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RESEARCH ARTICLE

Lung epithelium injury biomarkers in workers exposed to sulphur dioxide in a non-ferrous smelter

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Abstract

Serum Clara cell protein (CC16) and surfactant-associated protein D (SP-D) were measured in 161 workers exposed to sulphur dioxide (SO_2) in a non-ferrous smelter. Seventy workers from a blanket manufacture served as referents. Exposure to SO_2 and tobacco smoking were associated with a decrease of CC16 and an increase of SP-D in serum. Tobacco smoking and exposure SO_2 interacted synergistically to decrease serum CC16 but not to increase serum SP-D. While further illustrating the potential of serum CC16 and SP-D, our study confirms that SO_2 can cause airways damage at exposure levels below current occupational exposure limits.

Keywords: *Clara cell protein; surfactant-associated protein D; sulphur dioxide exposure; lung epithelium; pneumoprotein*

Introduction

Sulphur dioxide (SO_2) is a corrosive gas released primarily during the burning of fossil fuels, the manufacture of sulphuric acid and the roasting of sulphide ores in non-ferrous smelters. The short-term effects of this pulmonary irritant are well documented especially by controlled-exposure experiments on volunteers. Respiratory effects of SO_2, which develop very quickly, consist in irritation symptoms, lung function decrements, decreased mucociliary clearance and an inflammatory response (Schlesinger 1999, Lippmann 2000). Exercise, oral breathing and co-exposure to other pollutants such as particulate matter have been shown to aggravate the respiratory effects of SO_2 (Schlesinger 1999). Asthmatics are particularly sensitive to SO_2 and show a significant decrease in forced expiratory volume in 1 s (FEV_1) even from concentrations of about 0.5 ppm (Sheppard et al. 1980).

Much less is known about the long-term respiratory effects of SO_2. Even in industrial workers exposed to relatively high concentrations of SO_2, the exact levels from which this gas can chronically damage the airways and impair lung function are still uncertain. Studies in occupational settings, most of which date to the 1970s, have provided conflicting results. Smith et al. (1977) found a significant decrease in FEV_1 in a group of copper smelter workers ($n=113$) exposed to more than 1 ppm SO_2. The study of a larger cohort in the same plant ($n=430$) did not confirm these findings as no change in pulmonary function was detected in workers exposed to more than 5 ppm SO_2 (Lebowitz et al. 1979). Lowe et al. (1970) also found no increase in respiratory symptoms and no reduction in respiratory performance in a survey of two large cohorts of steel plant workers ($n=4506$ and 5943) exposed to 0.8–1.2 ppm SO_2. However, in another study of copper smelter workers ($n=953$), a reduction of the forced vital capacity (FVC) and FEV_1 was found in

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workers who had been exposed to 0.4–3.0 ppm SO_2 for more than 20 years (Archer & Gillan 1978). This reduction correlated with the duration of exposure, both in smokers and in non-smokers. More recently, Osterman et al. (1989) reported similar observations in silicon carbide workers who had been exposed to an average of 1.5 ppm SO_2 or less and momentarily to peaks up to 4 ppm SO_2. These authors found highly significant relations between cumulative exposure to SO_2 and symptoms of phlegm, wheeze and dyspnoea. Interestingly, they found a synergistic interaction between tobacco smoking and exposure to SO_2 in the development of most respiratory symptoms. These findings suggest that exposure to 1.5 ppm SO_2 or less can be detrimental to airways and that the current occupational exposure limit for SO_2 (American Conference of Governmental Industrial Hygienists (ACGIH 2008) threshold limit value (TLV)–time weighted average (TWA), 2 ppm) may not adequately protect workers.

Over the past few years, novel approaches have been developed to assess non-invasively airway damage or inflammation caused by inhaled pollutants. Such an approach, referred to as pneumoproteinaemia, consists of measuring plasma lung-specific proteins (pneumoproteins) reflecting the cellular integrity or the permeability of the bronchoalveolar blood barrier (Hermans & Bernard 1998, 1999). Among these proteins, the most validated as a lung biomarker is the Clara cell protein (CC16, CC10 or secretoglobin 1A1), an anti-inflammatory protein secreted along the tracheobronchial tree, and the surfactant-associated protein D (SP-D), which is secreted by the alveolar epithelium in the deep lung. The aim of the present study was to evaluate whether or not these two lung markers can detect airway damage in workers chronically exposed to SO_2 in a non-ferrous smelter.

