducted by Christiani *et al* (1993) in the 1990s, and from measurements made subsequently in a survey that we conducted in three cotton factories in 2002 (Astrakianakis *et al*, 2006a). The cotton dust samples were assayed using the kinetic limulus amoebocyte lysate assay using standard protocols described by Thorne (2000). Results were reported in endotoxin units (EU) per cubic metre (m^3). The assay is sensitive and generally yields a limit of detection in the range of 1 per 100 EU per ml of analyte or $\sim 0.5\,\mathrm{EU\,m^{-3}}$ based on standard air sampling volumes.

We estimated cumulative exposures $\mathrm{EU\,m^{-3}} \times$ years, by summing the products of period, factory, and major process unit and specific process unit endotoxin concentrations with durations of employment (Astrakianakis *et al*, 2006b) for subjects according to their work histories. Workers from non-cotton units or factories were assigned no exposure. Exposures could not be estimated for 53 cases and 11 non-cases without work history data. We also eliminated from the analysis 50 cases and 145 non-cases who worked in endotoxin-exposed jobs—machinists, wool processing, and sanitation—for which we could not assess quantitative exposures.

Statistical analysis. The analysis was restricted to 1456 lung cases and 3022 non-cases for which we had quantitative exposure data. Cox proportional hazards modelling, adapted for the case–cohort design (Langholz and Jiao, 2007), was applied to estimate relative risks (hazard ratios), and 95% confidence intervals associated with cumulative exposure to endotoxin. The period of risk was from entry into the cohort until diagnosis of lung cancer, death, date of last known follow-up, or end of follow-up on 31 December 2006. Person-years of follow-up for the 20 cases who were in the subcohort were included in the person-time calculations for the subcohort until dates of diagnoses; thereafter, their person-time was included with that for the cases. Exposure strata were defined as non-exposed, and quintiles of increasing cumulative exposure in which the strata contained approximately equal numbers of cases to maximise statistical efficiency. Trend tests were calculated on the basis of median values in each exposure category, treating the never exposed as the referent group. To account for possible disease latency, we conducted analyses in which cumulative exposure was sequentially lagged by 0, 10, and 20 years. We also conducted exposure time window analyses in which exposure–response trends were estimated separately for time periods defined as $\leqslant 15$, >15 years since first exposure in an attempt to identify time periods of potentially heightened response to endotoxin-related effects on lung carcinogenesis. Separate analyses were performed with adjustment for parity (parous *vs* nulliparous) and time since termination of employment (0, <5, 5–10, 11–15, 16–20, >20 years). All analyses included adjustment for age and smoking (ever *vs* never).

Ethics approval. All study procedures were approved by Institutional Review Boards at the University of Washington, the Fred Hutchinson Cancer Research Center, the University of California, San Diego, and Zhong Shan Hospital, Shanghai.

RESULTS

Demographic, work history, smoking, and parity. As summarised in Table 1, cases and non-case subcohort workers had similar distributions of age and years employed in the textile industry. As expected, relatively more cases than non-cases had ever smoked (11.3% *vs* 4.6%). A larger proportion of smokers among cases than among non-cases smoked for >30 years (5.6% *vs* 1.7%). Cases included a somewhat larger percentage of nulliparous women than non-cases (5.4% *vs* 4.0%).

Exposure–response trends. There was no association between cumulative endotoxin exposure and lung cancer risk, irrespective of lag period (Table 2). The exposure–response trend results were only minimally changed when analyses were restricted to the 564 histologically confirmed cases (data not shown). Findings from analyses by exposure time windows (Table 3) indicate no associations with cumulative exposure within the first 15 years of exposure onset, but slightly elevated relative risks (~ 1.27) at the highest cumulative exposures experienced subsequently. The trends were neither monotonically increasing nor statistically significant. The results only changed minimally when the analyses included adjustments for parity and time since work termination (data not shown).

