Blood antioxidant status in coal dust-induced respiratory disorders: a longitudinal evaluation of multiple biomarkers

Roel P. F. Schins, Soedjajadi Keman and Paul J. A. Borm

To investigate the involvement of oxidative stress in coal dust-induced respiratory disorders, red blood cell and serum antioxidants in 66 coal miners were related to 5-year changes in coal workers' pneumoconiosis (CWP), chronic bronchitis, and lung function decrease (n = 40). Reduced (GSH) and oxidized (GSSG) glutathione concentrations, glutathione peroxidase (Gpx), glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase activities were measured in erythrocytes and vitamin A, vitamin E and iron were determined in serum. Changes in CWP were determined by chest radiography, chronic bronchitis was determined from a validated questionnaire and lung function decline was calculated by linear regression for a 10 year interval before blood sampling. SOD activity was increased in miners with progression of CWP (2308 ± 156 vs 1703 ± 155 U g^{-1} Hb, $p < 0.05$), and GSH was reduced in those with chronic bronchitis at follow-up (3.53 ± 0.16 vs 4.05 ± 0.09 mmol g^{-1} Hb, $p < 0.01$). Stepwise discriminant analysis showed that for both pneumoconiotic and non-pneumoconiotic respiratory disease in this cohort, increased enzymatic antioxidants (i.e. Gpx, Catalase, SOD) were high risk factors, while increased 'non-enzymatic' antioxidants (i.e. vitamin E, GSH) indicated reduced risk. GST activity showed discriminative power in two ways, i.e. decreased activity in those at risk for CWP, but increased in those with rapid decline in FEV_1. We conclude that the multiple marker approach applied here shows the relevance of interpretation of total 'antioxidant status' versus single antioxidant measurements in health screening of individuals at risk for respiratory impairments.

Keywords: *blood antioxidants, coal workers' pneumoconiosis, airway obstruction, multiple markers, follow-up.*

Abbreviations: *CR, chest radiograph; CWP, coal workers' pneumoconiosis; Gpx-Se, selenium-dependent glutathione peroxidase; Gpx-To, total glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione-S-transferase; SOD, superoxide dismutase.*

Introduction

It is well known that occupational exposure to coal dust can lead to coal workers' pneumoconiosis (Crystal *et al.* 1991). However, chronic inhalation of coal dust may also cause other respiratory effects such as emphysema, chronic bronchitis and airflow obstruction (Wouters *et al.* 1994). The role of oxygen radicals in these diseases has been discussed extensively over the last decade (Cantin and Crystal 1985, Barnes 1990, Kehrer 1993, Halliwell and Cross 1994). Because of the intrinsic physicochemical characteristics of mineral dusts and their complex behaviour in the lung, the significance of 'oxidative stress' in the development of mineral dust-related respiratory disorders has received special attention (Janssen *et al.* 1992, Kamp *et al.* 1992). Previously, we showed that many components of the blood antioxidant system of coal miners were related to the severity of coal workers' pneumoconiosis (CWP) (Engelen *et al.* 1990). More recently, altered antioxidant status in coal dust exposure and stage of pneumoconiosis was also reported by others, in the blood (Perrin-Nadif *et al.* 1996), and in bronchoalveolar lavage (Vallyathan *et al.* 1995). Since antioxidant status has also been related to obstructive disease (Morabia *et al.* 1989, Schwartz and Weiss 1994, Britton *et al.* 1995), one could suggest that the impaired oxidant/antioxidant balance observed in coal workers (Engelen *et al.* 1990) may also play a role in the non-pneumoconiotic respiratory effects in these subjects.

Although the presence of 'oxidative stress' in coal dust-exposed subjects may be evident, its significance in the development or progression of coal dust-induced respiratory disorders remains to be elucidated. To determine the significance of antioxidant measurements with regard to the development or progression of a disease, a longitudinal design is necessary (Schulte *et al.* 1993). However, follow-up studies dealing with the prognostic power of antioxidant status are scarce. In the present study several blood antioxidant parameters previously measured in coal workers are related to prospective changes in pneumoconiosis (chest radiography) and the presence of chronic bronchitis, as well as to retrospective analysis of lung function decline. The aim of this study is to determine the relevance of blood antioxidant status in surveillance and screening of (retired) coal workers. For this purpose discriminant analysis is used to determine to what extent multiple blood antioxidants as indicators of 'oxidative stress' may identify coal workers at increased risk for coal dust-induced respiratory disorders.