Materials and methods

Study population

The study population included 161 apparently healthy workers, all male, who had been exposed to SO_2 in the non-ferrous smelter of Ghazaouet in Algeria. The smelter annual production is 36 000 tons of zinc and 72 000 tons of sulphuric acid. The production of cadmium ceased in 1999. Exposed workers were recruited out of a total population of 235 workers (participation rate of 69%). They were divided into a low- and high-exposure group according to the level of SO_2 at the worksite. The low-exposure group included 42 workers who had been exposed on average to less than 0.5 ppm (0.2–0.3 ppm SO_2). These subjects were members of the administrative staff or were workers not directly involved in smelting operations. The high-exposure group included 119 workers exposed to SO_2 concentrations higher than 0.5 ppm and ranging from 0.7 to 2.2 ppm. These workers were directly engaged in the smelting operations or in the production of sulphuric acid. The referents group was constituted of 70 male workers from an acrylic blanket factory located in Tlemcen, a city 60 km from Ghazaouet.

Protocol

The protocol included a questionnaire, a spirometric test and the collection of urine and blood for metals or pneumoprotein analyses. All workers were examined in the morning between 7:30 and 8:30. A Schiller type Sp1 spirometer (Schiller, Baar, Switzerland) was used to assess the FVC (% of predicted values) and the FVE_1 (% of predicted values). The FVC and the FEV1 were the means of the best acceptable values obtained in accordance with the guidelines of the American Thoracic Society (1995). Blood samples were obtained by venipuncture and collected in dry tubes. Each sample was allowed to clot and after centrifugation, the serum was decanted and stored at -18°C. CC16 was determined in serum by latex immunoassay using rabbit anti-Clara cell protein (Dakopatts, Glostrup, Denmark) and as standard CC16 purified in our laboratory (Bernard et al. 1992a). This assay has been validated by comparison with a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (Hermans et al. 1998). The concentration of SP-D in serum was determined by the SPD ELISA kit from the Yamasa Corporation, Tokyo, Japan. Urinary cadmium and zinc were quantified by means of inductively coupled argon plasma mass spectrometry (ICP-MS) with an Agilent 7500 instrument. Briefly, urine specimens (500 µl) were diluted quantitatively (1 + 9) with a HNO_3 1%, HCl 0.5% solution containing Sc, Ge, Rh and Ir as internal standards. Cd was analysed using no-gas mode while helium mode was selected to quantify Zn. Using this validated method, the laboratory has obtained successful results in external quality assessment schemes organized by the Institute for Occupational, Environmental and Social Medicine of the University of Erlangen, Germany (G-EQUAS programme) and by the 'Institut National de Santé Publique', Québec (PCI and QMEQUAS programmes). Creatinine was determined using a Beckman Synchron LX 20 analyser (Beckman Coulter GmbH, Krefeld, Germany). Urine samples with a creatinine concentration lower than 0.3 g l^{-1} or higher than 3 g l^{-1} were excluded. The concentration of SO_2 in the air of the smelter was measured at different stationary sites using the transportable Testo 350 M/XL control unit (Testo N.V/S.A. Schapenbaan 1, B-1741 Ternat, Belgium).

Statistics

Continuous variables were described as the mean with standard deviation and compared between groups by the ANOVA test followed by the Dunett's multiple comparison test. Categorical variables were compared by the χ^2 test. We used two-way ANOVA to assess the changes in serum pneumoproteins associated with exposure to SO_2, smoking status (never-, ex- and current smoker categorized as 1, 2 and 3) and the possible interactions between these factors. Factors influencing the serum levels of pneumoproteins were identified by a stepwise regression analysis testing as potential predictors the age and the smoking status in referents and the duration of employment and smoking status in the low- and high-exposure groups analysed separately. Independent variables in multiple regression analyses were entered at a p-value of 0.25 and kept in the model at $p<0.05$. The relations between serum CC16 and duration of employment in the exposed groups were assessed by Pearson's correlation. With the exception of age, all variables were normalized by log transformation. The level of statistical significance was set at $p<0.05$. Statistical analyses were performed by using SAS version 9.1.3 (SAS International, Cary, NC, USA).

