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Exploring occupational toxicant exposures in patients with metabolic dysfunction-associated steatotic liver disease: A prospective pilot study

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ABSTRACT

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) has been traditionally associated with insulin resistance and obesity. Recently, pollutants have been shown to contribute to the development of MASLD. Given the global burden of MASLD, understanding whether pollutants are merely associated with steatosis or contribute to its progression to advanced chronic liver disease (ACLD) and hepatocellular carcinoma (HCC) is critical. Workers exposed to occupational toxicants represent an ideal population for assessing the potentially hazardous consequences of professional exposure. Confirming a link between occupational exposure and ACLD/HCC may not only provide further elements in understanding MASLD, but also contribute to preventive strategies for exposed workers.

Objective: This study aimed to assess the prevalence of self-reported occupational exposure to toxicants in patients with MASLD.

Methods: This hospital-based prospective pilot study included 201 patients with MASLD. Data on workplace toxicant exposure were collected systematically using a structured questionnaire. Subsequently, patients with ACLD and/or HCC ($n = 55$) were compared to controls ($n = 146$). Logistic regression analysis and propensity score models were used to investigate the associations between self-reported occupational exposure and ACLD and/or HCC.

Results: Patients with ACLD/HCC reported exposure to metals, halogenated refrigerants, pain/resins, and fuel emissions more often than the controls. After controlling for confounders, durations of 21–30 years and >30 years of occupational exposure to toxicants showed odds ratios (ORs) of 2.31 (95 % confidence interval [CI]: 1.09–4.88, $p = 0.029$) and 4.47 (95 % CI: 2.57–7.78, $p<0.001$), respectively.

Conclusions: In this pilot study, patients with MASLD complications were more likely to report workplace toxicant exposure. Our results warrant future multicentre confirmatory studies, as implementing prevention policies may reduce the risk of life-threatening diseases among exposed populations.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as nonalcoholic fatty liver disease, is the most common liver disease in both adults and adolescents, and is projected to become the predominant cause of hepatocellular carcinoma (HCC) in many countries by 2030 [1]. The diagnosis of MASLD requires evidence of hepatic steatosis (either by imaging or histology) and the presence of cardiometabolic criteria [2]. Histology offers some advantages, including the possibility of evaluating inflammatory infiltrates and degenerative changes in hepatocytes (i.e. ballooning). However, liver biopsy is associated with known risks, and its systematic use outside pharmaceutical clinical trials may pose ethical problems. Therefore, its systematic use is not supported by the current guidelines. [2] In clinical

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practice, imaging evidence of steatosis is typically obtained by using ultrasonography, which is sometimes regarded as being dependent on the operator's experience. In fact, the inter-rater variability for the diagnosis of steatosis is limited and comparable with that of biopsy data, as documented in large meta-analyses [3]. Moreover, misdiagnosis of MASLD remains extremely unlikely, as an ultrasonographic diagnosis of liver steatosis has a specificity as high as 95–99 % [4,5]. The presence of at least one of the following five cardiometabolic criteria is required to link hepatic steatosis to metabolic dysfunction and allow a diagnosis of MASLD: overweight/obesity, impaired glucose tolerance/type 2 diabetes mellitus, arterial hypertension, hypertriglyceridaemia, and low high-density lipoprotein cholesterol. [2]

As MASLD is becoming a leading global healthcare problem [6,7], increasing efforts are being directed towards understanding its pathophysiology. Lifestyle factors including sedentary lifestyle and a high-calorie diet rich in sugary beverages, processed foods, and saturated fats contribute to the development of obesity, dyslipidaemia, and insulin resistance [2]. However, other factors have also been implicated in the development and eventual progression of MASLD. Alterations in gut microbiota composition and function have been linked to impaired gut barrier function, increased gut permeability, and consequently, a higher degree of liver inflammation and fibrosis [8,9]. Genetic risk factors have also been identified. Carriers of specific polymorphisms of the patatin-like phospholipase domain containing 3 (PNPLA3) are more susceptible than non-carriers to develop HCC, whereas other genetic factors are likely involved in the development of liver fibrosis [10–12].

Although MASLD occurs in up to 25–30 % of the global population, its progression to advanced chronic liver disease (ACLD) or HCC is rare. As mentioned previously, some factors involved in generating MASLD have been suggested to contribute to its eventual progression. However, the exact mechanism leading from complicated MASLD to ACLD/HCC remains unclear.

Recently, exposure to pollutants and other toxicants has gained substantial attention as a possible MASLD pathogenic factor, both as a cause of abnormal lipid accumulation within hepatocytes and as an independent cause of oxidative stress, theoretically favouring fibrosis development [13]. In 2010, Cave et al. [14]. coined the term toxicant-associated steatohepatitis to describe steatohepatitis in human vinyl chloride (VC) workers. Liver biopsies from highly exposed workers were indistinguishable from those of obese individuals or alcoholics, although these workers were neither obese nor consumed alcohol [14].

