Relation between renal function tests and a retrospective organic solvent exposure score

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ABSTRACT In case-control studies on glomerulonephritis and organic solvents several authors have assessed exposure to organic solvents with a score based on the self reported occupational history. The underlying principle is that all exposures can be weighted by an intensity factor and then added to get one number representative of lifetime exposure. As this score has hitherto not been validated the relation between this exposure score and kidney function tests in a population of workers with past or present exposure to organic solvents was examined. The results suggest that such relations do exist for the N-acetyl- β -D-glucosaminidase (NAG) activity, erythrocyturia, and, perhaps, albuminuria but not for the protein creatinine ratio or for leucocyturia.

In their case-control studies on glomerulonephritis and organic solvents Bell *et al*¹ and Ranskov *et al*² assessed the exposure to organic solvents with an exposure score based on the occupational history. The relation between a quantitative exposure score and kidney function tests in a group of workers has not hitherto been examined.

This was accomplished in the present study by comparing the possible relation between this exposure score and clinical kidney function tests in a group of workers with definable past or present exposure to organic solvents. As Bell's exposure score is a summary measure of the organic solvent exposure which supervenes in different jobs, our main purpose was its validation by the comparison of the kidney function tests in different exposure score groups and not between different occupational categories.

Three hypotheses were examined: firstly, a search of correlation between exposure score and kidney function tests, secondly, the role of the duration of the exposure, and, finally, the possibility of a healthy worker effect.

Subjects and methods

Altogether 148 workers were examined as a part of a larger epidemiological study programme on the nephrotoxicity of organic solvents. Seven could not be examined because of holidays, leaving the job, refusal, foreign language, or organisational problems. The

calculation of the exposure score was not possible in one case. The N-acetyl- β -D-glucosaminidase (NAG) activities are missing for 25 further workers because of technical problems at the beginning of the study.

To avoid a recall bias, subjects did not know that we were interested in the effects of organic solvents. The medical examination was always presented either as a periodic or preplacement medical examination or as a general evaluation of the possible occupational risks. All participants were working at the time of the examination and patients referred to us for possible occupational disease were not eligible.

Solvent exposure was assessed with the questions and the intensity factors proposed by Bell *et al*.¹ Briefly, the exposure duration is assessed and then multiplied by an independently determined intensity factor. The resulting scores are then added to get the total lifetime score. The units are then years weighted by the exposure intensity factor.

The questionnaire suggested by Bell *et al*¹ was used with some slight adaptations taking into account the characteristics of the different occupations with a potential solvent exposure in the study region. The intensity of solvent exposure was then graded as follows.

Heavy exposure (intensity factor 2) consisted of occupational house painting indoors, industrial spray painting without protection devices, carpet and floor covering cleaning with cleaning agents, floor laying or impregnating, or both, production of paint, production of glue, polyester resin application with heavy contact with styrene,³ glue sniffing, tank cleaning

without protection devices, and paint stripping with organic solvents (if not already a painter).

Moderate exposure (intensity factor 1) consisted of non-occupational house painting indoors, spray painting with protective devices, industrial degreasing of metal, printing work, occupational gluing, anaesthetic work, dry cleaning, varnishing (if not already a painter), use of hair sprays, use of pesticides, and polyester resin application with low exposure to styrene.

Slight exposure (intensity factor 0.5) consisted of outdoor painting, motor repairing, handling of petrol fuels, tank cleaning with protective devices, hobby gluing, drawing with felt tipped pens, and exposure to exhaust fumes outdoors.

An open question that was coded blindly by an industrial hygienist asked for "other jobs with organic solvents." Care was taken to distinguish between solvent and water based cleaning agents, glues, and so on.

As the results of Bell *et al.*¹ compatible in this regard with those of Ranskov *et al.*² showed that non-occupational exposures do not seem important and correspond to much smaller score values we simplified the calculations. The exposure durations were then expressed in years and months instead of hours. A month was defined as a minimal exposure time of one hour a workday or five hours a week or 20 hours a month. Shorter exposure times were considered as insignificant. Work breaks of more than two months were considered as unusual and the exposure duration was then proportionally reduced, as it was by part time jobs. The dates of beginning and ending jobs were precisely determined.

Written instructions were given to the examining doctors and all codings of the answers were reviewed by the same occupational health practitioner. The exposure durations were recalculated by this second observer and differences of more than two months were not accepted.

