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## Liver function assessment in workers exposed to vinyl chloride

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**Abstract** *Objective:* To investigate liver function in vinyl chloride workers and assess its relation with current/past occupational exposure to vinyl chloride monomer (VCM). *Methods:* A medical examination including the execution of liver function tests (LFTs) and liver ultrasonography was executed in a group of 757 workers with a long-standing service in the production of sVCM/polyvinylchloride (PVC). Cumulative and maximum VCM exposures were calculated. History of viral hepatitis and alcohol intake were carefully investigated. Regression analysis explored the association between abnormal LFTs and a group of possible determinants (VCM cumulative and maximum exposure, BMI, age, history of viral hepatitis, alcohol and triglyceride levels). Also, synergistic effect between VCM and a history of hepatitis was analysed, as well as the possible association between VCM exposure and aspartate aminotransferase/alanine amino transferase (AST/ALT) ratio >1. Distribution of abnormal LFTs was also assessed in relation to the results provided by liver ultrasonography. *Results:* The most frequently abnormal serum parameters were, in decreasing order: total cholesterol (27.3%), triglycerides (12.2%), total bilirubin (9.1%), gamma glutamyl transpeptidase (GGT; 9.0%) and ALT (8.2%). The AST/ALT ratio >1 was present in 28.1% of workers. Abnormal LFTs were not found to be associated with current or past VCM exposure. High ALT resulted positively associated with BMI, AST with alcohol intake, GGT with alcohol intake and triglycerides. No synergistic effect on LFTs of exposure to VCM and a history of hep-

atitis was observed. The AST/ALT ratio >1 was not found to be associated with VCM exposure. The prevalence of abnormal LFTs was higher in case of liver steatosis (ALT) or periportal fibrosis (GGT), but not in case of pure hepatomegaly, as documented by ultrasonography. *Conclusions:* Liver function assessment only including LFTs is not able to detect VCM-induced liver damage, but reveals alterations due to non-occupational factors, such as dietary and/or metabolic disfunctions. The LFTs are however of importance to detect conditions that could recommend avoidance of exposure to VCM and are useful for medical counselling and health promotion purposes.

**Keywords** Vinyl chloride monomer · Liver function assessment · Liver ultrasonography · Medical surveillance program · Health promotion

### Introduction

The clinical assessment of liver conditions in workers exposed to hepato-toxicants can be made through the use of serum liver function tests (LFTs) and diagnostic imaging techniques.

The LFTs can reveal the presence of liver damage due to occupational toxicants but they are also useful to evidence liver alterations due to non-occupational factors, such as f.i. viral hepatitis, excessive alcohol intake, overweight and metabolic disfunctions. Liver ultrasonography, a non-invasive, low-cost, risk-free imaging technique, enables the detection of focal (cysts, haemangiomas, malignancies) as well as diffuse (hepatomegaly, steatosis, fibrosis/periportal fibrosis, cirrhosis) liver lesions.

As vinyl chloride monomer (VCM) toxicity affects the liver as a primary target, liver function modifications have been investigated in a number of studies on workers with occupational VCM exposure. Abnormalities of liver function tests were documented on workers exposed to

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VCM in some studies going back to the 1960s and 1970s, when workplace concentrations were extremely high compared to current levels (Harris and Adams 1967; Lilis et al. 1975; Makk et al. 1974), even if a liver function test specific for VCM toxicity was never identified (Lilis et al. 1975).

More recent surveys carried out in the 1980s and 1990s generally failed to demonstrate LFTs alterations in VCM workers (Du et al. 1995; Sugita et al. 1986; Huang et al. 1997; Hsieh et al. 2003), although abnormal liver findings were attributed to VCM in a 1991 study (Ho et al. 1991). The interpretation of this study made by the authors is however questionable, due to the lack of consideration of all potential confounders.

A synergistic action of exposure to VCM and viral hepatitis in inducing LFTs changes in workers was suggested in a recent study (Hsieh et al. 2003) and such a synergism was also proposed to increase the risk of hepatocellular carcinoma development (Du and Wang 1998). Additive effects on LFTs modifications were also suggested in case of combined exposure to moderate levels of dichloroethane and VCM (Cheng et al. 1999).

In another recent study, exposure to VCM was suggested to be responsible for an increased aspartate aminotransferase/alanine amino transferase (AST/ALT) ratio (Di Lorenzo et al. 2003), a condition that generally occurs in alcoholic hepatitis (Harrison 1998) and has been associated with liver fibrosis (Sorbi et al. 1999).

Although the use of liver ultrasonography in VCM exposed workers was first proposed in 1975 (Taylor et al. 1975) to improve the efficacy of the medical surveillance of the workers employed in the production of polyvinylchloride (VCM/PVC), this examination has been seldom carried out in a systematic way among large groups of VCM exposed workers until recently (Maroni et al. 2003; Hsiao et al. 2004). This fact has probably limited a complete and correct interpretation of the liver function findings in most of the studies above mentioned.