According to the United States National Institute for Occupational Safety and Health, 33 % of the 677 most common workplace chemicals are associated with hepatotoxicity [15]. These include but are not limited to solvents and other halogenated hydrocarbons, volatile organic mixtures, pesticides, and nitroorganic compounds [16,17]. A myriad of pathologic liver lesions are associated with chemical exposure and include hepatitis, fibrosis, and cirrhosis [14]. However, steatohepatitis remains the most common pathophysiological finding and often occurs with normal serum aminotransferases levels [14]. Workplace toxicants can induce liver steatosis and steatohepatitis through various mechanisms, including lipotoxicity, mitochondrial dysfunction, autophagy, altered intestinal microbiota with decreased intestinal barrier function, oxidative stress, altered hepatic lipid metabolism, and insulin resistance [16].

Despite compelling experimental evidence, clinical investigations of the hepatotoxic effects of chemicals in population studies are still limited and have focused on the environment rather than workplace toxicants. In 2022, Guo et al. [18]. reviewed a large prospective multi-ethnic dataset (>90,000 patients) to identify a possible correlation between MASLD and exposure to four different groups of air pollutants (PM1, PM2.5, PM10, and NO2). The authors reported a positive correlation between air pollution levels and MASLD. Interestingly, the risk of MASLD was particularly increased among subgroups with other risk factors for steatosis (males, smokers, and individuals with central obesity) [18]. Despite benefitting from a large-scale human cohort allowing robust analyses and subgroup studies, this study did not explore whether air pollution increased the risk of life-threatening complications of MASLD (including ACLD and HCC). In the same year, Sen et al. [19]. characterised the liver and circulating metabolomes of 105 patients who underwent laparoscopic bariatric surgery. In this small but well-characterised human MASLD cohort, per- and polyfluoroalkyl substances (PFAS) exposure was associated with perturbation of key metabolic pathways previously found altered in MASLD, particularly bile acids, triacylglycerols, and ceramides [19]. The strengths of this study included a very well-characterized human cohort and elaborate metabolomics techniques. However, the small sample size and peculiar clinical setting in which the patients were recruited limited the generalisability of these findings to the entire MASLD population.

Interestingly, PFAS were related to the development of liver cancer for the first time in a recent proof-of-concept study that included 50 non-viral HCC cases and 50 matched controls [20]. Although inspiring and provocative in its main findings, the study had a small sample size. Consequently, the effect of known risk factors, such as age, sex, obesity, and diabetes, could not be estimated.

Both PFAS and airborne pollutants are pervasive environmental contaminants, and the expeditious implementation of preventive measures poses formidable challenges, necessitating a profound re-evaluation of the current paradigms of industrialisation. Conversely, managing and quantifying exposure to hazardous occupational substances is more feasible. However, very few studies have investigated the role of occupational toxicants in HCC, and only from an epidemiologic perspective [21]. Clinical studies exploring the potential interplay between metabolic dysfunction and workplace toxicants are limited. Consequently, it remains unknown whether the dual steatogenic mechanisms of metabolic dysfunction and workplace toxicants translate into a more aggressive MASLD phenotype.

Workers are of particular interest because they can be exposed to high concentrations of toxicants for prolonged periods. Thus, the potentially hazardous effects of workplace toxicants can be detected more easily than those of widespread environmental pollutants. Confirming (or disproving) the hypothesis that exposure to workplace toxicants increases the risk of more severe MASLD phenotypes can help understand the possible mechanisms of disease progression. More importantly, demonstrating the link between occupational exposure and the risk of liver cirrhosis and cancer would open the door to preventive strategies aimed at identifying and surveying high-risk populations to reduce the burden of these diseases. Therefore, this study aimed to verify whether exposure to workplace toxicants could contribute to a more severe MASLD phenotype by exploring whether these toxicants are hyperexpressed in patients with ACLD/HCC.

2. Methods

2.1. Ethics statements

This study was approved by the Institutional Review Board of Vasta Emilia Centro (protocol number: 141/2018/Oss/AOUBo) and conducted in accordance with the ethical guidelines of the latest Declaration of Helsinki. All patients provided written informed consent before participation.

2.2. Clinical setting

The Unit of Internal Medicine, Hepatobiliary and Immunoallergic Diseases of the IRCCS Azienda Ospedaliero-Universitaria di Bologna has long experience in managing different chronic liver diseases, including MASLD. It is also a referral centre for patients with liver cirrhosis and HCC.

2.3. Study population and design

Consecutive patients with steatotic liver disease were enrolled in this prospective pilot study between March 2018 and February 2021. The inclusion criteria were based on the previous non-alcoholic fatty liver disease definition, that is, imaging or histological evidence of hepatic steatosis and exclusion of other causes, including alcohol consumption of >20 g/day [22]. As a result of the new steatotic liver disease nomenclature in June 2023 [2], all patients were reclassified as having single-aetiology MASLD (since all of them had one or more cardiometabolic factors).

