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Cross sectional study on cytokine production (TNF-α, IL-8) in German coalminers with progressive massive fibrosis and in control miners using a rapid whole-blood assay

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Whole-blood release of tumour necrosis factor (TNF-α) and interleukin 8 (IL-8) was studied in 26 German ex-coalminers with progressive massive fibrosis ($\geq$ A, ILO 1980; cases) and 26 ex-miners free of pneumoconiosis ($\leq 0/1$; controls) using a simple whole-blood assay. Cases and controls were matched individually by age and duration of coalmine dust exposure (5-year window). Whole-blood cytokine release was determined (blinded to case control status) in incubations without additions (spontaneous) and with endotoxin (LPS, 3 ng ml^{-1}) or with coalmine dust (CMD; 5 mg ml^{-1}). CMD-stimulated TNF-α release was significantly increased and LPS-induced IL-8 release was significantly decreased in cases (matched t-tests: $p < 0.01$). No effect of duration of exposure was detectable in an unmatched analysis. No clear relationship with lung function parameters independent from case/control-status was observed, although a possible positive association with central airway resistance was indicated by multiple regression for both CMD stimulated TNF-α and LPS-stimulated IL-8. This study on individually matched coalminers validates previous findings on monocyte TNF release as a marker for pneumoconiosis using a method (whole-blood assay) that is more feasible for epidemiological studies. The different response of TNF-α and IL-8 may be useful in studying the occurrence of different endpoints like pneumoconiosis and lung function decrease.

Keywords: Tumour necrosis factor (TNF) α, interleukin (IL) 8, coalminers, progressive massive fibrosis, lung function.

Introduction

Cytokines are believed to play an important role in the pathogenesis of coal worker's pneumoconiosis (CWP) and obstructive lung diseases (for a review Vanhee *et al*. 1995, Schins and Borm 1999). They may serve as relevant biomarkers in epidemiological studies and in individual health screening (Borm 1994, Preat 1997, Schulte 1997). Research on biomarkers in coalminers has been focused on assessing effect markers or susceptibility markers. Cytokines as

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mediators of particle-induced pulmonary responses have been used extensively as a pool to generate candidate makers and tumour necrosis factor-α (TNF-α) as well as interleukin 8 (IL-8) have been suggested to be related to respiratory impairment in workers exposed to coal dust (Schins and Borm 1995a, Keman *et al.* 1997). IL-8 is a key chemo-attractant for neutrophils and as such is considered as a crucial factor in lung inflammation which also may occur during chronic inhalation of particles (Schins and Borm 1999). In addition to their role in inflammation, both TNF-α and IL-8 have been implicated in fibrotic lung disorders (e.g. Smith *et al.* 1995, Martinet *et al.* 1996, Coker and Laurent 1998). In studies on coalminers, higher TNF-α levels were observed in subjects developing coal worker's pneumoconiosis (Borm *et al.* 1988, Porcher 1994), as well as in miners with airway obstruction (Jorna *et al.* 1994); higher IL-8 levels were observed in coalminers with better lung function (Keman *et al.* 1997a).

However, these previous studies suffer from shortcomings including definition of disease and uncontrolled covariables (see the Discussion). Moreover, an elaborate method using isolated blood monocytes from each individual was used for assessment of cytokine release.

The aim of this study is to perform a more homogeneous comparison in TNF-α release and IL-8 release between groups of coalminers with clear and valid differences in the degree of coalworker's pneumoconiosis and/or lung function deficit using the whole-blood method to determine the cytokine concentrations. In addition, the study should validate or falsify previous findings about these cytokines as biomarkers of susceptibility or effect and evaluate the use of the whole-blood assay to perform this type of biomarker application.

Material and methods

Sample size, enclosure criteria and recruitment

The association of CWP and TNF-α, found in previous studies, was taken as a basis to calculate the necessary sample size in a matched and unmatched *t*-test (Rahlfs 1987, Armitage and Berry 1988). Schins (1996) reported the results of two studies (1987 and 5-year follow-up in 1992) about monocyte TNF-α release in coalminers in response to coal mine dust (CMD) stimulation. Based on these results it was decided to sample at least 25 cases and 25 controls in this cross sectional study with matching ($\alpha = \beta = 0.05$).