METHODS

Study design

Figure 1 shows the design of the study. A group of 91 coal workers from the 'Kempen' coal-mining region in Belgium previously involved in cross-sectional studies (1987) to evaluate blood antioxidant parameters in relation to severity of coal workers' pneumoconiosis (Engelen *et al.* 1990, Evelo *et al.* 1993) was recruited for follow-up. In the cross-sectional studies, reduced (GSH) and oxidized (GSSG) glutathione concentrations, total (Gpx-To) and selenium-dependent (Gpx-Se) glutathione peroxidase activities, glutathione-S-transferase activity (GST), superoxide dismutase activity (SOD) and catalase activity were measured in red blood cells and concentrations of vitamin A, vitamin E and iron (Fe) were determined in serum as described previously (Engelen *et al.* 1990). GST levels were measured only in 64 subjects of this cohort (Evelo *et al.* 1993). Ultimately, 66 out of 91 coal miners previously screened for antioxidant parameters (73% of

Roel P. F. Schins *(author for correspondence),* **Soedjajadi Keman** *and* **Paul J. A. Borm** *are in the Department of Health Risk Analysis and Toxicology, University of Limburg, PO Box 616, 6200 MD Maastricht, The Netherlands.*

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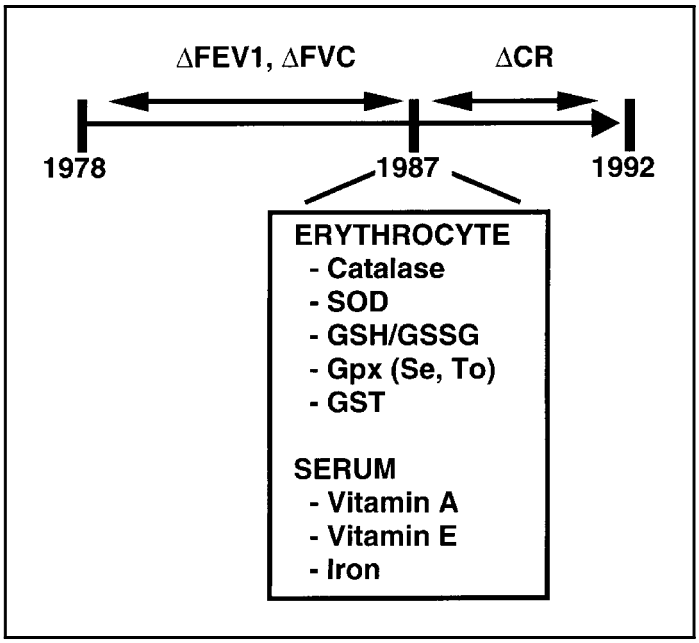

Figure 1. Design of the study. Antioxidants measured in red blood cells or plasma of coal workers were related to lung function decline over a 10-year interval (retrospective, $n = 40$), and to development ($n = 52$) or progression ($n = 14$) of coal workers' pneumoconiosis, and chronic bronchitis ($n = 66$) determined at 5-year follow-up (prospective). Lung function decline was determined by longitudinal analysis of spirometry data (FEV_1 and FVC) determined between 1978 and 1987. Progression of CWP was evaluated by paired comparison of chest-radiographs (CR) made in 1987 and 1992. Job-history (including calculation of cumulative dust exposure), smoking, medication and medical history (including chronic bronchitis) were determined at follow-up from questionnaires, personal interviews and medical files (see text for details).

the original cohort) participated in our-follow-up. Meanwhile all miners were retired due to the closure of the mines in Belgium (Schins and Borm 1995). GST levels were only known for 46 subjects of this group. Upon written informed consent, a new chest radiograph was made. A questionnaire was combined with a personal interview to determine job-history, smoking and medical status. Furthermore, all medical files were screened retrospectively, for a 10-year period to determine longitudinal lung function decline (see Figure 1). Lung function decline (retrospective analysis of medical files) was obtained from 40 out of 91 subjects. With regard to these different subgroups, no selection bias due to loss to follow-up was observed for age, smoking habits, exposure, medication, pneumoconiotic stage and antioxidants (data not shown).

