Modified nucleosides in asbestos workers at high risk of malignant disease: results of a preliminary study applying discriminant analysis

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ABSTRACT Patients with asbestos related malignant mesothelioma excrete high levels of modified nucleosides in their urine. The purpose of the present report was to explore further the usefulness of measuring these breakdown products of transfer RNA (tRNA) in male asbestos insulation workers who are at high neoplastic risk but without clinical signs of malignancy. Modified nucleoside levels (Ψ , m'A, m'I, m₂G, and ac₄C) were used as discriminator variables in a computer generated discriminant function in which 96% of the controls and 95% of the insulation workers were correctly classified. It was also found, using a similar multiple regression model, that 10 of 13 were correctly classified as having normal chest radiographs and 27 of 30 asbestos exposed subjects as exhibiting alterations in either the parenchyma, pleura, or both. The results suggest that measuring modified nucleosides levels in the urine of asbestos exposed workers, and perhaps others exposed to carcinogenic agents, has the potential for identifying, through multivariate statistical techniques, individuals who are at high neoplastic risk.

Potential carcinogenic agents are being identified with increasing frequency in various occupational settings. A need exists, therefore, to explore new diagnostic techniques which can detect the presence of antecedent pathophysiological changes that may be present before the appearance of tumours.

Patients with certain cancers have been found to excrete high levels of purines, pyrimidines, and their ribosides, which are breakdown products of transfer RNA (tRNA) in their urine^{1,2}; and we have reported that individuals with malignant mesothelioma, an asbestos related neoplasm, also excrete raised levels of modified nucleosides.³ By contrast, normal adults exhibit relatively low and constant levels of modified nucleosides.⁴

The inhalation of asbestos fibres has been associated with a wide range of diseases including interstitial pulmonary fibrosis and pleural abnormalities (thickening and calcification)—namely, pleuropulmonary asbestosis.⁵ Of even greater concern, however, is the strong association between exposure to asbestos and the increased risk of developing vari-

ous types of cancer, especially carcinoma of the lung and malignant pleural and peritoneal mesothelioma. A latent period of several decades between the onset of exposure and the clinical manifestations characterises these diseases. Insulation workers with a long history of occupational exposure to asbestos constitute an appropriate population in which to study the biochemical events occurring during the latent period.

As the first part of a nationwide, longitudinal study in the United States we have examined a large group of male asbestos insulation workers.

The purpose of this brief communication is to present some preliminary data, from a small subset of these subjects, in order to evaluate the possibility that the biochemical alterations observed in patients with cancer also occur in individuals at high neoplastic risk but without the clinical manifestations of malignancy. Specifically, we have applied a multiple regression approach to investigate whether nucleoside levels had predictive value in differentiating a group of subjects at risk of developing cancer from a group of normal subjects. It was also of interest to determine whether nucleoside levels were predictive of radiograph alterations in the exposed individuals.

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Modified nucleosides in asbestos workers

Every insulation worker participating in the national survey underwent a comprehensive clinical examination—namely, review of symptoms; complete physical examination, pulmonary function tests, standard 14" × 17" chest radiographs, and clinical biochemical tests. As part of this pilot study, however, urinary nucleoside levels were measured for only 47 subjects (median age 59). The modified nucleosides, determined according to previously reported procedures,⁶ were pseudouridine (Ψ), 1-methyladenosine (m'I), 1-methylguanosine (m'G), N₂-methylguanosine (m₂G), N₂N₂-dimethylguanosine 2-pyridone-5-carboxamide-N'-ribofuranoside (PCNR), and 4-acetylcytidine (ac₄C). One DNA breakdown product, β-aminoisobutyric acid (BAIB), was also measured. Radiographic abnormalities were identified according to the ILO International Classification of Radiographs of Pneumoconioses and recorded for computer analysis as involving parenchymal or pleural alterations or both.

The control subjects consisted of 44 healthy adult men (median age 39). Even though the difference in age between the controls and asbestos workers was significant (Wilcoxon two sample test $S = 1248$, $p < 0.0001$), we have reported elsewhere that nucleoside levels are not dependent on age and are unaffected by smoking history in this study group.⁷

The first objective of the analysis was to determine the accuracy of a computer generated linear regression model in discriminating between the control subjects and the asbestos exposed workers based on their nucleoside profile.⁸ The actual data analyses were performed using the packages provided by the Statistical Analysis System with an alpha level of 0.05 stipulated for significance.⁹ (Multiple correlational techniques require that several measurements be available on each subject and only individuals with complete nucleoside profiles are included in the calculation of any particular discriminant function. This explains the slight numerical discrepancy in the tables to follow.) The rationale of discriminant analysis is to develop a classification algorithm that determines group membership based on a set of discriminator variables, in this case nuc-

leoside level. Recently, it has been suggested that especially Ψ and perhaps m'A were crucial biochemical markers that adequately characterised patients with malignant lymphomas.¹⁰

Table 1 presents the results of the discriminant analysis using only Ψ and m'A to classify the subjects in the present study. There was a 98% "hit rate" for the normal controls (31 correct out of 32), whereas the model correctly predicted 86% of the asbestos workers, or a 14% "miss rate," on the bases of Ψ and m'A levels alone (see the note in the table for explanations of hits and misses). Subsequent inclusion of m'I, m₂G, and ac₄C substantially improved the classification of the latter group; and the results of this discriminant function are shown in table 2. It should be noted that, with the addition of these nucleosides, the hit rate for the normal subjects was not substantially altered, and 95% of the asbestos workers were correctly classified. The apparent need to include a broader range of biochemical markers, in the absence of clinically confirmed malignancy, may reflect the existence of an underlying biological gradient that requires more information in order to increase the accuracy of a multivariate prediction model.

