Early Changes in Clinical, Functional, and Laboratory Biomarkers in Workers at Risk of Indium Lung Disease

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Abstract

Rationale: Occupational exposure to indium compounds, including indium–tin oxide, can result in potentially fatal indium lung disease. However, the early effects of exposure on the lungs are not well understood.

Objectives: To determine the relationship between short-term occupational exposures to indium compounds and the development of early lung abnormalities.

Methods: Among indium–tin oxide production and reclamation facility workers, we measured plasma indium, respiratory symptoms, pulmonary function, chest computed tomography, and serum biomarkers of lung disease. Relationships between plasma indium concentration and health outcome variables were evaluated using restricted cubic spline and linear regression models.

Measurements and Main Results: Eighty-seven (93%) of 94 indium–tin oxide facility workers (median tenure, 2 yr; median plasma indium, 1.0 μg/l) participated in the study. Spirometric abnormalities were not increased compared with the general population, and few subjects had radiographic evidence of alveolar proteinosis (n = 0), fibrosis (n = 2), or emphysema (n = 4). However, in internal comparisons, participants with plasma indium concentrations ⩾ 1.0 μg/l had more dyspnea, lower mean FEV_1 and FVC, and higher median serum Krebs von den Lungen-6 and surfactant protein-D levels. Spline regression demonstrated nonlinear exposure response, with significant differences occurring at plasma indium concentrations as low as 1.0 μg/l compared with the reference. Associations between health outcomes and the natural log of plasma indium concentration were evident in linear regression models. Associations were not explained by age, smoking status, facility tenure, or prior occupational exposures.

Conclusions: In indium–tin oxide facility workers with short-term, low-level exposure, plasma indium concentrations lower than previously reported were associated with lung symptoms, decreased spirometric parameters, and increased serum biomarkers of lung disease.

Keywords: occupational lung disease; pulmonary alveolar proteinosis; fibrosis; Krebs von den Lungen-6; surfactant protein-D

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Indium lung disease is a potentially fatal condition that can occur in workers exposed to indium compounds including indium–tin oxide (1). The disease is characterized by pulmonary alveolar proteinosis that may progress to fibrosis with or without emphysema with relatively short latency (1–13 yr from first exposure to diagnosis) (2). Epidemiologic studies of indium–tin

oxide production facilities where indium lung disease cases occurred have demonstrated subclinical disease in coworkers (3–7). In one indium–tin oxide workplace, high-resolution computed tomography (HRCT) of the chest showed interstitial changes in 21% of workers and emphysematous changes in 13% (3). A multisite study in Japan found increases in nonspecific serum biomarkers of lung damage, including Krebs von den Lungen (KL)-6 and surfactant protein (SP)-D associated with increases in serum indium levels; the authors recommended keeping serum indium, which appeared to be a marker of cumulative exposure, below 3 μg/l to prevent lung disease (5).

We previously reported two cases of pulmonary alveolar proteinosis, including one fatality, among workers at a United States indium–tin oxide production and reclamation facility (8). Indium compounds encountered at the facility included indium metal, indium salts, indium oxide, and indium–tin oxide. Review of corporate medical surveillance data demonstrated a 4-fold excess of spirometric restriction after hire and greater than expected declines in FEV_1 on serial examination (7). After-hire diffusing capacity defects were common. Significant associations between lung function abnormalities and blood indium concentration were not seen, but lung function test quality was inconsistent, and an insensitive blood indium analytical method with a detection limit of 5 μg/l had been used. Although more recently hired workers had fewer abnormalities, the available data suggested that additional efforts to reduce exposures and prevent adverse health effects were needed.

The facility introduced a variety of engineering controls and expanded respiratory protection. To inform further preventive interventions, we sought to better understand the relationship between respiratory health and exposure to indium compounds in the current workforce. Our primary question was: Are current exposures in the facility low enough to prevent lung damage? To answer this question, we focused on current workers whose experience would best reflect recent conditions. Given our previous observations, we chose to use spirometry and diffusing capacity tests, although they appeared to be of limited utility in other workplaces (3–5). For serum biomarkers, KL-6 and SP-D, as well as general markers of inflammation, were included. In addition, we explored the potential roles of YKL-40, a chitinase-like protein produced by macrophages that was recently reported to be a predictor of disease progression in pulmonary alveolar proteinosis unrelated to indium (9), and granulocyte-macrophage colony-stimulating factor (GM-CSF) auto-antibodies, which have been demonstrated to mediate the pathogenesis of idiopathic pulmonary alveolar proteinosis (10). GM-CSF autoantibodies had been detected in one of the present facility's cases of pulmonary alveolar proteinosis, raising the possibility of an exposure-related autoimmune response (8). HRCT of the chest was used because of its greater sensitivity for subclinical changes in indium–tin oxide workers compared with plain chest radiography (3). Finally, we recognized the need to use an analytical method for indium comparable in sensitivity to that used in Japanese studies (3–5), which previously had not been available in the United States.

