



## Serum lactate dehydrogenase and its isoenzyme pattern in ex-coalminers

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Serum lactate dehydrogenase (LDH) activity, a marker of cell damage, is increased in several pulmonary disorders, especially when fibrosis is involved. In rats exposed to silica, high levels of LDH activity were found. A rise of serum LDH<sub>3</sub> has been associated with lung tissue injury. The aim of this study was to investigate the serum LDH isoenzyme pattern after coal-dust exposure and the possible relation to pulmonary function tests.

Ex-coalminers ( $n=201$ ), with a history of coal-dust exposure more than 20 yr ago, were admitted to the authors' hospital for a medical check-up and were included in the study.

The serum LDH activity was found to be elevated in 79.1% of the ex-coalminers ( $634 \pm 245 \text{ U l}^{-1}$ ). Moreover, in 196 of the 201 cases (97.5%), a high LDH<sub>3</sub> level ( $31 \pm 4\%$ ) was demonstrated. A moderate negative relation was found between the forced expiratory volume in 1 s (FEV<sub>1</sub>) and the LDH activity ( $r = -0.26$ ;  $P < 0.001$ ), as well as between FEV<sub>1</sub> and LDH<sub>3</sub> activity ( $r = -0.23$ ;  $P < 0.001$ ), even in the subgroup ( $n=42$ ) with a normal LDH. All other liver function tests were within normal limits.

These results suggest that coal-dust, even many years after the actual exposure, causes an increase in the total serum LDH activity and changes in the LDH-isoenzyme pattern, mainly characterized by a high LDH<sub>3</sub> activity.

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### Introduction

Occupational exposure to mineral dusts can cause pulmonary fibrosis. Several animal studies described the pathogenesis of pulmonary silicosis (1,2). Moreover, it was demonstrated that this cytotoxic effect for alveolar macrophages (AMs) persists after cessation of exposure. It is now evident that coalworkers' pneumoconiosis (CWP) can become apparent or progress further after cessation of exposure of silica. Besides pneumoconiosis accumulation of coal dust in the lungs, other non-pneumoconiotic effects such as chronic airway obstruction, bronchitis and emphysema may occur, both in smokers and non-smokers, irrespective of the extent of possible pneumo-

coniotic abnormalities. Until now, however, no clinical parameter was reported to identify the relation between pulmonary function impairment and silica exposure (3).

In the pathogenesis of lung fibrosis, complicated processes are involved. These processes include intercellular communication by peptides released from and to various immune cells and lung target cells in a phenomenon referred to as the 'cytokine network'. It has been shown that silica, as well as coal dust, can stimulate the release of monocyte/macrophage-derived pro-inflammatory cytokines, such as tumour necrosis factor (TNF)- $\alpha$ , IL-1 $\beta$ , IL-6 and the macrophage inflammatory proteins 1a and 2 (4). More recently, elevated messenger ribonucleic acid (mRNA) levels of TNF and IL-6 have been observed in bronchoalveolar lavage fluid (BALF) obtained from pneumoconiosis patients (4,5).

Cellular enzymes in the extracellular space can serve as indicators for disturbances of the cellular integrity induced by pathological conditions (6).

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TABLE 1. Characteristics of the studied cases of ex-coalminers

Group	<i>n</i>	Age (years)	Weight (kg)	Height (cm)	Underground years	Smoking (pack years)
Total group	201	72 (52–92)	76 (46–117)	170 (153–194)	25.8 (10–42)	29.9 (3–90)
Group I	42	70 (56–85)	77 (46–102)	171 (157–184)	26.2 (12–41)	31.0 (10–80)
Group II	159	72 (50–92)	75 (77–117)	169 (153–194)	25.7 (10–42)	29.6 (3–90)

Data are expressed as means with ranges in parentheses.

Group I, normal serum lactate dehydrogenase (LDH) activity; Group II, high serum LDH activity. Note: no significant differences between the groups.