Results

Table 1 shows the characteristics of the two exposure groups of smelter workers and of the acrylic blanket factory workers who served as referents. There were no significant differences between the three groups with respect to age, body mass index, duration of employment and the urinary excretion of zinc and cadmium, even though the latter was slightly higher in the higher SO_2 exposure group. The proportions of never-, ex- and current smokers as well as the mean pack-years were also very similar between the three groups of workers.

The results of lung function tests and of pneumoprotein determination in serum are given in Table 2. All referents performed the spirometry whereas only 62% of the workers in the low and 54% in the high SO_2 exposure group agreed that their lung function be evaluated. While the FVC did not differ between the groups, the FEV_1 was significantly higher in workers with the greatest SO_2 exposure compared with the referents. While lung function tests did show, if any, an apparent improvement with SO_2 exposure, tobacco smoking and exposure to SO_2 in the smelter were associated with a decrease of serum CC16 and an increase of serum SP-D. The serum CC16/SP-D ratio, integrating both types of changes, was markedly decreased, on average by 42% in the most exposed workers. The comparison of serum pneumoprotein levels among all workers, including those who did not perform lung function tests, revealed the same pattern of changes in serum CC16 and SP-D as well as in the CC16/SP-D ratio. The concentration of serum CC16 correlated negatively with that of serum SP-D in the whole population ($r=-0.31$, $p<0.0001$, $n=231$), in the referents ($r=-0.27$, $p=0.02$, $n=70$) as well as in the high SO_2 exposure group ($r=-0.23$, $p=0.01$, $n=119$).

As tobacco smoking is known to strongly influence the circulating levels of CC16 and SP-D, we assessed by a two-way analysis of variance the effects of SO_2 exposure and of the smoking status (never-, ex- and current smokers) and the possible interactions between both factors. As illustrated in Figure 1, exposure to SO_2 was associated with a highly significant decrease of serum CC16 and increase of serum

Table 1. Characteristics of smelter workers and their referents.

Parameter	Referents (blanket factory workers)	Workers exposed to sulphur dioxide: Low exposure (0.2–0.3 ppm SO_2)	Workers exposed to sulphur dioxide: High exposure (SO_2 0.7–2.2 ppm)	*p*-Value
n	70	42	119	
Age (years)	40.5 (8.6)	40.0 (9.1)	39.2 (10.7)	0.82
Body mass index (kg m^{-2})	24.5 (4.9)	25.9 (3.8)	25.5 (3.9)	0.17
Duration of employment (years)	8.4 (7.0)	12.6 (9.1)	9.1 (8.2)	0.06
Zinc in urine (μg g^{-1} creatinine)	351 (300)	299 (171)	365 (305)	0.34
Cadmium in urine (μg g^{-1} creatinine)	0.75 (0.53)	0.74 (0.41)	1.10 (1.32)	0.22
Never-smokers, *n* (%)	28 (40.0)	13 (31.0)	34 (28.6)	0.47
Ex-Smokers				
n (%)	15 (21.4)	14 (33.3)	34 (28.6)	0.35
Pack-years (*n*)	14.1 (8.9)	16.7 (8.4)	15 (4.9)	0.57
Current smokers				
n (%)	27 (38.6)	15 (35.7)	51 (42.9)	0.68
Pack-years (*n*)	18.1(10.3)	17.5 (2.0)	18.0 (7.4)	0.96

Data are represented as mean (SD). *p*-Values indicate the level of statistical significance in χ^2 test (prevalences) or in the ANOVA test (mean values).

Table 2. Lung function and serum pneumoproteins in workers exposed to sulphur dioxide and their referents.

			Workers exposed to sulphur dioxide				
	Referents (blanket plant)		Low exposure (0.2–0.3 ppm SO_2)		High exposure (0.7–2.2 ppm SO_2)		*p*-Value
Parameter	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	
FVC (% of predicted value)	70	86.8 (6.2)	26	94.5(5.4)	64	93.7 (6.7)	0.11
FEV_1 (% of predicted value)	70	90.3(12.8)	26	91.7 (6.4)	64	93.5 (6.2)*	0.0005
CC16 in serum (μg l^{-1})							
Workers with LFTs	7070	7.5 (2.3)	26	5.7 (1.9)	64	5.7(2.7)*	<0.001
All workers		7.5 (2.4)	42	6.2 (2.6)	119	5.8 (3.0)*	<0.001
SP-D in serum (μg l^{-1})							
Workers with LFTs	7070	54.5 (37.1) 54.5	26	73.8 (53.4) 74.9 (44.4)	64	74.2 (34.8)* 77.6 (40.9)*	<0.002
All workers		(37.2)	42		119		<0.001
CC16/SP-D in serum							
Workers with LFTs	7070	0.19 (0.15) 0.1	26	0.17 (0.34) 0.14 (0.27)	64	0.11 (0.10)* 0.10 (0.09)*	<0.001
All workers		9 (0.15)	42		119		<0.001

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; LFTs, lung function tests. *p*-Values indicate the level of statistical significance in the χ^2 test (prevalences) or in the ANOVA test (mean values,). *Significantly different from referents $p < 0.05$.

