**INTRODUCTION**

Recently, a multicentric Italian case–control study (Corrao et al., 1998) demonstrated that, despite the high prevalence of viral infection in our country (De Bac et al., 1994), alcohol is the risk factor with the highest impact on symptomatic liver cirrhosis. However, in both clinical practice and research, major attention is given to viral infection but not to alcohol consumption in subjects with chronic liver disease (CLD). Only 4–5% of all manuscripts submitted to Hepatology, a leading journal on the liver, deal with alcohol-related liver cirrhosis. Although, reviewed in report, the major cause of liver disease in the USA (Hartford, 1992; Bach, 1996). Others reported a low prevalence of alcoholics in a population of outpatients with CLD (Byron and Minur, 1996). This is probably due to the lack of attention that practitioners and researchers have given to alcohol use, especially in subjects positive for markers of viral infection. It might also be due to the difficulty in gathering valid information on alcohol intake (Orrego et al., 1979) and underestimating the role of ethanol in social and occasional drinkers. It is important to assess alcohol use in these individuals, because ethanol, by interacting with hepatitis B and C viruses, induces liver cirrhosis and hepatocellular carcinoma (HCC) and affects the response to interferon (IFN) therapy (Pares et al., 1990; Nalpas et al., 1991; Okazaki et al., 1994; Ohnishi et al., 1996; Ikeda et al., 1998; Ostopowicz et al., 1998).

Mabee et al. (1998) stressed that not one controlled trial on the efficacy of IFN treatment of patients with hepatitis C virus (HCV)-related chronic hepatitis determined alcohol intake prior to therapy. Alcohol intake has been shown to correlate directly with gamma-glutamyl transpeptidase (γGT) levels (Lieberman et al., 1995), which also predicted the response to treatment (Camps et al., 1993). Recently, a retrospective analysis of a large cohort of drug users with HIV and chronic hepatitis C infections demonstrated that alcohol influences their γGT plasma levels (Pol et al., 1998).

The pattern of HCV-related chronic hepatitis is characterized, at a histological level, by a variable degree of steatosis and fibrosis (Scheuer et al., 1991). These alterations are considered typical of ethanol-induced damage (International Group, 1981). In addition, Pessione et al. (1998), during the evaluation of our data, documented that alcohol also affects liver fibrosis in chronic hepatitis C patients.

In Italy, HCV infection is common throughout the country and there is a high alcohol consumption pro capite, even allowing for the fact that drinking has decreased in the last 10 years (Stud ‘Progetto Europa’, 1994). In this study, we intended to: (1) estimate how many subjects in our country misused alcohol before and after being diagnosed as having HCV-related CLD; (2) determine if their drinking habits affected the principal aspects of this disease: routine laboratory data (particularly γGT plasma levels), histological pattern (particularly liver steatosis and fibrosis), HCV RNA levels, and response to IFN therapy; (3) compare results from this and a previous study of ours (Aricò et al., 1994) to determine if CLD subjects have modified their drinking habits since a decrease was observed in the general population.

**METHODS AND SUBJECTS**

Two hundred and forty-five inpatients with HCV-related CLD were enrolled consecutively. All subjects were HBsAg-negative and human immunodeficiency virus (HIV)-negative and had not taken any medication/drugs for at least 1 month prior to enrolment. Other causes of chronic liver disease, such...
as genetic or autoimmune conditions, were excluded. Diagnosis was made by liver biopsy, which had been performed during the 3 years preceding the study. The majority of our subjects were sporadic cases, only 13% having had a blood transfusion in the past, and none was an intravenous drug user. Two pathologists (M.P.D.M. and F.B.), who were unaware of clinical and virological data, evaluated the histology according to the Ishak et al. (1995) score. This score was chosen because it describes the type and extent of liver fibrosis more clearly by staging the architectural changes from 0 to 6. We also arbitrarily graded liver steatosis on a scale from 0 to 3: 0 = absent; 1 = mild (<20%); 2 = moderate (21–50%); 3 = severe (>50%). We found an inter-observer concordance in about 90% of cases and, if there was any disagreement, we obtained a further evaluation of the histological samples.