Lactate dehydrogenase (LDH) is a cytoplasmatic enzyme present in essentially all major organ systems. The extracellular appearance of LDH is used to detect cell damage or death and is abnormal in a host of disorders (6–9). The total serum LDH activity is elevated in several pulmonary disorders associated with fibrosis, and has been suggested to be a useful monitor of disease activity (8,10–16). Elevation of LDH activity in BALF has been described after exposure to silica, in humans as well as in rats (17–19). However, the serum LDH activity is a highly sensitive, but non-specific, parameter for pulmonary disease. Besides the elevated serum LDH activity, a high LDH<sub>3</sub> activity has also been reported to be related to lung injury (12). Although the serum LDH-isoenzyme pattern has a slightly better specificity, the clinical value is still rather low. The LDH isoenzyme pattern of the lung is characterized by proportional increases in isoenzymes 3, 4 and 5 as compared to the serum isoenzyme pattern (7,12,16,20–22).

The aim of this study was to investigate whether the serum LDH activity and its isoenzyme pattern was related to pulmonary damage caused by silica exposure. Therefore, the relationship between serum LDH activity, its isoenzyme pattern and pulmonary function impairment was investigated in patients with a history of coal-dust exposure.

## Methods

### PATIENTS

The study was performed in a population of ex-coalminers ( $n=201$ ), all men. They were admitted to the authors' hospital for a medical check-up. All had a history of coal-dust exposition, more than 20 yr ago. Their medical history revealed no other relevant pulmonary disorders. The majority of the ex-coalminers ( $n=182$ , 90%) were smokers, having had a smoking history of many years. The chest X-ray was classified as normal in 52 cases (26%). The

chest X-ray was classified as abnormal showing abnormalities varying between few nodules, normal lung markings visible and numerous opacities, and normal markings totally obscured ( $n=149$ ; 74%) (23). The characteristics of the studied population are summarized in Table 1. The population of ex-coalminers was divided into two categories, based on the serum LDH activity. Group I consisted of ex-coalminers with a normal serum LDH activity ( $n=42$ ; LDH  $\leq 450$  U l<sup>-1</sup>), and Group II consisted of cases with an elevated serum LDH activity ( $n=159$ ; LDH  $>450$  U l<sup>-1</sup>).

A group of 48 healthy control subjects, all men (age  $58 \pm 13$  years), without a relevant medical history, was used to assess normal values of serum LDH and its isoenzyme activity pattern. Of this latter group, the total protein, albumin,  $\gamma$ -glutamyl transferase ( $\gamma$ -GT), alanine aminotransferase (ALT) and creatine kinase (CK) were within normal limits.

### RESPIRATORY FUNCTION TESTS

Pulmonary function tests were assessed. Forced expiratory volume capacity (FVC) and forced expiratory volume in 1 s (FEV<sub>1</sub>) were determined using a spirometer (Jaeger, Masterlab, Wuerzburg, Germany). Diffusion capacity (DCO) was obtained by the single breath method and corrected for haemoglobin. The predicted values for each subject, based on sex, age and height, were obtained from standard tables (24). Data were expressed as percentages of the predicted values.

### LABORATORY TESTS

Simultaneously with pulmonary function tests, blood samples were taken and serum was stored frozen at  $-70^{\circ}\text{C}$  until actual measurement of LDH and its isoenzyme pattern. In the authors' laboratory, LDH activity is measured at  $37^{\circ}\text{C}$  by an enzymatic rate

