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Bronchial dysplasia induced by radiation in miners exposed to ^{222}Rn progeny

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Abstract

Objectives—To investigate whether sputum cytology can be used to monitor epithelial cell changes in groups at high risk of lung cancer from exposure to radiation.

Methods—Dysplasia of bronchial cells was investigated by means of sputum cytology in a group of 434 underground miners. 100 of them were not exposed, and 334 were exposed to ^{222}Rn progeny at cumulative exposures < 450 working level months.

Results—The frequency of dysplasia in

the exposed group was significantly higher than that in the not exposed group ($P < 0.0001$), and an exposure-response relation was found. This relation was different for smokers and non-smokers.

Conclusions—Possibly the frequencies of dysplasia could be used to assess past exposures of groups of miners. This approach could be applied to cases where data on radiation monitoring are not available or are very scarce.

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A typical case of dysplasia as seen through a transmitted light microscope. Original magnification (A) 400 $\times$, (B) 1000 $\times$.

Assessment of the risk of developing lung cancer as a result of inhalation of Rn daughters is now considered to be an important problem in occupational and environmental medicine. Recently, an increased mortality in miners exposed to low doses of Rn progeny has been reported.^{1,2} Similar risks may be expected for a proportion of the total non-mining population, as a result of exposure to Rn indoors.³

The development of bronchogenic cancer involves progressive changes in the bronchial epithelium, from cell metaplasia to malignant transformation.⁴ Smoking, toxic inhalants, and acute or chronic pulmonary inflammatory diseases contribute to occurrence and progress of squamous metaplasia.⁵⁻⁷ The stages that precede the development of carcinoma are reversible in a proportion of affected people, especially if smoking or the exposure to toxic inhalants stops⁶ or with treatment of the inflammatory process.⁷ Usually lung cancer develops only in a small proportion of the cases of epithelial cell transformations. The examination of exfoliated metaplastic cells, found in the sputum, has shown that cell transformation occurs in the bronchial epithelium long before the development of clinical symptoms. These findings have stimulated interest in sputum cytology as a method for monitoring high risk groups and prevention of lung cancer.⁸

We present the results of sputum cytology in a group of 434 underground miners (334 exposed and 100 not exposed to Rn progeny). The frequency of appearance of atypical cells (to the level of notable dysplasia, figure) in sputum specimens is significantly higher for the exposed group than for the controls. This frequency follows an exposure response

relation that has been found to be different in smokers and non-smokers.

The present findings suggest that the information about the proportion of miners with abnormal cells in their sputum samples, could be used as an indicator of the exposure conditions in the past, if retrospective information about levels of radiation is not available.

Materials and methods

COHORT

The study was carried out on a cohort of active miners who still worked underground, selected at random from the workers in five uranium and six metal mines in Bulgaria. The dust and silica concentrations in each of these mines were similar, but concentrations of Rn and Rn progeny differed significantly. In one of the metal mines, Rn gas was virtually absent and the miners there were used as controls. The exposure to other toxic gases (diesel exhaust, explosion gases) is similar in all mines.

The miners were classified into three categories of smokers: the smokers group included only people who smoked >10 cigarettes/day for at least five years (the overall distribution in this group was: 0.5–1 pack/day 21%, 1 pack 62%, 1–2 packs 15%, and >2 packs 2%). The group of non-smokers included people who had never smoked. Ex-smokers and moderate smokers who were exposed to Rn were considered as a third group (there were no ex and moderate smokers among controls).

In all the uranium mines and in three of the metal mines the data from long term monitoring of radiation were available. The 233 miners (smokers and non-smokers) from these mines were defined as group A. For each of them individual exposure was determined. Three exposure categories were selected. The lowest category was of 0–50 working level months (WLM). (The WLM is a unit of cumulative exposure defined as $1.3 \times 10^5 \text{ MeV l}^{-1}$ of the potential α energy concentration of Rn daughters in air endured for 170 hours.) A proportion of the total non-mining population may be exposed to these low levels (Rn indoors). The second category was of 50–150 WLM. This was the group of high occupational exposure. The third group was of exposures >150 WLM. With the limit of 5 WLM/year, as recommended by the International Commission on Radiological Protection,⁹ the cumulative exposure, for 30 years of work, should not exceed 150 WLM. Therefore, the group of exposures above 150 WLM, is representative for overexposed people.

