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Relative Lymphocytosis-Disorder Caused by Occupational Exposure to Vinyl Chloride Monomer

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Vinyl chloride monomer (VCM) is a gas used in the plastic industry, which employs several million workers (Uzych 1988). Twenty years ago, it was recognized as a substance with mutagenic and carcinogenic potential (Infante et al. 1976; Anderson et al. 1981; Laplanche et al. 1987; Wu et al. 1989; Fucic et al. 1990; Giri 1995). At the end of 1973 threshold limit values (TLV) in the working environment ranged from 100 ppm in the German Federal Republic to 500 ppm in Sweden. Association between exposure to this gas and development of malignant neoplasia has been well documented in epidemiologic and laboratory studies. The recommended concentration in most countries is 1 ppm. Despite regulations, however, concentrations of VCM in the working environment may rise briefly as a consequence of specific requirements in the technological process.

Masked by severe health disorders, such as neoplasia, angiosarcomas, brain and lung tumors or acroosteolysis which develop after exposure to high concentrations of VCM, hematological disorders have not been sufficiently studied. Lymphocytosis has been reported in animal models after exposure to VCM in experimental conditions (Suciu et al. 1975). Lymphocytosis combined with lymphoproliferative disorders has been reported in persons occupationally exposed to VCM (Infante et al. 1981; Smulevich et al. 1988; Doll 1988).

An absolute lymphocytosis conventionally defined as a lymphocyte count above 4.0 x 10^9/l, may be followed by jaundice, lymphadenopathy, hepatosplenomegaly or signs of infection. It is possible, however, to intially diagnose malignant lymphocytosis by a routine blood count before specific symptoms develop. The borderline between benign lymphocytosis and the incidence of lymphoproliferative malignancy in patients with lymphocytosis is still a matter for discussion (Macintyre and Linch 1988 ; Batata and Shen 1993). Relative lymphocytosis (% lymphocytes) can also reveal exposure to VCM which may cause a deviation from reference limit of 35 %.

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The aim of this study was to evaluate the incidence of absolute and relative lymphocytosis after occupational exposure to low and high concentrations of VCM in the work place.

MATERIALS AND METHODS

Our study comprised 121 employees from two plastic factories who were monitored between May 1995 and June 1996. Subjects were devided into two groups; the first of which consisted of 81 male subjects occupationally exposed to VCM concentration of 1 ppm and periodically (every three months) exposed to concentrations of 50 ppm. The second group consisted of 40 male subjects occupationally exposed to an average VCM concentration of 300 ppm (+ /- 100ppm). All subjects had been employed in the plastics industry for an average of 18 years, 8 hours per day (range 1-33 years).

Vinyl chloride monomer concentrations in ambient air were measured with a Beckman 6700 gas chromatography system. Samples were taken continuously from 20 points in the working environment. Evaluation of measurements (approx. 4000,000 per year) was performed on a PDP 11 computer.

The control group consisted of 60 age-matched, male subjects from the clerical staff. Subjects in the control group had never been exposed to ionizing radiation or any known chemical mutagen in the working environment.

The exposed subjects and controls had not undergone any diagnostic procedure using ionizing radiation or taken any medications during the 6 months prior to sampling. Subjects did not differ in dietary habits. Data on the number of cigarettes smoked per day were available for the examinees, while for the control group only information on smoking/non smoking habit was obtained.

Blood samples were collected at the work place during the morning by venopuncture and automatically analyzed by ABX-Agros (Hoffman-Laroche). As a control measurement, lymphocyte counting was also carried out in an independent hematological laboratory. Variation in the results of the lymphocyte count among the referent laboratories was less than 3%.

Statistical analysis comprised standard descriptive procedures. Distribution of lymphocyte counts in all groups was shown graphically using the Box-and-Whisker plots (Frank and Todeschini 1994). Hypotheses on the effect of exposure were tested by analysis of covariance (ANCOVA) with duration of exposure as a covariate. Both absolute and relative lymphocyte counts were analysed. Relative counts

were transformed using the standard arcus sine square root transformation (in order to stabilise the variance for proportions). Homogeneity of variances was tested by Chi-square test (Weisberg 1985). This test consists of performing ANOVAs with the squared scaled residuals from our model as the dependent, and each factor as the independent variable. The residuals are squares divided by the number of observations. The regression sum of squares in these ANOVAs divided by two is asymthotycally chi-square distributed with the degrees of freedom equal to 1 for quantitative and the number of levels minus 1 for class predictors. Since there was a significant relationship between the predicted values and the residual variance, both the absolute counts and the transformed relative counts were additionally logarithmically transformed. Pairwise comparison was done using Bonferroni adjustment for assessing the experimentwise significance. Since the model for the absolute lymphocyte counts was designed for logarithms, it is a multiplicative and not an additive model of the original counts. The parameters of the model can be used to estimate the ratio of the lymphocyte counts between each of the exposed groups and the control group, i.e. the relative risk (RR). This is calculated as 10beta, where beta is the appropriate parameter. Confidence limits for the parameters can be estimated from the standard errors using normal approximation. All analyses were performed using the SAS/LAB Ver. 6.12 for PC Windows.