TABLE 2. Results of laboratory tests of the studied cases of ex-coalminers

Parameters	Reference value	Total group (n=201)	Group I (n=52)	Group II (n=159)
Albumin (g l <sup>-1</sup> )	35.0-55.0	39 ± 3 (38.9)	38 ± 3 (38.9)	38 ± 3 (38.8)
Ureum (mmol l <sup>-1</sup> )	3.0-7.0	6.0 ± 2 (6.0)	6.0 ± 1.6 (6.0)	6.3 ± 2 (6.0)
Creatine (mmol l <sup>-1</sup> )	71-110	100 ± 60 (95)	93 ± 19 (16.0)	102 ± 67 (94)
ALT (U l <sup>-1</sup> )	5-40	19 ± 10 (17)	19 ± 7 (19.0)	19 ± 10 (17)
γ-GT (U l <sup>-1</sup> )	2-50	31 ± 43 (23)	30 ± 37 (22)	31 ± 44 (23)
CK (U l <sup>-1</sup> )	40-240	96 ± 78 (76)	80 ± 43 (69)	100 ± 84 (77)
LDH (U l <sup>-1</sup> )	361 ± 59	633 ± 245 (573)§	371 ± 55 (372)	703 ± 229 (632)*
LDH <sub>1</sub> (%)	21 ± 3.4	15 ± 4 (15.0)§	19 ± 3 (19.5)	14 ± 3 (13.9)‡,§
LDH <sub>2</sub> (%)	39.7 ± 2.5	37 ± 4 (37.0)§	40 ± 4 (40.9)	36 ± 3 (36.4)‡,§
LDH <sub>3</sub> (%)	18.6 ± 1.9	31 ± 4 (31.9)§	26 ± 4 (25.4)§	33 ± 3 (32.5)‡,§
LDH <sub>4</sub> (%)	8.7 ± 1.4	11 ± 3 (11.2)§	9 ± 2 (8.6)	12 ± 3 (11.9)‡,§
LDH <sub>5</sub> (%)	11.8 ± 3.2	5 ± 2 (4.6)§	6 ± 2 (5.4)§	5 ± 2 (4.4)‡,§

Data are expressed as means ± SD, with median values in parentheses. Group I, normal serum lactate dehydrogenase (LDH) activity; Group II, high serum LDH activity; ALT, alanine aminotransferase; γ-GT, γ-glutamyl transferase; CK, creatine phosphokinase; LDH<sub>1-5</sub>, LDH isoenzymes.

*t*-test Group I vs Group II: \**P*<0.05, †*P*<0.01, ‡*P*<0.001.

*t*-test vs control subjects: §*P*<0.0001.

method, using pyruvate as a substrate. The test is performed on a Beckman Synchron CX-7 system with Beckman reagents (test kit 442660) and is optimized according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (DGKC recommendations). The system monitors the reduction of pyruvate to L-lactate with the concurrent oxidation of β-nicotinamide adenine dinucleotide (NADH; reduced form) at 340 nm. The change in absorbency at 340 nm, caused by the disappearance of NADH, is measured over a fixed time interval and is directly proportional to the LDH activity. Lactate dehydrogenase activity is expressed in micromoles of substrate (pyruvate) converted per minute (U), per litre of serum at 37°C. The measuring range is 1800-3800 U l<sup>-1</sup> and for higher concentrations, the samples are automatically diluted with saline and re-analysed. The reference range for LDH is 200-450 U l<sup>-1</sup> in serum. The surface charge difference is the basis on which the five LDH isoenzymes are separated by electrophoresis. After the LDH isoenzymes have been separated by electrophoresis, the agarose gel is incubated with a reaction mixture, containing the LDH substrate lactate, the coenzyme NAD<sup>+</sup> and a tetrazolium salt. During this incubation, NADH is formed at the zones on the gel, where the LDH isoenzymes are present. The NADH generated is detected by its reduction of the tetrazolium salt to the coloured bands, which can be

quantitated by scanning the gel at 600 nm. Blood samples were also analysed for ureum, total protein, albumin, γ-glutamyl transferase (γ-GT), alanine amino transferase (ALT) and creatine kinase (CK). Serum ureum, LDH, ALT, CK total protein and albumin have been determined on a Synchron CX-7 analyser (Beckman Instruments Inc, California, U.S.A.), using test kits from Beckman Instruments Inc.

#### STATISTICAL METHODS

Pearson coefficient of correlation (*r*) was estimated in order to test against a relation between serum LDH activity and the performed pulmonary function tests. A *P* value of less than 0.05 was considered to be significant. Differences between groups were statistically analysed by Student's *t*-test.