Aims of this study were to analyse the alterations of liver function tests in a cohort of workers with a long work history in the production of VCM/PVC, to assess their relation to liver morphological changes detected by liver ultrasonography and to identify the factors—occupational or non-occupational—responsible for such LFTs alterations. In addition to the relation of LFTs to current and past occupational exposure to VCM, this study also examined the presence of synergistic effects between VCM exposure and a history of hepatitis and the possible association between VCM exposure and AST/ALT ratio >1.

## Methods

A total of 757 workers (735 males and 22 females) employed at four VCM/PVC plants in Italy underwent a clinical liver examination in 1998 after an informed consent was signed. The data presented in this paper have already been partly described in a former study (Maroni et al. 2003).

The examination program included:

- collection of information concerning past medical history and lifestyle
- assessment of individual past and present VCM exposure
- a general physical examination with focus on liver
- execution of serum liver function tests
- execution of liver ultrasonography.

The occurrence of viral hepatitis and/or other liver diseases in the history was investigated with care. Information about lifestyle included alcoholic beverage consumption and smoking habit. Alcohol consumption was collected by direct interview during the examination; the subjects were then classified into three groups according to daily alcohol intake (0, <40, >40 g/day).

Height and weight of each worker were measured and body mass index (BMI) was calculated according to the formula: (weight in Kilogram)/(height in metre)<sup>2</sup>. Subjects were then grouped into three classes of BMI (<25, 25–30, >30).

A careful assessment of individual past and present occupational VCM exposure was also part of the study. For the period before 1983, VCM exposure levels were assigned according to the results of a survey made by the Italian National Institute of Health (Pirastu et al. 1991). For the period 1983–1998, exposure levels were provided by fixed environmental monitoring systems. Maximum yearly average concentrations were calculated for each worker and five classes of exposure were identified (0, 1–10, 50, 200, 500 ppm). Cumulative VCM exposures (ppm × years) were also computed and the workers grouped into five classes (0, 1–10, 11–100, 101–1000, >1000 ppm × years). For more details on exposure assessment see Maroni et al. 2003.

The LFTs battery included: AST, ALT, GGT, alkaline phosphatase (AP), total and direct bilirubin. Other hematochemical tests performed were total cholesterol, triglycerides and complete blood cell counts.

Serum and blood tests were analysed at four different laboratories located at the four plant sites. Results were considered to be abnormal when exceeding the upper reference limits provided by each laboratory.

Liver ultrasonography examination was executed according to a standardised procedure (for more details, see Maroni et al. 2003). In particular, in addition to focal liver lesions, the presence of hepatomegaly, steatosis, liver fibrosis and fibrosis of the portal vein (periportal fibrosis) was investigated.

## Statistical analysis

Statistical analysis was performed using SPSS 11.5 for Windows

Multiple logistic regression analyses were carried out to investigate the association between each single liver function test, assumed as dependent variable, and possible determinants, assumed as independent variables.

Two models were run. In Model 1, the independent variables were represented by cumulative VCM exposure, alcohol consumption, BMI, history of hepatitis, age classes and serum triglyceride contents. In Model 2, cumulative VCM exposure was replaced with maximum yearly average VCM exposure. In addition to individual effects of the independent variables, the interaction between VCM exposure and history of hepatitis was also tested in the regression models.

The AST/ALT ratio  $>1$  was also analysed as dependent variable in the regression Models 1 and 2 above described.

Cumulative and maximum yearly average VCM exposures were considered to be a five-level variable as previously described; and the odds ratios refer to the unexposed odds value. Alcohol intake was considered to be a three-level variable as previously described as well as BMI as previously described; and the odds ratios refer to non-drinkers and to  $<25$  BMI class odds values, respectively.

Age was considered to be a four-level variable as previously described; and the odds ratios refer to the youngest class odds value.

History of hepatitis was considered to be a two-level variable (absent, present) and serum triglyceride contents also a two-level variable (normal, abnormal).

For every statistical analysis, a two-tailed  $P \leq 0.05$  was the level of significance chosen.

## Results

The mean age of examined workers was 44 years (SD 10) and the mean length of employment 19 years (SD 10).

Some general features of the cohort of PVC workers are shown in Table 1.

The frequencies of normal/abnormal liver function tests and serum parameters are shown in Table 2. The most frequently elevated parameters were, in decreasing order: total cholesterol (27.3%), triglycerides (12.2%), total bilirubin (9.1%), GGT (9.0%) and ALT (8.2%). The AST/ALT ratio was  $>1$  in 28.1% of workers.

Table 3 shows the frequency of abnormal LFTs according to daily alcohol intake, BMI, history of hepatitis and age classes. As regards alcohol consumption, statistically significant differences in prevalence were found for AST, GGT, triglycerides and cholesterol; as for BMI, the difference was significant for ALT, cholesterol and AST/ALT ratio; as for history of hepatitis, the difference was statistically significant for ALT; as for age classes, statistically significant differences were found for ALT, GGT, total bilirubin, cholesterol and triglycerides.