Cases were defined as male German ex-coalminers with a high degree of CWP. Both, the diagnosis of progressive massive fibrosis (PMF), i.e. CWP with large opacities in a present radiograph (ILO $\geq$ A), and the occurrence of profusion category 1/1, ILO 1980 within the first 15 years of work underground were necessary for inclusion. Controls were defined as male German ex-coalminers without CWP: on a present radiograph, the profusion category had to be $\leq$0/1, ILO 1980. In addition, cases and controls were matched individually on age and duration of exposure (time underground) within a window of ~5 years each.

Ex-coalminers compensated for coal worker's pneumoconiosis were examined routinely by two of us (RD at Ewald colliery medical department, HL at Sophia-Jacoba colliery medical department) between June 1995 and May 1996 to see whether they fulfil the enclosure criteria. Subjects identified by this procedure were asked to attend a detailed medical examination with a posterior–anterior radiograph of the thorax and a collection of peripheral blood by venepuncture. Coalminers were included as definite cases for study after informed consent to this procedure and a confirmed diagnosis of a large opacity (ILO $\geq$ A) in new radiograph (full size, 110 kV). During the study period, RD recruited 24 cases, HL two cases. All ex-coalminers who fulfilled the enclosure criteria agreed to attend the additional examinations.

Using the data of the personnel department of colliery Sophia-Jacoba coalminers were identified fulfilling the control and homogeneity criteria. A profusion category not higher than 0/0 must be read on the last existing radiograph (in Germany active coalminers are to be screened radiographically about each second year). Coalminers identified by this procedure where invited personally to attend a medical examination like it was given to the cases. If the new radiograph was assessed as < 0/1 (ILO 1980) the

coalminer was recruited as a definite control for study. Out of 125 potential controls (seven were deceased, 20 could not be followed up, 43 did not attend the medical examination) 55 ex-miners were examined between November 1995 and October 1996 resulting into 26 controls fulfilling the criteria.

Exposure history, medical data and cytokine assays

During the medical examination the following data was sampled for analysis: year of birth, height, weight, smoking status (non-smoker, ex-smoker, smoker), begin of work underground, end of work underground, years of having stopped working underground in between, sum of years having worked underground, job history, colliery history, year of first occurrence of profusion category 1/1 according to ILO 1980, ILO-reading result of the new radiograph, lung function values (FEV_1, VC, R_t, ITGV) in spirometry and bodyplethysmography, degree of being disabled, current infection (yes, no), chronic bronchitis according to WHO criteria (yes, no), clinical indication of obstruction (yes, no), clinical indication of emphysema (yes, no), cancer (yes, no), all medical diagnoses, medication, blood sentimentation rate, number of leukocytes, liver function data, creatinine data.

Blood (10 ml) was collected from each person under study into a sterile tube precoated with heparin. Quantities of 1 ml heparinized blood were transferred into three incubation tubes. This transfer was duplicated to allow a repeated measurement (measurements A and B). Whole blood was incubated without additions (spontaneous SPT) and with endotoxin of *E. coli* (lipopolisaccharide LPS, 3 ng ml^{-1}) or with coalmine dust (CMD, 5 mg ml^{-1}). Following incubation, the cell free supernatants of the whole-blood incubations were stored at -30°C during the recruitment period until analysis. Two subjects (no coalminers) were used as long-term controls to evaluate intrasubject variation, as well as the effect of differences in storage times. Peripheral blood was collected six times from one subject and three times from the other during the recruitment period of cases and controls. Preparation of the whole-blood assays was done in the laboratory where later assessment of cytokines (TNF-α, IL-8) was done (PB, RS). Cytokine determinations were performed in random order and blinded to case/control status, blinded to repetition and to all other covariables in January 1997 by two of us (PB, RS) at the Department of Health Risk Analysis & Toxicology, University of Maastricht. The details of the method are described elsewhere (Schins *et al.* 1996). In the following, the different outcome combinations are abbreviated as TNF-SPT, TNF-CMD, TNF-LPS, IL-8-SPT, IL-8-CMD and IL-8-LPS.

The distribution of differences between first (A) and second (B) measurements was rather symmetrically around 0 for most parameters. A significant ($p < 0.05$) difference between A and B measurement was only observed for LPS induced IL-8 release but without relevance when compared with the level of mean values. A simple measure of reproducibility (σ) was calculated from the repeated measurement. With one exception (TNF-CMD levels in one subject), no monotone or significant trend was observed in any of the cytokine values. Since this exceptional trend was against expectation, we found no indication that differential storage times influence the results of cytokine level determination systematically.