#### Results

The laboratory results of the studied group of ex-coalminers as well as the normal values are given in Table 2. Serum LDH activity was found to be elevated in 159 of the 201 ex-coalminers (79.1%). The total group of ex-coalminers showed a significant different LDH isoenzyme pattern compared to the control group (Table 2). The LDH isoenzyme pattern

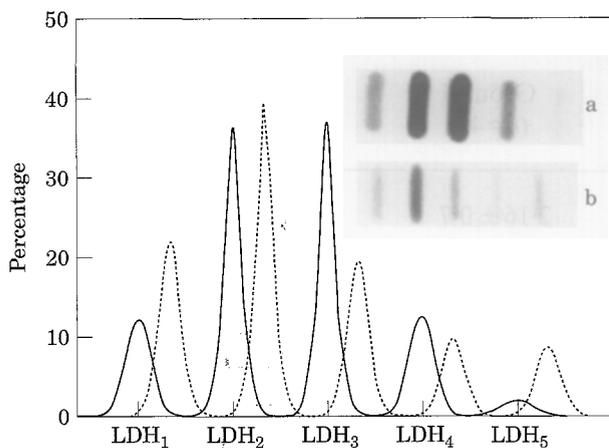


FIG. 1. Lactate dehydrogenase isoenzyme (LDH) pattern of (a) an ex-coalminer with a high LDH<sub>3</sub> and (b) a patient with a normal isoenzyme pattern, visualized by electrophoresis and after scanning the gel at 600 nm.

was mainly characterized by an increase of the LDH<sub>3</sub> fraction ( $31 \pm 4\%$ ;  $P < 0.0001$ ). Group II (LDH high,  $703 \pm 229 \text{ U l}^{-1}$ ,  $n = 159$ ) was characterized by a high LDH<sub>3</sub> ( $33 \pm 3\%$ ) in all cases, whereas in Group I (LDH normal,  $371 \pm 55 \text{ U l}^{-1}$ ,  $n = 42$ ), in 37 of 42 cases (88.1%), a high LDH<sub>3</sub> ( $26 \pm 4\%$ ) was found. So, in 196 out of the 201 studied cases of ex-coalminers (97.5%), a high LDH<sub>3</sub> activity was demonstrated. Also, the LDH<sub>4</sub> fraction was high in Group II ( $12 \pm 3 \text{ U l}^{-1}$ ) vs Group I ( $9 \pm 2\%$ ;  $P < 0.001$ ), as well as the absolute LDH<sub>4</sub> activity ( $87 \pm 41$ , respectively,  $33 \pm 9 \text{ U l}^{-1}$ ;  $P < 0.001$ ). LDH<sub>4</sub> was only increased in four of 42 (9.5%) ex-coalminers with a normal LDH activity (Group I) and in 101 of 159 (63.5%) with high LDH activity (Group II). So, in 105 of the 201 studied cases (52.2%), a high LDH<sub>4</sub> activity was demonstrated.

No differences were found in the serum LDH activity and its isoenzyme pattern between smokers ( $n = 182$ ) and non-smokers ( $n = 19$ ) in either of the pulmonary parameters. When comparing the group of ex-coalminers with normal chest X-ray ( $n = 52$ ) and the group with abnormal chest X-ray ( $n = 149$ ), no statistical differences were found in the serum LDH activity and its isoenzyme pattern, or in pulmonary function parameters, except for the DCO which showed a slight negative correlation ( $P < 0.05$ ). This same correlation was found between the duration of working underground and the DCO in smokers as well as in non-smokers ( $P < 0.05$ ).

An example of the serum LDH isoenzyme pattern of an ex-coalminer as well as a control subject is given in Fig. 1. All other laboratory values measured (liver

function tests, creatine kinase and creatinine) were within normal ranges (Table 2).

In Table 3, the results of the pulmonary function tests of the different groups are summarized. Negative relations between the FEV<sub>1</sub> and the total LDH activity ( $r = -0.26$ ,  $P < 0.001$ ), LDH<sub>3</sub> activity ( $r = -0.23$ ,  $P < 0.001$ ; Fig. 2) and LDH<sub>4</sub> activity ( $r = -0.25$ ,  $P < 0.001$ ) were found in the total population of ex-coalminers. The FEV<sub>1</sub> was low in Group II compared to Group I ( $P < 0.05$ ). Of the 201 ex-coalminers, 190 managed to complete the single breath method for DCO measurement. In these latter cases, no relation was found between LDH activity and DCO. Moreover, no difference between the two groups with regard to the DCO or FVC were found (Table 4).