In the remaining two metal mines, Rn progeny have been measured since 1991. The results have shown a substantial presence of Rn progeny in the atmospheres of the mines, but the real past exposures could not be determined directly. Their range was estimated by extrapolation with the assumption that ventilation during the past 15–20 years had not changed dramatically. The miners (smokers and non-smokers) in these mines were defined as group B. The ex and moderate smokers were defined as group C. The results

for groups B and C were interpreted in view of semiquantitative support from the findings obtained for group A and controls. Table 1 shows a detailed description of all the groups.

CYTOLOGY

Sputum samples were collected by direct expectoration in the early morning. From each sputum sample at least three specimens were prepared with conventional techniques described elsewhere.^{10,11} Different numbers were attached to the specimens, and under these they were further analysed for metaplasia with a transmitted light microscope.

A specimen was classified as having notable dysplasia, if the metaplastic cells vary in size and shape, the nucleus is enlarged and hyperchromatic, the nuclear to cytoplasmic ratio is increased considerably, and obvious pleomorphism or dyscariosis are present. The figure is typical of this stage of squamous metaplasia. These criteria of dysplasia support the definition given by Risse *et al.*,⁷ and the earlier classification of Fulmer *et al.*¹²

When a specimen was moderately atypical, suggestive but not conclusive of notable dysplasia, the sputum was resampled at six to 12 months. If the new specimen could not be reliably classified as dysplasia, or if the sputum sample could not be repeated, the case was excluded from further analysis (only five people were excluded because of this).

STATISTICAL METHODS

A retrospective cohort study was performed with data from group A (exposed) and controls (not exposed).¹³ The risk of dysplasia in relation to exposure to Rn progeny, smoking, and underground work experience was studied by logistic regression analysis.¹⁴ The determination of the 95% confidence intervals (95% CIs) was based on the likelihood ratio criterion. Levels of significance (P) and χ^2 were determined by the likelihood χ^2 method. For multiple comparisons Kruskal-Wallis non-parametric analysis of variance was used. The data were analysed by the package SPSS/PC+, version 4.01.

LUNG DISEASES

About 46% of non-smokers and 62% of smokers in our cohort showed some degree of chronic inflammatory lung disorders. The higher frequency for smokers was significant ($\chi^2 = 8.86$, $P = 0.003$), but the differences between exposed and not exposed groups, considered separately for smokers and non-smokers, were not significant.

After the end of this study one person from group A (a 40 year old with 18 years of work experience, exposure of 316 WLM and a smoker of one pack/day for 20 years) developed bronchial carcinoma (further confirmed by necropsy). He died 10 months after the first clinical symptoms. His last sputum sample was taken seven months before the first symptoms. The record showed that chronic bronchitis and severe dysplasia had been diagnosed, but without indications of carcinoma. There is no information on other lung cancers

Table 1 Description of the groups of miners

	Group A (n = 233)	Group B (n = 79)	Group C (n = 22)	Controls (n = 100)
Smokers	140	55	—	42
Age (mean (SD, range) y)	40.3 (6.0, 20–59)	40.0 (8.2, 23–56)	37.3 (6.2, 25–44)	42.9 (5.3, 24–51)
Work duration (mean (SD, range) y)	9.5 (5.6, 1–24)	7.4 (5.3, 2–28)	8.1 (5.7, 2–21)	11.7 (7.5, 1–25)

among the studied groups during the past two years.

Results and discussion

The study showed 87 cases of dysplasia among the 334 miners in groups A, B, and C (frequency 0.26, 95% CI 0.21–0.31) *v* three cases among the 100 controls (0.03, 95% CI 0.007–0.077). Thus, the higher occurrence of dysplasia in exposed miners was significant ($P < 0.0001$).

The data in table 1 indicate similar distributions of age and underground work practice for exposed and not exposed groups—that is, the exposures to silica, dust, and other toxic inhalants (not Rn) were expected to be similar. The stepwise logistic regression analysis showed the significance of exposure to Rn progeny ($P < 0.0001$) and smoking ($P = 0.0016$). The underground work experience did not fit the criteria for significance. These results support the opinion that the prevalence of the frequency of dysplasia in the exposed group is probably related to the inhalation of Rn and Rn progeny.

In the model used, the risk of dysplasia was estimated as:

$$\exp(z)/(1 + \exp(z)).$$

In this expression $z = -2.79 + 0.012 \text{ WLM} + 1.089 \text{ Sm}$, where smoking (Sm) is treated as a dichotomous variable (0 = non-smoker, 1 = smoker). The numerical coefficients for smoking (1.089), exposure in WLM (0.012), and constant (–2.79) are the maximum likelihood estimates derived by logistic regression. The analyses suggest an exposure-response relation, which was different for smokers and non-smokers (table 2).