Chest radiography and lung function

Chest radiographs taken in 1987 and in 1992 were scored according to the classification rules of the International Labour Organization (ILO 1980) by an experienced panel of three physicians. Scoring was done in plenary sessions where consensus had to be reached based on individual scores. Miners were grouped as either 'healthy coal workers' with ILO classification of 0/0 or as miners with CWP with ILO classification of $\geq$0/1. Five-year progression or new development of CWP was determined from a paired reading session of each individuals' chest radiograph of 1987 and 1992 (Schins *et al.* 1994).

Lung function decline (ΔFEV_1 and ΔFVC) was calculated by linear regression for a 10 year interval before blood sampling (1978–1987). Lung function had been measured periodically at medical screening using a Vitalograph TM spirometer (Vitalograph Ltd, UK) at two mine-pits and a Vicatest 4 (Mijnhardt, Odjik, Holland). Lung function decline of coal miners was calculated as the regression slope obtained with least-square analysis through minimally eight out of 10 yearly measurements. Lower and upper quartiles of ΔFEV_1 were used as cut-off point to define rapid versus slow decliners.

Exposure, smoking, medication, and medical history

Occupational history from each subject was gathered from the personel and medical files. Individual dust exposure was calculated from each individuals' job exposure matrix obtained from a personal interview at follow-up and the medical file. Exposure parameters used for this study were cumulative dust exposure, cumulative quartz exposure, years underground in 1987, and years underground in 1992. Cumulative exposure was calculated as described elsewhere (Schins and Borm 1995).

A validated questionnaire (Cotes *et al.* 1987) was sent to all participants in which medical symptoms, medical history, smoking habits and medication were asked. Answers were verified during personal interviews and by comparison with records from the preceding study (Engelen *et al.* 1990). Smoking amounts (pack years) were calculated as described elsewhere (Jorna *et al.* 1994). The questionnaire was used to identify respiratory respiratory symptoms at follow-up. When bronchitis-like symptoms (i.e. presence of both cough and pleghm) were reported for most days of the week and at least 3 months per year, the subject was classified as 'chronic bronchitis'.

Statistical evaluations

Correlations between antioxidant levels and (cumulative) exposure, age, smoking, lung function were evaluated by Spearman Rank correlation. Unpaired differences between 1987 and 1992 (sub) groups were tested by the Mann–Whitney *U*-test. Differences between miners without or with CWP progression, obstruction, and respiratory symptoms were evaluated by the Mann–Whitney test. Finally, stepwise discriminant analysis was used to determine whether each individuals' 'antioxidant status', i.e. the use of multiple antioxidant parameters would allow classification of coal miners into mutually exclusive groups (Dillon and Goldstein 1984). Therefore, all subjects were classified in the following groups: subjects (1) with or without development of CWP, (2) with or without progression of already existing CWP, subjects with (3) slow or rapid longitudinal FEV_1-decline, and (4) subjects with or without chronic bronchitis. Stepwise discriminant analysis was performed on natural logarithm-transformed data. Antioxidants tested in the discriminant function were: catalase, SOD, GSH, GST, Gpx (total), Fe, vitamin A and vitamin E. Missing values were replaced with the group average. All statistical evaluations were made using SPSS 6.1 (SPSS Inc. Chicago, IL).

Results

Single marker analysis

None of the antioxidants listed in Table 1 was significantly related to individual cumulative (dust/quartz) exposure nor to any other exposure estimate (i.e. years since first exposure or years exposed). Furthermore, no antioxidant was significantly related to age or smoking habits (pack years).

Based on the paired chest radiograph reading sessions, it was observed that three out of 52 miners newly developed CWP, while eight out of 14 miners had progression of already existing CWP. None of the initial antioxidant data of these subgroups (Table 1) was significantly different for miners with or without disease development or progression, only red cell SOD activity was significantly higher in miners with subsequent progression of CWP ($p < 0.05$). As can be seen in Table 1, this finding was not biased by differences in