Statistical methods

The main analysis (comparing cytokine levels of cases and controls) was based on the average of both measurements (A, B). Outliers were identified and calculations were done with and without these outliers. Distributions of all relevant variables including the cytokine levels were explored by histograms, box and whisker plots, average values and standard deviations. Matched and unmatched *t*-tests were performed to check for significant differences in quantitative variables between the groups of cases and controls. The matched *t*-tests were performed for cytokine levels with and without accounting for a derived measure of reproducibility. Differences in qualitative variables between cases and controls were analysed by (unmatched) Mantel–Haenszel and (matched) McNemar χ^2-tests (Hartung 1995). Paired and unpaired multiple linear regression models were fitted to the cytokine data adjusting for subsets of covariates (Neter *et al.* 1985). A significance level of 5% were chosen for all tests.

Graphics and statistics were done with Excel 5.0 (Microsoft Corporation 1993), ORIGIN 4.0 (Beneke and Schwippert 1997), and BMDP Release 7 (Dixon *et al.* 1992).

Results

General characteristics

An overview on the distribution of important characteristics of the study group is given in table 1.

Ex-coalminers included in this study had a mean age of 69.4 years and had worked underground for 21.6 years on the average. The mean difference in age or

Table 1. Mean standard deviation of basic covariables and distribution of qualitative data in 26 cases and 26 controls.

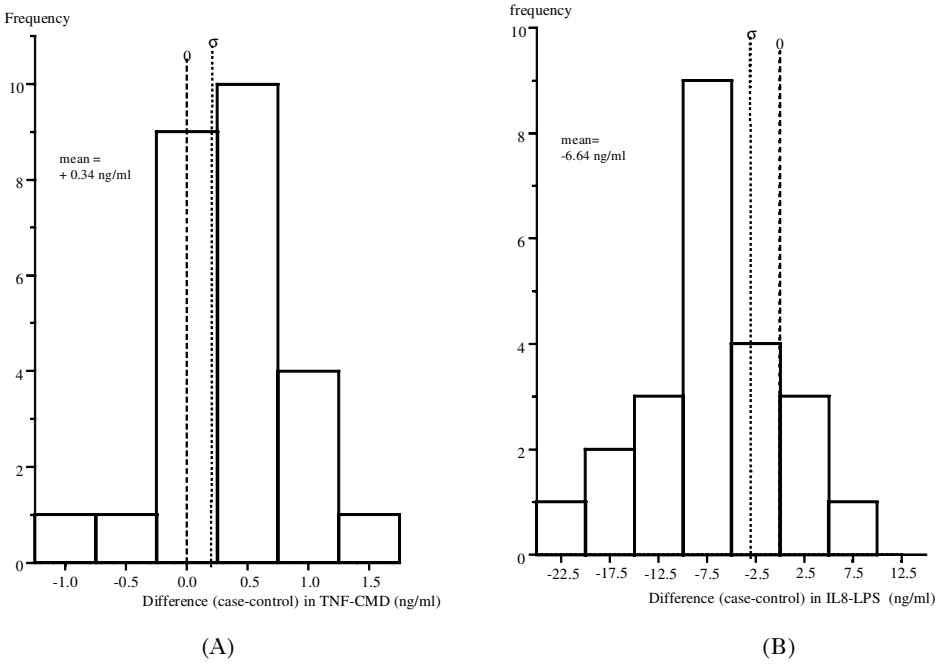
	Cases, ILO ≥ A		Controls, ILO ≤ 0/1	
	Mean	SD	Mean	SD
Quantitative data				
Age (years)	69.6	4.12	69.3	4.48
Time underground (years)	21.3	9.58	21.8	9.91
First exposure (years)	1946.7	4.13	1948.2	5.76
Time since last exposure (years)	26.9	8.91	24.7	8.69
Weight (kg)	78.5	10.32	77.5	12.36
Height (cm)	169.7	5.85	172.5	5.79
Blood sedimentation rate ((1 h) mm^{-1})	11.5	9.39	12.3	12.95
Leukocytes ($G\,l^{-1}$)	7.6	1.71	5.6	1.06
γ-Globulin ($U\,l^{-1}$)	25.9	21.64	15.7	8.82
Creatinine (mg dl^{-1})	0.95	0.19	1.03	0.09
VC (l)	3.1	0.74	3.2	0.81
FEV_1 ($l\,s^{-1}$)	1.9	0.67	2.3	0.76
FEV_1/VC (%)	59.3	14.5	71.9	14.5
R_t (hPa· $s\,l^{-1}$)	0.41	0.19	0.31	0.20
ITGV (l)	4.25	1.4	4.37	0.99
Qualitative data	No	Yes	No	Yes
Smoking 1 (no = never smoker)	2	24	0	26
Smoking 2 (no = non-smoker)	24	2	19	7
Infection	23	3	26	0
Cancer	25	1	22	4
Inhalation drugs	7	19	23	3
Cardiovascular drugs	8	18	11	15
Anti-inflammatory drugs	12	14	26	0
Antibiotics	26	0	26	0