The urinary cell counts were executed on uncentrifuged urine within one hour of micturition with a phase contrast microscope in a Bürker's cell. Two chambers of 0.9 mm³ were counted each time. Leucocytes and tubular cells were counted together according to Askergren.⁴ The difference between the two chamber counts was small (mean = 0.93 cells for 15 counts with less than 11 erythrocytes, mean = 1.5 cells for 15 counts with less than 10 leucocytes). The interobserver error was greater for leucocytes (mean = 2.1 cells for the same counts) than for erythrocytes (mean = 1 cell per count).

NAG activity was assayed as described by Powell *et al.*⁵ The mean intra-assay coefficient of variation was 2.5% (n = 20). Creatinine in blood and urine was assayed by the Jaffé method (Boehringer Diagnostica,

Hotz, Pilliod, Söderström, Rey, Boillat, Savolainen, Mannheim, Germany). Albuminuria was measured by radioimmunoassay (Pharmacia, Uppsala, Sweden). Shaw's index—that is, the protein: creatinine ratio in urine sample—is described as a semiquantitative assessment of the 24 hour excretion of protein.⁶ It was assayed according to Shaw *et al.* with the method described by Iwata *et al.*⁷ The mean intra-assay coefficients of variation for 20 samples were 0.8% for the protein and 0.9% for the urinary creatinine determinations.

The workers came fasting for examination and ate their breakfast at the institute to reduce the individual variations in fluid intake. Total urine samples were collected on waking (urine 1) and after breakfast at the institute (urine 2). They were asked not to urinate between collections. Blood sampling was performed before breakfast. Assays of NAG, albuminuria, creatinine, and the cell counts were made on urine 2.

As drugs or diseases could be important confounding factors, all subject who were regularly taking drugs at the time of the study or who had a clinical history with past or present diseases that could have some repercussion on the kidney or the urinary tract (the so called patient group) were excluded in the second step of the analyses. This patient group comprised 48 people.

Hypertension means a diastolic blood pressure ≥ 96 mm Hg, a systolic blood pressure ≥ 141 mm Hg, or both at the time of the clinical examination. Twenty (25%) of the patient group were hypertensive; remaining eight hypertensive workers belong to the patient group without kidney or urinary tract diseases (past or present) and did not take drugs.

Statistics

The normality of the distributions were tested by the Kolmogorov-Smirnov test and a mathematical transformation was made when necessary. If this was not successful non-parametric tests were used. One and multiple way variance analysis, Kruskal-Wallis one way variance analysis, Kendall's or Pearson's correlations, and chi-squared tests were used. All calculations were done with the statistical package for social sciences.⁸

Results

The median exposure score was 5.3 units with a 90th percentile at 33.0 (range 0–72.6; n = 140). In Bell's study this corresponds to a median of 9240, a mean ($\pm$ SEM) of 21 612 (2328) (range: 0–127 776). For comparison Bell *et al.* reported a mean value ($\pm$ SEM) of 13 186 (3716) (range 0–108 195) in the glomerulonephritis group (n = 50).¹

The exposure score was correlated with age

($r = 0.35$; $n = 140$; $p:0.000$), as were exposure duration and age ($r = 0.40$; $n = 140$; $p:0.000$). In the study group duration of exposure explains the major proportion of the variance of the exposure score ($r^2 = 0.767$; $n = 140$).

There is a weak but statistically significant correlation between raw exposure score and NAG activity ($r = 0.21$; $n = 114$; $p:0.001$) or erythrocyturia ($r = 0.12$; $n = 136$; $p:0.05$). After exclusion of the patient group, the correlations between raw exposure score and NAG activity ($r = 0.26$; $n = 68$; $p:0.002$) or erythrocyturia ($r = 0.20$; $n = 84$; $p:0.01$) were slightly better but the explained variances were still small.

There was no correlation between raw exposure and

and albumin excretion rate, Shaw's index, or leucocy-turia even after excluding the patient group.

As it is possible that the exposure score is a better estimate of exposure in a group than on an individual basis the whole group was subdivided into four quartiles (Q1 to Q4 in order of increasing exposure score). There is an increase in the NAG activity ($p = 0.006$; $n = 114$) and in the number of workers with an erythrocyturia above the median as defined in their control group by Askergren *et al* ($p = 0.08$; $n = 136$).⁴

After exclusion of the so called patient group the significance levels are 0.01 in both cases ($n = 68$ or 84 respectively) (tables 1 and 2, figure). A similar analysis was performed for leucocy-turia but did not show a clear tendency. As there is no detailed publication about the normal distribution of erythrocyturia or leucocy-turia in a healthy non-exposed worker group, a comparison with the normal ranges was not possible.