Patients were classified as having MASLD-related HCC and/or ACLD (Group A) or MASLD without advanced liver disease or cancer (Group B). The composite definition of Group A was necessary because liver cirrhosis acts as an independent risk factor for HCC, but a significant proportion of MASLD-related HCC arise in non-cirrhotic livers [23]. A structured questionnaire was administered to all the patients to investigate their occupational exposure to potentially hepatotoxic compounds. Questionnaire data were subsequently integrated with clinical, laboratory, and imaging data collected during routine clinical examinations.

2.4. Assessment of MASLD

The diagnosis of MASLD was based on ultrasonographic evidence of fatty liver in patients with at least one cardiometabolic criterion [2]. Only patients with single-aetiology MASLD were included; therefore, all patients were investigated for prior or current alcohol intake, hepatitis B virus and hepatitis C virus infections, use of drugs known to induce chronic liver damage, and other known causes of chronic liver disease (e.g. autoimmune liver disorders, glycogenosis, and other storage diseases).

Hepatic steatosis was defined according to the Hamaguchi criteria (abnormally intense, high-level echoes arising from the hepatic parenchyma, liver-kidney difference in echo amplitude, echo penetration into the deep portion of the liver, and clarity of liver blood vessel structure) [4]. Steatosis was assessed using real-time imaging, whereas the examination of previously acquired static images was not allowed. The ultrasonographic presence of steatosis was confirmed by members of the Unit of Internal Medicine, Hepatobiliary, and Immunoallergic Diseases with a minimum expertise of 10 years in ultrasound imaging who have performed at least 1000 ultrasound examinations per year.

2.5. Definition of ACLD and HCC

Patients were divided into groups according to the presence (Group A) or absence (Group B) of MASLD liver complications. ACLD was defined on the basis of a combination of clinical, laboratory, and elastography data [24,25]. HCC was diagnosed by histology or imaging in the presence of a typical hallmark (arterial phase hyperenhancement, followed by mild and late washout in the portal venous or delayed phases) [26].

2.6. Exposure assessment of occupational toxicants

The questionnaire was divided into the following sections: Section 1, general information (demographics, marital status, and education level); Section 2, lifestyle habits (smoking status and alcohol consumption); Section 3, work history (retirement status and previous and current job titles, together with task descriptions and length of employment) along with workplace-related toxicants (these were reported in a predefined list based on those proposed in the literature [27], including arsenic, colourants-paints-pigments-resins, carbon disulphide, dioxin, ethanol, explosives [picric acid and trinitrotoluene], fuels emissions, halogenated refrigerants, ketones, insecticides, laboratory reagents [chloronaphthalene and tetrabromomethane], metals [beryllium, cadmium, copper, lead, and thallium], pesticides, phosphorus, plastic industry emissions, selenium, solvents [carbon tetrachloride, dichloromethane, dichloropropane, dichloropropanol, dimethylacetamide, dimethylformamide, dioxane, nitropropane, styrene, tetrachlorethylene, trichloroethylene, trichloroethane, tetrachloromethane, trichloropropane, and trifluoro-dichloroethane], synthetic rubber production emissions, and vinyl chloride); and Section 4, residential history (together with detailed information on proximity to major traffic roads, gas station, and farms with potential exposure to pesticides), leisure time activities involving chemical exposure, and other non-occupational exposure.

The duration of occupational exposure was coded as follows: i) no exposure, ii) 1–10 years, iii) 11–20 years, iv) 21–30 years, and v) >30 years. To include potential non-occupational exposure to pesticides, residential data were coded as follows: i) no proximity to farms, ii) proximity to farms without pesticide use, iii) proximity to farms with possible pesticide use, and iv) proximity to farms with probable pesticide use. The job titles of workers reporting at least one at-risk exposure were coded according to the International Standard Classification of Occupations using a two-digit code [28]. Notably, an *ad hoc* analysis based on job titles was the purpose of this ongoing study. The job-specific plausibility of each self-reported occupational exposure to toxicants was reviewed by two trained occupational epidemiologists (SM and SC) who were blinded to the clinical data of the enrolled patients. Self-reported occupational exposure to toxicants that were considered implausible were reclassified as 'no exposure'; any implausible absence of exposure was also amended. These re-evaluated data on occupational exposure to toxicants were used for the subgroup analysis of only HCC.

2.7. Statistical analysis

Statistical analyses were aimed at i) describing the occupational exposures to known hepatotoxic agents and their duration; ii) exploring with a crude estimate whether long-term exposures were more frequently reported by patients who had developed ACLD and HCC, using a multivariable regression model; iii) confirming the previous findings after addressing any known confounders, using propensity score techniques and two different weighted regression models; and iv) establishing the strength of the previously explored aspects, considering the effects of potentially unknown confounders by means of a sensitivity analysis, performing subgroup analyses, and validating our results by recalling a proportion of patients and readministering our questionnaire to check the soundness of their memory.

Continuous variables are expressed as medians and interquartile ranges. Categorical variables are expressed as frequencies and percentages.