Methods

Study Design

The Institutional Review Board of the National Institute for Occupational Safety and Health (NIOSH) approved this study. All individuals employed at an indium–tin oxide production facility when this cross-sectional study was initiated (July 16, 2012) were invited to give written informed consent for an interviewer-administered questionnaire (available in the online supplement), spirometry, measurement of diffusing capacity, HRCT scan of the chest, and blood draw. The questionnaire addressed respiratory symptoms and diagnoses, smoking history, work history, and demographic information. The respiratory questions were adapted from validated survey instruments (11–13). We used job title and department to classify participants into the following department categories: production (including indium oxide production, indium–tin oxide production, grinding, reclaim, and research and development), production support (e.g., maintenance, shipping/receiving, engineer, and janitorial), laboratory, and office.

Pulmonary Function Tests

Standard spirometry and diffusing capacity testing was performed and interpreted according to published guidelines (14–18). We conducted spirometry testing using a dry rolling-seal spirometer (14). Test results were interpreted using reference values generated from the Third National Health and Nutrition Examination Survey (15, 16). We defined obstruction as FEV_1 and ratio of FEV_1 to FVC below their respective lower limits of normal (fifth percentiles) with a normal FVC. We defined restrictive pattern as a normal FEV_1/FVC ratio with FVC below the lower limit of normal. We classified participants with both FEV_1/FVC ratio and FVC below the lower limit of normal as having mixed obstructive and restrictive abnormalities. Participants were considered to have any spirometric abnormality if they met the definition of obstruction, restrictive pattern, or mixed pattern.

We measured the diffusing capacity of the lung for carbon monoxide ($D_{L_{CO}}$) using the single breath technique with helium as the tracer gas (17). Test results were interpreted using reference values generated from a stratified random sample of a state population (18). For alveolar volume (V_A), we adjusted the predicted values by a factor of 0.88 for Black and Asian participants. We defined low $D_{L_{CO}}$ and V_A on the basis of their respective lower limits of normal.

Chest HRCT

Chest HRCT scans were offered to all nonpregnant participants and were conducted at a local radiology center using a low-dose (~1 mSv per scan), noncontiguous protocol. One participant declined HRCT but provided digital images from a chest CT done 3 months before the survey; we accepted these images for our analyses. All scans were systematically reviewed by a thoracic radiologist according to an international classification scheme (19), with attention to changes described in workers with indium lung disease (i.e., pulmonary alveolar proteinosis, fibrosis, and emphysema).

Plasma Metal and Serum Biomarker Analyses

We collected blood for measurement of metal concentrations and biomarkers of inflammation and interstitial lung disease. Ten milliliters of blood were collected using

trace metal–free evacuated containers and processed to isolate plasma. Another 10 ml of blood were collected using serum-separator evacuated containers and processed to isolate serum. Samples were stored frozen at −80°C until analysis.

Plasma samples were analyzed by a commercial laboratory for indium and tin using inductively coupled plasma mass spectrometry assays. The indium analytical method had a limit of detection of 0.03 μg/l and a limit of quantification of 0.1 μg/l. The tin method had a limit of detection of 2.0 μg/l.

The serum concentrations of C-reactive protein (CRP), YKL-40, KL-6, and SP-D were measured using ELISA kits (R&D Systems, Minneapolis, MN). GM-CSF autoantibodies were measured by ELISA as described previously (20), and lactate dehydrogenase (LDH) was measured by colorimetric assay (R&D Systems). Reference values for CRP, LDH, KL-6, and YKL-40 were determined from serum samples from 30 healthy individuals (15 male and 15 female subjects). Reference values for SP-D and GM-CSF autoantibodies were determined from serum samples from 101 healthy individuals (44 male and 57 female subjects). The upper limits used to define a test result as abnormally elevated were as follows: CRP, 14,365.5 ng/ml; LDH, 101.8 IU/ml; YKL-40, 109.8 ng/ml; KL-6, 466.7 U/ml; SP-D, 375.2 ng/ml; GM-CSF autoantibodies, 3.0 μg/l.

Statistical Analyses

Numeric data were evaluated for normality by the Shapiro-Wilk test, summarized using descriptive statistics, and presented as mean ± SEM if normally distributed and as median (interquartile range [IQR]) if nonnormally distributed. Plasma indium was the primary exposure metric. Results below the limit of detection, which were few, were assigned a value of 0.015 μg/l (half of the limit of detection). For results falling between the limit of detection and the limit of quantification, we used the measured value.

Standardized morbidity ratios (SMRs) of symptoms, diagnoses, and lung function abnormalities were calculated using data obtained from the United States adult population adjusted for race/ethnicity (white, black, Hispanic), sex, age, and smoking status (13). For spirometric restriction, we also examined the effect of body mass index.

Relationships between exposure and health outcomes were evaluated after stratifying by plasma indium concentration on the basis of the median and tertile values. Normally distributed data were evaluated for equal variance by the O'Brien test and analyzed by Student's *t* test (for two groups) or ANOVA (for more than two groups). Nonnormally distributed data were analyzed by the Mann-Whitney U test (for two groups) or by the Kruskal-Wallis test (for more than two groups). For categorical data, χ^2 test or Fisher's exact test were used. Recursive partitioning also was used to create plasma indium categories that achieve the maximum association between plasma indium and the health outcome of interest.

Logistic regression was used to assess the relationship between plasma indium (categorized by the median value) and dichotomous health outcomes. Spline and linear regression models were used to assess the relationship between plasma indium (or the natural log of plasma indium) and continuous health outcomes. For the spline regression models, we developed restricted cubic splines using a published SAS macro (21). Restricted cubic splines are a type of spline regression in which polynomials are defined over adjacent intervals and joined together at a small number of "knots." We specified five knots and modeled the difference between the value of the health outcome for any given plasma indium concentration and the value of the health outcome for a reference plasma indium concentration. We set the reference plasma indium concentration at half of the limit of detection because it was the lowest value assigned to any participant.