	Miners without CWP		Miners with CWP	
	NP (n = 49)	P (n = 3)	NP (n = 6)	P (n = 8)
Age, years (in 1992)	47.6 (0.7)	45.3 (3.8)	48.6 (1.4)	52.6 (1.3)
Underground in 1987, years	21.8 (0.6)	23.0 (4.6)	24.2 (1.1)	26.0 (1.1)
Total underground (1992), years	22.9 (0.6)	24.0 (4.0)	25.0 (1.4)	26.6 (1.4)
Cumulative dust, gh m^{-3} (in 1992)	95 (8)	135 (33)	141 (4)	123 (25)
Smoking, pack years (in 1992)	124 (17)	143 (34)	198 (71)	190 (45)
FEV_1 decline (ml $year^{-1}$)[a]	–30.1 (9.9)	–69.8 (43.2)	–36.1 (14.3)	–82.1 (41.5)
Chronic bronchitis, yes / no[b]	8/41	1/2	1/5	2/6
Hb, mg Hb ml^{-1}	15.1 (0.28)	13.9 (0.33)	14.4 (0.31)	15.8 (0.46)
SOD, U g^{-1} Hb	1979 (74)	1740 (190)	1703 (156)	2308 (155)*
Catalase, μmole H_2O_2 min^{-1} g^{-1} Hb[c]	0.45 (0.01)	0.50 (0.04)	0.43 (0.02)	0.46 (0.02)
GSHpx-To, μmole NADPH min^{-1} g^{-1} Hb	9.67 (0.09)	9.79 (0.15)	9.64 (0.20)	9.77 (0.13)
GSHpx-Se, μmole NADPH min^{-1} g^{-1} Hb	9.25 (0.11)	9.39 (0.28)	9.20 (0.33)	9.39 (0.16)
GST, U g^{-1} Hb[d]	3.11 (0.23)	2.35 (0.50)	3.63 (0.42)	2.54 (0.34)
GSH, μmole g^{-1} Hb	3.99 (0.09)	3.38 (0.42)	3.92 (0.25)	3.84 (0.20)
GSSG, μmole g^{-1} Hb	0.036 (0.001)	0.033 (0.004)	0.037 (0.003)	0.035 (0.002)
Fe, μmole ml^{-1} plasma	18.3 (1.1)	21.0 (5.8)	18.5 (1.9)	14.7 (1.3)
Vitamin A, μg ml^{-1} plasma	9.4 (0.4)	7.9 (0.6)	11.2 (1.3)	8.9 (0.9)
Vitamin E, μg ml^{-1} plasma	14.6 (0.7)	11.7 (2.8)	20.9 (4.3)	14.0 (1.7)

Table 1. Blood antioxidant parameters, age, exposure, smoking and the 5-year progression of pneumoconiosis in coal workers with and without CWP.

Data are mean (SEM). * Significantly different from NP, $p < 0.05$, Mann–Whitney U-test.

Key: P, progression (or development) of pneumoconiosis; NP, no progression of CWP.

[a] FEV_1 decline was obtained from 35 miners (i.e. n = 26, n = 2, n = 4, n = 3 subjects respectively).

[b] Odds ratios for chronic bronchitis and progression were 2.6 in controls and 1.7 in CWP group, both not significant at 90% CI.

[c] Upon exclusion of one catalase deficient subject, the average of NP controls becomes 0.46 (0.01).

[d] GST was related to progression in 46 miners (i.e. n = 33, n = 2, n = 6, n = 5 subjects respectively).

distributions of age, smoking or (cumulative) exposure. In the miners without development of CWP, one subject was identified with red blood cell catalase deficiency. Statistical analysis was repeated upon exclusion of this subject (see legend of Table 1).

The average decline in FEV_1 was– 34.1 ml $year^{-1}$ (n = 40)), and based on upper and lower quartiles subjects were divided into rapid (cut off – 68.4 ml $year^{-1}$) or slow decliners (cut off – 6.9 ml $year^{-1}$). However, no significant correlations were observed between longitudinal lung function decline (ΔFEV_1 or DFVC) and individual antioxidant parameters, nor was any significant difference seen between slow and rapid decliners for any antioxidant (see Table 2). FEV_1-decline was not different between subjects with or without progression of pneumoconiosis (see Table 1). Based on the questionnaires, 12 subjects were classified as having chronic bronchitis at follow-up. Both development or progression of CWP were independent of the presence of chronic bronchitis (see Table 1), and FEV_1-decline was not different between subjects with or without chronic bronchitis (see Table 2). Interestingly, in subjects with chronic bronchitis red cell GSH concentration was significantly reduced compared with normal ($p < 0.01$).

