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RESEARCH ARTICLE

Serum pneumoproteins in firefighters

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Abstract
Serum Clara cell protein (CC16) and surfactant-associated protein A (SP-A) were measured in a cross-sectional study in 402 firefighters. For the population as a whole, no associations were detected between serum pneumoproteins and smoke exposure. SP-A levels were increased in symptomatic subjects exposed to fire smoke within 2 days before testing. SP-A levels were higher after an inhalation incident ever. CC16 was negatively associated with the number of fires fought in the last 12 months in current nonsmokers. These associations between pneumoprotein levels reiterate the importance of adequate use of self-contained breathing apparatus by firefighters.
Keywords: Bronchial hyperresponsiveness, Clara cell protein, surfactant-associated protein A

Introduction

Fires produce a complex mixture of airway irritants. Occupational exposure to fire smoke among firefighters should be avoided by the use of self-contained breathing apparatuses. Still, exposure to toxic hazards remains a concern, because devices are often not used in overhaul situations, and not permanently during firefighting itself, especially owing to the visual impression of low smoke concentration (Brandt-Rauf et al., 1988; Burgess et al., 2001).

The evidence on respiratory effects of smoke inhalation relies mostly on evaluation of symptoms and lung function testing (Haponik, 1993; Scannell and Balmes, 1995; Bernard et al., 1997). Previous studies have indicated that smoke exposure may result in acute respiratory obstruction (Brandt-Rauf et al., 1989; Large et al., 1990) sometimes accompanied by an acute increase of airway responsiveness (Chia et al., 1990). Furthermore, studies have suggested that firefighters are at risk of developing chronic respiratory symptoms and obstructive airway changes (Mustajbegovic et al., 2001; Miedinger et al., 2007; Ribeiro et al., 2009). Miedinger et al. (2007) found increased bronchial hyperresponsiveness (BHR) in a population of 101 firefighters compared with the Swiss general population (Miedinger et al., 2007). We previously reported a positive association between the number of fires fought in the last 12 months and BHR, which we interpreted as an indication of an increased risk for irritant-induced asthma (Greven et al., 2011a).

Recently, several lung-specific proteins, pneumoproteins, have been proposed as new biomarkers for lung epithelial injury after exposure to airway irritants (Hermans and Bernard, 1999). Clara cell protein (CC16), produced in Clara cells along the tracheal–bronchial tree, and surfactant-associated protein A (SP-A), predominantly produced in alveolar type II cells, are normally found in small amounts in the blood circulation (Doyle et al., 1997; Robin et al., 2002). Their presence is explained by leakage through the air–blood barrier (Hermans and Bernard, 1999). Therefore, toxicants that affect the integrity of this barrier have an effect on the concentration of these pneumoproteins in serum. Serum pneumoproteins have been used as biomarkers in studies of exposure to respiratory irritants, including tobacco smoke (Bernard et al., 1994b; Robin et al., 2002), asbestos (Lesur et al., 1996), silica (Bernard et al., 1994a), bioaerosols (Steiner et al., 2005),

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general air pollution (Berthoin et al., 2004), bitumen fume (Ulvestad et al., 2007), trichloramine exposure in swimming pools (Carbonnelle et al., 2002), and fire smoke (Bernard et al., 1997; Burgess et al., 2001, 2002, 2003).

To investigate the nature of respiratory health effects caused by fire smoke, serum pneumoproteins were assessed in firefighters. The current study was performed in a sample from a source population in which we previously examined the effect of smoke inhalation on spirometry and bronchial responsiveness in relation to atopy (Greven et al., 2011a). Furthermore, studies found associations between chronic exposures to agents such as foundry (Broeckaert et al., 2000), crystalline silica (Bernard et al., 1994a), and sulfur dioxide (Haddam et al., 2009). Therefore, we hypothesized that exposure to fire smoke could be assessed by serum CC16 and SP-A as markers of lung–blood leakage and cytotoxicity.