Final adjusted models included the following covariates: cigarette smoking status (current/former/never), facility tenure (years), and age (years). Age was not included as a covariate in models of percent predicted values of lung function parameters because the percent predicted values account for age. The effect of prior occupational exposures was also examined.

Analyses were conducted using SAS software version 9.3 and JMP software version 10.0.1 (SAS Institute, Inc., Cary, NC). All *P* values reported are two-sided. We considered $P \leq 0.05$ to be significant.

Results

Participant Health Outcome Variables

Eighty-seven (93%) of 94 eligible indium–tin oxide facility employees participated in the study (Table 1). Median facility tenure was short (2 yr), and approximately half of the participants worked in production. Nineteen (22%) of the 87 participants were included in the previous review of corporate medical surveillance records (7). All 87 participants completed the questionnaire, and most underwent lung function testing (n = 75; 86%), serum biomarker analysis (n = 80; 92%), and a chest HRCT scan (n = 70; 80%).

The majority (56%) of participants were asymptomatic; 6 to 22% reported a chest symptom or a prior or current diagnosis of asthma (Table 2). Most (n = 14; 74%) asthma diagnoses were made before employment at the facility. Two participants reported a diagnosis of lung scarring or fibrosis; none reported a current diagnosis of chronic obstructive lung disease. Few participants had an abnormality of spirometry or gas diffusion (Table 2). There were no associations between spirometric or diffusing capacity abnormalities and smoking status, but the FEV_1% and FEV_1/FVC ratio were significantly lower in current smokers (not shown). In adjusted comparisons with the United States adult population, participants had significantly higher-than-expected prevalence of wheeze in the last 12 months (SMR 1.6; 95% confidence interval [CI], 1.0–2.5), lifetime asthma diagnosis (SMR, 3.2; 95% CI, 2.1–5.1), and current asthma diagnosis (SMR, 2.7; 95% CI, 1.4–5.2). Other symptoms and spirometric abnormalities were not in excess.

Of the 70 participants who had a chest HRCT, none had radiologic evidence of pulmonary alveolar proteinosis, two had evidence of early fibrosis, and four others had evidence of emphysema (centrilobular in one and paraseptal in three). The signs of emphysema occurred in current smokers between 26 and 42 years of age, with 6 to 29 pack-year smoking histories. Most (n = 5) of these radiographic abnormalities occurred in participants who reported prior exposure to asbestos, silica, or other lung hazards ($P = 0.3971$).

Of the 80 participants who provided blood samples for biomarker analysis, KL-6 was elevated in 46 (58%), YLK-40 in 15 (19%), SP-D in 8 (10%), and CRP in 2 (3%). None had elevation of LDH or GM-CSF autoantibodies. Median KL-6 and SP-D were significantly lower in current smokers than in other participants (not shown).

Table 1. Demographic and employment data of the participating indium–tin oxide facility employees*

	All Participants	Plasma Indium < 1 μg/l	Plasma Indium ⩾ 1 μg/l	*P* Value†
Number	87	40	40	
Age, yr (range)	44 (35–52)‡	43 (33–50)	47 (40–52)	ns
Male	75 (86)	31 (78)	37 (93)	0.055
Race				ns
White	63 (72)	31 (78)	27 (68)	
Asian	9 (10)	4 (10)	5 (13)	
Black	3 (3)	0	2 (5)	
Other/unknown	12 (14)	5 (13)	6 (15)	
Hispanic	8 (9)	3 (8)	4 (10)	ns
Smoking status				0.088
Current	22 (25)	12 (30)	8 (20)	
Former	20 (23)	5 (13)	13 (33)	
Never	45 (52)	23 (58)	19 (48)	
Tenure, yr§	2 (1.3–5.0)	1.3 (1.0–2.0)	2.8 (1.9–8.2)	<0.0001
Job category				0.007
Production	44 (51)	15 (38)	27 (68)	
Production support	23 (26)	10 (25)	9 (23)	
Laboratory	8 (9)	5 (13)	3 (8)	
Office	12 (14)	10 (25)	1 (3)	
Past work with asbestos, silica, or other lung hazards	53 (61)	24 (60)	25 (63)	ns

Definition of abbreviation: ns = nonsignificant.
*Eighty seven (93%) of 94 individuals employed at the facility participated in the study.
†*P* values are based on comparison of the two subgroups defined by plasma indium concentration (<1 and ⩾1 μg/l) using χ^2 test or Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. $P \leqslant 0.05$ was considered significant; *P* values ⩽ 0.10 are presented.
‡Data are presented as median (interquartile range) or n (%).
§Tenure as a facility employee was calculated from self-reported hire date.

Plasma Indium and Tin

Indium was detected in nearly all of the 80 participants evaluated (n = 76; 95%), and concentrations were quantifiable in most of the participants (n = 70; 88%). Fourteen (18%) had plasma indium concentrations ⩾ 3 μg/l, with a maximum value of 37 μg/l. The median plasma indium concentration was 1.0 μg/l (Table 3). The median plasma indium concentration was higher in participants with tenure of ⩾2 years compared with <2 years (Table 3). The median plasma indium concentration also varied by job category (Table 3).