## Discussion

Serum LDH<sub>3</sub> activity was high in almost all ex-coalminers (196 out of 201), whilst the total serum LDH activity was demonstrated to be high in 80% of the study population (159 out of 201). So, even in 88% of the ex-coalminers with a normal LDH activity, a high LDH<sub>3</sub> activity was found. Since all other laboratory tests were normal (which excluded the liver, heart and muscles to be a potential source of serum LDH activity increase) and silica induces cell damage resulting in LDH release, these results indicate that the LDH most likely originates from the lung. Moreover, a negative relationship between the serum LDH<sub>3</sub> activity, as well as LDH activity, and the FEV<sub>1</sub> was found. Adequate diagnosis of coal-dust-related respiratory effects requires evaluation of several influences on the respiratory system. Until now, no reliable clinical parameter has been identified to assess clinical deterioration including pulmonary damage. To the authors' knowledge, serum LDH activity related to silica exposure in human has not received emphasis in the literature. The increased levels of serum LDH, especially LDH<sub>3</sub>, in persons with a history of silica exposure, might reflect more cell damage and greater access of LDH to the circulation from pulmonary cells. The character and severity of lung tissue reaction to mineral dust is not predictable, because of differences in the variation in individual susceptibility that possibly involves immunologic mechanisms, lung structure, and/or clearance capacity (25). However, higher cumulative dust exposure does not necessarily lead to higher profusion score on a chest radiograph. It is well documented that chest radiographs and high resolution computed tomography (HRCT) have value in the assessment of interstitial disease, but cannot

TABLE 3. Results of pulmonary function tests of the studied cases of ex-coalminers

Flow volume (BTPS)	Total group (n=201)	Group I (n=42)	Group II (n=159)
FEV <sub>1</sub> (l)	1.91 ± 0.69	2.16 ± 0.7	1.84 ± 0.67*
FEV <sub>1</sub> of norm (%)	69 ± 22	75 ± 21	68 ± 22†
FVC (l)	3.38 ± 0.78	3.65 ± 0.74	3.31 ± 0.78*
FVC of norm (%)	96 ± 31	98 ± 16	96 ± 34
FEV <sub>1</sub> /FVC (%)	56 ± 14	58 ± 11	55 ± 15
	n=190	n=40	n=150
Dco (%)	68 ± 20	70 ± 21	68 ± 20

Data are expressed as means ± SD. Group I, normal serum lactate dehydrogenase (LDH) activity; Group II, high serum LDH activity; BTPS, body temperature and pressure, saturated with water vapour; FEV<sub>1</sub>, forced expiratory volume in 1 s; FVC, forced expiratory volume; Dco, diffusion capacity measured by single breath method.

\* $P < 0.05$  Group I vs II, † $P < 0.001$  Group I vs Group II.

adequately differentiate the contribution of dust exposure as cause of the pulmonary function impairment (23). In line with this, differences were not found

between ex-coalminers with a normal chest X-ray vs those with an abnormal chest X-ray concerning the LDH activity and its isoenzyme pattern.

In several pulmonary disorders associated with cell death or cell damage, elevated serum LDH activities have been reported. De Remeé (14) reported elevated serum LDH in five cases of interstitial pneumonitis. Matusiewicz *et al.* (8) reported serum LDH to be a simple, though non-specific, test which appears to reflect changes of disease activity in patients with cryptogenic fibrosing alveolitis (CFA), extrinsic allergic alveolitis (EAA) and hypersensitivity pneumonitis, but not in sarcoidosis. Recently, Drent *et al.* (22) described a similar increase of the LDH isoenzyme pattern in serum as well as in BALF obtained from a patient with a lipid pneumonia. Moreover, a high LDH activity has been reported in *Pneumocystis carinii* pneumonia (22,28), pulmonary alveolar proteinosis (10) and desquamative interstitial pneumonitis (11,13,14). So, several pulmonary disorders have been associated with elevated serum LDH activity. An increase in airway LDH activity might arise from diverse sources, including rupture (necrosis) of the airway and/or alveolar epithelial cells, AMs or other pulmonary cell types, increased flux of plasma-derived LDH through an air/blood barrier rendered more permeable by pulmonary injury (e.g. oedema, haemorrhage), and elevated plasma LDH concentration resulting in an increased plasma/alveolus concentration gradient, with a consequent increased rate of passage of LDH across the air/blood barrier of a normal lung (16,29). Thus, LDH might be released