Several studies have investigated associations between CC16 and SP-A and determinants, such as sex (Robin et al., 2002; Burgess et al., 2003), age (Bernard et al., 1994b; Robin et al., 2002; Hermans et al., 2003), body mass index (BMI) (Nomori et al., 1998), diurnal variation (Blomberg et al., 2003; Helleday et al., 2006), and exercise (Nanson et al., 2001). Up till now, adjustments in studies on associations between serum pneumoproteins and exposure to environmental factors have mostly been limited to a few potential confounders with seemingly arbitrary selection procedures. As an additional goal of our study, we therefore analyzed associations between exposure to fire smoke and serum pneumoproteins and systematically considered all potential confounders mentioned earlier in the literature, i.e. sex, age, atopy, BMI, diurnal variation, and smoking behaviour and lung function. Furthermore, we analyzed associations between serum pneumoproteins and respiratory endpoints (asthma symptoms, atopy, and BHR). To assess variability in serum pneumoproteins over time, we compared results from blood samples obtained on two different occasions from a subset of the current study for CC16 and for SP-A.

Methods

Population and design

The current cross-sectional study was performed in a randomly chosen subset of 402 firefighters of 23 fire brigades of a previous survey in The Netherlands (Greven et al., 2011a). All tests were performed at the fire stations between December 2008 and June 2009.

The institutional review board for human studies of the University Medical Centre Utrecht (Utrecht, The Netherlands) approved the protocol, and written consent was obtained from all participants.

Questionnaire and exposure variables

Personal exposure variables were obtained by questionnaire items involving questions on working years, inhalation incidents (have you ever inhaled a large amount of smoke?), the use of self-contained breathing apparatus, the number of days since the last fire preceding the test, the perception of exposure to fire smoke during the last fire preceding the test, the presence of respiratory symptoms after the last fire preceding the test, and the number of fires fought during the past 12 months. Details of the questionnaire can be found elsewhere (Greven et al., 2011b).

Spirometry and methacholine challenge

Spirometry was obtained by experienced technicians according to European Respiratory Society standards (Pellegrino et al., 2005) and presented as percentage of predicted (Quanjer et al., 1993) as described (Greven et al., 2011a). Bronchial hyperresponsiveness to methacholine was measured using a previously described protocol (Greven et al., 2011a). Bronchial hyperresponsiveness was considered to be present if $PD_{20} \leq 1.92$ mg methacholine (BHR_{20}). To make optimal use of all available data, we also calculated the dose–response slope (DRS) as the percent decrease in FEV_1 per milligram inhaled methacholine (O'Connor et al., 1987).

Serology

Blood was obtained from each subject by venipuncture between 8:00 AM and 9:30 PM. Each sample was processed within 4 h, and serum aliquots were stored at –80°C until analysis. Specific IgE (the common panel of allergens consisted of house dust mite, cat, dog, grass pollen mixture, and birch pollen [Allergon AB, Angelholm, Sweden]) and total IgE (DAKO A00094 and DAKO PO364, Dakopatts, Copenhagen, Denmark) were assessed in our laboratory based on previously published methods (Doekes et al., 1996). We defined atopy as a positive reaction to the specific IgE panel or total IgE exceeding 100 kU/l.

Serum pneumoproteins

CC16 was measured with a BioVendor Human Clara Cell Protein enzyme-linked immunosorbent assay (ELISA) kit (Brno, Czech Republic) as described in the manufacturer's protocol. We used two plates for the CC16 assay. A between-run variability of 7.4% was found (%CV).