The frequency of dysplasia for non-smokers at the lowest exposure (0–50 WLM) was not significantly above the controls, whereas for smokers the prevalence was significant ($\chi^2 = 5.91$, $P = 0.015$). In this lowest exposure subgroup the greater frequency of dysplasia in smokers over non-smokers was significant ($P = 0.033$). The results for the group exposed to 50–150 WLM showed

similar frequency of dysplasia for smokers and non-smokers. The frequency of dysplasia for non-smokers in this high exposure subgroup was significantly above the controls ($\chi^2 = 9.83$, $P = 0.007$). A greater frequency of dysplasia occurred in smokers than in non-smokers, but it was not significant ($P = 0.40$). The frequency of dysplasia was further increased in the highest exposure group (>150 WLM) and the prevalence of dysplasia in the smokers *v* the non-smokers was significant ($P = 0.02$).

The results in groups B and C (table 2) were considered in view of exposure-response relations derived for group A. The results for group B did not conflict with those assessed by extrapolation of a range of exposures (2–70 WLM). The 95% CIs for group B were consistent with low (< 50 WLM) and occupational (50–150 WLM) exposures.

With the information about the exposures of group C (range 2.5–346 WLM) and the logistic regression coefficients derived for group A, expected frequency of dysplasia for this group was estimated. The results showed that if group C is considered as non-smokers the expected frequency of dysplasia is 0.19, but if group C is considered to consist of smokers the expected value is 0.35. The observed frequency was 0.27 (95% CI 0.11–0.48) between both the expected frequencies (for smokers and non-smokers). Due to the broad CI the differences between observed and expected ratios for smokers and non-smokers were not significant. In general the results for groups B and C gave semiquantitative support to the exposure-response relation found for group A.

According to the results of our study, when the exposure was >50 WLM the risk of dysplasia was >0.1 (non-smokers) and >0.25 (smokers). Above 150 WLM the risks were >0.3 and >0.5 respectively. These results indicated that the frequency of dysplasia may be used for a retrospective estimation of the range of exposures for groups with unavailable or very scarce data on radiation monitoring. This could be the case in parts of the third world.

Besides these benefits, some limitations

Table 2 Frequency of dysplasia by exposure

Group	Exposure (WLM)	Non-smokers		Smokers	
		Dysplasia/ total	Frequency (95% CI)	Dysplasia/ total	Frequency (95% CI)
Group A	0–50	2/45	0.044 (0.007–0.132)	7/37	0.19 (0.085–0.335)
	50–150	7/27	0.26 (0.12–0.44)	26/77	0.34 (0.24–0.45)
	>150	8/21	0.38 (0.19–0.59)	19/26	0.73 (0.54–0.88)
Group B	2–70	4/24	0.167 (0.054–0.348)	8/55	0.145 (0.069–0.255)
Group C	2.5–346			6/22	0.27 (0.11–0.48)
Controls		2/58	0.034 (0.006–0.10)	1/42	0.024 (0.001–0.10)

have become obvious with this approach. The exposure-response relation was for active miners. Former miners could be unsuitable, because of a possible disappearance of dysplasia in a proportion of them, thus making the interpretation of the results unclear. In our cohort both smokers and non-smokers showed exposure-response relations, but the group of smokers showed relatively uniform smoking habits (about two thirds of them smoke one pack/day). This could not be expected in every case. Different smoking habits may result in different dysplasia outcomes. Therefore, it is our belief that the non-smokers group gives a better opportunity for the retrospective exposure assessment. In either case, to avoid possible strong differences due to the different way of life, smoking habits,¹⁵ or individual sensitivity of miners in different areas of the world, a preliminary investigation of a group analogous to our group A may be necessary for reliability in such studies.

Conclusion

We conclude that exposure of underground miners to ^{222}Rn progeny results in a significantly increased frequency of squamous cell metaplasia. At the level of notable dysplasia, this frequency follows an exposure-response relation. Sputum cytology could be used for a retrospective assessment of the range of exposures for groups for which this range could not be assessed directly. In the arrangement of such studies the limitations of this approach

should be recognised. Further investigations of metaplasia in miners are needed to clarify the exposure-response relation under different conditions.

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