A similar analytical approach was then applied to explore the predictive value of nucleoside level in the classification of asbestos related radiographic abnormalities. The exposed subjects were subdivided into one group that exhibited normal chest radiographs and a second having at least one radiographic abnormality—for example, alterations in either parenchyma, pleura, or both. The outcome of the classification model is presented in table 3. Interestingly, ten of 13 (77%) and 27 of 30 (90%) were correctly identified as having normal and abnormal radiographic findings, respectively. Taken together with the previous results, it could be hypothesised that there exists an underlying gradient of biochemical events in the course of asbestos related diseases since differentiation was shown between controls and asbestos insulation workers and within the exposed group with respect to radiographic findings.

Table 1 Classification of asbestos workers and normal controls on the basis of Ψ and m'A levels

Actual group	Computer predicted group	
	Controls	Workers
Controls	31* 97%	1† 3%
Workers	6† 14%	38* 86%

*Individuals correctly classified, "hit."

†Individuals misclassified, "miss."

Table 2 Classification of asbestos workers and normal controls on the basis of Ψ, m'A, m'I, m₂G, and ac₄C levels

Actual group	Computer predicted group	
	Controls	Workers
Controls	27* 96%	1† 4%
Workers	2† 5	42* 95%

*Individuals correctly classified, "hit."

†Individuals misclassified, "miss."

Table 3 Classification of radiographic findings in asbestos workers on the basis of modified nucleoside levels

Actual group	Computer predicted group	
	Normal x ray results	At least one abnormal
Normal x ray results	10* 77%	3† 23%
At least one abnormal	3† 10%	27* 90%

*Individuals correctly classified, "hit."

†Individuals misclassified, "miss."

In conclusion, the application of discriminant analysis has been applied as an effective multivariate statistical tool that, in the present investigation, has used the characteristics of several biological markers as predictors of criterion variables—for example, control subjects v asbestos exposed workers or the presence of radiographic abnormalities. The importance of this technique can be better appreciated when the nature of the underlying biochemical events involve complex inter-relationships that may not be apparent when considering simpler bivariate models; this statistical approach has been used extensively in other scientific disciplines. In the present communication, through the application of discriminant analysis in which linear regression functions used modified nucleoside levels as discriminator variables, we have shown that it was possible to predict accurately (1) a group of asbestos insulation workers at high neoplastic risk (but without clinical evidence of malignancy) from a group of normal controls and (2) radiographic abnormalities in the asbestos exposed subjects. Whether a similar pattern of biochemical change occurs, albeit of a more subtle nature, in individuals also at risk of cancer with normal radiographic findings is currently

being explored. It is possible that, as the manifestations of asbestos related diseases develop during the characteristic period of "clinical latency" and then advance through various stages of severity, the concomitant biochemical sequelae become more specific for certain biological markers.

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References

- Borek E, Baliga BS, Gehrke CW, *et al*. High turnover rate of transfer RNA in tumor tissue. *Cancer Res* 1977;37:3362-6.
- Sharma OK, Waalkes TP, Gehrke CW, Borek E. Application of urinary nucleosides in cancer diagnosis and cancer management. *Cancer Detect Prev* 1983;6:77-85.
- Fischbein A, Sharma OK, Selikoff IJ, Borek E. Urinary excretion of modified nucleosides in patients with malignant mesothelioma. *Cancer Res* 1983;43:2971-4.
- Gehrke CW, Kuo KC, Waalkes TP, Borek E. Patterns of urinary excretion of modified nucleosides. *Cancer Res* 1978;39:1150-3.
- Selikoff IJ, Lee DHK, eds. *Asbestos and disease*. New York: Academic Press, 1978.
- Gehrke CW, Kuo KC, Davis GE, Suits RD, Waalkes TP, Borek E. Quantitative high performance liquid chromatography of nucleosides in biological materials. *J Chromatogr* 1978;150:455-76.
- Fischbein A, Sharma OK, Solomon S, *et al*. Transfer RNA breakdown products in the urine of asbestos workers. *Cancer Detect Prev* 1984;7:247-52.
- Klecka WR. In: Nei NH, Hadlaihull C, Jenkins JG, Steinbrenner K, Bent DH, eds. *Statistical package for the social sciences*. New York: McGraw-Hill Book Co, 1975:434-67.
- Sarle WS. In: Helwig JT, Council KA, eds. *SAS users guide*. Raleigh, NC: SAS Institute Inc, 1979:183-90.
- Rasmuson T, Björk GR, Damber L. Evaluation of carcinoembryonic antigen, tissue polypeptide antigen, placenta alkaline phosphatase, and modified nucleosides as biological markers in malignant lymphomas. Recent results. *Cancer Res* 1983;43:331-43.