Lung function data: vital capacity, VC forced expiratory volume in 1 s FEV_1; Tiffeneau index, FEV_1/VC airway resistance, R_t; intra thoracic gas volume, ITGV. Classification of medical treatment was done according to Schins and Borm (1995b).

duration of exposure between cases and controls was less than half a year. Since there was a systematic lag in date of examination between cases and controls, this involves a difference in duration of storage of the frozen blood until cytokine level determination (reflected by significant differences in e.g. time since last exposure). This potential bias was explored by analysing the data of the long-term controls (see the Materials and methods). Number of leukocytes and γ-globuline values were clearly elevated among cases ($p \leq 0.05$). After excluding one control miner with renal disease ($n = 25$) the difference in creatinine levels was still of borderline significance ($p = 0.08$).

With the exception of Tiffenau index (FEV_1/VC) lung function values point at deficits in both cases and controls compared with normal values of the European Community (Quanjer *et al.* 1993; data not presented). Airway resistance (R_t) and intrathoracic gas volume (ITGV), both determined by bodyplethysmography, were elevated in cases and controls compared with normal values (Ulmer *et al.* 1991; data not shown). Thus, deficits in lung function were found in both groups on the average, irrespective of CWP-status (Morfeld and Piekarski 1996).

Whereas there was no clear difference in vital capacity (VC) the forced expiratory volume in one second (FEV_1) was smaller in cases ($p = 0.02$). Most pronounced is the difference in FEV_1/VC with and without standardization to



(A) (B)

Figure 1. Distribution of the differences of coal mine dust stimulated TNFα release (TNF-CMD, Fig. 1A) and endotoxine stimulated IL-8 release (IL8-LPS, Fig. 1B) between cases (ILO $\geq$ A) and controls (ILO $\leq$ 0/1). Analysis of 26 (TNF) and 23 (IL8) matched pairs. σ indicates the estimated measure of reproducibility (TNF: $\sigma = 0.2$ ng/ml, IL8: $\sigma = 3.0$ ng/ml).

normal values according to Quanjer *et al.* 1993: the values in cases were significantly lower than in the controls ($p < 0.001$). Correspondingly, airway resistance R_t, measured by bodyplethysmography, was higher among cases on the average ($p = 0.10$). No difference was observed in the intrathoracic gas volume (ITGV) with or without taking normal values according to Ulmer *et al.* 1991 into account. Hence, as expected, lung function values were better among controls on the average. As a consequence, degree of pneumoconiosis and lung function values have to be analysed simultaneously to separate their association with cytokine levels.

The number of ex-smokers was slightly higher among cases (22 versus 19), as was the number of subjects with acute infection (3 versus 0). Significant differences in medical treatment were obvious: cases were treated more often with inhalation drugs (19 versus 3) and anti-inflammatory drugs (14 versus 0). Thus, medical treatment is a potential confounder.

Evaluation of biomarkers

The TNF levels induced by coalmine dust were higher in cases (mean $= 0.53\,\mathrm{ng\,ml^{-1}}$) than among controls (mean $= 0.19\,\mathrm{ng\,ml^{-1}}$) while the opposite was observed for LPS induced IL-8 release (cases: mean $= 6.21\,\mathrm{ng\,ml^{-1}}$, controls: mean $= 14.24\,\mathrm{ng\,ml^{-1}}$).

Cytokine levels varied systematically with the kind of incubation (table 2): lowest concentrations were found in spontaneous release, higher values after

Table 2. Group size (n), mean and standard deviation of cytokine levels for cases and controls and two sided p of matched t-tests against zero. Because of outliers, additional analyses with restricted numbers were performed. No results are reported for TNF-SPT because nearly all measurements show levels below detection limit.