One hundred and thirty-eight subjects had chronic hepatitis without cirrhosis (79 men and 59 women; median age 49 years; range 21–74) and 107 presented with liver cirrhosis (52 men and 55 women; median age 61 years; range 36–78), all without HCC.

All patients, once the diagnosis of their liver disease was made, were advised to abstain completely from alcohol use. We retrospectively evaluated alcohol intake between 1 and 3 years after the diagnosis, by using standardized questionnaires (Norton et al., 1987; Corrao and Aricò, 1998) in the presence of their relatives. We calculated the average amount of ethanol (g/day for the 5 years preceding diagnosis and at enrolment). In particular, our participants were asked to specify what type of alcoholic beverage they usually consumed, the number and frequency of drinks (daily, weekly or occasionally), when they had begun and/or had stopped consuming alcohol. The relatives were asked to correct or confirm the information that we had gathered. The reliability of the interview technique was evaluated in a sample of 30 patients and indicated a good degree of concordance between our subjects and their relatives. The correlation coefficient between the information obtained from the interview of the patients and from the interview of their respective relatives was, according to others (Corrao and Aricò, 1998), 0.85 for the daily alcohol intake and 0.89 for the duration of alcohol consumption. We subdivided the patients into: abstainers, those who used to drink, and those who still drank. Ethanol consumption was divided according to daily intake: 0, ≤40, 41–80 or >80 g independently from the type of alcoholic beverage specified. The total alcohol intake was determined for the group and subdivided by sex and age; age was divided into three groups: <30, 31–50, >50 years.

Routine liver function tests (aminotransferases, alkaline phosphatase, bilirubin, prothrombin activity, plasma protein electrophoresis, etc.) were performed in the morning, after an overnight fast, on the second day after admission.

Sixty-five patients who had received interferon treatment (αIFN; 6 MU on alternate days), were observed continuously for 36 months. Forty-two of them were men and 23 women (aged between 21 and 62 years; median age 46 years) all having chronic hepatitis and no cirrhosis. We determined, both under basal conditions and during therapy, HCV RNA levels using an improved branched DNA (bDNA) signal amplification assay (Quantiplex 2.0; Chiron Corp.; Emeryville, CA, USA). This assay has a detection limit of 0.2 × 10^6 genome equivalents per milliliter (Eq/ml) and is virtually unaffected by the genotype variability of the virus (Detmer et al., 1996). The IFN schedule and response were defined according to serum aminotransferase levels and viral clearance (Hoofnagle and Di Bisceglie, 1997). Sustained responders were those with persistently normal aminotransferase levels (≤40 U/l) and HCV RNA-negative throughout the follow-up period. Relapers were patients who exhibited an increase of aminotransferase levels when IFN treatment was stopped. Non-responders or relapers were considered as a single group, because they did not differ for any of the aspects studied.

Statistical analysis

The differences between groups and correlation have been analysed by non-parametric statistical methods. We utilized the Wilcoxon rank test to evaluate the differences between groups and the Mann–Whitney procedure of testing by ranks for the correlation analysis. Alcohol consumption and age were considered as a continuous variable. We determined the correlations between the amount of ethanol intake (g/day) or the age (years) with the absolute values of γGT, histological score and HCV RNA plasma levels. In addition, we also performed the statistical analysis by arbitrarily dividing patients into various ethanol intake and age classes. Differences of \( P < 0.05 \) were considered significant.