SP-A was measured using a sandwich ELISA technique. Therefore, immunoassay plates (immune plates Medisorp 96 well NUNC, Roskilde, Denmark) were coated and incubated for 2 h at 37°C with a polyclonal goat antibody for human SP-A (AB3422, Millipore, Billerica, MA) diluted 1:1100 in phosphate-buffered saline (PBS). The plates were washed three times with PBS/0.05% Tween-20 (PBST) and blocked with 5% gelatine in PBST. After washing, standards and samples were added. As standard, we used pooled serum samples with high SP-A. Samples were tested undiluted and, if necessary, diluted in PBST. The plates were incubated at 4°C overnight. The following day, the plates were washed, and detection was performed with 1:1100 diluted biotin-labeled monoclonal SP-A antibody (HYB 238-04B, BioPorto, Gentofte, Denmark). After washing, avidin–horseradish peroxidase conjugate (P0364,

1:2000, Dako, Glostrup, Denmark) was added for 2 h at 37°C. For color development, OPD/H_2O_2 (Dako) was added, and the reaction was stopped with 2 M HCl. The optical density was read at 492 nm with a spectrophotometer (Molecular Devices, Versamax, tunable microplate reader Softmax Pro 4.1). SP-A results were expressed in units/milliliter, with the highest point in our serum pool standard set at 100 U/ml. The detection limit was 5 U/ml. We used six plates for the SP-A assay. A between-run variability of 6.8% was found (%CV).

Statistical analyses

Serum pneumoprotein concentrations, the DRS and the exposure variables, working years, the number of days since the last fire preceding the test, and the number of fires fought in the past 12 months were log-transformed for analysis. SP-A levels below the limit of detection were given a value of 5 U/ml. Associations between exposure variables (log-transformed) and serum pneumoprotein data were calculated using a linear regression analysis using SAS version 9.1 statistical software (SAS Institute, Cary, NC). We investigated the influence of the potential confounders, sex, age, atopy, BMI, diurnal variation, smoking behaviour, and lung function variables forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) on associations between pneumoproteins and exposure. If the regression coefficient of this association changed more than 10% when a potential confounder was included in the model, associations were adjusted for the confounder involved. Furthermore, associations were analyzed between serum pneumoproteins and respiratory endpoints (asthma symptoms, atopy, and BHR). An analysis of variance was applied on results from blood samples obtained on two different occasions from a subset of the population study for CC16 and for SP-A. The level of statistical significance was set at $P < 0.05$.

Results

Population characteristics

The randomly chosen subset comprised 402 firefighters. One fire brigade consisted solely of professional firefighters, and two fire brigades consisted of both professional and volunteer firefighters. The remaining fire brigades comprised the majority of volunteers. All tests were performed at fire stations between December 2008 and June 2009.

General characteristics of the study population and exposure variables are shown in Table 1. Of the 402 firefighters, 305 worked as volunteers, 60 as professionals, and 37 as both. Women worked significantly shorter as a firefighter ($P < 0.0001$), were younger ($P = 0.002$), fought their last fire preceding the examination longer ago ($P = 0.0036$), and used self-contained breathing apparatus more often during the last fire they fought preceding the examination compared with men ($P = 0.006$).

Serum pneumoproteins

In Table 2, serum concentrations of CC16 and SP-A are presented. Serum SP-A concentrations were below the detection limit in 168 subjects (39.9%). Serum SP-A was significantly lower in current smokers compared with currently nonsmokers ($P = 0.018$) and never smokers ($P = 0.036$). No differences were found between male and female firefighters.

The associations between potential confounders and log-transformed serum pneumoproteins are presented in Table 3. Serum CC16 were higher in male firefighters, positively associated with FEV_1 and FVC ($\beta = 0.034$, $P = 0.04$ and $\beta = 0.023$, $P = 0.03$, respectively), and negatively

Table 1. Descriptive characteristics of the firefighters.

	Total	Male	Female
Sex, *n* (%)	402 (100)	356 (88.6)	46 (11.4)
Smoker, *n* (%)	111 (27.7)	100 (28.1)	11 (24.4)
Ex-smoker, *n* (%)	115 (28.7)	102 (28.7)	13 (28.9)
Age (year, mean ± SD (min–max))	41.3 ± 8.1 (20–60)	41.8 ± 8.0 (20–60)	37.9 ± 7.8 (22–53)*
Working years as firefighter (year, mean ± SD (min–max))	12.5 ± 8.5 (0-40)	13.3 ± 8.6 (0-40)	6.1 ± 4.8 (0-19)*
Fires fought in the past 12 months (*n*, mean ± SD (min–max))	16.7 ± 18.9 (0-200)	17.1 ± 19.6 (0-200)	13.0 ± 11.8 (0-51)
Time since last fire (d, median [25th, 75th percentile])	14.0 [9.0, 100.0]	14.0 [7.5, 100.0]	35.5 [14.0, 100.0]*
Inhalation incident cases ever, *n* (%)	139 (34.8)	122 (34.6)	17 (37.0)

*$P < 0.05$.