RESULTS AND DISCUSSION

Descriptive statistics for apsolute and relative lymphocyte counts, age and years of exposure are presented in Table 1. Both absolute and relative lymphocyte counts appeared to increase with mean VCM concentration. The group exposed to higher concentrations was also exposed for a longer period. There was no statistically significant difference ($p > 0,005$) in lymphocyte counts in relation to smoking habits within the group and between the groups of examinees.

Distribution of lymphocyte counts in the analysed groups are shown graphically in Box-and-Whisker plots in Figure 1. The distributions of lymphocyte counts appear to slightly skew to the right. The skewness is more pronounced in the group exposed to higher VCM concentrations. The range and the interquartile range appear to be larger in the groups with higher mean values.

The effect of exposure was assessed by analysis of covariance, with duration of exposure as a covariate. The fit of the models for the absolute and relative lymphocyte counts are reported in Table 2. For both variables, the effect of the group is highly significant, and the effect of the years of exposure is not. Since duration of exposure was higher in the group exposed to higher VCM concentrations, the effect of the duration might not have been significant due to confounding with the

Table 1. Descriptive statistics for absolute and relative lymphocyte counts, years of exposure, smoking habit and age (sample size, mean and standard error).

Variable	Control			1 ppm			300 ppm		
	N	Mean	S.E.	N	Mean	S.E.	N	Mean	S.E.
Lymphocyte absolute counts (10^9/l)	60	1.8	0.1	81	2.1	0.1	40	2.7	0.1
relative counts (%)	60	29.6	0.7	81	39.7	0.9	40	42.0	1.4
Exposure(years)	60	0	0	81	8.4	0.7	40	18.9	1.3
Cigarettes/day	60	-	-	81	11.4	1.5	40	8.1	1.7
Age (years)	60	37.5	1.2	81	32.9	0.8	40	44.0	1.3

Table 2. Analysis of covariance, the fit of the model

Source	d.f.	F	p	R^2	Adj. R^2
Absolute lymphocyte counts (10^9/l)					
Model	3	13.59	0.0000	0.1872	0.1734
Group	2	10.73	0.0000		
Years of exposure	1	0.59	0.4403		
Error	177				
Relative lymphocyte counts (%)					
Model	3	31.40	0.0000	0.3474	0.3363
Group	2	29.99	0.0000		
Years of exposure	1	1.54	0.2155		
Error	177				

Table 3. Estimated parameters of the ANCOVA model for absolute and relative lymphocyte counts

Variable	Parameter	S.E.	t	p
Absolute lymphocyte counts (10^9/l)				
Intercept	0.249	0.017	14.63	0.0000
Group				
1 ppm	0.071	0.027	2.64	0.0090
300 ppm	0.196	0.043	4.59	0.0000
Years of exposure	-0.001	0.002	-0.773	0.4403
Relative lymphocyte counts (%)				
Intercept	-0.244	0.007	-36.34	0.0000
Group				
1 ppm	0.080	0.011	7.612	0.0000
300 ppm	0.104	0.017	6.196	0.0000
Years of exposure	-0.001	0.001	-1.243	0.2155