Cytokine Release (ng ml^{-1})	Cases (ILO ≥ A)			Controls (ILO ≤ 0/1)			
	n	Mean	SD	n	Mean	SD	p
TNF-CMD	26	0.53	0.38	26	0.19	0.31	0.002
TNF-LPS	25	3.50	1.40	25	3.38	1.96	0.81
IL-8-SPT	26	0.17	0.19	26	0.48	0.52	0.009
	25	0.17	0.20	25	0.42	0.40	0.10
IL-8-CMD	26	6.19	7.91	26	9.09	14.12	0.39
	23	4.22	3.23	23	7.14	9.12	0.16
	22	4.34	3.25	22	5.66	5.87	0.32
IL-8-LPS	25	6.21	4.38	25	14.43	8.71	0.0001
	23	6.59	4.37	23	13.23	7.38	0.0001

TNF and IL-8 are tumour necrosis factor-α and interleukin 8; SPT, CMD and LPS are spontaneous (without additions), coal mine dust (5 mg ml^{-1}) and LPS (3 ng ml^{-1}) induced cytokine release in whole blood.

stimulation with coal mine dust CMD (5 mg ml^{-1}) and the highest values were determined after stimulation with endotoxin LPS (3 ng ml^{-1}). In general, TNFα levels were higher, but IL-8 levels were lower among cases.

This effect was significant when CMD-stimulation was used but not when LPS-stimulated TNF-α release was analysed as the outcome measure. The suppression was significant in IL-8-SPT and IL-8-LPS and was most pronounced when LPS stimulation was used. Although the effect was greater on the average when CMD stimulation was applied than in spontaneous IL-8 release, clearly no significance was reached for IL-8-CMD. This limitation was no result of distortions due to outliers. These results remained unchanged even when the restricted reproducibility was taken into account explicitly.

Simple linear regression models were used separately for cases and controls to study the relation between TNF or IL-8 release and duration of dust exposure (Derwall *et al.* 1998). Despite a wide range of duration of exposure, no trend of rising levels of TNF-CMD with increasing duration of exposure was detected. t-Tests of the regression coefficients gave $p = 0.42$ (cases ILO ≥ A) and 0.91 (controls ILO ≤ 0/1). Similar results were found for LPS induced IL-8 release: testing the regression coefficients against zero resulted into $p = 0.65$ (cases) and 0.84 (controls). The analyses of cytokine levels after other stimulation also showed no trend with duration of exposure.

To explore whether cytokine data were related to lung function independent from case/control status (i.e. from pneumoconiosis) regression analyses were performed separately for cases and controls.

Figure 2A and B demonstrate the higher airway resistance among cases. When restricting the analyses to controls no trends in cytokine levels with airway resistance could be detected (TNF-CMD $p = 0.78$, IL-8-LPS $p = 0.23$). Interestingly, a borderline significant positive association between airway resistance and coal mine dust stimulated TNF-α release was found in cases ($p = 0.052$), while no (significant) trend was found in IL-8-LPS simultaneously ($p = 0.36$).

We fitted a series of unpaired and paired multiple linear regression models to estimate and control the influence of the whole set of covariables (table 1) on