Only two participants had quantifiable concentrations of tin in plasma.

Relationship between Plasma Indium and Health Outcomes

Stratification by the median plasma indium concentration of 1 μg/l demonstrated multiple associations between plasma indium and health outcomes (Table 2). Participants in the higher plasma indium group had a higher prevalence of dyspnea, wheeze in the absence of an upper respiratory infection, and lifetime asthma diagnosis (Table 2). These associations remained evident in logistic regression models adjusted for smoking status (not shown). In particular, dyspnea remained associated with plasma indium concentration ⩾ 1 μg/l in a model adjusted for age, smoking status, facility tenure, and prior exposure to asbestos, silica, or other lung hazards (odds ratio, 5.3; 95% CI, 1.2–30.5; $P = 0.0267$).

The mean FEV_1% and mean FVC% were lower in the higher plasma indium group (Table 2). Although the prevalence of specific spirometric abnormalities did not differ, the prevalence of any spirometric abnormality was increased in the higher plasma indium group (Table 2). Although similar trends in means and prevalence of abnormalities were observed for the gas diffusion parameters, the differences were not significant (Table 2). Chest HRCT abnormalities consistent with indium lung disease did not differ by plasma indium group (not shown). None of the health outcomes associated with plasma indium in these unadjusted analyses was associated with prior exposure to asbestos, silica, or other lung hazards (not shown).

The median serum KL-6 and median serum SP-D were higher in the higher plasma indium group (Table 2). Furthermore, the prevalence of participants with an elevated KL-6 was higher in the higher plasma indium group (75% vs. 40%; $P = 0.001$). In contrast, the difference in prevalence of participants with an elevated SP-D (15% vs. 5%) was not significant.

Stratification by tertile of plasma indium concentration (i.e., <0.5, 0.5 to <1.6, ⩾1.6 μg/l) also demonstrated increases in adverse health outcomes with increasing plasma indium concentration (not shown). Significant differences were observed for FEV_1% and KL-6. Mean FEV_1% was 104 ± 1.9 for the lowest tertile, 93 ± 3.5 for the middle tertile, and 93 ± 2.6 for the highest tertile ($P = 0.001$). Median KL-6 (U/ml) was 406 (IQR, 303–606) for the lowest tertile, 493 (IQR, 370–577) for the middle tertile, and 818 (IQR, 609–1,360) for the highest tertile ($P < 0.0001$). These observations were consistent with the outcomes of recursive partitioning, which identified plasma indium concentrations of 0.5 μg/l for FEV_1% (indium <0.5 μg/l: n = 24, mean = 103.9; indium ⩾0.5 μg/l: n = 51, mean = 93.0) and 2.1 μg/l for KL-6 (U/ml) (indium <2.1 μg/l: n = 58, mean = 464; indium ⩾2.1 μg/l: n = 22, mean = 1,115) as cutoffs for maximal association between plasma indium concentration and these health outcomes.

Adjusted restricted cubic splines for FEV_1%, FVC%, KL-6, and SP-D by plasma indium concentration showed nonlinear relationships (Figure 1) and demonstrated that the estimated FEV_1% and FVC% were lower and the estimated KL-6 and SP-D were higher than the corresponding values for the reference plasma indium concentration of 0.015 μg/l. Significant differences were seen for FEV_1% starting at a plasma indium concentration of 1.0 μg/l, for FVC% and KL-6 at 1.5 μg/l, and for SP-D at 7.0 μg/l (Table 4).

In unadjusted linear regression models, FEV_1% and FVC% were not associated with plasma indium concentration (not shown) but were associated with the natural log of plasma indium concentration (Table 5). KL-6 and SP-D were associated with both plasma indium concentration (not shown) and the natural log of plasma

Table 2. Clinical characteristics, pulmonary function, and serum biomarkers of the participating indium–tin oxide facility employees