Table 1. Demographic characteristics of lung cancer cases and non-cases

	Cases ($n = 1456$)		Non-cases ($n = 3022$)	
Characteristics	**No.**	**%**	**No.**	**%**
Year of birth, *n* (%)				
1925–1929	512	(36.0)	884	(29.6)
1930–1934	473	(33.2)	890	(29.3)
1935–1939	173	(12.2)	340	(11.3)
1940–1944	54	(3.8)	145	(4.8)
1945–1949	88	(6.2)	267	(8.8)
1950–1954	77	(5.4)	304	(10.0)
1955–1958	46	(3.2)	189	(6.3)
Years worked, *n* (%)				
<10	35	(2.5)	110	(3.4)
⩾10 to <20	284	(20.0)	568	(18.8)
⩾20 to <30	600	(42.2)	1263	(41.8)
⩾30	504	(35.4)	1088	(36.0)
Smoking status, *n* (%)				
Never	1262	(88.7)	2882	(95.4)
Ever	161	(11.3)	140	(4.6)
Parity, *n* (%)				
Nulliparous	76	(5.3)	122	(4.0)
1 live birth	233	(16.4)	707	(23.3)
⩾2 live births	1114	(78.3)	2193	(72.7)

Table 2. Exposure-response trends for endotoxin exposure and lung cancer risk, by exposure lag period

Cumulative exposure (EU m^{-3} × years)[a] lag period (years)	Cases	Non-cases	HR[b]	95% CI
Lag = 0				
Non-exposed (0)	460	912	1.00	Referent
>0–1469	200	499	0.81	0.66–1.00
1470–2282	198	472	0.82	0.66–1.01
2283–3024	200	416	0.87	0.71–1.08
3025–4529	199	376	0.95	0.76–1.17
>4529	199	347	0.98	0.79–1.23
			P-trend = 0.57	
Lag = 10				
Non-exposed (0)	462	913	1.00	Referent
>0–1469	214	521	0.81	0.66–1.00
1470–2282	198	463	0.82	0.67–1.01
2283–3024	189	413	0.85	0.69–1.06
3025–4529	194	367	0.96	0.77–1.20
>4529	199	345	1.00	0.81–1.25
			P-trend = 0.42	
Lag = 20				
Non-exposed (0)	490	940	1.00	Referent
>0–1469	254	635	0.79	0.66–0.95
1470–2282	199	454	0.81	0.66–1.00
2283–3024	169	353	0.86	0.69–1.07
3025–4529	164	319	1.02	0.81–1.28
>4529	180	321	1.02	0.81–1.28
			P-trend = 0.20	

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a]Endotoxin units per m^3 air.
[b]Hazard ratio, adjusted for year of birth, smoking (ever/never).

DISCUSSION

Findings from this update of the case–cohort study of Shanghai women textile workers no longer support an anti-carcinogenic effect of endotoxin exposure for lung cancer, as had been reported previously from the initial follow-up (Astrakianakis *et al*, 2007, 2010). We observed a null association overall, although there was a possible small excess risk associated with relatively recent exposures. Somewhat complex patterns of time-varying association were suggested from previous analyses of the initial follow-up of this cohort. Using exposure time windows, Agalliu *et al* (2011), found a stronger inverse exposure–response gradient associated with exposures that occurred 20 or more years before lung cancer incidence diagnosis than with exposures during the 20 years preceding diagnosis. The latter time period includes more inactive person-time time since leaving work. Consistent with this, Applebaum *et al* (2013) observed some evidence that the reduced risk dissipated after 15 years since cessation of exposure, suggesting that the protection from exposure to endotoxin is reduced over time. The potential for diminishing protective effects of endotoxin on lung cancer risk with the passage of time since exposure cessation was previously described by Mastrangelo *et al* (2012).

Most of the occupational epidemiology literature on endotoxin and lung cancer indicates an inverse association with risk. However, modest relative risk excesses (~1.3) observed at the highest cumulative exposures >15 years since first exposure are similar in magnitude to findings from the recently reported SYNERGY case–control study (Peters *et al*, 2012). Moreover, contrary to findings presented for the extended follow-up of our Shanghai textile worker cohort, prior analyses of data from the initial follow-up (1989–1998) indicated an inverse dose–response trend with cumulative exposure to endotoxin (Astrakianakis *et al*, 2007, 2010).