Table 4 shows the frequency of abnormal LFTs according to cumulative or maximum VCM exposure. As regards cumulative exposure, a statistically significant difference was found for GGT that showed an increasing trend consistent with exposure and for AP,

**Table 1** General features of the studied workers

Feature	Class	<i>n</i>	Percentage
Gender	Female	22	2.9
	Male	735	97.1
History of hepatitis	Present	53	7.0
	Absent	704	93.0
Daily alcohol consumption	None	203	27.1
	$<40$ g	489	65.2
	$>40$ g	58	7.7
Body mass index	$<25$	306	41.7
	25–30	344	46.9
	$>30$	84	11.4
	Age		
	$<30$ years	87	11.8
	31–40 years	183	24.7
	41–50 years	232	31.4
	$>50$ years	238	32.2
Cumulative exposure (ppm $\times$ years)	0	86	11.4
	1–10	281	37.1
	11–100	214	28.3
	101–1000	104	13.7
	$>1000$	72	9.5
Maximum average yearly exposure (ppm)	0	86	11.4
	1–10	485	64.1
	50	65	8.6
	200	54	7.1
	500	67	8.8

**Table 2** Frequency of normal/abnormal biochemical tests

Test	Normal	Abnormal
Aspartate amino transferase	719/730	11/730
	98.5%	1.5%
Alanine amino transferase	669/729	60/729
	91.8%	8.2%
Gamma glutamil transpeptidase	660/725	65/725
	91.0%	9.0%
Alkaline phosphatase	713/720	7/720
	99.0%	1.0%
Total bilirubin	609/670	61/670
	90.9%	9.1%
Conjugated bilirubin	523/552	29/552
	94.7%	5.3%
Triglycerides	567/646	79/646
	87.8%	12.2%
Cholesterol	468/644	176/644
	72.7%	27.3%
AST/ALT ratio $>1$	521/725	204/725
	71.9%	28.1%

that however was represented by very few elevated cases. As for maximum exposure, a significant increase was noted only for AP above 500 ppm. However, the small total number of elevated cases of AP makes the significance of the comparison among groups of uncertain validity.

The prevalence of abnormal liver function tests according to ultrasonography diagnosis of liver damage is shown in Table 5. The highest frequency of abnormal LFTs was almost always found among subjects diagnosed with two or more ultrasonography-detected liver alterations. Considering the individual tests, abnormal ALT, GGT, triglycerides, cholesterol and AST/ALT

**Table 3** Abnormal hematochemical results according to presence of a history of hepatitis, alcohol intake, BMI and age. *AST*: Aspartate amino transferase, *ALT*: alanine amino transferase, *GGT*: gamma glutamyl transpeptidase, *AP*: alkaline phosphatase, *TB*: total bilirubin, *CB*: conjugated bilirubin, *TRI*: triglycerides, *CHOL*: cholesterol. Statistical analysis by  $\chi^2$  test: hepatitis/*ALT*  $P = 0.045$ , alcohol/*AST*  $P = 0.000$ , alcohol/*GGT*  $P = 0.000$ , alcohol/*triglycerides*  $P = 0.012$ , alcohol/*cholesterol*  $P = 0.002$ , *BMI*/*ALT*  $P = 0.031$ , *BMI*/*cholesterol*  $P = 0.002$ , *BMI*/*AST-ALT* ratio  $P = 0.000$ , age/*ALT*  $P = 0.000$ , age/*GGT*  $P = 0.008$ , age/*TB*  $P = 0.006$ , age/*triglycerides*  $P = 0.023$ , age/*cholesterol*  $P = 0.000$ , age/*AST-ALT* ratio  $P = 0.042$