Table 2. Serum pneumoproteins.

	All ($n = 402$)	Current smokers ($n = 110$)	Current nonsmokers ($n = 277$)	Never smokers ($n = 170$)
CC16 (ng/ml)	2.065 (0.99–18.62)	2.006 (0.99–12.83)	2.089 (1.11–18.62)	2.104 (2.00–18.62)
SP-A (U/ml)†	3.224 (5.0–3217.41)	2.703 (5.0–1015.63)	3.455 (5.0–3217.41)	3.761 (5.0–3217.41)

Data are presented as geometric means (min–max)
†Data less than DL set at 5.0 U/ml.
CC16, Clara cell protein; SP-A, surfactant-associated protein A.

associated with BMI ($\beta = -0.007$, $P = 0.01$) and sampling time ($\beta = -0.009$, $P = 0.0006$). Serum CC16 levels tended to be lower in ever smokers compared with never smokers ($\beta = -0.033$, $P = 0.09$), and CC16 did not differ between current smokers and ex-smokers ($P > 0.10$). The number of cigarettes per day was not significantly associated with serum CC16 levels ($P > 0.10$) among smokers. SP-A was negatively associated with age, smoking behaviour, and FEV_1. The association with FVC was statistically borderline significant (Table 3).

In addition, we analyzed associations of serum pneumoproteins with respiratory endpoints (asthma symptoms, atopy, and BHR). Firefighters with diagnosed asthma as defined by the questionnaire tended to have lower CC16 levels ($\beta = -0.055$, $P = 0.10$). Firefighters who were bronchial hyperresponsive or had a higher DRS had lower CC levels (Table 4). These associations were hardly affected when adjusted for smoking and atopy. No associations were found between SP-A and BHR.

When the analysis was stratified for atopy, a weak association was found between CC16 and DRS ($\beta = -0.507$, $P = 0.07$) in atopic subjects, which grew stronger when the association was adjusted for smoking ($\beta = -0.582$, $P = 0.04$). These associations were weaker in nonatopic subjects ($P > 0.10$).

Stratification for smoking showed the following associations between CC16 and the DRS adjusted for atopy: current nonsmokers ($\beta = -0.336$, $P = 0.054$) and never smokers ($\beta = -0.433$, $P = 0.11$). In never smoking atopic subjects, a strong association was found with both DRS ($\beta = -1.379$, $P = 0.008$) and BHR_{20} (OR = 0.02 (95% confidence interval, <0.001–0.88)).

Table 3. Factors associated with serum pneumoprotein levels.

	CC16		SP-A	
Variable	Coefficient	*P*	Coefficient	*P*
Sex	−0.065	0.03*	−0.019	0.85
Age	0.002	0.08	−0.011	0.01*
BMI	−0.007	0.01*	−0.011	0.26
Smoking (0/1)	−0.033	0.09	−0.270	<0.0001*
Pack-years†	−0.001	0.22	−0.012	<0.01*
FEV_1 (L)	0.034	0.01*	−0.133	<0.01*
FVC (L)	0.023	0.03*	0.069	0.06
Sampling time	−0.009	0.0006*	−0.003	0.75
Atopy (0/1)	0.011	0.60	0.086	0.22

Serum pneumoprotein levels and DRS are log-transformed.
*$P < 0.05$.
†Analyses restricted to ever smokers.
BMI, body mass index; DRS, dose–response slope; smoking, never compared with ever smoking.