The mechanisms by which endotoxin influences carcinogenesis are undoubtedly complex, and remain incompletely understood. Possible anti-carcinogenic effects are generally thought to be mediated by interactions between the innate and acquired immune systems in which inflammatory cytokines, including interleukins and tumour necrosis factor-alpha (TNF-α), have pivotal roles (Lundin and Checkoway, 2009). There is, however, evidence that pro-inflammatory cytokines, especially TNF-α, may have both cancer-inhibiting and -promoting effects that depend on the exposure timing (Balkwill, 2009).

Noteworthy strengths of our study are the large, well-characterised cohort that served as the study base, identification of incident lung cancer cases, large case and reference groups that ensured good statistical power, our quantitative endotoxin exposure assessment, and control for important potential confounders, smoking and reproductive history. The study also has some limitations that deserve mention. Some exposure misclassification is inevitable, especially for an agent with a high degree of variation, such as endotoxin. The cohort was originally enroled as a cross section of actively employed and retired textile workers with previous histories of employment in the industry. As such, study subjects might represent workers who were resistant to endotoxin toxicity, possibly leading to underestimated exposure–response trends. However, job mobility in this workforce historically has been very low, and employment patterns were predictable with women having been hired at early ages (20s) and retiring in the mid-to-late 40s. Thus, job transfers and early retirements were not likely to have caused substantial healthy worker survivor bias.

Table 3. Exposure–response trends for endotoxin exposure and lung cancer risk, by years since first exposure

Cumulative exposure (EU m^{-3} × years)[a] Years since first exposure	Cases	Non-cases	HR[b]	95% CI
⩽15 Years				
Non-exposed (0)	460	912	1.00	Reference
>0–959	200	516	0.74	0.60–0.91
960–1372	199	431	0.90	0.73–1.11
1373–1660	198	386	0.93	0.75–1.16
1661–2387	200	419	0.89	0.72–1.10
>2387	199	358	0.98	0.78–1.21
			P-trend = 0.82	
>15 years				
Non-exposed (0)	604	1298	1.00	Reference
>0–959	299	685	1.19	0.93–1.53
960–1372	129	307	1.00	0.74–1.36
1373–1660	88	155	1.28	0.90–1.82
1661–2387	145	257	1.27	0.93–1.73
>2387	191	320	1.27	0.91–1.77
			P-trend = 0.13	

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a]Endotoxin units per m^3 air.
[b]Hazard ratio, adjusted for year of birth, smoking (ever/never).

In fact, 55% of cases and 46% of non-cases held only one textile industry job; corresponding percentages for two jobs were 29% and 35%, respectively. Although only a small proportion of study subjects were smokers, we did not have data on some relevant non-occupational risk factors that affect risks in non-smokers. These include environmental tobacco smoke, outdoor air pollution, and fossil fuel heating and cooking sources (Couraud *et al*, 2012), which undoubtedly were widespread in Shanghai. Nonetheless, these exposures were unlikely to be have been correlated with endotoxin exposure, and thus were probably not important confounders.

Results from this updated case–cohort study do not indicate any strong associations of cumulative endotoxin exposure with lung cancer risk, although there is the possibility that modestly elevated risks may occur with greater time since first exposure. However, chance cannot be discounted as an explanation for the changing exposure–response patterns of exposure observed in the original and extended follow-ups of this cohort.

Insofar as endotoxin is a widespread occupational and environmental exposure throughout the world, an understanding of its potential role in lung carcinogenesis could have important disease prevention implications. As pointed out by Mastrangelo *et al* (2013), quantitative exposure–response analyses that address time-varying associations should be encouraged in other endotoxin-exposed cohorts to clarify further the possible effects of endotoxin in lung cancer aetiology.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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