Preliminary group comparisons between patients with ACLD/HCC and controls were performed using Mann–Whitney's U test for continuous variables and the two-tailed Pearson's chi-square test or Fisher's exact test for categorical variables. Consequently, we modelled the crude estimates for ACLD/HCC (Model 0). A backward stepwise multivariable logistic regression model was used to investigate the association between self-reported

occupational exposure to toxicants and the odds of complicated MASLD. Only the referred exposures that occurred before the outcome (ACLD or HCC, whichever occurred first) were analysed. This initial model included the duration of occupational exposure, residential data (coded as reported in the previous paragraph), and the following clinical variables: age, sex (male/female), tobacco smoking (non-smoker/current smoker/former smoker), obesity (yes/no), diabetes (yes/no), arterial hypertension (yes/no), and alcohol consumption (non-drinkers versus low-dose drinkers). The choice of these clinical variables was based on both established risk factors for ACLD/HCC and recent literature on pollutants and liver diseases. Odds ratios (ORs) and the corresponding 95 % confidence intervals (CIs) were calculated.

Models 1 and 2 were created to balance the possible differences between complicated and uncomplicated MASLD using an inverse probability weighting (IPW) approach. Treatment weights were calculated as 1/propensity score for patients with complicated MASLD and 1/(1–propensity score) for uncomplicated MASLD. Model 1 was adjusted for sex and age. Model 2 was adjusted for tobacco smoking, low-dose alcohol consumption, diabetes mellitus, arterial hypertension, and obesity.

Additional analyses were also performed. First, sensitivity analysis was performed to evaluate the strength of an unmeasured confounder and disprove the possible relationship between exposure to workplace-related toxicants and the presence of ACLD/HCC. Sensitivity analysis was performed by calculating the E-value and 95 % CI closest to the null hypothesis, as previously described [29,30]. The E-value is the minimum strength of association on the risk ratio scale that an unmeasured confounder would need to have with both the treatment and outcome, conditional on the measured covariates, to explain a treatment–outcome association [29,30]. Second, to rule out results driven by single subgroups, we reran the fully adjusted analyses (Model 2) stratified by age, sex, low-dose alcohol consumption, smoking status, diabetes, arterial hypertension, and obesity. Third, a more conservative subgroup analysis was performed and included i) only HCC cases; ii) controls selected from Group B frequency matched to HCC cases (1:1) by age (±5 years), sex, and residence area (i.e. Bologna including suburbs, Bologna province located in the plain, Bologna province located in the Apennines, other provinces of the Emilia-Romagna region, and other Italian regions); and iii) re-evaluated exposure to workplace-related toxicants. Univariate and multivariate unconditional logistic models adjusted for frequency-matched variables were run, and ORs and 95 % CIs were estimated.

Finally, questionnaires were readministered to random patients to verify the soundness of memory and rule out a significant recall bias. The concordance between the first and subsequent answers was evaluated using Cohen's kappa, a statistical coefficient that represents the degree of accuracy and reliability of statistical classification. The agreement was classified as previously recommended; kappa values of 0.01–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.00 indicated slight, fair, substantial, and (almost) perfect agreement, respectively [31]. The minimum number of patients to retest was estimated on the basis of the proportion of patients reporting long-term exposure and the expected kappa, as previously reported [32].

All statistical tests were two-sided, with an alpha error of 0.05. Statistical analyses were performed using STATA/SE 17 software (StataCorp).

3. Results

3.1. Study population

Two hundred one consecutive patients were included in this study. Among them, 55 had liver-related complications (HCC and ACLD, $n = 37$; HCC without ACLD, $n = 12$; ACLD without HCC, $n = 6$), whereas 146 had MASLD without complications. The study groups differed in age and the prevalence of males (Table 1).

Table 1
Characteristics of the enrolled population ($n = 201$). Group A: patients with advanced chronic liver disease/hepatocellular carcinoma; Group B: remaining patients. All values given are n (%).

Variables	Group A ($n = 55$)	Group B ($n = 146$)	p-value
Age			
<50 years	4 (7.3)	37 (25.3)	<0.001
50–59 years	8 (14.5)	42 (28.8)	
60–69 years	24 (43.6)	39 (26.7)	
70–85 years	19 (34.5)	28 (19.2)	
Sex (male)	43 (78.2)	83 (56.8)	0.005
Education level			
Non-graduated	27 (49.1)	54 (37.0)	0.119
Graduated	28 (50.9)	92 (63.0)	
Smoking habits			
Non-smokers	20 (36.4)	72 (49.3)	0.217
Former smokers	27 (49.1)	54 (37.0)	
Current smokers	8 (14.5)	20 (13.7)	
Low-dose alcohol*	29 (52.7)	92 (63.0)	0.184
Obesity	19 (34.5)	65 (44.5)	0.201
Diabetes	28 (50.9)	71 (48.6)	0.773
Hypertension	34 (61.8)	77 (52.7)	0.249

* Defined as alcohol consumption <20 g/day vs no alcohol consumption.