RESULTS

Alcohol intake

The continuous use of ethanol was documented in 94/138 patients with chronic hepatitis (68%; 58 men and 36 women) and 70/107 cirrhotics (65.4%; 41 men and 29 women). After diagnosis, 34/94 chronic hepatitis (36.1%; 20 men and 14 women) and 45/70 cirrhotics (64.2%; 27 men and 18 women) stopped drinking. The percentage of patients consuming alcohol in the different categories is shown in Fig. 1 and median doses of ethanol before and after diagnosis of liver disease in Table 1. It is evident that most patients with viral liver disease drank regularly, with about 20% consuming >80 g/daily. The diagnosis of liver disease did not significantly influence their daily consumption.

Seventy-six per cent of men and 61% of women habitually drank alcoholic beverages, without significant differences between the chronic hepatitis and cirrhotic groups. Furthermore, men drank more than women: 63% had a daily ethanol intake of >40 g, while the majority of the women (84%) consumed ≤40 g/daily. The percentage of alcohol users increased with age in both sexes (≤30 years: 2.8%; 31–50 years: 34.3%; >50 years: 62.4%). The median daily intake among the classes was similar.

We compared the results of this study with those of a previous study reported in Aricò et al. (1994), performed on 63 subjects (22.0% with alcoholic, 15.8% with HBsAg- and 62.2% with HCV-related liver cirrhosis). About 86% of those with viral chronic liver disease drank regularly, of whom 52% drank >80 g/daily. Only 14% of alcoholic and 17% of viral cirrhotics stopped drinking after diagnosis.

γGT plasma levels

γGT plasma values were above the normal range (≥50 U/l) in 39/138 (28.2 %) chronic hepatitis (median value 70 U/l; range
52–186) and in 29/107 (27.1%) cirrhotics (median value 72.5 U/l; range 51–247) (Table 2). The GT increase was not significantly affected by ethanol intake in subjects when considered as a whole, nor in those with chronic hepatitis; however, cirrhotics having an ethanol intake of >40 g/day had a greater increase of this enzyme ($P < 0.01$ vs others; see Table 2).

Age had no effect on γGT plasma levels, but a statistically significant difference was observed between sexes (Table 3). The γGT level in males progressively increased as their daily intake of alcohol increased, and was independent of the degree of liver damage (Table 3).

No statistically significant differences were observed between either liver damage, liver function tests and ethanol consumption.

**Histological data**

We evaluated the global histological index (grading and staging) separately at first, then the activity index, the entity of liver steatosis and fibrosis according to alcohol intake. Ethanol intake had not influenced liver histology when we considered the global score, the grading, or the presence and the entity of liver steatosis. Fibrosis was apparently unaffected by ethanol ingestion in subjects globally considered, but its entity was significantly different in women than in men. In fact, fibrosis significantly increased in men according to their alcohol intake ($P < 0.01$ between <40 and >40 g of ethanol), whereas fibrosis was greater in women ($P < 0.001$), even at low ethanol levels, and was not modified by the increase in ethanol consumption (Table 4).

No statistically significant correlation was observed between the degrees of steatosis, fibrosis, γGT or HCV RNA plasma levels, and age. Only the degree of fibrosis was slightly significantly related to age in all patients ($r = 0.38; P < 0.05$).

### Table 1. Percentage of patients drinking before and after diagnosis of liver disease and median actual daily ethanol intake (g)

<table>
<thead>
<tr>
<th>Daily alcohol intake (g)</th>
<th>Hepatitis Before (n = 94)</th>
<th></th>
<th>Hepatitis After (n = 60)</th>
<th></th>
<th>Cirrhosis Before (n = 70)</th>
<th></th>
<th>Cirrhosis After (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>median</td>
<td>%</td>
<td>median</td>
<td>%</td>
<td>median</td>
<td>%</td>
</tr>
<tr>
<td>≤40</td>
<td>59.5</td>
<td>28.5</td>
<td>70.1</td>
<td>15</td>
<td>47.1</td>
<td>26</td>
<td>50.0</td>
</tr>
<tr>
<td>(7–40)</td>
<td>(10–30)</td>
<td>(9–130)</td>
<td>(10–40)</td>
<td>(7–130)</td>
<td>(10–40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41–80</td>
<td>22.3</td>
<td>52.0</td>
<td>17.8</td>
<td>57</td>
<td>27.1</td>
<td>57</td>
<td>23.0</td>
</tr>
<tr>
<td>&gt;80</td>
<td>18.0</td>
<td>98.0</td>
<td>12.5</td>
<td>98</td>
<td>25.7</td>
<td>98</td>
<td>23.0</td>
</tr>
<tr>
<td>(95–150)*</td>
<td>(95–100)*</td>
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</tr>
</tbody>
</table>