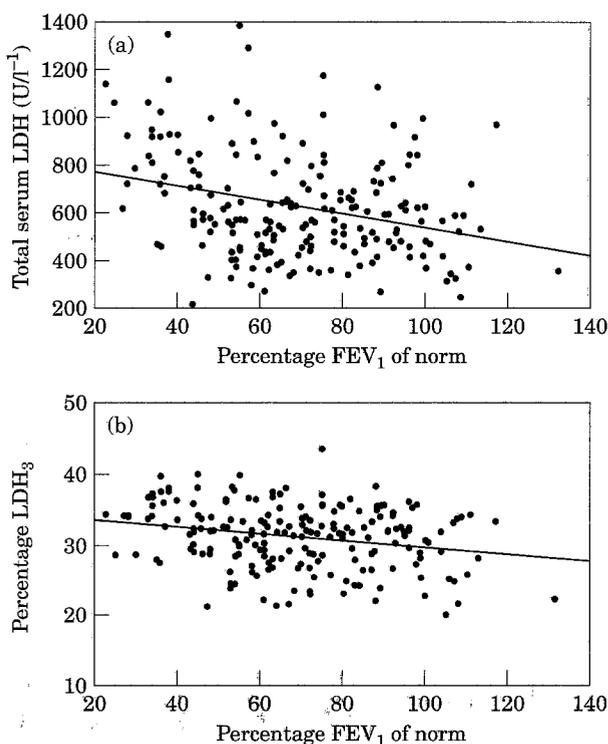


FIG. 2. Relations between (a) the forced expiratory volume in 1 s (FEV<sub>1</sub>) and serum lactate dehydrogenase isoenzyme (LDH), and (b) FEV<sub>1</sub> and LDH<sub>3</sub> activity in the total group of ex-coalminers.

TABLE 4. Pearson coefficient of correlation testing a relation between the total lactate dehydrogenase (LDH) activity in serum and pulmonary function tests of ex-coalminers

	FEV <sub>1</sub> of norm (%)	FVC of norm (%)	Dco (%)
Correlation with total LDH	$r = -0.26$ $P < 0.001$	$r = -0.06$ NS	$r = -0.06$ NS
Correlation with LDH <sub>3</sub>	$r = -0.23$ $P = 0.001$	$r = -0.06$ NS	$r = -0.07$ NS

NS, not significant; FVC, forced expiratory volume; FEV<sub>1</sub>, forced expiratory volume in 1 s; Dco, diffusion capacity measured by single breath method;  $r$ , coefficient of correlation.

from injured cells of the lung into the pulmonary interstitium and alveoli, or from damaged inflammatory cells that infiltrate the lung after treatment. However, less is known about the utility of the LDH isoenzyme pattern in the assessment of pulmonary function impairment.

The LDH isoenzyme pattern of the lung is characterized by proportionally high LDH<sub>3</sub> and LDH<sub>4</sub> compared to the normal serum isoenzyme pattern. A high serum LDH<sub>3</sub> activity was reported just after pulmonary embolism (12). Release of LDH<sub>3</sub> from injured pulmonary parenchyme presumably produces the observed rise in serum LDH<sub>3</sub> activity. However, during acute rejection of a pulmonary graft in man, serum LDH<sub>4</sub> and LDH<sub>5</sub> were increased (30). Bansal *et al.* (17) found that AMs contained all five LDH isoenzymes, with LDH<sub>5</sub> being the most prominent.