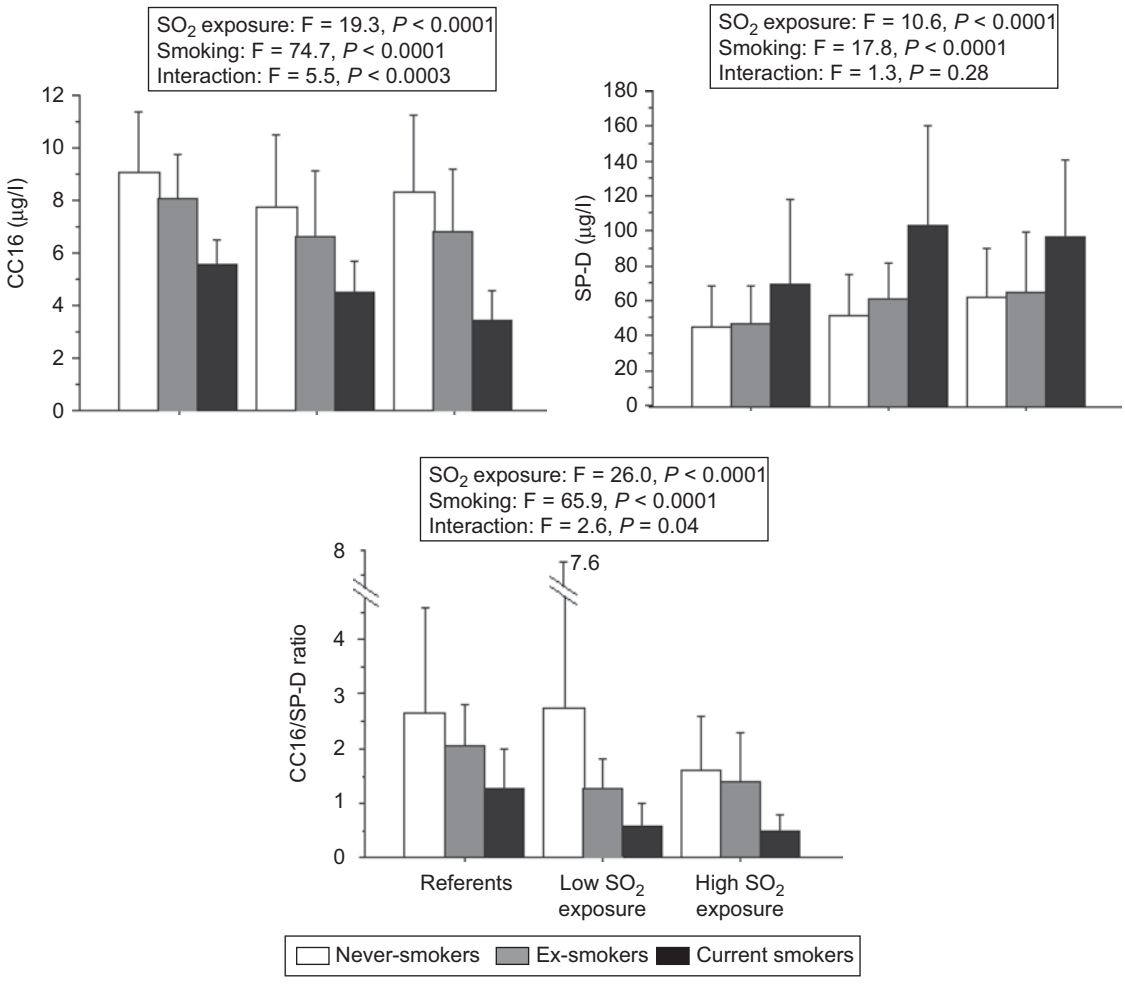

Figure 1. Effects of exposure to sulphur dioxide (SO_2), smoking status and their possible interactions on the serum concentrations (mean and SD) of Clara cell protein (CC16), surfactant-associated protein D (SP-D) and the serum CC16/SP-D ratio. Results were assessed by two-way analysis of variance. The numbers in the different exposure and smoking categories are given in Table 1.