Subject Characteristics	All Participants	Plasma Indium < 1 μg/l	Plasma Indium ⩾ 1 μg/l	*P* Value*
Symptom or diagnosis	(n = 87)	(n = 40)	(n = 40)	
Asymptomatic	49 (56)†	25 (63)	20 (50)	ns
Cough	14 (16)	7 (18)	7 (18)	ns
Chronic cough‡	9 (10)	4 (10)	5 (13)	ns
Sputum production	10 (11)	4 (10)	6 (15)	ns
Chronic sputum production‡	4 (5)	3 (8)	1 (3)	ns
Dyspnea	14 (16)	3 (8)	10 (25)	0.030
Wheeze§	19 (22)	7 (18)	10 (25)	ns
Wheeze without URI§	5 (6)	0	5 (13)	0.055
Chest tightness§	10 (11)	7 (18)	3 (8)	ns
Asthma (self-reported)				
Past or current	19 (22)	5 (13)	12 (30)	0.053
Current	9 (10)	2 (5)	6 (15)	ns
Spirometry	(n = 75)	(n = 37)	(n = 37)	
$FEV_{1\%}$ predicted (mean ± SEM)	96 ± 1.7	102 ± 1.9	92 ± 2.6	0.002
FVC % predicted (mean ± SEM)	99 ± 1.6	103 ± 1.9	95 ± 2.3	0.012
FEV_1/FVC %	79 (73–85)	79 (74–85)	81 (68–85)	ns
Obstructive pattern‖	3 (4)	0	3 (8)	ns
Restrictive pattern¶	8 (11)	2 (5)	5 (14)	ns
Mixed pattern**	1 (1)	0	1 (3)	ns
Any abnormality††	12 (16)	2 (5)	9 (24)	0.018
Diffusing capacity‡‡	(n = 74)	(n = 36)	(n = 37)	
D_{LCO} % predicted (mean ± SEM)	91 ± 1.7	94 ± 2.3	89 ± 2.6	ns
V_A % predicted (mean ± SEM)	91 ± 1.4	94 ± 1.9	89 ± 1.9	0.056
Low D_{LCO}	8 (11)	2 (6)	6 (16)	ns
Low V_A	9 (12)	3 (8)	6 (16)	ns
Serum biomarker	(n = 80)	(n = 40)	(n = 40)	
CRP, mg/l	0.9 (0.5–2.2)	0.9 (0.5–2.5)	0.7 (0.5–1.8)	ns
LDH, IU/ml	53 (47–60)	52 (48–58)	54 (46–61)	ns
YKL-40, ng/ml	62 (40–96)	63 (40–100)	59 (37–92)	ns
KL-6, U/ml	542 (372–730)	433 (334–560)	649 (469–984)	<0.0001
SP-D, ng/ml	163 (113–233)	147 (94–216)	187 (146–302)	0.027
GM-CSF autoantibodies, μg/ml	0.5 (0.3–0.7)	0.5 (0.4–0.7)	0.4 (0.3–0.6)	0.075

Definition of abbreviations: CRP = C-reactive protein; D_{LCO} = diffusing capacity of the lungs for carbon monoxide; GM-CSF = granulocyte-macrophage colony stimulating factor; KL = Krebs von den Lungen; LDH = lactate dehydrogenase; ns = nonsignificant; SP-D = surfactant protein-D; URI = upper respiratory infection; V_A = alveolar volume.

*P values are based on comparison of the two subgroups defined by plasma indium (<1 and ⩾1 μg/l) using χ^2 test or Fisher's exact test for categorical variables. For continuous variables, Student's t test was used for normally distributed data and the Mann Whitney U test for nonnormally distributed data. $P \leqslant 0.05$ was considered significant; P values ⩽ 0.10 are presented.

†Data are presented as median (interquartile range) or n (%) unless otherwise noted.

‡Chronic symptoms defined as occurring most days for ⩾3 consecutive mo/yr.

§Any occurrence in the 12 mo preceding evaluation in this study.

‖Defined as FEV_1/FVC and FEV_1 below their respective lower limits of normal (fifth percentiles) and FVC above the lower limit of normal (fifth percentile).

¶Defined as FVC below the lower limit of normal (fifth percentile) and FEV_1/FVC above the lower limit of normal (fifth percentile).

**Defined as FEV_1/FVC and FVC below their respective lower limits of normal (fifth percentiles).

††Defined as having obstructive, restrictive, or mixed pattern.

‡‡Test results for one participant were excluded because they were inadequate.

indium concentration (Table 5). Including smoking status, facility tenure, and age as covariates had little effect on the coefficients for the indium variable or the significance of the models (Table 5). In addition, including prior exposure to asbestos, silica, or other lung hazards as a covariate in the models had little effect (not shown).

Discussion

In an indium–tin oxide production and reclamation workforce with a median tenure of 2 years, median plasma indium concentration below the current Japanese standard of 3.0 μg/l (22), and few radiographic abnormalities on chest HRCT, we found consistent associations between plasma indium concentration and clinical, functional, and laboratory biomarkers. Higher plasma indium concentrations were associated with more dyspnea, reduced lung function, and increased serum KL-6 and SP-D concentrations. These differences were not explained by age, smoking status, length of employment at the facility, or prior occupational exposures and were observed at lower plasma indium concentrations than previously reported (5). On the basis of these findings, we recommend efforts to further reduce exposure to indium compounds in this industry and the use of medical surveillance in at-risk workforces, which includes, at a minimum, a sensitive plasma indium test and high quality spirometry.

Our results indicate that spirometry is a sensitive measure of the early adverse effects of indium exposure on the lungs. Although specific spirometric abnormalities were not increased for the entire group

Table 3. Plasma indium concentrations of the participating indium–tin oxide facility employees*

Group	Number	Plasma Indium (μg/l)	*P* Value†
All participants	80	1.0 (0.2–2.3)‡	
Tenure§			<0.0001
⩽2 yr	40	0.4 (0.1–1.1)	
>2 yr	40	1.6 (0.9–5.5)	
Job category‖			<0.0001
Production	42	1.6 (0.8–3.3)	
Production support	19	0.8 (0.2–1.4)	
Laboratory	8	0.6 (0.2–1.8)	
Office	11	0.03 (<0.03–0.3)	

*Eighty (85%) of 94 individuals employed at the facility had plasma indium concentration measured.
†*P* values are based on comparisons within groups (tenure or job category) using the Mann-Whitney U test. $P \leqslant 0.05$ was considered significant.
‡Data are presented as median (interquartile range).
§Tenure as a facility employee was calculated from self-reported hire date.
‖Participants in production jobs spent most of their time in production areas where indium compounds were handled in large quantities. Participants in production support jobs spent some of their time in production areas. Participants in laboratory jobs spent most of their time in the quality control laboratory where indium compounds were handled in smaller quantities. Participants in office jobs spent most of their time in administrative or other nonproduction areas where indium compounds were not handled.