	Hepatitis			Alcohol			BMI			Age						
	No	Yes	Total	No	<40	>40	Total	<25	25-30	>30	Total	<30	31-40	41-50	>50	Total
<i>AST</i>	9/679 1.3%	2/51 3.9%	11/730 1.5%	2/192 1.0%	6/478 1.3%	3/55 5.5%	11/725 1.5%	6/302 2.0%	5/341 1.5%	0/82 0.0%	11/725 1.5%	2/87 2.3%	5/179 2.8%	1/230 0.4%	3/234 1.3%	11/730 1.5%
<i>ALT</i>	52/678 7.7%	8/51 15.7%	60/729 8.2%	17/191 8.9%	35/478 7.3%	8/55 14.5%	60/724 8.3%	22/302 7.3%	25/340 7.4%	13/82 15.9%	60/724 8.3%	3/87 3.4%	30/179 16.8%	16/230 7.0%	11/233 4.7%	60/729 8.2%
<i>GGT</i>	60/675 8.9%	5/50 10.0%	65/725 9.2%	4/192 2.1%	52/474 11.0%	8/54 14.8%	64/720 8.9%	23/301 7.6%	30/337 8.9%	10/82 12.2%	63/720 8.3%	0/87 0.0%	15/178 8.4%	28/228 12.3%	22/232 9.5%	65/725 9.0%
<i>AP</i>	6/670 0.9%	1/50 2.0%	7/720 0.9%	2/191 1.0%	4/471 0.8%	1/53 1.9%	7/715 1.0%	2/298 0.7%	4/336 1.2%	1/81 1.2%	7/715 1.0%	0/86 0.0%	1/177 0.6%	2/226 0.9%	4/231 1.7%	7/720 1.0%
<i>TB</i>	59/623 9.5%	2/47 4.3%	61/770 7.9%	21/181 11.6%	38/432 8.8%	2/52 3.8%	61/655 9.2%	32/282 11.3%	24/305 7.9%	5/78 6.4%	61/655 9.2%	14/85 16.5%	18/170 10.6%	21/215 9.8%	8/200 4.0%	61/670 9.1%
<i>CB</i>	27/510 5.3%	2/42 4.8%	29/552 5.2%	12/145 8.3%	17/353 4.8%	0/50 0.0%	29/548 5.3%	15/224 6.7%	8/253 3.2%	6/71 8.5%	29/658 5.3%	4/65 6.2%	10/130 7.7%	12/172 7.0%	3/185 1.6%	29/552 5.3%
<i>TRI</i>	70/599 11.7%	9/47 19.1%	79/646 12.2%	16/178 9.0%	52/420 12.4%	11/43 25.6%	79/641 12.3%	23/261 8.8%	45/309 14.6%	10/71 14.1%	78/641 12.2%	3/78 3.8%	12/138 8.7%	31/296 15.0%	33/224 14.7%	79/646 12.2%
<i>CHOL</i>	159/597 26.6%	17/47 36.2%	176/644 27.3%	31/179 17.3%	130/417 31.3%	14/43 27.4%	175/639 27.4%	53/261 20.3%	103/308 33.4%	18/70 25.7%	174/639 27.2%	3/78 3.8%	31/138 22.5%	61/205 29.8%	81/223 36.3%	176/644 27.3%
<i>AST/ALT</i> ratio >1	194/674 28.8%	10/51 19.6%	294/725 40.5%	59/190 31.1%	129/45 27.2%	15/55 27.3%	203/720 28.2%	109/302 36.1%	84/337 24.1%	10/81 12.3%	203/720 28.2%	31/87 35.6%	47/178 26.4%	51/227 22.5%	75/233 32.2%	204/725 28.1%

**Table 4** Abnormal hemochemical results according to maximum and cumulative VCM exposure. Statistical analysis by  $\chi^2$  test: cumulative exposure/GGT  $P = 0.024$ , cumulative exposure/AP  $P = 0.085$ , maximum exposure/AP  $P < 0.001$ 

	VCM cumulative exposure (ppm × years)						VCM maximum yearly average (ppm)					
	0	1–10	11–100	101–1000	> 1000	Total	0	1–10	50	200	500	Total
AST	1/85 1.2%	6/273 2.2%	1/205 0.5%	2/97 2.1%	1/70 1.4%	11/730 1.5%	1/88 1.1%	7/465 1.5%	2/60 3.3%	0/52 0.0%	1/65 1.5%	11/729 1.5%
ALT	8/85 9.4%	25/273 9.2%	17/205 8.3%	6/96 6.3%	4/70 5.7%	60/729 8.2%	8/88 9.1%	41/465 8.8%	3/59 5.1%	4/51 7.8%	4/65 6.2%	60/728 8.2%
GGT	6/85 7.1%	15/272 5.5%	22/202 10.9%	10/96 10.4%	12/70 17.1%	65/725 9.0%	6/88 6.8%	36/461 7.8%	5/59 8.5%	6/51 11.8%	12/65 18.5%	65/724 9.0%
AP	1/85 1.2%	1/269 0.4%	1/200 0.5%	1/96 1.0%	3/70 4.3%	7/720 1.0%	1/88 1.1% <sup>c</sup>	2/456 0.4% <sup>c</sup>	0/60 0.0%	0/50 0.0%	4/65 6.2%	7/719 1.0%
TB	4/66 6.1%	26/255 10.2%	17/191 8.9%	8/89 9.0%	6/69 8.7%	61/670 9.1%	5/69 7.2%	42/433 9.7%	3/56 5.4%	5/46 10.9%	6/65 9.2%	61/669 9.1%
CB	1/39 2.6%	10/205 4.9%	10/159 6.3%	4/81 4.9%	4/68 5.9%	29/552 5.3%	1/42 2.4%	20/352 5.7%	1/49 2.0%	3/44 6.8%	4/64 6.3%	29/551 5.3%
Tri	15/82 18.3%	23/237 9.7%	18/182 9.9%	15/88 17.0%	8/57 14.0%	79/646 12.2%	28/83 33.7%	98/407 24.1%	24/61 39.3%	12/41 29.9%	14/51 27.5%	79/645 12.2%
Chol	27/82 32.9%	50/237 21.1%	54/181 29.8%	30/88 34.1%	15/56 26.8%	176/644 27.3%	15/83 18.1%	40/408 9.8%	8/61 13.1%	8/41 19.5%	8/52 15.4%	176/643 27.4%
AST/ALT ratio >1	27/84 32.1%	80/271 29.5%	48/205 23.4%	30/95 31.6%	19/70 27.1%	204/725 28.1%	26/87 29.9%	125/463 27.0%	19/59 32.2%	15/50 30.0%	18/65 27.7%	203/724 28.0%