SP-D, the ratio between the two proteins being also markedly decreased. Almost exactly the same pattern of changes in the serum levels of the two pneumoproteins and their ratio was induced by tobacco smoking. When tobacco smoking and SO_2 exposure were combined, these two factors interacted synergistically to decrease serum CC16 and the CC16/SP-D ratio in serum. Tobacco smoking and SO_2 exposure did not interact, however, when increasing serum SP-D.

The influence of age in referents or of the duration of employment in the two exposure groups on the circulating levels of pneumoproteins were assessed by a multiple regression analysis adjusting for the smoking status. In referents and in the low-exposure group, tobacco smoking was the only significant predictor of serum CC16, SP-D and CC16/SP-D ratio; the age (in referents) and the duration of employment (low exposure) exerted no significant influence (results not shown). In the high SO_2 exposure group, serum SP-D correlated only with smoking status but interestingly an inverse association emerged between serum CC16 and both smoking status (log serum CC16, partial r=-0.75, p<0.0001) and duration of employment (log serum CC16, partial r=-0.126, p=0.046). The association of serum CC16 (r=-0.28, p=0.04) persisted after adjustment for the number of pack-years, which indeed correlated with both age and duration of employment. As illustrated in Figure 2, this influence of duration of employment on serum CC16 was notable only in current smokers.

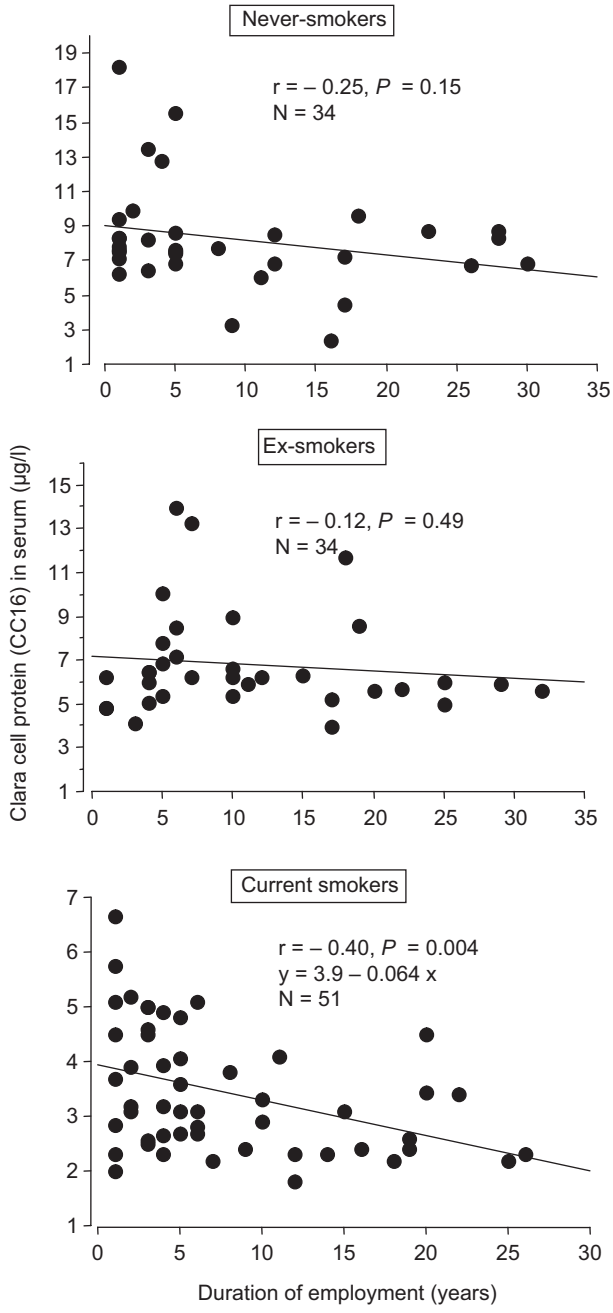

Figure 2. Associations between serum Clara cell protein (CC16) and duration of employment in workers exposed to sulphur dioxide (n=119, high-exposure group, SO_2, 0.7–2.2 ppm) categorized by smoking status.

