Serum type III procollagen peptide in asbestos workers: an early indicator of pulmonary fibrosis

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ABSTRACT Serum type III procollagen peptide (PIIIP) concentrations were determined in 36 male workers exposed to asbestos fibres in the production of asbestos cement items and in 13 healthy male controls. Mean (SD) PIIIP serum concentrations were 9.3 (1.5) ng/ml (range 7–12) in the controls and 13.7 (3.5) ng/ml (range 7–20) in the asbestos workers; the difference was statistically significant (p < 0.01). The exposed workers were subdivided according to presence or absence of radiological signs of asbestosis and intensity and duration of exposure. PIIIP serum values of workers with asbestos related interstitial fibrosis were the highest of the groups at 14.6 (2.3) ng/ml. In workers with heavy exposure the PIIIP values were significantly related to duration of exposure (r = 0.95; p < 0.01). PIIIP serum values may be a useful index for the early diagnosis of asbestos induced pulmonary fibrosis and its use should be considered as part of the biological monitoring of exposed workers.

Exposure to asbestos may induce several non-malignant and malignant lung diseases. Among the former, hyaline plaques of parietal pleura and asbestosis are the most important. The diagnosis of asbestos induced non malignant lung disease is based on the presence of abnormal physical signs, impairment of lung function, and radiographic abnormalities. Such symptoms and signs, however, are not likely to detect early pulmonary effects and other methods may be required to detect these and prevent progression to a more severe stage of interstitial fibrosis.

Techniques such as bronchoalveolar lavage (BAL) and 67-gallium scintigraphy have been reported to be useful in detecting pulmonary effects before the appearance of radiological or functional changes, but are not suitable for use in large groups of occupationally exposed workers; moreover their specificity has still to be assessed.

Recent studies suggest that the pathogenesis of pulmonary fibrosis may be related to changes in the structure and function of pulmonary collagen rather than to an increase in its absolute amount. At least five collagen isotypes are present in the human lung, the most abundant are type I and type III collagen and their normal ratio is 66:33.

An increase in type III collagen was recently observed in lung biopsy specimens taken from patients in the early stages of cryptogenic pulmonary fibrosis and from subjects with active fibrotic disease. By contrast, type III collagen was reduced in postmortem lung samples taken from patients who had died from pulmonary fibrosis and in those from patients with a longer duration of the disease. These observations suggest that an increase in type III collagen occurs in the early stages of the disease and is followed by a decrease in the later stages. This hypothesis is in agreement with findings obtained in other tissues, including skin scar tissues. Type III collagen is synthesised by the collagen producing cells as a precursor form, procollagen which has specific N-terminal and C-terminal extension peptides at the ends of the molecule. These peptides are cleaved in stoichiometric amounts by specific peptidases during the secretion of newly formed collagen from the cell. N-terminal peptides may be measured in serum and other body fluids with a radioimmunoassay technique developed by Rohde et al.

Increased concentrations of type III procollagen N-terminal peptides (PIIIP) were observed in BAL fluids and in sera of patients with idiopathic pulmonary fibrosis. This increase is considered to reflect type III collagen changes in lung tissue.

Increased serum PIIIP concentrations were recently reported in silicotic subjects and in some workers occupationally exposed to silica dust who had no
Table 1  Airborne asbestos fibre concentrations (ff/ml) determined by stationary or personal sampling method or both, in the examined factory