The same statistical analyses were carried out for albuminuria and Shaw's index with the upper limits of normal as cut off points, $20 \mu\text{g}/\text{min}^2$ for the albumin excretion rate or 127 for Shaw's index.⁶ The results did not show statistically significant tendencies.

It has been suggested that chronic organic mental disorders could appear after exposure to organic solvents for 10 years or more¹⁰ but it is not known whether this limit has any meaning for kidney function tests or whether the exposure intensity still has any influence beyond this ten year limit. Therefore, to examine the medical significance of this 10 year limit we built three subgroups with an exposure duration equal to 0 (group A1 or controls), between 0.01 and 9.9 years (group A2), and equal to or greater than 10 years (group A3). NAG activity and the number of people with an erythrocyturia above the median were greater in group A3 than in the other subgroups (table 3, figure). Table 1 shows that the exposure score is greater in this subgroup as well. A similar trend was

NAG activity and exposure score. ■ Exposure score quartiles (groups Q1–Q4), ● ten year limit (groups A1–A3) (details: see text), △ ten year limit and exposure intensity factor (groups C1–C3) (details: see text). Results calculated by variance analysis after correction for belonging to patient group and to “hypertensive group” (see methods). Factor exposure group is significant at $p = 0.05$ for Q1–Q4 and 0.02 for A1–A3 and C1–C3. For comparison, effect of factor “hypertensive group” is significant at $p = 0.03$, 0.03, and 0.007, respectively. Number is 110, 110, and 60 respectively.

Table 1 Age and exposure to organic solvent

Variables	Subgroups by									
	Exposure score quartiles				10 year limit			10 year limit and intensity factor		
	Q1	Q2	Q3	Q4	A1	A2	A3	C1	C2	C3
Age (y):										
$\bar{x}$	33.0	28.6	39.0	46.5	33.3	30.1	45.5	33.3	48.55	43.3
SD	11.4	8.6	11.1	9.8	11.8	9.5	9.4	11.8	9.9	9.4
No	38	32	35	35	32	53	55	32	24	17
Exposure duration (y):										
Median/No	0.0/38	1.8/32	12.8/35	23.0/35	0.0/32	3.0/53	21.2/55	0.0/32	22.1/24	16.5/17
Range	0.0–0.1	0.1–8.4	3.0–29.0	10.3–44.0	0.0–0.0	0.1–9.8	10.3–44.0	0.0–0.0	13.2–44.0	10.3–36.3
Exposure score:										
Median/No	0.0/38	1.8/32	11.5/35	30.4/35	0.0/32	3.3/53	22.8/55	0.0/32	22.1/24	33.0/17
Range	0.0–0.1	0.2/5.2	5.3/20.2	20.6/72.6	0.0–0.0	0.05–17.6	5.5–72.6	0.0–0.0	13.2–44.0	20.6–72.6

Table 2 Erythrocyturia and exposure score quartiles

No of cases with values	Subgroups*			
	Q1	Q2	Q3	Q4
Below the median†	19	13	10	9
Above the median†	17	18	24	26

*Difference statistically significant at $p = 0.08$ and after excluding the patient group at $p = 0.01$ ($n = 84$) (chi-squared test).

†As defined in his control group by Askergren.⁴

Table 3 Erythrocyturia and exposure of duration

No of cases with values	Subgroups*		
	A1	A2	A3
Below the median†	17	19	15
Above the median†	13	32	40

*Difference statistically significant at $p = 0.03$ and after excluding the patient group at $p = 0.07$ ($n = 84$) (chi-squared test).

†As defined in his control group by Askergren.⁴

observed for the albuminuria but not for Shaw's index or for leucocyturia. Excluding the patient group did not alter the results.

The same variables were then compared between three other subgroups: the first with no exposure at all, the second with all workers who stayed 10 years or more in the same exposure category, and the third with all other workers. The purpose was to see whether people who stayed a longer time in the same exposure category behave in a way different from workers with one or several short durations of exposure. The results were identical.

To test the respective roles of the chronic exposure and of the intensity factor we built three homogeneous subgroups. The first (C1) comprised workers with no exposure.