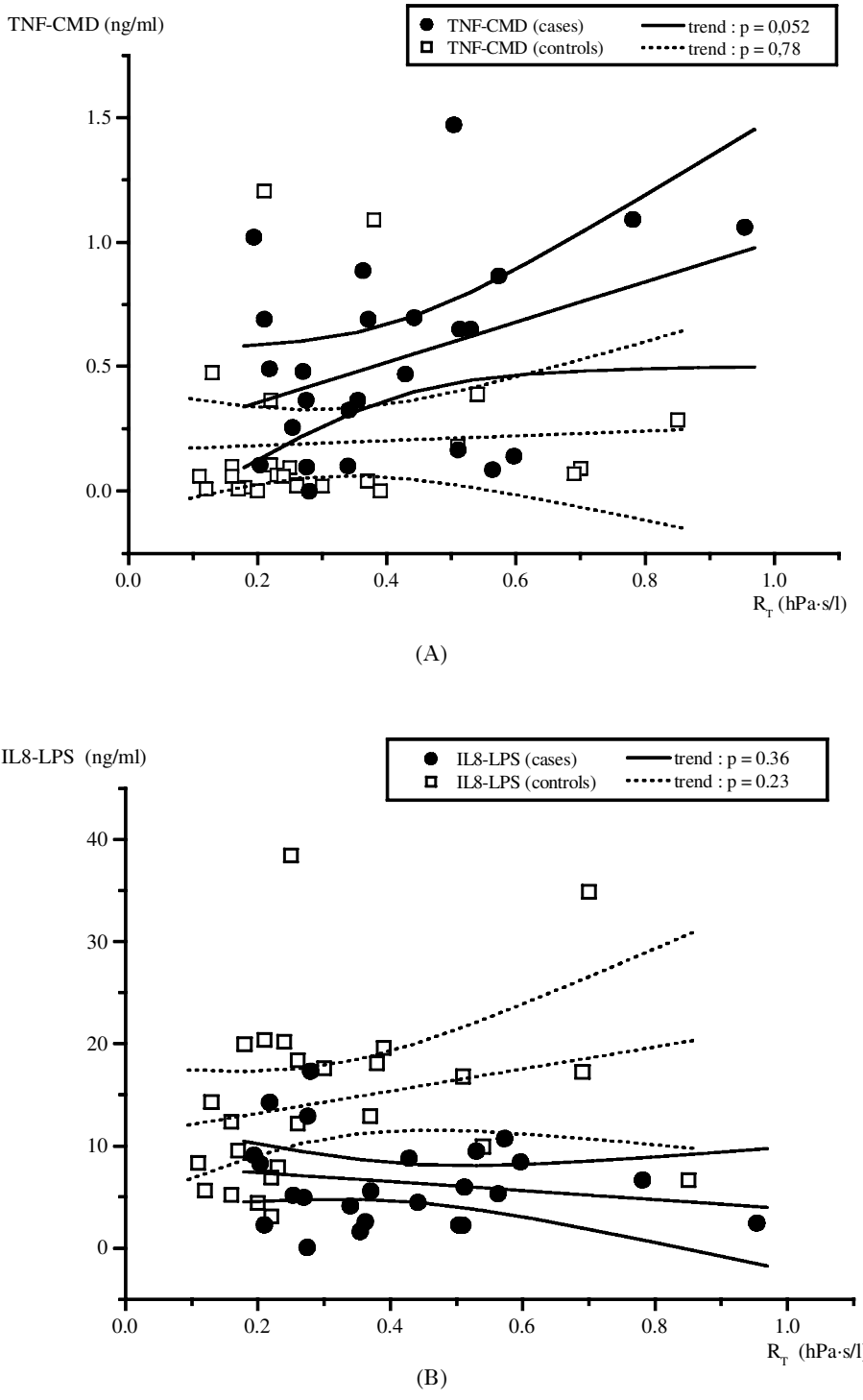


Figure 2. Coal mine dust stimulated TNFα-release (TNF-CMD) by airway resistance R_t in 25 cases (ILO ≥ A ■) and 25 controls (ILO ≤ 0/1 ●) (Figure 2A). Endotoxin stimulated IL8-release (IL8-LPS) by airway resistance R_t in 24 cases and 25 controls (Figure 2B). Separate regression lines with 0.95-confidence regions are shown.

Table 3. Coefficients and p in unpaired and paired multiple linear regression of coalmine dust stimulated TNF release (TNF-CMD) and endotoxin-stimulated IL-8 release (IL-8-LPS) on coalworker's pneumoconiosis CWP (yes: ILO $\geq$ A, no: ILO $\leq$ 0/1), airway resistance, R_t (measured by bodyplethysmography), Tiffeneau index, FEV_1/ VC (measured by spirometry) and time underground, i.e. duration of exposure. The TNF-model is adjusted additionally for age, smoking status and number of leukocytes. The IL-8-model is adjusted additionally for age, blood sedimentation rate and medication.