Multiple marker analysis

To determine the involvement of multiple antioxidants in specific disease a discriminant analysis was performed. Due to the distribution skewness of several antioxidant parameters, analysis was performed on logarithm-transformed data. Antioxidants tested in the discriminant function were: catalase, SOD, GSH (i.e. reduced glutathione only), GST, GPX (i.e. total), Fe, vitamin A and vitamin E. Missing values (for GST, vitamin A and vitamin E) were replaced with the (ln-transformed) group average. Furthermore, a catalase 'outlier' subject was excluded from all multiple statistical analyses. The results of the stepwise analysis are shown in Table 3, for several discriminating criteria along with the percentage of correct grouped subjects and its significance (χ^2), and the antioxidants significantly attributing to the correct grouping.

Eighty six per cent of the subjects were correctly grouped into progression (n = 8) or no progression (n = 6) of already existing CWP, when erythrocyte GSH concentration, SOD and GST activities, and serum vitamin E status were included in the discriminant function (df = 4, $\chi^2 = 14.2$, $p < 0.01$). The discriminant function indicated a negative association with disease progression for GSH, vitamin E and GST, while SOD was higher in those with progression. Furthermore, a significant discrimination of subjects classified according to new development of CWP (3 versus 48 subjects) was observed with GSH, vitamin E (both negative) and catalase and iron (positive) in the discriminant model. A discrimination between slow and rapid decliners in FEV_1 was obtained with SOD, catalase, glutathione-S-transferase and glutathione

	FEV_1 decline		Chronic bronchitis	
	Rapid (n = 10)	Slow (n = 10)	Yes (n = 12)	No (n = 53)[a]
Age, years (in 1992)	48.2 (1.7)	46.1 (1.2)	49.5 (1.6)	47.9 (0.6)
Underground in 1987, years	23.0 (3.9)	22.1 (1.3)	23.2 (1.3)	22.5 (0.6)
Total underground (1992), years	23.8 (1.0)	23.5 (1.4)	23.8 (1.3)	23.6 (0.6)
Cumulative dust, gh m^{-3} (in 1992)	100 (19)	93 (18)	89 (17)	108 (8)
Smoking, pack years (in 1992)	199 (51)	136 (35)	161 (38)	135 (17)
FEV_1 decline (ml $year^{-1}$)[b]	–100.4 (9.5)	17.0 (10.0)**	–10.1 (29.3)	–44.6 (7.8)
Chronic bronchitis, yes/no[c]	1/9	2/8		
Hb, mg Hb ml^{-1}	15.8 (0.6)	14.7 (0.6)	15.2 (0.5)	15.0 (0.2)
SOD, U g^{-1} Hb	2079 (287)	1989 (128)	2171 (126)	1941 (71)
Catalase, μmole H_2O_2 min^{-1} g^{-1} Hb	0.46 (0.02)	0.43 (0.01)	0.43 (.01)	0.47 (0.01)
GSHpx-To, μmole NADPH min^{-1} g^{-1} Hb	9.86 (0.12)	9.48 (0.29)	9.78 (0.16)	9.65 (0.08)
GSHpx-Se, μmole NADPH min^{-1} g^{-1} Hb	9.35 (0.13)	9.22 (0.27)	9.36 (0.21)	9.24 (0.10)
GST, U g^{-1} Hb[d]	3.09 (0.27)	2.87 (0.57)	3.32 (0.27)	2.99 (0.21)
GSH, μmole g^{-1} Hb	4.00 (0.18)	4.12 (0.15)	3.53 (0.16)	4.05 (0.09)**
GSSG, μmole g^{-1} Hb	0.035 (0.003)	0.038 (0.003)	0.035 (0.003)	0.035 (0.001)
Fe, μmole ml^{-1} plasma	16.9 (1.3)	18.1 (2.0)	18.7 (3.5)	18.0 (1.0)
Vitamin A, μg ml^{-1} plasma	10.5 (1.3)	10.6 (1.2)	10.6 (0.9)	9.2 (0.4)
Vitamin E, μg ml^{-1} plasma	16.6 (2.3)	16.7 (1.8)	17.7 (1.7)	14.5 (0.8)

Table 2. Blood antioxidant parameters, age, exposure, smoking and FEV_1 decline in coal workers with or without bronchitis and lung function decline.