compared with national rates, participants with higher plasma indium concentrations had a higher prevalence of any spirometric abnormality than participants with lower plasma indium concentrations. A restrictive pattern was the most common abnormality, which is consistent with prior reports (2, 7). Furthermore, results for FEV_1% and FVC% suggest that indium compound exposures corresponding to plasma indium concentrations as low as 0.5 μg/l are associated with adverse effects on lung function. Restricted cubic spline analyses revealed a maximum effect at a plasma indium concentration of only 1.5 μg/l, a value at which FEV_1% and FVC% were 18 and 11 percentage points lower, respectively, than corresponding reference values. Our results differ from previous reports that either did not identify any relationship between serum indium concentration and spirometric parameters or only identified differences at high serum indium concentrations (⩾20 μg/l) (3–5). One explanation is that our approach extended beyond simple linear analysis and included spline regression and log transformation of the exposure metric, which were important in detecting the associations. High-quality spirometric measurements and the use of robust reference equations may also have contributed (15, 16). It is also possible that the exposure–response relationship varies by plasma indium concentration and is steeper at the lower end of exposure.

The serum biomarkers KL-6 and SP-D appear to be sensitive indicators of early adverse effects of indium compound exposure on the lungs. KL-6 was elevated in >50% of participants and SP-D was elevated in 10%, and both biomarkers were positively associated with plasma indium concentration. Results for KL-6 were particularly striking: participants in the highest tertile of plasma indium (⩾1.6 μg/l) had a median KL-6 more than double that of participants in the lowest tertile of plasma indium (<0.5 μg/l), and significant effects were noted with spline regression at a plasma indium concentration of 1.5 μg/l. SP-D appeared less sensitive than spirometric parameters and KL-6, with significant effects noted with spline regression at a plasma indium concentration of 7.0 μg/l. Although other explanations are possible, the associations between plasma indium and these biomarkers of lung disease are likely to reflect the effect of exposure to indium compounds. These associations have been observed consistently in indium-exposed workforces globally (5, 6, 23), and a recently reported 5-year follow-up of indium workers found that serum indium, KL-6, and SP-D declined to similar degrees with cessation or reduction of exposure (24).

In contrast, LDH and CRP, two widely used clinical laboratory tests, were rarely elevated and were not associated with plasma indium concentration. YKL-40 was elevated in nearly 20% of participants, but levels were not associated with plasma indium concentration. GM-CSF autoantibody concentration was not increased in any participant and was not associated with plasma indium concentration. If indium compound exposure can provoke an anti–GM-CSF autoimmune response, as suggested previously (8, 25), these results do not support the monitoring of serum GM-CSF autoantibody levels in at-risk individuals in the absence of signs or symptoms of pulmonary alveolar proteinosis.

The excesses of wheeze and asthma and their association with plasma indium concentration were unanticipated. Because asthma is a common diagnosis, it is possible that some indium–tin oxide workers with indium compound exposure–related respiratory symptoms were misdiagnosed with asthma. However, most asthma diagnoses preceded employment. Further evaluation of these associations using diagnostic tests for asthma may be useful.

The prevalence of restrictive spirometry in this study (11%) was lower than that found at the same facility in a previous report (31%), in which half of the participants had plasma indium concentrations ⩾ 5 μg/l, one participant was a sentinel case, and most participants were no longer employed at the facility at the time of the current study (7, 8). This difference could be related to exposure reduction within the facility over time or to the introduction of new hires and/or the inclusion of nonproduction workers in the current study. Indeed, nearly 80% of the participants in the current study were not included in the previous evaluation. Half of the participants in our 2012 study had been hired in the preceding 2 years and 75% had been hired in the preceding 5 years, so few of the participants had worked at the facility when the cases of pulmonary alveolar proteinosis occurred (1999–2005) or when high rates of restrictive spirometry were documented (2002–2010). Systematic longitudinal exposure assessment is lacking, but the intervening introduction of engineering controls (e.g., machine enclosures, isolation of some dusty processes, and expanded use of task-based respiratory protection) likely reduced