<table>
<thead>
<tr>
<th></th>
<th>1976</th>
<th>1982*</th>
<th>1983*</th>
<th>1987*</th>
</tr>
</thead>
<tbody>
<tr>
<td>High exposure tasks:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stationary sampling</td>
<td>6-11</td>
<td>0-1-1</td>
<td>0-3-0.6</td>
<td>—</td>
</tr>
<tr>
<td>Personal sampling</td>
<td>—</td>
<td>0-2-0.8</td>
<td>0-4-1-4</td>
<td>0.03-0.1</td>
</tr>
<tr>
<td>Low exposure tasks:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stationary sampling</td>
<td>0-1-0.6</td>
<td>0-1-0.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Personal sampling</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.01-0.025</td>
</tr>
<tr>
<td>Crocidolite % of total asbestos</td>
<td>5-20</td>
<td>10-20</td>
<td>10-25</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data obtained according to 1979 AIA reference method.44

clinical or radiographic signs of pneumoconiosis, suggesting that the assay may be useful for the early detection of silicosis.21-23 Furthermore, serum PIIIP concentrations observed in a group of asbestos insulation workers with interstitial pulmonary fibrosis tended to be higher than those of a control group composed of asbestos exposed workers without opacities or pleural fibrosis on chest x ray films, although this difference was not statistically significant.4 On the other hand, Begin et al observed significantly raised PIIIP concentrations in bronchoalveolar lavage fluids of workers with asbestos associated alveolitis or asbestosis.5

Our study aimed to measure PIIIP serum concentrations in subjects with different concentrations and duration of exposure to asbestos to investigate their value in the biological monitoring of asbestos exposure.

Methods

Thirty six male workers occupationally exposed to asbestos fibres during the production of asbestos cement items were examined; all were currently exposed at the time of study. Exposure was evaluated by stationary or personal sampling, or both, according to the AIA 1979 reference method.44 The results of environmental monitoring performed in 1976, 1982, 1983, and 1987 are reported in table 1. The main exposure was to chrysotile but, up to 1984, an exposure to crocidolite (5-25% of total airborne asbestos) was also present.

According to job evaluation and environmental monitoring data, workers exposure was divided into high and low (high exposure jobs: sack opening, asbestos cement powder mixing, and dry finishing; low exposure job: moulding). Duration of previous exposure to asbestos was checked by questionnaire.

All chest x ray films of exposed subjects were blindly and independently classified according to the ILO 1980 International Classification of Radiographs of Pneumoconiosis55 by two radiologists experienced in occupational lung diseases. Only concordant results were accepted. According to exposure data and radiological findings, workers were then classified into four groups.

Group 1—Workers exposed to asbestos with radiological signs of asbestosis (1/1 or more according to the 1980 ILO classification).

Group 2—Workers employed in tasks with low exposure to asbestos for 10 years or more without radiological signs of asbestosis.

Group 3—Workers employed in tasks with low exposure to asbestos for five years or less without radiological signs of asbestosis.

Group 4—Workers employed in tasks with high exposure to asbestos regardless of duration or exposure and with no radiological signs of asbestosis.

Ventilatory function was assessed with the subjects sitting using a rubber sealed spirometer (Vitalograph). Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were recorded and classified according to Cotes.26

Cases were compared with a control group composed of 13 healthy male subjects not exposed to asbestos, other dusts, or toxic substances. Table 2 shows the mean age of the controls and the exposed workers and the mean duration of exposure. A questionnaire concerning tobacco and alcoholic use, health status, and previous diseases was completed for all subjects. To exclude incidental liver or collagen diseases, or both, in cases and controls erythrocyte and leucocyte counts, haemoglobin, packed cell volume, mean corpuscular volume, serum aminotransferases (AST, ALT), erythrocyte sedimentation rate (ESR), rheumatoid factor, and protein electrophoresis tests were performed. Serum creatinine and urine analysis were also undertaken to rule out an increase in PIIIP