Data are mean (SEM). ** $p < 0.01$ (Mann–Whitney U-test).

[a] The catalase-deficient subject was excluded from analysis.

[b] FEV_1 decline determined in n = 10, n = 10, n = 5, n = 35 subjects respectively.

[c] Odds ratio = 0.44, for chronic bronchitis vs lung function decline; not significant at 90% CI.

[d] GST subgroup numbers are 7 vs 9, and 7 vs 38 respectively.

peroxidase in the model. Interestingly, these parameters were all increased in those with rapid decline in FEV_1 in comparison with those with a low decline. The presence or absence of chronic bronchitis was significantly discriminated with GSH, SOD and vitamin A, i.e. explained by lower GSH and increased SOD and vitamin A levels in the symptomatic group.

Discussion

As stated by Halliwell and Cross (1994), the major issue is not whether oxidative stress can be demonstrated in a disease, but whether it makes a significant contribution to the disease activity. Previously, we and others elucidated the first question for subjects chronically exposed to coal dust by showing different antioxidant status in stages of coal workers' pneumoconiosis (Engelen *et al.* 1990, Evelo *et al.* 1993, Perrin-Nadif *et al.* 1996). Glutathione concentrations as well as glutathione-S-transferase activities were significantly decreased at early stage pneumoconiosis, while at later stages erythrocyte GSH and GST were back to normal (Engelen *et al.* 1990, Evelo *et al.* 1993). In these cross-sectional studies, our original hypothesis was that subjects who develop pneumoconiosis are 'less well equipped to deal with reactive oxygen species' (Engelen *et al.* 1990), and that erythrocytes could be considered as important circulating antioxidant carriers in this disease. Further support for this hypothesis is provided by our recent observation that oxidative DNA

Classification criterion	Yes/no	Correct grouped (%)	χ^2	df	p-value	Variables included in discriminant model
Development of CWP	3/48	86.0	12.3	4	< 0.02	GSH (–), vit.E (–), Fe (+), cat (+)
Progression of CWP	8/6	85.7	14.2	4	< 0.01	GSH (–), vit.E (–), GST (–), SOD (+)
Lung function decline	10/10	85.7	10.2	4	< 0.04	Gpx (+), SOD (+), GST (+), cat. (+)
Chronic bronchitis	12/53	70.3	12.6	3	< 0.01	GSH (–), SOD (+), vit.A (+)

Table 3. Discriminant analysis of antioxidants in erythrocytes and plasma in relation to coal dust-induced respiratory disorders.

All subjects were classified as follows: (1) presence or absence of development of CWP; (2) presence or absence progression of already existing CWP; (3) rapid (= 'yes') or slow (= 'no') decline in lung function (i.e. lower versus upper quartile of ΔFEV_1). The catalase outlier was excluded from analysis. For each classification criterion, the table shows the percentage of correct grouped subjects, the significance of the canonical correlation and the antioxidants significantly attributing to the correct grouping, i.e. positive (+) or negative (–) in the canonical correlation function.

damage (i.e. 8-hydroxydeoxyguanosine residues) is increased in peripheral blood lymphocytes of coal workers compared with non-exposed controls (Schins *et al.* 1995). However, whether the variation in oxidative stress observed in coal workers is significantly related to respiratory disease, would necessitate a different experimental approach.

In the present study, prospective analysis of the above cohort since 1987 has shown that red blood cell SOD activity was significantly higher in miners with subsequent progression of pneumoconiosis as compared with those without progression at 5-year follow-up. Previously we reported that SOD activities were not increased in coal workers with pneumoconiosis compared with healthy miners (Engelen *et al.* 1990). However, increased erythrocyte SOD levels were found recently in underground coal miners compared with surface workers (Perrin-Nadif *et al.* 1996). This might be a consequence of differences in coal dust exposures but one could also consider these findings to reflect the higher pneumoconiosis risk for underground versus surface workers. Previously, it has been shown that macrophage superoxide anion ($O_2^{\bullet -}$) generation was increased in coal workers compared with non-exposed controls (Rom *et al.* 1987, Wallaert *et al.* 1990), and the activity of SOD may therefore reflect the extent of a respiratory $O_2^{\bullet -}$ burst from macrophages and neutrophils in the lung. This is underscored by animal data, demonstrating a direct upregulation of manganese SOD expression and immunoreactive protein in the lung by mineral dusts, which was related to the inflammatory response (Janssen *et al.* 1992).