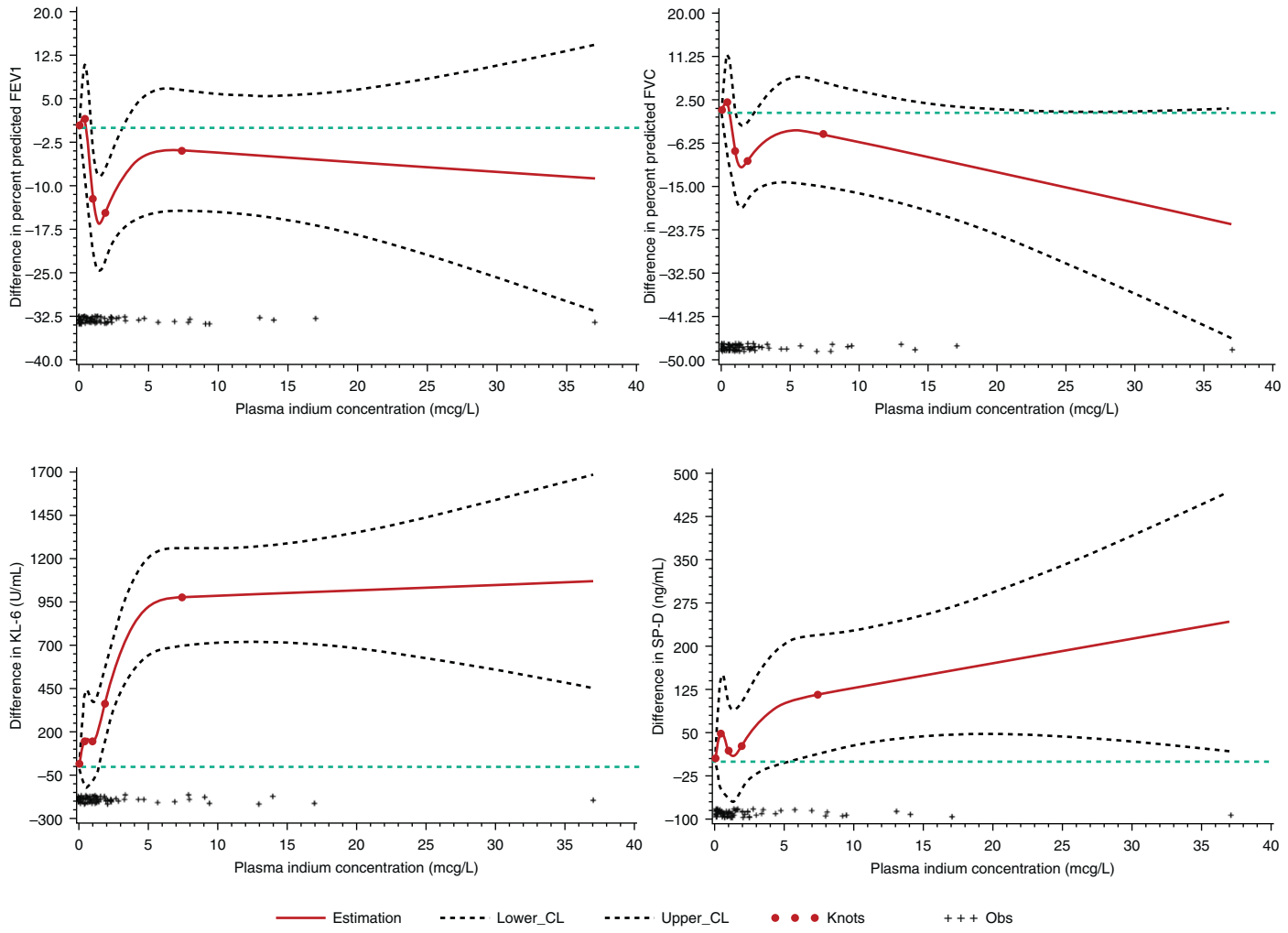

Figure 1. Restricted cubic splines show the relationship between percent predicted FEV_1, percent predicted FVC, Krebs von den Lungen (KL)-6, surfactant protein (SP)-D (*vertical axis*), and plasma indium (*horizontal axis*), adjusted for smoking status (current/former/never) and facility tenure (years). Splines for KL-6 and SP-D are also adjusted for age (years). The splines model the estimated difference in the value of the health outcome compared with a referent (value in the health outcome for plasma indium concentration of 0.015 μg/l, which is half of the limit of detection). CL = confidence limit; Obs = observation.

exposures for these more recently hired workers. We chose to include nonproduction workers (e.g., accountants, human resources specialists, customer service coordinators, and managers) due to concerns that they may have had unappreciated exposures. Four of these participants had plasma indium concentrations above the limit of quantification, confirming that even some office-based participants were exposed to indium compounds. Nonetheless, their inclusion contributed to expanding the range of exposures experienced by the study participants and to enabling the evaluation of an exposure–response relationship.

Associations between interstitial or emphysematous abnormalities on HRCT and plasma indium concentration have been previously reported (3, 5). The lack of such associations in the present study may be because these abnormalities were uncommon among participants. In addition, not all participants underwent HRCT, which may have hindered our ability to detect associations between plasma indium concentration and radiographic abnormalities. Nonetheless, the relatively young ages (26–42 yr) and limited smoking histories (6–29 pack-yr) of the participants with emphysema on HRCT make it difficult to attribute these abnormalities solely to smoking.

The lack of association between diffusing capacity and plasma indium concentration was also unexpected and differs from a prior report in which D_{LCO} was reduced in participants with serum indium concentrations of 22 to 127 μg/l (3). It is possible that, compared with changes in spirometric parameters and serum biomarkers, changes in diffusing capacity occur with higher exposure or later in the course of disease. The lack of contemporary population-based reference equations may also have contributed. We used reference equations developed from a representative sample of an industrial state, but those equations are more than 30 years old and were based on analyses that included only white adults and relatively few nonsmokers (18). Thus, comparing our measurements to the predicted values may have introduced some bias.