	Unpaired coefficient	p	Paired coefficient	p
Multiple linear regression for TNF-CMD ($ng\,ml^{-1}$)				
CWP (yes/no)	0.46	<0.01	0.44	0.01
R_t ($hPa \times s\ l^{-1}$)	0.92	<0.01	1.6	<0.01
FEV_1/VC (%)	0.0073	0.08	0.0209	0.04
Time underground (years)	−0.0023	0.65	0.0032	0.95
Multiple linear regression for IL8-LPS ($ng\,ml^{-1}$)				
CWP (yes/no)	−6.1	<0.01	−7.2	0.04
R_t ($hPa \times s\ l^{-1}$)	9.4	0.10	6.0	0.43
FEV_1/VC (%)	0.15	0.04	0.01	0.93
Time underground (years)	−0.11	0.25	−2.98	0.03

cytokine levels. The final models for TNF-CMD and IL-8-LPS are presented in table 3. Many other combinations of covariables were tried, but those covariables were dropped from the final models, if they showed no relevant impact on CWP and lung function coefficients.

Table 3 demonstrates that the elevation of TNF-CMD as well as the suppression of IL-8-LPS among cases with ILO $\geq$ A cannot be explained by confounding. Moreover, no effect of duration of exposure was found in the unmatched analysis. Interestingly, independent effects of lung function were indicated: A positive correlation with airway resistance measured by bodyplethysmography and a simultaneously borderline significant positive correlation with the Tiffenau value FEV_1/ VC were found for both cytokines. Note, that higher values of airway resistance measured by Rt are accompanied by lower values of FEV_1/VC normally. Accordingly, Rt and Tiffenau index were negatively correlated in the data ($r = -0.58$, $p < 0.01$). Thus, these results may point at a positive association of both cytokines with central airway resistance, independent from CWP-status. As an exception the paired multiple analysis of IL-8-LPS was unable to demonstrate this relationship with lung function clearly. Here we have to take into account the improbable result of a significant effect of duration of exposure in an exposure matched study indicating this paired analysis as unreliable (possibly due to the small numbers because the same result was found without adjusting for time underground).

Discussion

Release of tumour necrosis factor (TNF-α) and interleukin 8 (IL-8) was studied in 26 German ex-coalminers with progressive massive fibrosis (≥A, ILO 1980; cases) and 26 ex-miners free of pneumoconiosis (≤ 0/1, ILO 1980; controls) using a simple whole-blood assay. Cases and controls were matched individually on age and duration of coalmine dust exposure (5-year window). Whole-blood cytokine release was determined (blinded to case/control-status) in incubations without additions (spontaneous) and with endotoxin (LPS, $3\,ng\,ml^{-1}$) or with coalmine dust (CMD, $5\,mg\,ml^{-1}$). CMD-stimulated TNF-α release was significantly increased and LPS-induced IL-8 release was significantly decreased in cases (matched t-tests: $p < 0.01$). No effect of duration of exposure was detectable in an

unmatched analysis. No clear relationship with lung function parameters independent from case/control-status was observed, although a possible positive association with central airway resistance was indicated by multiple regression for both cytokines, CMD stimulated TNF-α and LPS-stimulated IL-8.

In previous studies on cytocines as biomarkers in CWP the disease severity was achieved differently. Schins and Borm (1995a) considered a profusion category of 0/0, ILO 1980 (Internationales Arbeitsamt 1980) as 'healthy coal workers'. A profusion category $\geq 0/1$, ILO 1980 was considered as evidence for coal worker's pneumoconiosis since one was interested in changes at early stage of disease. However, category 0/1 on the ILO 1980 scale does not correspond to a definite disease, or even necessarily to a specific reaction to inhaled dust. In a clinical context, category 0/1 may be interpreted cautiously as an early biological sign of dust retention or perhaps as a premorbidity indicator (Borm 1994, Morfeld *et al.* 1997a,). In addition, no multivariable control or matching on potential confounders was performed (Borm *et al.* 1988, Schins and Borm 1995a, Keman *et al.* 1997a). In other studies, control groups were often clearly different from study groups in age or other important covariables (Porcher *et al.* 1994, Hadnagy and Idel 1997). In this study, retired coalminers with severe pneumoconiosis (progressive massive fibrosis, cases) and without pneumoconiosis (ILO $\leq 0/1$, controls) were compared. Thus, a clear and valid difference in the degree of coal worker's pneumoconiosis between comparison groups was ensured. By design, cases and controls were matched on age and duration of dust exposure. The mean difference in age and duration of exposure between cases and controls was less than half a year, ruling out major distortions of the results due to these variables. In addition, multiple regression was used to adjust for a set of potential confounders simultaneously.