The two others comprised either printers (intensity factor 1) (group C2) or floor layers (intensity factor 2) (group C3). In both groups the duration of exposure in this industry branch is equal to or greater than 10 years. It may be seen in table 1 that exposure duration and score diverge. Group C2 has a longer exposure time but a lower score than group C3 (there were unfortunately not enough people with the same occupation in the low exposure category for building a third homogeneous group).

Again, NAG activity and the number of workers with an erythrocyturia above the median are highest in the group with the greatest exposure score (table 4, figure). Exclusion of the patient group did not alter the trend but for erythrocyturia the numbers were too small for a valid chi-squared test. Testing the exposed groups taken together against the control group,

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Table 4 Erythrocyturia, 10 year duration of exposure score

No of cases with values	Subgroups*		
	C1	C2	C3
Below the median†	17 (14)	7 (3)	2 (0)
Above the median†	13 (11)	17 (9)	15 (6)

*Difference statistically significant at $p = 0.006$; after excluding the patient group chi-squared is no more valid (because of too many cells with expected frequencies < 5). The numbers after this exclusion are given in parentheses. The comparison of C1 against C2 + C3 taken together is significant at $p = 0.02$ ($n = 43$) after excluding the patient group.

†As defined in his control group by Askergren.⁴

however, did reach statistical significance, even after excluding the patient group ($p = 0.02$; $n = 43$).

The albumin excretion rates are compatible with this trend but the number of pathological values is small (1/24 in the non-exposed and 2/16 in the exposed groups, after exclusion of the patient group) which precludes statistical examination. Leucocyturia and Shaw's index were again not clearly related to the exposure score.

As diuresis could alter our results the variance analysis for NAG activity was made with the uncorrected enzyme activity and by taking diuresis as an additional independent variable. The effect of the exposure score remains evident but the explained variance increased to 41%. If the erythrocyte excretion rates are used instead of the erythrocyte concentrations similar trends are obtained.

Finally, it should be mentioned that the number of workers with an erythrocyturia above the median is not greater in the group with a raised blood pressure ($p = 0.50$; $n = 136$). After excluding the patient group, there is no relation between NAG activity of erythrocyturia and age either in the whole group or in the control group ($p \geq 0.22$).

The serum creatinine concentrations were comparable in all subgroups.

Discussion

The interpretation of the relation between exposure score and kidney function tests is made difficult by the presence of confounding factors such as hypertension and kidney or urinary tract disease. As the prevalence of these disturbances increases with age which is itself associated with the exposure score, the influence of such confounding factors should be ruled out.

A significant role of age is unlikely for erythrocyturia^{11,12} or albuminuria.¹³⁻¹⁵ For NAG activity the age factor seems to play a part in one study¹⁶ but was considered as insignificant or weak by other workers.¹⁷⁻¹⁹

In this study we found no statistically significant

association between age and NAG activity, increased albumin excretion rate, or erythrocyturia above the median either in all workers or in the controls after excluding the patient group. Furthermore, the results observed in subgroups C1–C3 cannot be explained by a hypothetical influence of age. Therefore, it seems unlikely that age has biased our results.

Taking account of blood pressure and clinical history in the analysis did not alter our results which can therefore not be ascribed merely to an association between age, higher exposure score, and pathological states.

Some NAG activities were lost at the beginning of the study but the missing cases came from the same plants as the valid cases. Furthermore, the loss was due to technical problems and not to selection. Consequently a bias seems unlikely.

Evidently, our sample is not representative of the most frequent jobs with exposure to organic solvents because printers and floor layers are overrepresented. These results should not be generalised before more extensive studies have been completed.

Obviously no unifying mechanism for the kidney effects can be presented in populations with such differing exposure conditions. Transient microalbuminuria is associated, for example, with generalised traumatic capillary permeability but this recedes within hours of the event.²⁰ On the other hand, microalbuminuria seems to be associated with an increased risk of diabetic nephropathy⁹ and of vascular disease in non-diabetic subjects.¹³ There is also an association between increased NAG activity and kidney disease.^{16–19}

The renal changes permitting erythrocyturia are not clear. No consistent pathological alterations have been found in glomerular basement membrane in idiopathic microscopic haematuria.²¹ A distinction between glomerular and non-glomerular erythrocytes may shed some light on this equation.

In conclusion, we found a connection between the retrospective organic solvent exposure score as assessed with a slightly modified Bell's questionnaire and kidney function tests in a working population. We think that this is an interesting finding in view of the results of those authors who observed an association between this exposure score and the relative risk of glomerulonephritis.

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