Table 4. Difference from reference values for pulmonary function parameters and serum biomarkers by plasma indium concentration*

Plasma Indium†	Difference (95% CI)‡			
	FEV_1, %	FVC, %	KL-6 (*U/ml*)	SP-D (*ng/ml*)
0.5 μg/l	0.3 (−10 to 11)	1.2 (−9.3 to 12)	158 (−123 to 439)	50 (−52 to 153)
1.0 μg/l	**−12 (−21 to −3.9)**	−7.9 (−16 to 0.7)	143 (−83 to 369)	19 (−64 to 101)
1.5 μg/l	**−17 (−25 to −8.4)**	**−11 (−19 to −2.8)**	**232 (17 to 447)**	12 (−67 to 90)
2.0 μg/l	**−14 (−22 to −6.3)**	**−9.5 (−17 to −1.6)**	**395 (189 to 601)**	30 (−45 to 105)
3.0 μg/l	**−9 (−18 to −0.6)**	−6.3 (−15 to 2.4)	**658 (427 to 888)**	63 (−20 to 147)
5.0 μg/l	−4.5 (−15 to 6.0)	−3.7 (−14 to 6.8)	**920 (638 to 1,202)**	101 (−2.3 to 204)
7.0 μg/l	−3.9 (−14 to 6.6)	−4.2 (−15 to 6.4)	**976 (692 to 1,259)**	**115 (11 to 218)**
9.0 μg/l	−4.2 (−14 to 6.0)	−5.4 (−16 − 4.9)	**982 (707 to 1,258)**	**123 (23 to 224)**

Definition of abbreviations: CI = confidence interval; KL = Krebs von den Lungen; SP-D = surfactant protein-D.
*Pulmonary function data are for 74 (85%), and serum biomarker data are for 80 (92%) of 87 participants in this study.
†The reference group comprised the 4 (5%) participants with assigned plasma indium concentration of 0.015 μg/l (half of the limit of detection).
‡Differences were calculated using restricted cubic spline regression. Differences with 95% CIs that exclude 0 are in bold.

Two thirds of the previously studied production workers (7) were no longer employed at the facility, and their exclusion from this study may have obscured (for HRCT and diffusing capacity) or altered (for spirometry and serum biomarkers) relationships between plasma indium concentration and adverse health outcomes variables. Current workers with longer tenure and higher plasma indium concentrations may comprise a "survivor" group that is healthier than the initial cohort. There is evidence from the spline regressions (Figure 1) of such a survival effect (26). For FEV_1% and FVC%, there is a nadir in the difference from the reference at 1.5 μg/l, and the difference is smaller at higher plasma indium concentrations. This pattern could be interpreted, erroneously in our opinion, as indicating that a higher indium exposure is protective. Furthermore, the slope of KL-6 (or SP-D) versus plasma indium concentration is steep initially but flattens out at higher plasma indium concentrations. Although this pattern could be interpreted as suggesting that a level of plasma indium exists above which no further adverse effects occur, a more constant slope may have been observed if former workers had been included in the present study. Nonetheless, our findings remain robust even if a survival effect led us to underestimate health effects of exposure to indium compounds.

A previous evaluation (7) and air sampling conducted at the time of this study indicate that exposures in this facility exceeded the Japanese standard for respirable indium of 0.0003 mg/m^3 throughout the facility and exceeded the NIOSH-recommended exposure limit for total indium of 0.1 mg/m^3 in most production areas. Little is known about the relationship between airborne levels of indium and plasma indium concentration. Our observation that participants with longer tenure had higher mean plasma indium concentration is consistent with the conceptualization of plasma indium as a metric of cumulative exposure. One study examined workers exposed to indium oxide, indium hydroxide, and indium chloride at an indium ingot production facility. Plasma and urine indium concentrations were stable over the workweek, and there were no correlations between measured air and plasma or urine indium concentrations (27). Nakano and colleagues showed that mean serum indium concentration was comparable (8.4 vs. 9.6 μg/l) between current workers and former workers whose indium exposure ended an average of nearly 5 years before the study (5). Although information on the former workers' earlier serum indium levels was lacking, this observation is consistent with accumulation of a lung burden of indium and slow clearance from the body. Indeed, in hamsters exposed to indium–tin oxide or indium oxide by intratracheal instillation, serum indium concentration increased consistently during the follow-up period, up to 78 weeks after the final exposure (28).

Conclusion

In this indium–tin oxide workforce with short tenure, low median plasma indium concentration, and few abnormalities on HRCT, we found consistent associations between plasma indium concentration and

Table 5. Linear regression models of log-transformed plasma indium concentration and health outcome variables*

Dependent Health Outcome Variable	Covariate(s)†	Coefficient (β)	*P* Value‡
FEV_1%	None	−2.31	0.015
FEV_1%	Smoking status, tenure	−2.14	0.025
FVC%	None	−2.00	0.021
FVC%	Smoking status, tenure	−2.10	0.022
KL-6, U/ml	None	133	<0.0001
KL-6, U/ml	Smoking status, tenure, age	146	<0.0001
SP-D, ng/ml	None	23.8	0.007
SP-D, ng/ml	Smoking status, tenure, age	17.9	0.040

Definition of abbreviations: KL = Krebs von den Lungen; SP-D = surfactant protein-D.
*Pulmonary function data are for 74 (85%) and serum biomarker data are for 80 (92%) of 87 participants in this study.
†Smoking status was defined as current/former/never; facility tenure and age were in years.
‡*P* value is based on F test.

adverse respiratory health effects. These differences were not explained by age, smoking status, tenure, or previous occupational exposure and were observed at lower plasma indium concentrations than previously reported. Until more is known about long-term outcomes, ongoing medical surveillance of workers and preventive efforts aimed at lowering exposures are prudent. ■

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