ratio >1 were found to be the parameters significantly associated with ultrasonography patterns of liver damage. In particular, ALT was found to be more frequently elevated among subjects diagnosed with steatosis or hepatomegaly plus steatosis. High GGT was found to be more frequent among workers with ultrasonography diagnosis of periportal fibrosis or periportal fibrosis, steatosis and hepatomegaly all together. Elevated triglycerides were found to be particularly frequent in subjects diagnosed with periportal fibrosis, steatosis and hepatomegaly or in subjects diagnosed with hepatomegaly and periportal fibrosis. The highest frequency of abnormal cholesterol levels was found among subjects

diagnosed with steatosis and periportal fibrosis or in subjects with hepatomegaly, steatosis and periportal fibrosis.

When analysing the relation between frequency of abnormal LFTs and each single type of ultrasonography-detected liver alteration (hepatomegaly, steatosis, periportal fibrosis) after excluding the cases with two or more alterations, the following results were found (Table 5). Abnormal levels of ALT were associated particularly with steatosis and to a minor extent with periportal fibrosis. Abnormal GGT occurred more frequently in case of periportal fibrosis and less frequently in case of hepatomegaly and steatosis. Abnormal cho-

**Table 5** Abnormal hemochemical results within ultrasonography-detected liver diseases. Statistical analysis by  $\chi^2$  test: ALT  $P = 0.002$ , GGT  $P = 0.000$ , triglycerides  $P = 0.001$ , cholesterol  $P = 0.000$ , AST/ALT ratio  $P = 0.001$ 

	Normal liver	Hepatomegaly	Steatosis	Periportal fibrosis	Hepatomegaly and steatosis	Hepatomegaly and periportal fibrosis	Steatosis and periportal fibrosis	Hepatomegaly, steatosis and periportal fibrosis	Total
AST	4/343 1.2%	1/70 1.4%	1/57 1.8%	0/62 0.0%	4/140 2.9%	0/19 0.0%	0/14 0.0%	1/25 4.0%	11/730 1.5%
ALT	15/342 4.4%	3/70 4.3%	8/57 14.0%	6/62 9.7%	23/140 16.4%	1/19 5.3%	1/14 7.1%	3/25 12.0%	60/729 8.2%
GGT	14/338 4.1%	6/73 8.2%	5/56 8.9%	11/61 18.0%	20/140 14.3%	0/18 0.0%	2/14 14.3%	7/25 28.0%	65/725 9.0%
AP	4/336 1.2%	3/72 4.2%	0/55 0.0%	0/61 0.0%	0/140 0.0%	0/18 0.0%	0/14 0.0%	0/24 0.0%	7/720 1.0%
TB	28/321 8.7%	7/67 10.4%	4/53 7.5%	5/55 9.1%	11/122 9.0%	3/19 15.8%	2/13 15.4%	1/20 5.0%	61/670 9.1%
CB	12/263 4.6%	4/58 6.9%	2/46 4.3%	1/52 1.9%	5/84 6.0%	33/18 16.7%	1/13 7.7%	1/18 5.6%	29/552 5.3%
TRI	15/300 5.0%	8/64 12.5%	10/52 19.2%	7/57 12.3%	24/128 18.8%	3/11 27.3%	2/13 15.4%	10/21 47.6%	79/646 12.2%
CHOL	64/299 21.1%	14/64 21.5%	16/52 32.1%	12/56 21.1%	50/128 38.4%	3/11 27.3%	7/13 58.3%	10/21 52.2%	176/644 27.3%
AST/ALT ratio >1	113/340 33.2%	26/70 37.1%	10/57 17.5%	17/62 27.4%	21/138 15.2%	7/19 36.8%	3/14 21.4%	7/25 28.1%	204/725 28.1%

**Table 6** Statistically significant regression analysis outputs. Model 1 includes cumulative exposure and Model 2 includes maximum exposure