Associations between exposure and pneumoproteins

No crude associations existed between serum pneumoprotein levels (log-transformed) and any of the exposure variables. SP-A was positively associated with exposure to fire smoke within 2 days preceding testing among those who also had respiratory symptoms ($\beta = 1.118$, $P = 0.003$), although the group involved was small (Table 5). This association became clearly stronger ($\beta = 1.241$, $P = 0.0007$) after adjustment for smoking. When exposure took place within the last 24 h before testing, the association became stronger ($\beta = 1.910$, $P < 0.0001$), and when it took place within the last 3 days, it became weaker ($\beta = 0.412$, $P = 0.120$). This trend continued when the period was extended (data not shown). The association between exposure within 2 days preceding testing and SP-A grew stronger when we excluded SP-A levels below the detection limit ($\beta = 1.571$, $P = 0.0008$). Serum SP-A levels tended to be higher when subjects had ever inhaled a large amount of fire smoke, which became significant when adjusted for age ($\beta = 0.138$, $P = 0.04$), smoking ($\beta = 0.139$, $P = 0.04$), FEV_1 ($\beta = 0.135$, $P = 0.047$), and FVC ($\beta = 0.131$, $P = 0.056$). No associations were found between other exposure variables and SP-A.

No associations were found between any exposure variable and CC16 in the total population. A negative association between the number of fires fought in the last 12 months (log-transformed) and serum CC16 levels ($\beta = -0.054$, $P = 0.04$) was found in current nonsmokers (Figure 1). This association grew stronger when adjusted for FEV_1 ($\beta = -0.061$, $P = 0.02$). Analysis without sampling time in the model indicated some weak confounding. Association became slightly stronger after adjustment for sampling time ($\beta = -0.040$, $P = 0.01$). In addition, no associations were found between CC16 and SP-A ($\beta = -0.244$, $P = 0.15$).

Variability within and between individuals

In a subset of 45 subjects, blood samples were obtained with a 4-month interval. Analysis of variance revealed that for CC16 about 56% of the total variability was variability between individuals and 44% was variability over time. For SP-A, about 99% of the total variability was interindividual variability and only the remaining 1%

Table 4. Associations between serum pneumoprotein levels and bronchial hyperresponsiveness.

	BHR_{20}	$BHR_{20,\ adjusted}$*	DRS		$DRS_{adjusted}$*	
Serum pneumoprotein	OR (95% CI)	OR (95% CI)	*β*	*P*	*β*	*P*
CC16	0.23 (0.06–0.92)	0.22 (0.06–0.88)	−0.314	0.023†	−0.323	0.018†
SP-A	0.82 (0.52–1.28)	0.79 (0.50–1.25)	0.007	0.87	−0.002	0.96

Serum pneumoprotein levels and DRS are log-transformed
*Adjusted for atopy and smoking.
†$P < 0.05$..
BHR20, bronchial hyperresponsiveness; DRS, dose–response slope; OR, odds ratio; CI, confidence interval.

Table 5. Associations between exposure and serum pneumoprotein levels.

	logCC16		$logCC16_{adjusted}$*		logSP-A		$logSP\text{-}A_{adjusted}$†	
Exposure variables	β	P	β	P	β	P	β	P
Fires fought in the past 12 months (n)	−0.024	0.31	−0.029	0.22	0.078	0.34	0.061	0.44
Inhalation incident ever (0/1)	0.016	0.44	0.024	0.21	0.115	0.09	0.151	0.02‡
Working years as firefighter (year)	0.028	0.24	−0.033	0.20	−0.091	0.27	0.065	0.57
Time since last fire (d)	−0.008	0.63	−0.004	0.81	−0.011	0.83	0.000	0.996
Exposure during recent fire (0/1)	0.018	0.68	0.014	0.75	0.002	0.99	0.032	0.83
Exposure during recent fire accompanied with respiratory symptoms (0/1)	0.093	0.40	0.116	0.29	1.118	0.003‡	1.275	0.0004‡

Serum pneumoprotein levels, fires fought in the past 12 months, working years, and days since last fire are log-transformed
*Adjusted for sex, body mass index, FEV1, and smoking.
†Adjusted for age, FEV1, and smoking.
‡$P < 0.05$.
CC16, Clara cell protein; SP-A, surfactant-associated protein A.

was variation over time within subjects. Thus, levels of pneumoproteins in serum seemed to be relatively stable in each subject, but differences between individuals were relatively high.