Overall, 112 (55.7 %) patients reported at least one occupational exposure of any length to one or more toxicants (single and multiple exposures to toxicants were reported in 23 and 89 patients, respectively; single and multiple at-risk occupations were reported in 15 and 97 patients, respectively). Of these, 23 (11.4 %), 21 (10.4 %), 18 (9.0 %), and 50 (24.9 %) patients reported exposure durations of exposure 1–10, 11–20, 21–30, and >30 years, respectively.

Regarding workers who reported workplace-related exposure to toxicants, the most frequent occupational categories were as follows: '72-Metal, Machinery and Related Trades Workers' ($n = 33$, 16.4 %), '81-Stationary-plant and Machine Operators' ($n = 24$, 11.9 %), and '71-Extraction and Building Trades Workers' ($n = 13$, 6.5 %).

The most frequent self-reported occupational exposures to toxicants were solvents ($n = 48$, 23.9 %), metals ($n = 45$, 22.4 %), and colourants-pigments-pains-resins ($n = 35$, 17.4 %). Exposure to metals, halogenated refrigerants, colourants-pigments-paints-resins, fuel emissions, and pesticides was more frequently reported by patients in Group A than those in Group B (Table 2).

Additionally, 26 (12.9 %) and 17 (8.5 %) patients in Groups A and B, respectively, reported proximity to farms with probable or possible exposure to pesticides.

3.2. Crude estimates for ACLD/HCC (Model 0)

Patients in Group A were at higher risk than those in Group B for the most known demographic risk factors, including male sex (OR=4.08, 95 % CI: 1.68–9.87) and age >70 years (OR=5.94, 95 % CI: 1.42–24.81). Self-reported exposure to workplace-related toxicants of >30 years (OR=4.26, 95 % CI: 1.73–10.51) and proximity to farms with probable use of pesticides (OR=3.65, 95 % CI: 1.31–10.18) were also independently associated with HCC and/or ACLD in the multivariable model (Table 3).

3.3. Adjusted estimates for ACLD/HCC (Models 1 and 2)

Models 1 (partially adjusted) and 2 (fully adjusted) were constructed using the IPW method. After applying the propensity score, the standardised difference between the patients with com-

Table 2
Distribution of self-reported workplace-related toxicants. Group A: patients with advanced chronic liver disease/hepatocellular carcinoma; Group B: remaining patients.

Toxicants	Group A (n = 55)	Group B (N = 146)	Total (N = 201)	P
Arsenic	0	2 (1.4)	2 (1.0)	1.000
Colorants, pigments, paints, resins	15 (27.3)	20 (13.7)	35 (17.4)	0.024
Dioxin	1 (1.8)	2 (1.4)	3 (1.5)	1.000
Ethanol	11 (20.0)	22 (15.1)	33 (16.4)	0.400
Explosives	5 (9.1)	4 (2.7)	9 (4.5)	0.065
Halogenated refrigerants	7 (12.7)	1 (0.7)	8 (4.0)	<0.001
Fuels emissions	11 (20.0)	13 (8.9)	24 (11.9)	0.031
Insecticides	9 (16.4)	10 (6.8)	19 (9.5)	0.040
Ketones	5 (9.1)	6 (4.1)	11 (5.5)	0.176
Laboratory reagents	4 (7.3)	5 (3.4)	9 (4.5)	0.261
Metals	24 (43.6)	21 (14.4)	45 (22.4)	<0.001
Pesticides	7 (12.7)	6 (4.1)	13 (6.5)	0.027
Phosphorus	2 (3.6)	0	2 (1.0)	0.074
Plastics	9 (16.4)	15 (10.3)	24 (11.9)	0.235
Selenium	2 (3.6)	1 (0.7)	3 (1.5)	0.182
Solvents	18 (32.7)	30 (20.5)	48 (23.9)	0.071
Synthetic rubbers production	5 (9.1)	10 (6.8)	15 (7.5)	0.590
Vinyl chloride	2 (3.6)	1 (0.7)	3 (1.5)	0.182
Carbon disulphide	4 (7.3)	1 (0.7)	5 (2.5)	0.021

Table 3
Risk factors for advanced chronic liver disease and/or hepatocellular carcinoma.