Dependent variable	Independent variable	Regression coefficient	Standard error	Freedom degrees	p-value	Odds ratio	95.0% CI for odds ratio	
<b>Model 1</b>								
AST	Alcohol 0 g/day			2	0.033			
	Alcohol 40 g/day	-0.084	0.981	1	0.931	0.919	0.134364 6.288652	
	ALCOHOL >40 g/day	2.255	1.127	1	0.045	9.535	1.045434 86.97805	
ALT	BMI <25			2	0.082			
	BMI 25-30	0.003	0.389	1	0.992	1.003	0.468146 2.152773	
	BMI >30	0.989	0.498	1	0.047	2.690	1.012039 7.153973	
	Age ≤30			3	0.001			
	Age 31-40	1.762	0.787	1	0.025	5.827	1.244724 27.27889	
	Age 41-50	0.377	0.871	1	0.665	1.458	0.264145 8.052927	
GGT	Age >50	0.116	0.898	1	0.897	1.123	0.193243 6.529224	
	Alcohol 0 g/day			2	0.022			
	Alcohol 40 g/day	1.546	0.566	1	0.006	4.694	1.546016 14.25439	
	Alcohol >40 g/day	1.583	0.717	1	0.027	4.871	1.194362 19.86757	
TB	Triglycerides	1.183	0.350	1	0.000	3.266	1.643519 6.492437	
	Age ≤30			3	0.047			
	Age 31-40	-0.608	0.505	1	0.229	0.545	0.202 1.467	
	Age 41-50	-0.599	0.571	1	0.295	0.549	0.179 1.684	
AST/ALT ratio >1	Age >50	-1.705	0.669	1	0.011	0.182	0.049 0.675	
	BMI <25			2	0.000			
	BMI 25-30	-0.704	0.202	1	0.000	0.495	0.333 0.735	
Model 2	BMI >30	-1.561	0.394	1	0.000	0.210	0.097 0.455	
	<b>Model 2</b>							
	AST	Alcohol 0g/day			2	0.021		
Alcohol 40 g/day		0.107	0.963	1	0.912	1.113	0.169 7.346	
Alcohol >40 g/day		2.587	1.123	1	0.021	13.296	1.472 120.091	
ALT	BMI <25			2	0.090			
	BMI 25-30	0.028	0.387	1	0.941	1.028	0.481 2.197	
	BMI >30	0.976	0.494	1	0.048	2.656	1.007 7.004	
	Age ≤30			3	0.001			
	Age 31-40	1.841	0.779	1	0.018	6.303	1.366 29.071	
	Age 41-50	0.540	0.834	1	0.517	1.717	0.334 8.823	
GGT	Age >50	0.247	0.865	1	0.775	1.280	0.234 6.986	
	Alcohol 0 g/day			2	0.023			
	Alcohol 40 g/day	1.538	0.565	1	0.006	4.656	1.538 14.097	
	Alcohol >40 g/day	1.555	0.719	1	0.030	4.737	1.156 19.412	
TB	Triglycerides	1.147	0.349	1	0.001	3.151	1.589 6.249	
	Age ≤30			3	0.053			
	Age 31-40	-0.562	0.489	1	0.250	0.569	0.218 1.488	
	Age 41-50	-0.376	0.483	1	0.435	0.686	0.266 1.769	
AST/ALT ratio >1	Age >50	-1.500	0.594	1	0.011	0.223	0.069 0.715	
	BMI <25			2	0.000			
	BMI 25-30	-0.724	0.201	1	0.000	0.484	0.326 0.719	
	BMI >30	-1.575	0.395	1	0.000	0.206	0.095 0.448	

lesterol values were more frequently observed in case of steatosis; and abnormal triglyceride levels more frequently in case of steatosis and less frequently in case of hepatomegaly and periportal fibrosis.

### Regression analysis

The independent variables that resulted to be statistically significant in the multiple logistic regression analyses (Models 1 and 2) performed for each serum test as a dependent variable are shown in Table 6.

No association between abnormal liver function tests and either cumulative or maximum VCM expo-

sure was found. The main determinants of abnormal liver function tests were found to be overweight (as measured by BMI), alcohol intake and high triglyceride levels.

Abnormal AST levels resulted significantly associated with high alcohol intake (Model 1  $P = 0.045$ , Model 2  $P = 0.021$ ). An increased OR was also noticed in subjects with high triglyceride levels, but without statistical significance.

Increased ALT levels resulted significantly associated with BMI >30 (Model 1  $P = 0.047$ , Model 2  $P = 0.048$ ). Abnormal ALT were also found to be associated with age: a statistically significant increased OR was found in the age class 31-40 (Model 1  $P = 0.025$ , Model 2  $P = 0.018$ ),

while ORs decreased in the older age classes. Increased but not statistically significant ORs were observed among subjects with a history of hepatitis, among subjects with increasing cumulative VCM exposure classes and in those diagnosed with abnormal triglyceride levels.

Increased levels of GGT were significantly associated with low and high alcohol intake (Model 1  $P = 0.006$  and  $0.027$ , respectively, Model 2  $P = 0.006$  and  $0.031$ , respectively) and with high triglyceride levels (Model 1  $P = 0.000$ , Model 2  $P = 0.001$ ). An increased OR was noted for the highest cumulative VCM exposure class, but without statistical significance. A slightly increased OR was also observed in the highest BMI class, but the statistical significance was very low.

The AP result associated with alcohol consumption, but the association was not statistically significant.

Total bilirubin (TB) was found to be significantly lower in the oldest age class (Model 1  $P = 0.011$ , Model 2  $P = 0.011$ ). Increasing OR were observed in increasing cumulative VCM exposure classes, but the level of statistical significance was not reached.

As for conjugated bilirubin (CB), an increased OR was found in the highest BMI class, but the increase was not statistically significant.

The AST/ALT ratio  $>1$  was found to be significantly and inversely associated with BMI both for the class 25–30 and  $>30$  (Model 1  $P = 0.000$  for both classes, Model 2  $P = 0.000$  and  $0.000$  for both classes). It was also found to be associated with history of hepatitis and alcohol, but the level of statistical significance was not reached.