Table 2  Main characteristics of controls and workers exposed to asbestos

<table>
<thead>
<tr>
<th>No of subjects</th>
<th>Age (years) Mean (SD) (range)</th>
<th>Duration of exposure (years) Mean (SD) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>37.4 (12.1) (18-54)</td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>50.4 (1.7) (48-52)</td>
<td>17.2 (3.9) (12-20)</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>47.5 (4.6) (39-52)</td>
<td>20.0 (5.4) (10-27)</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>26.3 (10.5) (18-55)</td>
<td>3.2 (1.4) (2-5)</td>
</tr>
<tr>
<td>Group 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>37.6 (13.0) (25-55)</td>
<td>9.9 (7.5) (2-25)</td>
</tr>
<tr>
<td>All asbestos exposed subjects</td>
<td>39.1 (12.9) (18-55)</td>
<td>9.7 (7.7) (2-27)</td>
</tr>
</tbody>
</table>
due to impaired renal function. No subjects were excluded because of liver, collagen, or kidney disease. Serum PIIP measurements were performed by the radioimmunoassay method described by Rhode et al. Commercially available RIA-gnost Prokollagen-III Peptid kits (Behring) were used. Mean recovery at three different PIIP concentrations ranged from 89% to 104%; the interassay coefficient of variation ranged from 7% to 15% according to PIIP concentrations: these results are similar to data reported by others. Table 3 gives the reference values of serum PIIP previously reported in normal subjects and the concentrations measured in the present controls.

In a study on the predictive value of various diagnostic criteria for selecting asbestosis in evolution in workers exposed to low asbestos concentrations Murphy et al showed that reduced FVC has a high prognostic importance and concluded that measurement of FVC is a good measure for the health surveillance of workers exposed to asbestos. The same results were also reported by Becklake et al. For this reason, we classified the exposed subjects into two subgroups according to FVC results: workers with normal FVC and reduced FVC (more than 20% reduction of normal predicted values). Statistical analyses were performed using the SPSS package; the differences between the various groups were assessed with Student’s t test and the Mann-Whitney U test.

Environmental monitoring results obtained both by stationary and personal sampling showed a low exposure to asbestos: excluding 1976 data (not completely comparable with more recent determinations), all samples were within, or near, the present TLV-TWA ACGIH. Airborne asbestos concentrations measured in 1987 ranged from about 1/10 to 1/100 of the 1986 TLV. It must be emphasised that after 1984 the workers were exposed to chrysotile asbestos only but in the previous years crocidolite fibres had also been used.

PIIP values in exposed workers and controls are shown in Table 4 and in fig 1. Considering either the whole group or each subgroup, PIIP concentrations were significantly higher in the exposed workers than in the controls. The highest PIIP concentrations were observed in group 1 (14.6 ± 2.3, SD) whereas group 2 workers, whose duration of exposure was similar, showed the lowest mean values (13.6 ± 2.3, SD). PIIP mean concentrations for groups 3 and 4 were intermediate, although values observed in the group with higher exposure proved much more scattered than in the other groups. Nevertheless, in group 4 it was possible to show a significant relation between duration of exposure and PIIP serum concentrations (r = 0.95, p < 0.01; y = 6·5 + 0.53x) (see fig 2).

According to our results, PIIP serum concentrations were not influenced by alcoholic intake (all subjects were moderate drinkers) or by smoking habits. No significant correlations were observed between PIIP values and subjective respiratory symptoms or pleural changes (pleural fibrosis, pleural calcifications, and diaphragmatic plaques).

Serum PIIP values of exposed workers classified according to FVC measurement results are shown in Table 5: the concentrations in workers with reduced FVC were significantly higher, not only with respect to control subjects (p < 0.01) but also when compared with exposed workers with normal FVC (p < 0.02).

Table 3  Serum PIIP reference values previously reported and observed in the present study

<table>
<thead>
<tr>
<th>PIIP reference values (ng/ml)</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>No</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7±0.2</td>
<td>11±0.18</td>
<td>8±0.3</td>
<td>3±0.5</td>
<td>9±3.1</td>
</tr>
<tr>
<td>7±0.2</td>
<td>3±0.5</td>
<td>3±0.5</td>
<td>3±0.5</td>
<td>3±0.5</td>
</tr>
<tr>
<td>7±0.2</td>
<td>3±0.5</td>
<td>3±0.5</td>
<td>3±0.5</td>
<td>3±0.5</td>
</tr>
</tbody>
</table>

*p 0.05; **p 0.01.