Discussion

Changes in the serum levels of CC16 and SP-D observed in the present study are most likely the reflection of damage to the respiratory epithelium caused by tobacco smoking and exposure to SO_2 and perhaps also some other toxicants present in the atmosphere of the smelter. As demonstrated by several studies (Bernard et al 1992b, Bernard et al. 1994a, Shijubo et al 1997, Robin et al 2002), the reduction of serum CC16 caused by tobacco smoking mirrors a parallel reduction of the number of Clara cells and of the amount of protein secreted at the surface of airways. The reduction of serum CC16 associated with SO_2 exposure is probably also the consequence of a progressive loss of Clara cells as there is currently no evidence of an alternative mechanism that decrease the circulating levels of this lung-derived protein. The hypothesis of a chronic airways damage caused by SO_2 exposure is supported by the increased permeability of the pulmonary barrier as reflected by the elevation of serum SP-D. Loss of Clara cells is indeed frequently associated with a disruption of lung epithelial barrier both in acute or chronic lung damage in human and in experimental models of Clara cell damage (Hermans et al. 1999). This is the reason why the CC16/SP-D ratio, adjusting the level of CC16 for that of SP-D, has been proposed as a more accurate indicator of Clara cell damage. The calculation of this ratio relies, however, on the assumption that CC16 and SP-D similarly leak across the disrupted lung epithelial barrier (Robin et al. 2002). If CC16 and SP-D respond differently to changes in the permeability of the lung epithelial barrier, the CC16/SP-D ratio should be regarded as a more sensitive index of airway injury, integrating both cell damage and permeability changes of the lung epithelial barrier.

While all referents performed lung function tests, about 40% of the smelter workers refused to perform lung function tests, meaning that our observations might have been confounded by a participation bias. This participation bias has undoubtedly affected the results of the lung function tests, which paradoxically were significantly better in the most exposed group compared with the referents. Participation bias, however, does not seem to have affected the results of the serum pneumoproteins. The pattern of smoking and exposure-related changes in the circulating levels of these two proteins was indeed virtually the same whether considering the whole population or only the subjects who had their lung function tested. This observation confirms the great sensitivity of lung biomarkers, which can detect airway injury before lung function declines. This observation also highlights another interesting feature of lung biomarkers when used in population-based studies or surveys in the industry (Bernard 2008). Because they detect preclinical effects on the respiratory epithelium, which poorly correlated with lung function or symptoms and are normally unknown to the studied subjects, lung biomarkers might be less prone to participation biases than classical endpoints based on questionnaire or functional tests whose results are foreseeable or require an active participation of the subject. As shown in our study, this may be an advantage when study participants are not blinded to the tested hypothesis or when for different reasons it is not possible to achieve a satisfactory participation rate.

Although based on different endpoints, our results are in agreement with those of Osterman et al. (1989). Examining workers exposed to similar levels of sulphur dioxide, these authors found that SO_2 and tobacco smoking strongly interacted in the development of respiratory symptoms. Tobacco smoking and SO_2 also interacted synergistically in our study when decreasing the serum level of CC16. This interaction also emerged in the relationship between serum CC16 and duration of employment, which was highly significant in current smokers but failed to reach the level of statistical significance in never- or ex-smokers. There was however no significant interaction between tobacco smoking and SO_2 exposure when considering the increase of serum SP-D or the decrease of the serum CC16/SP-D ratio. The reason for this different response between CC16 and SP-D is unclear but it might be related to the site of secretion of these two pneumoproteins. While SP-D is mainly secreted by the alveolar epithelium in the deep lung, CC16 is predominantly secreted in the airways, precisely where most of the inhaled dose of tobacco smoke and of sulphur dioxide is deposited.

Our study further extends the list of occupational toxicants which have been shown to cause acute or chronic changes in the serum levels of CC16 or SP-D. Workers exposed to crystalline silica or suffering from silicosis show the same pattern of effects as observed here, with a decrease of serum CC16 (Bernard et al. 1994b) and an increase of serum SP-D (Wang et al. 2007). Serum CC16 has also been found to be reduced in workers exposed to the atmosphere of iron (Bergamaschi et al. 2003) and aluminium foundries (Halatek et al. 2006). The serum CC16 test has, however, a dual meaning. In some circumstances the protein is elevated because of an increased leakage across the disrupted pulmonary epithelial barrier. For instance, an increase of CC16 has been found in workers with acute or repeated exposures to irritants disrupting the epithelial barrier such as firesmoke (Bernard et al.1997, Burgess et al. 2001, Burgess et al. 1993), bioaerosols (endotoxin) (Steiner et al. 2005) or exposure to asphalt (Ulvestad et al. 2007). Similar variations of serum CC16 mirroring a loss of Clara cells or an increased epithelial permeability have been described recently in populations with short- or long-term exposures to gaseous or particulate pollutants (Arjomandi et al. 2008, Barregard et al. 2008, Madsen et al. 2008, Parvez et al. 2008).