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## Discussion

### VCM exposure and liver function test alteration

Abnormal LFTs on VCM exposed workers were reported in old studies going back to the 1960s and the early 1970s, when VCM exposures were extremely high, compared to current levels (Harris and Adams 1967; Lilis et al. 1975; Makk et al. 1974). In fact these studies also found other toxic signs of VCM toxicity (e.g. Raynaud's syndrome, loss of consciousness) that are known to be occurring only at extremely high VCM concentrations.

Harris and Adams (1967) registered persistent raised bilirubin levels and palpable liver on inspiration in one of two workers employed as autoclave cleaners and affected by acro-osteolysis. No information about other possible risk factors for liver damage was however available, which makes uncertain the association between bilirubin alteration and VCM exposure.

Lilis et al. (1975), whose study included 354 workers, found abnormal LFTs in a small percentage of subjects, although alkaline phosphatase was found to be higher, especially in workers with more than 5 years of exposure. A higher percentage of abnormal liver tests was found in workers with current exposure than in those

with past exposure, Abnormal AST and ALT values were also affected by alcohol intake.

Makk et al. (1974), in a cohort of 1183 workers, registered the presence of 6.3% subjects with one or more liver abnormalities. Subsequent liver scan and biopsy led to identify two cases of angiosarcoma. Even if the percentage of abnormalities seemed to be higher than expected, workers engaged in PVC production showed abnormal tests in a smaller percentage compared either to workers engaged in synthetic rubber production or to all other workers at the plant. Moreover, no other determinants of abnormal liver tests, such as alcohol consumption or viral hepatitis, were taken into consideration. The authors were also not able to identify a test specifically associated with angiosarcoma, as one of the two patients diagnosed with angiosarcoma only presented a slight elevation in LDH values.

A study by Du et al. (1995) did not find any association between VCM exposure and alteration of liver transaminases (AST, ALT). Only GGT activity was noticed to be associated with VCM exposure ( $>10$  ppm); however, the observation that GGT was not found to be influenced by alcohol consumption, which is known to be a major determinant of abnormal GGT levels, makes the validity of this result rather uncertain.

No association between current or past VCM exposure and liver function test alterations was generally found in more recent studies (Sugita et al. 1986; Huang et al. 1997; Hsieh et al. 2003).

Sugita et al. (1986) collected and analysed data from a group of 108 workers. Regression analysis revealed no association between VCM exposure and liver test abnormalities. The authors concluded that no disorder of liver function is specific for VCM-exposed workers.

Huang et al. (1997) analysed data of 251 workers and used ALT as an indicator of liver function. Using regression analysis, VCM exposure was not found to be associated with abnormal ALT levels. A strong interaction on LFTs elevation was instead noticed between exposure to VCM and CYP2E1 and GST T1 genotypes. In vivo, VCM is metabolised by cytochrome P450 2E1 to form the electrophilic metabolites chloroethylene oxide and chloroacetaldehyde which may either cause cell damage or be further metabolised and detoxified by glutathione S-transferase. The study investigated whether the genotypes CYP2E1, glutathione S-transferase  $\theta$  (GST 1) and  $\mu$  (GST M1) were correlated with ALT abnormal levels. At high VCM exposure, the c2c2 CYP 2E genotype was associated with increased OR of abnormal ALT, while positive GST T1 was significantly associated with decreased OR of abnormal ALT. Multiple linear and logistic regressions showed analogous results. The authors concluded that the two genotypes may play an important role in the biotransformation of VCM.

Hsieh et al. (2003) analysed data from 568 workers employed at PVC/VCM manufacturing plants. No association was found between abnormal AST or ALT and exposure to VCM. They suggested the presence of a

synergistic effect between exposure to VCM and viral hepatitis (see onwards).

Ho et al. (1991) analysed liver function tests in a group of 271 workers and attributed the abnormal findings observed in 12 workers to VCM exposure (six possible and six probable cases). The conclusion is however questionable, as the criteria for attribution were only circumstantial and not all confounders (HCV, BMI) were controlled.

Our results are consistent with the findings of most of the recent studies and confirm the lack of association between current or past VCM exposure levels and LFTs alterations. The main determinants of abnormal liver function tests in our VCM-exposed workers resulted to be extra-occupational risk factors such as high alcohol consumption and obesity (as expressed by BMI), while no association was found with VCM exposure (Table 6).

Moreover, a comparison with the results provided by liver ultrasonography examination indicated that abnormal liver function tests (AST, ALT) were associated with ultrasonographic pictures of steatosis and hepatomegaly, liver conditions that were found to be unrelated to VCM exposure in a previous study (Maroni et al. 2003). In fact, steatosis had been found to be dependent on lipid metabolism alterations, overweight and age, while hepatomegaly had been found to be dependent on increased BMI, alcohol consumption and hypertriglyceridaemia. Both steatosis and hepatomegaly did not show any association with VCM exposure, while periportal fibrosis was the only ultrasonographic picture that showed a significant association with a history of exposure to VCM concentrations equal to or greater than 200 ppm (Maroni et al. 2003). GGT, the only LFTs associated with periportal fibrosis, was in turn found to be dependent on alcohol intake, BMI and age (Maroni et al. 2003). In the present study, GGT was confirmed to be not dependent on VCM exposure but dependent on alcohol intake and triglycerides, the latter being strongly associated with BMI.