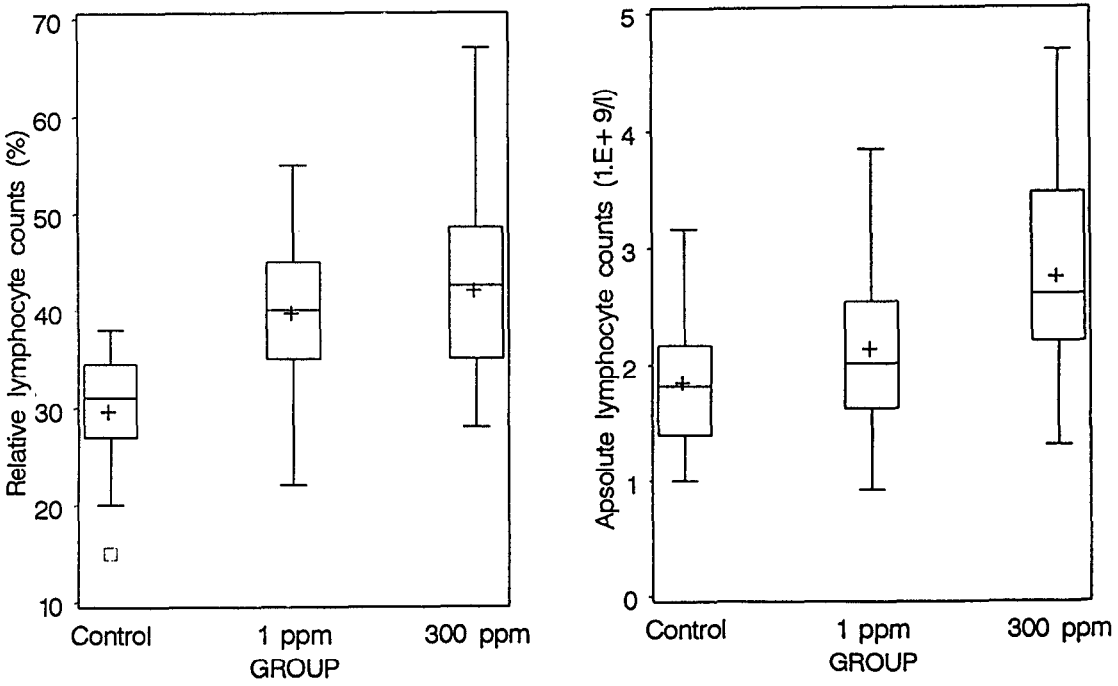

Figure 1. Distribution of relative and absolute lymphocyte counts in control and exposed groups presented as Box-and-Whisker plot. Both means and medians of relative and absolute lymphocyte counts in exposed groups (1 and 300 ppm) increases with VCM concentration in the work place. The bulk of the data is represented as a rectangle with the lower and the upper quartiles being the bottom and the top of the rectangle, respectively, and the median is portrayed by a horizontal line within the rectangle. Outliers, i.e. values outside the adjacent values, are plotted as individual points above and below the adjacent value line segment.

group effect. The ANCOVA model accounted for 17% of variation in absolute lyphocyte counts and 33% of variation in relative counts.

For both absolute and relative lymphocyte counts the effect of group was highly significant (Table 3). Multiple comparisons revealed that the group exposed to higher concentrations of VCM had significantly higher relative lymphocyte counts than both the control and the low exposure group. The group exposed to the lower concentrations did not differ significantly from the control group. For the absolute counts, however, all pairwise differences were significant. For the absolute counts the analysed model enabled expression of results as relative risks in relation to the control. The relative risk for the group exposed to lower concentrations was 1.18 (with 95% confidence limits 1.04-1.33), and for the group exposed to higher concentrations it was 1.57 (with 95% confidence limits 1.29-1.91).

Despite the fact that the risk of some severe neoplastic diseases associated with occupational exposure to high concentrations of VCM has been reduced to a minimum genome damage can still be detected even in low dose exposure if such exposure is accompanied by periodical elevated concentrations (Fucic et al. 1996). According to epidemiological studies, after occupational exposure to high concentrations of VCM the most frequently described neoplasia are hemangiosarcomas, brain tumors and lung tumors (Doll, 1993; Infante, 1981; Laplanche et al. 1987). One may speculate that the consequences of exposure to low doses of VCM will emerge through different distribution of neoplasia after exposure to high concentrations of VCM as indicated in some epidemiological studies (Smulevich et al. 1988).

Anemia and thrombocytopenia have been observed in VCM exposed subjects (Ward et al. 1976). Without a firmly established pathological definition for humans, lymphocytosis has only been described in experimental studies on rodents (Suciu et al. 1975). Lymphocytosis has never been interpreted as an indicator of lymphoproliferative disorder potential, while relative lymphocytosis was ignored in occupationally exposed populations, probably because it manifested as an asymptomatic phenomenon.

Chromosomal damage caused by exposure to VCM show that localization of breaks is not random and that the breaks are related to those detected in lymphoproliferative disorders (Fucic et al. 1995). Such regular patterns of chromosomal damage have also been described for some solvents, insecticides, and petroleum products (Mitelman et al. 1978). Together with splenomegaly and lymphocytic hyperplasia, the described disturbances of lymphocyte mitotic activity *in vitro* stimulated by phytohemagglutinin may additionally indicate VCM potential to affect the hematological system (Sharma et al. 1980; Lester et al. 1963; Fucic et

al. 1995). The results of our study on a population of 121 subjects occupationally exposed to VCM corroborate experimental data (Suciu et al. 1975). Both groups of examinees, despite differences in concentrations of VCM and different mode of exposures show the same disturbances in lymphocyte counts. Such prolonged effects have been described for thrombocytopenia caused by VCM (Ward et al. 1976). The long life of the lymphocytes can also mask fluctuation in VCM concentrations in the working environment.

Data on the correlation between lymphocytosis and lymphoproliferative disorders in populations affected by occupational or environmental mutagens remains to be collected. Workers occupational exposed to VCM for more than five years have been reported to manifest cancers of lymphocytic and hematopoietic tissues (Doll 1988). The twenty years of exposure to low doses of VCM eliminated the high risk of development of previously high dose related carcinogenesis and enables expression of a lymphoproliferative disorder with elevated rate of appearance. The persistance of lymphocytosis may be interpreted as an early manifestation of chronic lymphocytic leukemia (Ritis et al. 1997). In order to anticipate the new distribution of neoplastic diseases caused by exposure to low concentrations of VCM we suggest that periodical medical examinations include blood screening and assessments of maturation level of peripheral B-lymphocytes.

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