Table 4  Serum PIIP values observed in controls and in exposed workers

<table>
<thead>
<tr>
<th>PIIP values (ng/ml)</th>
<th>Mean (SD)</th>
<th>(Range)</th>
<th>Significance of differences v controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of subjects</td>
<td>(Range)</td>
<td>Student’s t test</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>13</td>
<td>(7–12)</td>
</tr>
<tr>
<td></td>
<td>Group 1</td>
<td>5</td>
<td>(12–17)</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>13</td>
<td>(8–20)</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td>12</td>
<td>(7–20)</td>
</tr>
<tr>
<td></td>
<td>Group 4</td>
<td>6</td>
<td>(7–20)</td>
</tr>
<tr>
<td></td>
<td>All asbestos exposed subjects</td>
<td>36</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01.
Discussion

Serum PIIIP values in the workers exposed to asbestos were significantly higher than in the controls (table 4) and all the values of workers with radiological signs of asbestosis (group 1) were higher than for control values. These results support the earlier observations of Begin et al3 and Okazaki et al6 on BAL fluids and serum respectively.

Bateman et al suggested that increased PIIIP serum levels are representative of active fibrosis4; our results are in keeping with those of Begin et al3 and Okazaki et al6 in indicating that high PIIIP serum concentrations in asbestotic subjects may be a marker of the extended "active" fibrotic process that characterises asbestosis.

A significant increase in serum PIIIP concentration was present in workers with no radiological signs of asbestosis and in subjects exposed to airborne asbestos concentrations of 0.2 fl/cc or less for periods not exceeding five years.

Begin et al found no significant increase in PIIIP concentrations in BAL fluids in exposed workers with no evidence of asbestos induced pulmonary damage.31 On the other hand, in the same study the author found significantly increased BAL fluid PIIIP concentrations in subjects with increased 67-Ga pulmonary uptake and rigid pulmonary pressure volume curve with no radiological signs of asbestosis.

Begin did not report any environmental data or give information on the time elapsed between the end of exposure and the date of the study. Our results suggest that changes in pulmonary collagen type III metabolism may be induced even by low exposures to asbestos. The exact meaning of these changes is not obvious but seem to imply a rearrangement of pulmonary collagen occurring at exposure levels considered "safe." These findings are not in agreement with other observations,32 33 but it should be emphasised that an increase in serum PIIIP concentrations was observed...
by Okazaki et al.\(^6\) in workers exposed to silica with no symptoms or signs of silicosis.\(^{21,22}\)

Furthermore, "negative" studies published so far are generally based on radiological or physiological changes that are not likely to detect the earliest pulmonary effects.\(^3\) On the other hand, cytological studies of BAL fluids or 67-Ga lung scan have shown appreciable changes in workers exposed to asbestos in the absence of radiological or physiological changes.\(^{4,31}\) All these observations suggest that the absence of fibrotic pulmonary effects below 1–2 fl/cc\(^3\) may be due to the lack of sensitive diagnostic methods: a definitive threshold for asbestos pulmonary effect has not yet been demonstrated.

A progressive impairment in FVC seems to be the most sensitive routine test for the early detection of asbestosis, followed by a restrictive syndrome due to increasing fibrotic change.\(^1,5,31\) PIIIP values in workers with a decreased FVC were significantly higher than in the controls and were also higher than those obtained in exposed subjects with normal lung function or with an obstructive pattern (p < 0.02).

It is commonly accepted that serum PIIIP values reflect type III collagen metabolism in the lung\(^9-20\): therefore raised PIIIP concentrations may be an early indication of a "pre"-fibrotic change.\(^19,34\) On the other hand, an increase in type I collagen and a pronounced reduction in type III occurs in the later stages of fibrotic processes.\(^13,15-17\) In agreement with these observations, Begin et al. reported an early transitory increase of PIIIP in BAL fluids associated with an asbestos induced alveolitis in sheep exposed intratracheally to chrysotile.\(^31\) Our results support the hypothesis that exposure to asbestos, at low levels is able to induce an increase in the synthesis of type III collagen even after short exposure: this increase is reflected by increased serum concentrations of PIIIP.