Discussion

We hypothesized that exposure to fire smoke is associated with serum pneumoproteins as markers of lung–blood leakage and cytotoxicity. Although this was not the case in the whole population, we did observe associations between serum pneumoprotein levels and several exposure variables. Serum SP-A levels were higher after exposure to fire smoke within 2 days before blood sampling among a small group of subjects who experienced respiratory symptoms after this exposure, after adjustment for smoking. Furthermore, serum SP-A levels tended to be higher in subjects who reported an inhalation incident ever. This association grew stronger adjusted for the confounders age, smoking, and lung function. CC16 levels in serum were lower for current nonsmokers who fought more fires in the past 12 months. This association also grew stronger when adjusted for sampling time and FEV_1. Furthermore, lower CC16 levels were associated with BHR, as expected.

Our finding of higher SP-A levels in recently exposed firefighters are supported by other studies, which described acute increases in serum SP-A levels after exposures to trichloramines (Carbonnelle et al., 2002) and fire smoke (Burgess et al., 2001, 2002). To investigate whether this association was influenced by the chosen time frame, we executed sensitivity analysis by shifting the days before testing. When exposure took place within the last 24 h before testing, the association became stronger, and when it took place within the last 3 days, it became weaker. This trend continued when the period was extended. This pattern of associations is indicative of a transient respiratory effect of smoke inhalation (Burgess et al., 2001). However, no associations were found between this exposure variable and CC16 levels. A possible explanation is that the half-life for serum CC16 levels is shorter than the half-life for serum SP-A levels. Serum CC16 levels are determined by three main mechanisms: (i) production of CC16 by the Clara cells in the airways; (ii) intravascular leakage of CC16 from the lung; and (iii) elimination by glomerular filtration (Hermans and Bernard, 1999; Broeckaert et al., 2000; Lakind et al., 2007). The serum half-life of CC16 is estimated to be 2–3 h due to glomerular filtration, whereas the clearance of SP-A is not associated with clearance through the kidney (Hermans et al., 2003). An alternative explanation is that serum CC16 levels are less sensitive to fire smoke exposure than serum SP-A levels. Researchers have observed higher serum SP-A levels after short-term exposures to chlorination products in swimming pools without changes in serum CC16 levels (Carbonnelle et al., 2002; Bernard et al., 2003). In addition, when exposure was not accompanied by symptoms, no associations between serum SP-A and acute exposure was found.

Higher serum SP-A levels in subjects who inhaled a large amount of fire smoke ever cannot be explained as the result of a transient response. At the moment, it is unclear whether this association might be caused by permanent damage to the pulmonary epithelial barrier. Burgess et al. (2003) described lower serum SP-A levels in firefighters compared with police officers. However, in this study, inhalation incidents were not described. As far as we know, associations between a fire smoke inhalation incident ever and serum SP-A levels have not been described before.

We found lower CC16 levels when subjects had fought more fires in the past 12 months. Chronic exposure to foundry (Broeckaert et al., 2000), crystalline silica (Bernard et al., 1994a), and low levels of sulfur dioxide (Haddam et al., 2009) is associated with lower serum CC16 levels. In addition, in a number of studies, lower serum CC16 levels are associated with smoking (Bernard et al., 1994b; Robin et al., 2002). In contrast, acute or repeated exposures to airway irritants such as bitumen fume (Ulvestad et al., 2007), bioaerosols (Steiner et al., 2005), and fire smoke (Bernard et al., 1997; Burgess et al., 2001) have been associated with higher serum CC16 levels. It has been suggested that increases of serum CC16 after exposure to irritants are caused by leakage through