Animal studies confirmed observations that the intrapulmonary instillation of silica particles results in an immediate severe inflammatory reaction. This early structural damage was followed by reparative processes that were manifested by replication of epithelial cells, endothelial cells and fibroblastic cells (31). Furthermore, a focal centrilobular necrosis of type I pneumocytes cells was accompanied by a fibrinous exudate. This focal necrosis was rapidly repaired by proliferation of type II pneumocyte cells. An increase in cuboidal cells after silica exposure was first described by Policard *et al.* (32). At present, type II cell hyperplasia is generally regarded as the standard reparative reaction after type I cell injury. Melloni *et al.* (33) showed LDH release from AMs in supernatants *in vitro* after 24 h of incubation with mineral dust. The latter group found that AMs were an important source of factors that normally stimulate type II cell proliferation, and that this proliferative activity was enhanced by *in vitro* silica exposure. The cytotoxicity of silica for AMs has been considered to be a major component in the development of fibrosis. Cell death induced the release of mediators

participating in the inflammatory and fibrogenesis processes (34). When AMs ingest silica, they release chemical attractants, thereby inducing the required amplification of macrophage production and release. This process is perpetuated by serial ingestion, cell killing, release of crystals and rephagocytosis (35–38). This accounts for the continuously high output of AMs, even after a brief exposure of silica. Brown *et al.* (39) demonstrated that supernatants of human AMs stimulated with silica contained large amounts of apparent growth factor activity for human lung fibroblasts. Since all miners in the present study were retired, acute effects of coal-dust exposure were excluded; repeated ingestion of old silica particles, still present in the lung, giving cell death after more than 20 yr, seems to be the cause of the elevated levels of serum LDH in this group of retired miners. Bowden *et al.* (40) studied the fibroblast proliferation of silica-stimulated human AMs *in vivo*. By determination of hydroxyproline, they found a significant increase in collagen. Taken in conjunction with the morphological data, it is reasonable to conclude that as the cellular granulomas subside, the residuum of collagen becomes incorporated into the peribronchiolar connective tissues. The localization of this excess of collagen, and the absence of significant scarring on the walls of peripheral air sacs, may account for the remarkable paucity of symptoms in many people who are exposed to silica, and for the poor correlation between the radiologic demonstration of peribronchial scars and the lack of significant impairment of pulmonary function. Moreover, simple mineral dust exposure, in the absence of smoking, pneumoconiosis, or both, did not increase the prevalence of emphysema, seen by HRCT (41).

In agreement with Dubar *et al.* (42), the present authors did not find differences between smokers and non-smokers. The latter group (42) studied the immediate affect of cigarette smoke on cell injury, cell viability and cytokine secretion by AMs from guinea

pigs and healthy human subjects. They measured LDH release in a culture medium after smoke exposure together with measurement of IL-6 and TNF- $\alpha$  activities. The release of LDH from AMs in the culture medium was unchanged both immediately after tobacco smoke exposure and at the time of the cytokine evaluation (18–20 h later). They demonstrated that the exposure to tobacco smoke produced significant changes in the AM secretory function without alterations of the cell viability. A study which compared BALF of non-smokers vs light and heavy smokers showed no differences in release of LDH by AMs (43). The present authors also found no differences between the LDH activity in BALF between smokers and non-smokers (44). Despite alterations of cell function, smoking causes no cell damage or death reflected by LDH release and elevated serum LDH activity.

In conclusion, coal-dust exposure, even many years after the actual exposure, is reflected by an increase in the total serum LDH activity, mainly characterized by a high LDH<sub>3</sub> activity. Since all other liver function test were within normal limits, and, moreover, silica exposure induces pulmonary cell damage resulting in LDH release, these results indicate that the increased LDH originates from the lung. The total LDH as well as the LDH<sub>3</sub> activity were found to be related to FEV<sub>1</sub> impairment in ex-coalminers. Future studies should focus on the LDH activity and its isoenzyme pattern in other relevant pulmonary disorders like pulmonary emphysema, and should aim to clarify the clinical relevance, especially whether LDH could be a promising parameter in monitoring pulmonary damage. Moreover, BALF analysis should be performed in a comparable population to confirm the results of the present study.

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