In conclusion, our study further illustrates the potential of non-invasive lung markers to detect early effects of air pollutants. Even in situations where the assessment can be confounded by participation bias and co-exposure to other lung toxicants, lung markers such as CC16 or SP-D still prove reliable tools to detect airway damage and to bring to light possible interactions between lung toxicants. Applied to workers exposed to SO_2 these two markers show that this irritant gas can cause chronic effects on airways at exposure levels which are lower than the occupational exposure limits still in application in most countries (TLV of 2 ppm recommended by ACGIH, 2008).

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References

ACGIH. American Conference of Governmental Industrial Hygienists. (2008). TLVs and BEIs.

American Thoracic Society. (1995). Standardization of spirometry. 1994 update. Am J Respir Crit Med 152:1107–38.

Archer VE, Gillam JD. (1978). Chronic sulfur dioxide exposure in a smelter. II. Indices of chest disease. J Occup Med 20:88–95.

Arjomandi M, Tager IB, Bastaki M, Chen C, Holland N, Balmes JR. (2008). Is there an association between lifetime cumulative exposure and acute pulmonary responses to ozone? J Occup Environ Med 50:341–9.

Bergamaschi E, Apostoli P, Catalani S, Festa D, Folesani G, Andreoli R, Manini P, Schenato S, DePalma G, Franchini I, Bernard A, Mutti A. (2003). Indicators of pulmonary epithelial damage among workers at a foundry exposed to airborne pollutants. G Ital Med Lav Ergon 25:104–6.

Barregard L, Sällsten G, Andersson L, Almstrand AC, Gustafson P, Andersson M, Olin AC. (2008). Experimental exposure to wood smoke: effects on airway inflammation and oxidative stress. Occup Environ Med 65:319–24.

Bernard A. (2008). Biomarkers of metal toxicity in population studies: research potential and interpretation issues. J Toxicol Environ Health A 71:1259–65.

Bernard A, Marchandise FX, Depelchin S, Lauwerys R, Sibille Y. (1992a). Clara cell protein in serum and bronchoalveolar lavage. Eur Respir J 5:1231–8.

Bernard A, Roels H, Buchet JP, Lauwerys R. (1992). Decrease of serum Clara cell protein in smokers. Lancet 339:1620.

Bernard AM, Roels HA, Buchet JP, Lauwerys RR. (1994a). Serum Clara cell protein: an indicator of bronchial cell dysfunction caused by tobacco smoking. Environ Res 66:96–104.

Bernard AM, Gonzalez-Lorenzo JM, Siles E, Trujillano G, Lauwerys R. (1994b). Early decrease of serum Clara cell protein in silica-exposed workers. Eur Respir J 7:1932–7.

Bernard A, Hermans C, Van Houte G. (1997). Transient increase of serum Clara cell protein (CC16) after exposure to smoke. Occup Environ Med 54:63–5.

Burgess JL, Nanson CJ, Bolstad-Johnson DM, Gerkin R, Hysong TA, Lantz RC, Sherrill DL, Crutchfield CD, Quan SF, Bernard AM, Witten ML. (2001). Adverse respiratory effects following overhaul in firefighters. Occup Environ Med 43:467–73.

Burgess JL, Witten ML, Nanson CJ, Hysong TA, Sherrill DL, Quan SF, Gerkin R, Bernard AM. (2003). Serum pneumoproteins: a cross-sectional comparison of firefighters and police. Am J Ind Med 44:246–53.

Hałatek T, Trzcinka-Ochocka M, Matczak W, Gruchała J. (2006). Serum Clara cell protein as an indicator of pulmonary impairment in occupational exposure at aluminum foundry. Int J Occup Med Environmental Health 19:211–23.

Hermans C, Bernard A. (1996). Clara cell protein (CC16): characteristics and potential applications as biomarker of lung toxicity. Biomarkers 1:3–8.