A combination of antioxidant parameters tested by discriminant analysis, showed that in addition to SOD, also GST, GSH and vitamin E status denominated the presence or absence of CWP progression. However, in contrast to SOD activity, these parameters were reduced in those with progression (see also Table 1). Interestingly, vitamin E and GSH were also reduced in the few ($n = 3$) healthy miners who developed pneumoconiosis during follow-up, while in those subjects catalase activity and plasma iron were increased. Therefore, reduced levels of red cell glutathione and plasma vitamin E status may be considered as risk factors for CWP. The concept of reduced GSH as a possible fibrotic risk factor was also discussed by Cantin *et al.* who showed that reduced extracellular GSH was associated with increased lung fibroblast proliferation (Cantin *et al.* 1990). The significance of reduced GST in fibrosis may be closely related to this observation. Its reduced activity may be related to oxidative stress during lipid peroxidation (Halliwell and Cross 1994), which is considered as an important feature of mineral dust-related lung toxicity. Other support for the involvement of GST in fibrosis comes from Smith *et al.* (1994), who recently showed that GST-m class (GST-M1) deficient subjects were at increased risk for asbestosis. Although the majority of GST activity in erythrocytes originates from GST-P class iosenzymes, GST polymorphism could, in addition to its role in (smoking-related) lung cancer (Seidegard *et al.* 1986, 1990), be significantly involved in the development of pneumoconiosis. Vitamin E in those at risk may be decreased because of its importance as chain reaction terminator in the lipid peroxidation process. Interestingly, erythrocyte GSH levels were not only associated with increased pneumoconiotic risk, but also dramatically reduced in subjects with chronic bronchitis symptoms reported at follow-up. Remarkably, all 'enzymatic' antioxidants (GST, Gpx, SOD and catalase) were increased in those with rapid decline in FEV_1 versus those with a slow decline. Since longitudinal decline was analysed retrospectively, our observations could be explained as a compensatory upregulation of enzymatic antioxidants in those with reduced 'non-enzymatic' antioxidant status. Reduced intake of vitamin A (Morabia *et al.* 1989), as well as vitamins C and E (Schwartz and Weiss 1994, Britton *et al.* 1995) is associated with increased risk of respiratory disease. Others have shown reduced GSH levels in bronchoalveolar lavage fluid in subjects with obstructive lung disease (Linden *et al.* 1993).

Finally, this study enabled us to test the relevance of concomitant evaluation of multiple parameters of oxidative stress in coal dust-induced respiratory disorders. We found that the risk for progression of CWP was related to reduced concentrations of erythrocyte GSH and serum vitamin E, as well as increased red cell activities of catalase and SOD. At the same time, an over-all increase in enzymatic antioxidant capacity identified subjects with a rapid retrospective lung function decline. GST activity showed a contrasting discriminative power; low GST activity was observed in subjects at increased risk for progression of coal workers' pneumoconiosis, but was also observed in those previously having relatively slow lung function decline. Although this finding should be interpreted with all the necessary reserves, due to the limited number of subjects involved in this analysis, recent findings (GST polymorphism) (Smith *et al.* 1994) provide support for further investigation of the role of GST in coal dust-induced lung disorders.

Although pneumoconiotic and non-pneumoconiotic endpoints were independent, it cannot be ruled out that both effects may be closely related to each other. On the one hand, a reduced intake of antioxidants associated with lung function loss may be a risk factor for coal dust-induced pulmonary fibrosis. On the other hand, coal dust exposure and its associated generation of ROS could also play a role in some of the non-pneumoconiotic effects such as chronic bronchitis or chronic lung obstruction. As such, measurement of multiple antioxidants in the peripheral blood may be relevant for surveillance and screening of (retired) coal workers. In our opinion, these observations should be extrapolated to other cohorts of coal (or other mineral) dust-exposed subjects, to validate whether antioxidant status patterns of the peripheral blood as reported above can be considered as useful biological markers in subjects chronically exposed to coal (i.e. mineral) dust.

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