MODELS	MODEL 0				MODEL 1*				MODEL 2*			
	CRUDE ESTIMATES				SEX AND AGE ADJUSTMENT				FULL ADJUSTMENT			
VARIABLES	OR	95 % CI		P	OR	95 % CI		P	OR	95 % CI		P
Age												
<50 years	Reference				Reference				Reference			
50–59 years	**2.09**	0.48	9.03	0.324	**1.31**	0.63	2.74	0.465	**1.40**	0.65	3.04	0.389
60–69 years	**4.95**	1.29	19.00	0.020	**0.80**	0.40	1.63	0.542	**0.83**	0.40	1.71	0.616
70–85 years	**5.94**	1.42	24.81	0.015	**0.93**	0.44	1.99	0.856	**0.93**	0.43	2.03	0.856
Sex (male)	**4.08**	1.68	9.87	0.002	**0.93**	0.55	1.57	0.786	**0.73**	0.45	1.17	0.191
Smoking habits												
Non-smokers	Reference				**Reference**				Reference			
Former smokers	**1.21**	0.53	2.75	0.656	**1.93**	1.15	3.24	0.013	**1.61**	0.97	2.67	0.068
Current smokers	**2.22**	0.63	7.86	0.217	**3.15**	1.49	6.63	0.003	**1.83**	0.90	3.74	0.097
Low-dose alcohol consumption**	**0.45**	0.20	1.03	0.057	**0.57**	0.35	0.92	0.022	**1.02**	0.61	1.71	0.934
Obesity	**0.82**	0.37	1.80	0.614	**0.97**	0.59	1.60	0.913	**0.85**	0.81	1.54	0.851
Diabetes	**0.92**	0.41	2.07	0.838	**1.11**	0.67	1.84	0.688	**1.51**	0.95	2.41	0.081
Hypertension	**1.12**	0.50	2.50	0.792	**1.59**	0.99	2.56	0.057	**1.30**	0.80	2.11	0.292
Self-reported exposure to workplace-related toxicants (duration)												
None	Reference				Reference				Reference			
1–10 years	**1.25**	0.32	4.87	0.745	**1.35**	0.63	2.89	0.435	**0.98**	0.44	2.19	0.954
11–20 years	**1.07**	0.27	4.22	0.927	**0.84**	0.35	1.99	0.691	**0.80**	0.34	1.87	0.608
21–30 years	**2.11**	0.56	7.90	0.270	**2.89**	1.30	6.44	0.009	**2.31**	1.09	4.88	0.029
>30 years	**4.26**	1.73	10.51	0.002	**5.38**	3.06	9.44	<0.001	**4.47**	2.57	7.78	<0.001
Residential exposure to pesticides												
No proximity to farms	Reference				Reference				Reference			
Proximity to farms without pesticides use	**0.86**	0.18	4.15	0.849	**0.55**	0.20	1.53	0.251	**0.67**	0.25	1.78	0.420
Proximity to farms with possible pesticides use	**2.17**	0.64	7.30	0.212	**1.97**	0.91	4.24	0.084	**2.26**	1.10	4.63	0.027
Proximity to farms with probable pesticides use	**3.65**	1.31	10.18	0.014	**4.99**	2.44	10.21	<0.001	**4.66**	2.36	9.19	<0.001

* Model 1 and 2 used an inverse probability weighting (IPW) approach.
** Defined as alcohol consumption <20 g/day vs no alcohol consumption.

plicated and uncomplicated MASLD was minor (Supplementary Table).

Both models suggested that occupational exposure to toxicants of >20 years (self-reported) and residential proximity to farms with probable/possible exposure to pesticides might be risk factors for patients in Group A compared with those in Group B (Table 3). In particular, the fully adjusted multivariable logistic regression model showed that patients with 21–30-year (OR=2.31, 95 % CI: 1.09–4.88) and >30-year self-reported occupational exposure to toxicants (OR=4.47, 95 % CI: 2.57–7.78) were at a higher risk for ACLD/HCC than others in Group A. Similar results were found for residential proximity to farms with possible (OR=2.26, 95 % CI: 1.10–4.63) or likely use of pesticides (OR=4.66, 95 % CI: 2.36–9.19).

3.4. Additional analyses: sensitivity analysis for Model 2

Sensitivity analyses showed that after adjusting for all known confounders (as shown in Model 2), only relevant unmeasured confounders explained the previous findings. The E-values for the