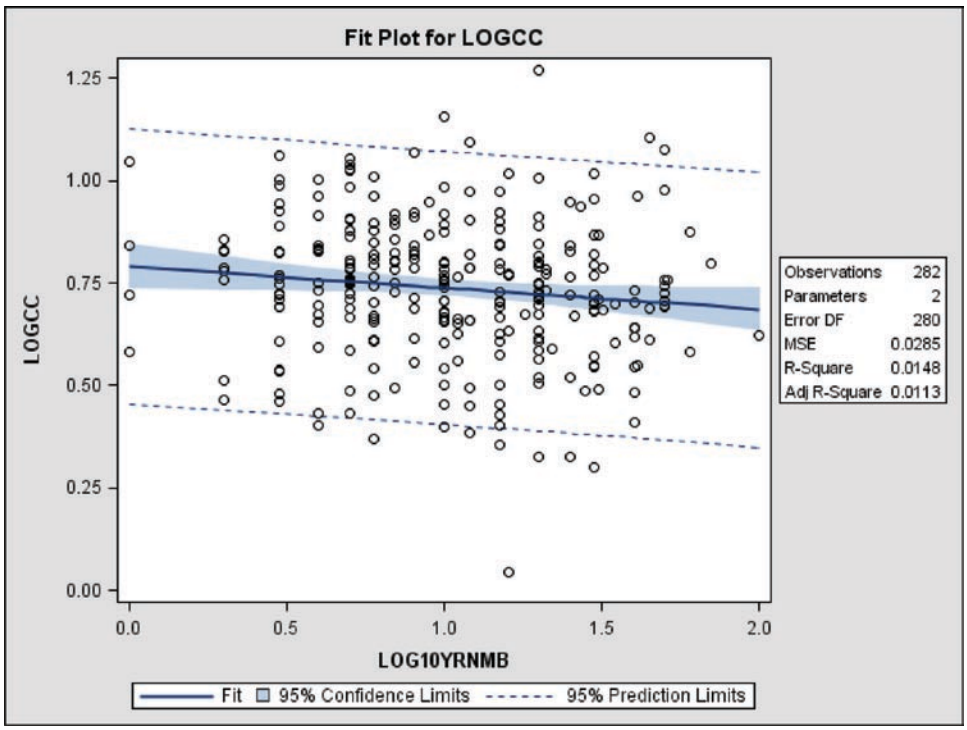

Figure 1. Relation between the number of fires fought in the past 12 months (log-transformed) and serum CC16 levels in currently nonsmoking firefighters.

a disrupted epithelial barrier (Hermans and Bernard, 1999), whereas a mechanism of reduced CC16 production by Clara cells are caused by cytotoxic effects of inhaled substances explains decreased serum CC16 levels (Robin et al., 2002; Haddam et al., 2009). A tentative explanation of our results might be that multiple exposures to fire smoke caused decreased CC16 production, which dominated increased lung–blood leakage. To the best of our knowledge, associations between frequency of exposure to fire smoke and lower CC16 levels have not been described before.

Interestingly, BHR was associated with lower levels of serum CC16. This is supported by our earlier result that BHR was associated with more frequent exposures to fire smoke (Greven et al., 2011a). Other investigators observed lower levels of CC16 in asthmatic subjects compared with healthy controls, as well for bronchoalveolar lavage fluid (Van Vyve et al., 1995) and serum (Shijubo et al., 2000; Ye et al., 2004). A possible explanation for the lower serum CC16 levels in asthmatic subjects is the markedly decreased number of CC16-positive cells in small airways of asthmatic subjects (Shijubo et al., 2000; Lakind et al., 2007).