Possible synergistic action on liver function tests of VCM with viral hepatitis infection or dichloroethane (DCE) exposure

Hsieh et al. (2003) reported on the presence of a synergistic effect on serum aminotransferase activity between hepatitis virus infection diagnosed by HBsAg and anti-HCVAb and occupational exposure to VCM and DCE. The authors suggested that viral infection could induce decreased levels of glutathione, enhancing the effects of the VCM toxic metabolites. The presence of a synergistic action between VCM exposure and viral hepatitis has also been proposed for hepatocellular carcinoma (HCC) development (Du and Wang 1998).

Our study did not indicate the presence of a synergistic action between VCM exposure and a history of hepatitis virus infection. However, in our study, sero-

logic information about hepatitis was not available for every subject and an underestimation of cases may have occurred. Further studies are hence advisable to examine this possible synergistic effect.

Cheng et al. (1999) found that combined exposure to low or moderate levels of DCE and VCM showed a dose-response relationship with abnormal ALT levels, concluding that relatively low concentrations of the two chemicals could synergise in causing liver damage (Cheng et al. 1999). However, this observation has not been so far confirmed in other groups of VCM-DCE-exposed workers.

In the large cohort of workers investigated in the present study, who were also exposed to DCE in addition to VCM, no effect on ALT or other LFTs was found in relation to occupational exposure. However, current levels of DCE exposure were rather low (1–2 ppm) and this makes our study not strictly comparable with the study of Cheng et al.

#### AST/ALT ratio and VCM exposure

In most liver diseases, the AST increase is less than that of ALT (AST/ALT ratio  $<1$ ), but in alcohol-related liver injury, the ratio frequently is  $>2$ , as a consequence of the deficit of pyridoxal 5'-phosphate (vitamin B6), a cofactor for ALT (Harrison 1998). The AST/ALT ratio  $>1$  has also been found in cases of liver fibrosis and cirrhosis, suggesting a correlation between AST/ALT ratio and the degree of fibrosis (Sorbi et al. 1999).

Recently, a statistically significant higher frequency of AST/ALT ratio  $>1$  was reported in a group of VCM-exposed workers compared to a control group. Results were however considered preliminary, as other possible determinants of AST/ALT ratio  $>1$ , namely BMI, blood lipid contents and hepatitis virus infection, were not included in the analysis (Di Lorenzo et al. 2003).

The results of our study do not provide evidence of an association between AST/ALT ratio  $>1$  and VCM exposure. Moreover, the AST/ALT ratio  $>1$  was not found to be more frequent in subjects with ultrasonography diagnosis of periportal fibrosis, compared to normal subjects. An increased AST/ALT ratio was found to be associated with a history of hepatitis and a high alcohol intake (RRs 1.19, 1.44, respectively), but the findings were not statistically significant. The proposal (Di Lorenzo et al. 2003) to consider AST/ALT ratio as a predictor of liver fibrotic degeneration in VCM exposed workers is hence not supported by our results.

#### The role of LFTs in the medical surveillance of VCM workers

Under conditions of low-level VCM exposures such as those currently present in most VCM/PVC plants, the detection of abnormal LFTs in the workers cannot be interpreted as a sign indicative of VCM-induced liver

toxicity, but it is rather indicative of liver injury due to non-occupational factors, namely overweight, high alcohol intake, high serum lipid levels and viral hepatitis.

Thus, in the medical surveillance of VCM workers, the execution of LFTs is recommendable not to reveal VCM-induced liver damage, but to detect other liver diseases that could suggest a precautionary avoidance of VCM exposure.

Total avoidance of exposure ought to be precautionarily recommended in cases of serious liver function damage documented by markedly abnormal of LFTs. A particular case concerns viral hepatitis, whose possible presence needs to be excluded when transaminases show markedly high or persistently elevated values.

The execution of LFTs is also of importance for medical counselling and health promotion purposes, as they can reveal the presence of congenital or acquired metabolic disorders, due to an improper diet, obesity and/or unhealthy life styles. A change in the subject's dietary habits, avoidance of alcohol consumption, weight loss and a moderate physical training can often re-normalise LFTs.

In order to detect VCM-induced liver damage either in the form of malignancies (angiosarcoma and, possibly, hepatocellular carcinoma) or periportal fibrosis, the early diagnostic value of LFTs is extremely low, as liver tumours do not precociously induce hepatocyte cytolysis or alteration of bile excretion and periportal fibrosis has only been shown to be associated with a GGT rise that however is a common and unspecific indicator. In contrast, liver ultrasonography has been elsewhere proved (Maroni et al. 2003) to enable a more accurate detection of liver lesions due to VCM exposure and should hence be used in combination with LFTs evaluation in the medical surveillance program of exposed workers.

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