In workers exposed to higher concentrations of asbestos we found a highly significant relation between duration of exposure and serum PIIIP concentrations. Furthermore, in subjects with lower exposure to asbestos, serum PIIIP concentrations were the lowest, whether in workers with radiological signs of asbestosis mean serum PIIIP values were the highest. All these data suggest a possible dose effect relation between "cumulative" asbestos dose and PIIIP serum concentrations.

This work was supported by a grant from Emilia Romagna Region (delibera No 1970, 13/5/1986). Statistical analyses were performed at Centro Interdipartimentale di Calcolo Automatico of Modena University.

### References

Serum type III procollagen peptide in asbestos workers: an early indicator of pulmonary fibrosis


30 American Conference of Governmental Industrial Hygienists. Threshold limit values for chemical substances in the work environment. Cincinnati: ACGIH, 1986.


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The journal now also publishes editorials which are normally specially commissioned. The Editor welcomes suggestions regarding suitable topics; those wishing to submit an editorial, however, should do so only after discussion with the Editor.
Serum type III procollagen peptide in asbestos workers: an early indicator of pulmonary fibrosis.

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question of the biological and toxic effects of the remaining group.4

Secondly, while PCHs are known to be ubiquitous it is not known yet what constitutes a normal background concentration, although systematic investigations are in progress.1 Sampling around the two incinerators, however, in sufficient detail, to allow the proper assessment of the potential role of PCHs as environmental toxins in that area has not been undertaken so far as we know. Nevertheless, one sampling study in the Bonnybridge area reported that the rise in PCB (as Aroclor 1242) was three to five times the background values, and the rise in PCB (as Aroclor 1254) was 16 times the background values.5 The general location of the soil samples that contained the raised PCB concentrations coincided with the "at risk" areas described in our paper.

"Concentrations of PCH have not changed since the Rechem chemical waste incinerator was closed in 1984" PCHs do not degrade readily in the environment; the half life of dioxins and furans, for example, has been estimated to exceed ten years6 (and Rechem International response to the Bonnybridge dossier, 1985). Consequently, the stability of the PCH concentrations during the past few years cannot be used to exclude, as a potential source of that pollution, any industrial activity which ceased as recently as 1984.

To conclude, all interpretations of findings—and particularly unusual types of finding—require to be viewed with caution and subjected to frequent reappraisal. We put forward our ideas in the spirit of the Maud Committee's report on the use of uranium for a bomb: "We should like to emphasise at the beginning of this report that we entered the project with more scepticism than belief, though we felt it was a matter which had to be investigated."

References

Notices
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The Second Summer Institute in Environmental Health Studies will present courses during a two week period for academic credit or for continuing education credit. It will be possible to register for more than one course. Subjects will include: risk assessment and risk management, toxicology, physiology, occupational health, and industrial hygiene. For further information contact: Dr Jacqueline Corn, Continuing Education Program, Johns Hopkins University, School of Hygiene and Public Health, 615 North Wolfe Street, Room 1003, Baltimore, MD 21205.

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Advanced workshop on occupational and environmental radiation protection, 17–21 July 1989

Focusing on current developments in radiation standards, radiation protection instrumentation, waste management, and methods for protection against occupational exposures. Emphasis will also be given to effluent controls and monitoring, and models for environmental surveillance. Fee: $900.

Correction
Serum type III procollagen peptide in asbestos workers: an early indicator of pulmonary fibrosis (1988;45:818–23). Two sentences report wrong statements due to a regrettable mistake made in editing the final version of the manuscript. On p820 section Results, line 19 "showed the lowest mean values (13.6 ± 2.3,SD). PIIP" should be replaced by "showed lower mean values (13.6 ± 2.3,SD). PIIP" and on the same page line 21 "intermediate, although values observed in the group with" should be substituted by "intermediate between group 1 and controls, although values observed in the group with."