Hermans C, Bernard A. (1998). Pneumoproteinaemia: a new perspective in the assessment of lung disorders. Eur Respir J 1:801–3.

Hermans C, Aly O, Nyberg BI, Peterson C, Bernard A. (1998). Determinants of Clara cell protein (CC16) concentration in serum: a reassessment with two different immunoassays. Clin Chim Acta 272:101–10.

Hermans C, Bernard A. (1999). Lung epithelium-specific proteins: characteristics and potential applications as markers. Am J Respir Crit Care Med 159:646–78.

Hermans C, Knoops B, Wiedig M, Arsalane K, Toubeau G, Falmagne P, Bernard A. (1999). Clara cell protein as a marker of Clara cell damage and bronchoalveolar blood barrier permeability. Eur Respir J 13:1014–21.

Lebowitz MD, Burton A, Kaltenbom W. (1979). Pulmonary function in smelter workers. J Occup Med 21:255–9.

Lippmann M. (2000). Sulfur oxides: acidic aerosols and SO2. In: Lippmann M, editor. *Environmental Toxicants. Human Exposures and Health Effects*. New York: Wiley-Interscience, John Wiley and Sons. p. 771–810.

Lowe CR, Campbell H, Khosla T. (1970). Bronchitis in two integrated steel works, III. Respiratory symptoms and ventilator-y capacity related to atmospheric pollution. Br J Ind Med 27:121–9.

Madsen C, Durand KL, Nafstad P, Schwarze PE, Rønningen KS, Håheim LL. (2008). Associations between environmental exposures and serum concentrations of Clara cell protein among elderly men in Oslo, Norway. Environ Res 108:354–60.

Osterman JW, Greaves IA, Smith TJ, Hammond SK, Robins JM, Theriault G. (1989). Work related decrement en pulmonary function in silicon carbide production workers. Br J Ind Med 46:708–16.

Parvez F, Chen Y, Brandt-Rauf PW, Bernard A, Dumont X, Slavkovich V, Argos M, D'Armiento J, Foronjy R, Hasan MR, Eunus HE, Graziano JH, Ahsan H. (2008). Nonmalignant respiratory effects of chronic arsenic exposure from drinking water among never-smokers in Bangladesh. Environ Health Perspect 116:190–5.

Petrek M, Hermans C, Kolek V, Fialová J, Bernard A. (2002). Clara cell protein (CC16) in serum and bronchoalveolar lavage fluid of subjects exposed to asbestos. Biomarkers 7:58–67.

Robin M, Dong P, Hermans C, Bernard A, Bersten AD, Doyle IR. (2002). Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. Eur Respir J 20:1152–61.

Schlesinger RB. (1999). Toxicology of sulfur oxides. In: Holgate S, Samet J, Koren H, Maynard R. *Air Pollution and Health*. New York: Academic Press. p. 585–602.

Sheppard D, Wong WS, Uehara CF, Nadel JA, Boushey HA. (1980). Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulfur dioxide. Am Rev Respir Dis 122:873–8.

Shijubo N, Itoh Y, Yamaguchi T, Shibuya Y, Morita Y, Hirasawa M, Okutani R, Kawai T, Abe S. (1997). Serum and BAL Clara cell 10 kDa protein (CC10) levels and CC10-positive bronchiolar cells are decreased in smokers. Eur Respir J 10:1108–14.

Smith TJ, Peters JM, Reading JC, Castle CJH. (1977). Pulmonary impairment from chronic exposure to sulphur dioxide in a smelter. Am Rev Respir Dis 116:31–9.

Steiner D, Jeggli S, Tschopp A, Bernard A, Oppliger A, Hilfiker S, Hotz P. (2005). Clara cell protein and surfactant protein B in garbage collectors and in wastewater workers exposed to bioaerosols. Int Arch Occup Environ Health 78:189–97.

Ulvestad B, Randem BG, Andersson L, Ellingsen DG, Barregard L. (2007). Clara cell protein as a biomarker for lung epithelial injury in asphalt workers. J Occup Environ Med 49:1073–8.

Wang SX, Liu P, Wei MT, Chen L, Guo Y, Wang RY, Tu ZG, Liang XC. (2007). Roles of serum clara cell protein 16 and surfactant protein-D in the early diagnosis and progression of silicosis. J Occup Environ Med 49:834–9.