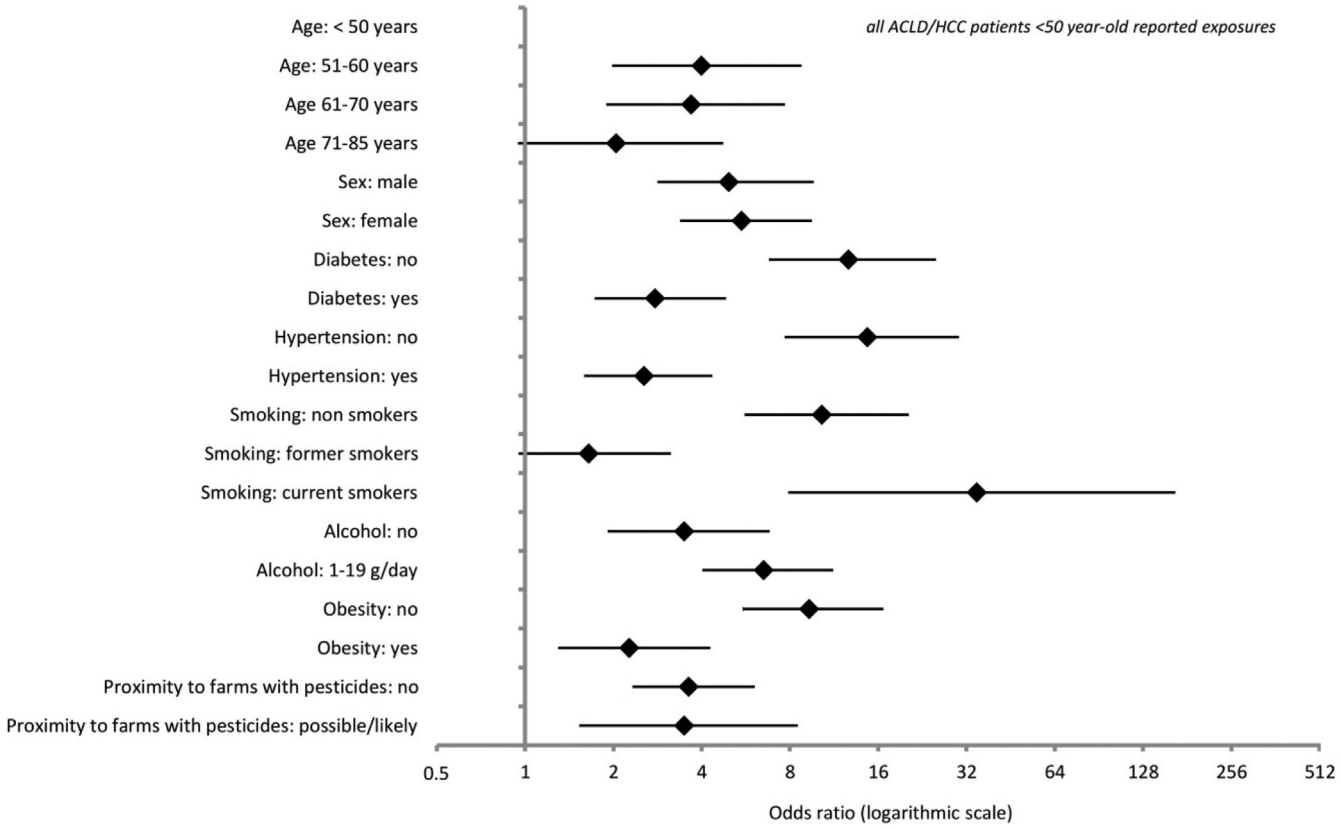

Fig. 1. Odds ratios for advanced chronic liver disease and/or hepatocellular carcinoma in patients reporting exposure to workplace toxicants for >20 years.

length of exposure were 4.04 (95 % CI: closest to the null 1.40) for 21–30-year exposure and 8.42 (95 % CI: closest to the null 4.59) for >30-year occupational exposure. Similar results were found for proximity to farms with possible (E-value=3.94, 95 % CI closest to null 1.43) or likely use of pesticides (E-value=8.78, 95 % CI: closest to null 4.19).

3.5. Additional analyses: re-run of Model 2 with stratification for covariates

On the basis of the results of Model 2, we adopted a threshold of >20 years to define long-term exposure in the stratified analysis. The increased odds of patients with ACLD/HCC having a self-reported history of long-term exposure to workplace-related toxicants in Group A compared with those in Group B were consistently confirmed across all study subgroups (Fig. 1).

3.6. Additional analyses: conservative subgroup analysis

The conservative subgroup analysis restricted to HCC cases using re-evaluated exposure to workplace-related toxicants provided similar results to those reported in the main analysis: re-evaluated occupational exposure to toxicants for >20 years was associated with HCC (OR=3.92, 95 % CI: 1.21–12.26) (Table 4).

3.7. Recall

Considering the prevalence of patients reporting *a* >20-year exposure (35 %) and estimating a kappa value of 0.80, re-testing of 39 patients yielded a precision of ±0.20 with a 95 % confidence level. Thus, 39 patients were randomly selected and readministered the exposure assessment questionnaire. The main clinical and demographic characteristics of these patients did not differ from those of the non-selected patients. There was an almost perfect concordance when patients were retested for the duration of exposure (kappa=0.91, 95 % CI: 0.87–0.95) and for exposure to single toxicants (kappa values ranging from 0.89 to 1.00, Supplementary Tables). The agreement was near perfect also for residential exposure to pesticides (kappa=0.83, 95 % CI: 0.77–0.90).

4. Discussion

This study aimed to verify whether professional exposure to workplace toxicants is associated with a more aggressive MASLD phenotype by verifying whether these exposures are more frequently reported in patients with ACLD/HCC. Our results support the hypothesis of harmful effects of long-term exposure to workplace toxicants in patients with MASLD for multiple reasons.

First, multivariate models adjusted for known confounders supported the association between long-term self-reported occupational exposure to toxicants and ACLD/HCC. Additionally, the sensitivity analysis suggested that very strong unknown confounders are needed to disprove this association, which is, therefore, less likely to be explained or biased. Additionally, subgroup analysis of HCC cases frequency matched by age, sex, and area of residence to controls showed an association between HCC and re-evaluated exposure to workplace-related toxicants for >20 years. Second, even if the toxicant doses could not be assessed, the odds of developing ACLD/HCC increased in parallel with the length of exposure. Third, the increased risk was consistent across the main study subgroups. Furthermore, confounding due to socioeconomic factors was unlikely considering that the association between ACLD/HCC and self-reported occupational exposure was still present in the stratified analyses among patients with low levels of education or blue-collar workers (data not shown). Fourth, our study only considered

Table 4
Risk factors for hepatocellular carcinoma (conservative subgroup analysis).