Several other potential determinants of CC16 levels have been investigated, such as sex, age, BMI, sampling time, and exercise (Lakind et al., 2007). We found that serum CC16 levels were higher in men as observed before (Burgess et al., 2003), whereas other studies found no effects (Bernard et al., 1994b; Steiner et al., 2005) or found the same pattern in a subset of smokers (Robin et al., 2002). The adjustment for sex had a marginal effect on the relationship between smoke exposure and CC16 levels. In accordance with other studies, we found that CC16 levels tended to be higher in nonsmokers (Bernard et al., 1994b; Robin et al., 2002; Berthoin et al., 2004) and in older age (Bernard et al., 1994b; Robin et al., 2002). Our finding of lower serum CC16 levels with higher BMI corresponds with results of a subset in a study by Steiner et al. (2005), whereas others found no association (Hermans et al., 1998; Widmeier et al., 2007), or, in contrast, a positive association between serum CC16 and BMI (Nomori et al., 1996) or body weight (Hermans et al., 2003). BMI did not confound the relationship between exposure and CC16. In accordance with recent studies, we observed a clear diurnal variation in CC16 levels (Blomberg et al., 2003; Helleday et al., 2006). The adjustment for sampling time strengthened the relation between repeated fire smoke exposure and CC16 levels in current nonsmokers, indicating weak confounding by this sampling time. We consider that our population was healthy and well trained, which may influence CC16 levels (Nanson et al., 2001). In addition, none of the subjects in this study was involved in firefighting tasks less than 12 h preceding the tests. Adjustment for FEV_1 changed the associations found between both serum SP-A and inhalation incidents, and serum CC16 and the number of fires fought. FVC influenced these associations in the same way, but for serum CC16, the change was smaller (9.6%). We decided to include adjustments for FEV_1 or FVC, although these variables have not been described earlier as determinants of SP-A or CC-16 and thus as potential confounders. Nevertheless, adjustment for lung function strengthened associations, and there were no indications that FEV_1 and FVC were intermediate effects. We have no

clear explanation for this association. It is unlikely that this association occurred because of correlations between FEV_1 or FVC with BHR. Adjustment for BHR has weaker effects than adjustment for lung function, although BHR is considered a hallmark of asthma and BHR has been mentioned to be associated with pneumoprotein levels. On the other hand, if we did not adjust for lung function, generally similar associations were observed, indicating that our findings are not dependent on the adjustments made.

In our study, serum SP-A levels were lower in smokers, whereas others found higher levels in smokers (Robin et al., 2002; Berthoin et al., 2004). The direction of associations remained unchanged when we analyzed smoking as pack-years, as ever smoking compared with never smoking, and as currently smoking compared with currently nonsmoking. We have considered variability in our measurements of pneumoproteins as contributing to lack of associations. Therefore, in a subset, we reanalyzed sera of firefighters on average 4 months later and found extremely stable levels within individuals (and much higher differences between individuals). This observation lends support to the robustness of our findings.

In our study, serum aliquots were stored between 12 and 18 months at –80°C until analysis. We investigated storage time as a potential confounder of the associations between fire smoke exposure and serum pneumoprotein levels. No associations were found between storage time and serum pneumoprotein levels. Furthermore, storage time did not confound the relationship between exposure and pneumoprotein levels when included in regression models.

The CC16 serum levels we found correspond with the levels described in other studies (Robin et al., 2002; Blomberg et al., 2003; Steiner et al., 2005; Helleday et al., 2006).

In this study, exposure variables were obtained by questionnaire. An inhalation incident ever was defined by the item "have you ever inhaled a large amount of smoke?" The possibility exists that this could lead to exposure misclassification. Other items such as the number of fires fought in the past 12 months seem less prone to misclassification. A second limitation was that we had no information about neither the number of cigarettes smoked per day in our study nor when the last cigarette was smoked before blood sampling. A third limitation was that exercise was not included in the questionnaire items. However, none of the firefighters fought a fire within 12 h before the tests.

Conclusions

In conclusion, we have found effects in serum pneumoprotein levels of both acute and repeated exposures. However, the influence of many factors on these protein levels complicates the possibility to use these markers as an easy tool to assess effects of exposure to fire smoke. Nevertheless, the short-lived increases in SP-A in blood and the long-lived changes in CC16 point to systemic consequences of exposure and reiterate the importance of adequate use of self-contained breathing apparatus by firefighters.

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