As in previous studies (Borm *et al.* 1988) cytokine concentrations were found to be skewed to the right in each comparison group which in case of TNF is possibly related to mutations in the TNF promoter site (Zhai *et al.* 1998). Owing to the matching procedure, the difference in cytokine release in pairs of cases and controls could be studied as the final measure of outcome in this investigation. As expected, the distribution of these differences was quite symmetrical and could be approximated by a Gaussian distribution readily. Hence, the analysis could be performed using simple parametric statistical procedures: a higher precision was gained and the results could be presented on a linear scale allowing easy interpretation.

As a main result, this study confirms previous findings on monocyte TNF release as a biomarker correlated with progressive massive fibrosis (Porcher *et al.* 1994, Vanhee *et al.* 1995) and is in line with an association to simple pneumoconiosis (Borm *et al.* 1988, Schins and Borm 1995). Coalmine dust stimulated TNF-α release in whole-blood assay was elevated among cases. This effect was significant for coal mine dust stimulated TNF-α release even when considering reproducibility of cytokine level measurement and when considering a set of potential confounders. Simultaneously, these findings about progressive massive fibrosis validate indirectly the outcomes using a simpler whole-blood method (Schins *et al.* 1996) in comparison to the more complicated isolated monocytes method (Borm *et al.* 1988). No clear relationship of TNF release with lung function was observed, albeit a multiple regression analysis pointed at a possible positive association with central airway resistance independent from pneumoco-

niosis. Although differing in detail this finding is in agreement with the results of Jorna *et al.* (1994) reporting an increased TNF-α secretion by blood monocytes upon *ex vivo* stimulation with silica and endotoxin in retired coalminers with airflow obstruction, defined by spirometry and impedance measurement (forced oscillation technique).

The suppression of LPS induced IL-8 release observed among miners with progressive massive fibrosis (cases) needs further investigation, but under these conditions a different direction of TNF-response (elevation) and IL-8-response (suppression) in coal worker's pneumoconiosis is seen. With respect to airway resistance no different associations of TNF-CMD and IL-8-LPS could be found; independent from case/control status multiple regression analyses indicated a possible positive association with central airway resistance of both cytokines, coal mine dust stimulated TNF-α and endotoxin stimulated IL-8. The positive association of endotoxin stimulated IL-8 with Tiffenau index FEV_1/VC indicated is in line with findings of Keman *et al.* (1997a) who reported higher IL-8 levels in coalminers with higher FEV_1 values.

TNF-α as found in stimulated whole blood is almost exclusively from blood monocytes, while neutrophils and lymphocytes produce only minor amounts of TNF-α (Schins and Borm 1999). On the other hand, neutrophils are a crucial source of IL-8 in whole blood (Strieter *et al.* 1992). In line with this, in whole blood stimulated with LPS, the releases of TNF-α and IL-8 are known to follow distinct kinetics (Cavaillon *et al.* 1995). But further studies are needed to explain the different 'priming' (Schins and Borm 1995a) of TNF-α and IL-8 sufficiently.

Similar to TNF-α, IL-8 values show no relationship with duration of dust exposure. Thus, IL-8 (like TNF-α: Borm, 1994) appears to be a potential biomarker of response but with a presumably different kinetic behaviour to TNF-α. An important caveat against the interpretation of TNF-α and IL-8 not being markers of exposure is that individual data on coalmine dust concentrations are not available in this study. Even if estimates of cumulative coal mine dust exposure were available a distinction between markers of exposure and markers of disease would not be straightforward or obvious (Schins and Borm 1995a)

Even if TNF-α and IL-8 are in fact disease markers the degree of variation of the difference in cytokine levels between matched cases and controls observed in this study (figure 1) limits the hope to apply these markers in individual health screening. In the case of best discrimination observed (IL-8-LPS) 17% of cytokine level differences were found on the 'wrong' side, i.e. differed from expectation in sign. However, the findings of this cross-sectional study on cytokine release in coalminers, based on a design of matched cases ($ILO \geq A$) and controls ($ILO \leq 0/1$), underscore the possible importance of biomarkers for epidemiological studies, even with a view to limit value assessment (Morfeld *et al.* 1997b). A combination of both markers (TNF-CMD, IL-8-LPS) should be tested in future studies as a more powerful index with possibly higher specificity and/or sensitivity than the single markers when studying different endpoints like pneumoconiosis and lung function diseases simultaneously.

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