	Group A HCC cases (n = 46)	Group B Controls* (n = 46)	Univariate** OR (95 %CI)	Multivariable** OR (95 %CI)
Smoking habits				
Non smokers	15	20	Reference	Reference
Former smokers	26	22	1.64 (0.66–4.10)	1.50 (0.54–4.19)
Current smokers	5	4	1.66 (0.34–8.06)	1.79 (0.26–12.47)
Alcohol consumption				
None	23	15	Reference	Reference
Low-dose	23	31	0.51 (0.20–1.32)	0.68 (0.22–2.09)
Body Mass Index				
Normal/Overweight	31	31	Reference	Reference
Obesity	15	15	0.98 (0.40–2.43)	1.01 (0.36–2.90)
Diabetes mellitus				
No	21	19	Reference	Reference
Yes	25	27	0.76 (0.32–1.79)	0.79 (0.28–2.21)
Arterial Hypertension				
No	16	20	Reference	Reference
Yes	30	26	1.40 (0.57–3.45)	1.90 (0.62–5.82)
Re-evaluated exposure to workplace-related toxicants (duration)				
None	14	29	Reference	Reference
1–20 years	11	7	3.72 (1.07–12.89)	3.38 (0.87–13.14)
>20 years	21	10	5.18 (1.77–15.14)	3.92 (1.21–12.66)
Residential exposure to pesticides				
No proximity to farms or Proximity to farms without pesticides use	26	39	Reference	Reference
Proximity to farms with possible or probable pesticides use	20	7	4.21 (1.51–11.72)	2.89 (0.92–9.07)

* Frequency matched to HCC cases by age (±5 years), gender, and residence area.
** (Multivariable) unconditional logistic model adjusted for frequency matching variables.
OR: Odds Ratio; CI:Confidence Interval.

exposures preceding the outcome, thus satisfying the temporality criterion for establishing associations.

These considerations add to the coherence with biological plausibility and existing literature, as pollutants have been shown to contribute to the abnormal accumulation of lipids within hepatocytes and oxidative stress, potentially favouring liver fibrosis [19]. Additionally, fibrosis and cirrhosis have been linked to workplace toxicants in pre-clinical studies [16]. Therefore it has been hypothesised that the additional disruption of glucose and lipid homeostasis mediated by toxicants can exacerbate the effect of high dietary fat [33] and explain the increased risk of the most severe complications of MASLD.

A direct comparison with other recent studies is not feasible, as our study is the first of its kind. Nevertheless, our results fit well with a very recent report by Goodrich et al. [20]. This nested case-control study provided a proof of concept showing that high levels of plasma PFAS metabolites are associated with an increased risk of nonviral HCC. In contrast to findings of Goodrich et al.'s study [20], our results did not rely on metabolomics but used questionnaire-collected data. Although this strategy did not allow laboratory analyses, it provided an easy instrument that could be used in everyday clinical practice. Despite the intrinsic differences in the study design, both our and Goodrich et al.'s results converge in the conclusion that toxicants may promote liver carcinogenesis in patients with nonviral liver disease. However, our study provided this evidence in the specific context of MASLD (whereas the previous study included a heterogeneous cohort of patients with alcoholic and cryptogenic liver diseases) and extended it to workplace toxicants.

Our study was limited by its single-centre hospital-based recruitment. The high prevalence of complicated MASLD reflects the typical cohort of a second-level hepatology clinic and may not represent the whole MASLD population with MASLD. However, hospital-based studies are often pivotal in detecting signals in selected groups of patients (in our case, the ACLD/HCC cohort) and should prompt future confirmatory studies. Additionally, the small sample size could be seen as another potential limitation, as it did not allow further analysis to dissect the role of single toxicants in a population often reporting multiple at-risk exposures. Nevertheless, the strengths of this study must be discussed. For instance, we featured a well-characterised human cohort with comprehensive medical data and detailed work histories collected through a structured questionnaire. These aspects allowed analyses that would have been difficult to perform in registry-based studies, in which the diagnosis of MASLD often relies on the International Classification of Diseases code for 'hepatic steatosis', which includes both alcohol-related and MASLD.

In conclusion, this is the first study to investigate the role of exposure to workplace toxicants in the development of ACLD/HCC in a population with MASLD. Patients with the most severe complications of MASLD (including ACLD and HCC) were more likely to be exposed to workplace toxicants for >20 years, independent of age, sex, and other potential confounders. Exposure to metals, halogenated refrigerants, colourants-pigments-paints-resins, and fuel emissions were reported more frequently in patients with ACLD/HCC than in those without. Our results warrant future multicentre collaborative studies to confirm our findings and to dissect the role of single toxicants. There is a potential for preventing severe complications from cirrhosis and cancer in exposed populations. Increased awareness of risks, adoption of personal protective equipment, periodic medical examinations in exposed populations, and implementation of other prevention policies can decrease the risk of severe liver disease.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2023.12.007.

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