No differences were observed between hepatitis and cirrhosis; *$P < 0.01$ vs others.

### Table 2. Relationship between ethanol consumption and plasma gamma-glutamyl transferase (γGT) levels in subjects with γGT above normal levels (>50 U/l)

<table>
<thead>
<tr>
<th>Ethanol use (g/day)</th>
<th>Chronic hepatitis (n = 39/138)</th>
<th>Cirrhosis (n = 29/107)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstainers</td>
<td>69.0 (53–170)</td>
<td>72.5 (53–176)</td>
</tr>
<tr>
<td>≤40</td>
<td>67.5 (52–186)</td>
<td>64.0 (56–141)</td>
</tr>
<tr>
<td>41–80</td>
<td>79.5 (62–148)</td>
<td>95.5* (51–247)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>76.5 (54–167)</td>
<td>118.0</td>
</tr>
</tbody>
</table>

*$P < 0.01$ vs all hepatitis values and vs abstainers and cirrhotics with <40 g.
Seventeen of the 65 (26.1%) subjects with chronic hepatitis showed a sustained response to IFN therapy. The remaining 48 (73.8%) did not respond (30) or relapsed (18). The percentage of subjects who responded while drinking ethanol can be seen in Fig. 2. The number of subjects showing a progressively decreased ethanol intake increased ($P < 0.01$ between abstainers and drinkers at all doses considered). In the non-responder group, the number of subjects abstaining was significantly different from those drinking (10.7% vs 63.1%, respectively; $P < 0.001$) when considered as a whole and for each group of drinkers.

Responders and non-responders had a similar histological pattern and plasma γGT levels at baseline, whereas plasma HCV RNA significantly differed between these two groups (0.6 ± 0.52 Eq/ml ×10⁶ in responders; 7.3 ± 6.0 in non-responders; $P < 0.01$). HCV RNA was also different in relation to alcohol use (abstainers 0.6 ± 0.3 Eq/ml ×10⁶, drinkers 6.9 ± 5.9; $P < 0.01$). The distribution of HCV RNA according to alcohol intake (g/day) and IFN response is shown in Fig. 3. Responders with an ethanol intake of >80 g/daily had significantly higher HCV RNA levels than the other ethanol classes ($P < 0.01$). Non-responders’ HCV RNA levels, in all classes of drinkers, differed quite significantly from abstainers, and in the >80 g/daily class were significantly higher than all other groups ($P < 0.001$).

**DISCUSSION**

It has been shown in another recent European population (Pessione et al., 1998) that ethanol is still regularly consumed by the majority of subjects with HCV-related chronic liver disease. Patients with hepatitis were more likely to drink regularly than cirrhotics. This indicates that insufficient attention is given to alcohol use, even though it has been documented that the consumption of ethanol favours the progression of liver damage and its complications (Pares et al., 1990; Nalpas et al., 1991; Ikeda et al., 1998; Ostapowicz et al., 1998; Pol et al., 1998). By comparing these data with those of a past study, we found fewer subjects who drank, both before and after diagnosis of liver disease, and a decrease in daily intake of ethanol. This may be because patients are better informed about alcohol toxicity in this situation. However, the observation that the percentage of drinkers increases with age, in our subjects as in the general population, suggests that the decrease in alcohol consumption is simply the expression of a reduced level of consumption in the general population during the last few years (Studio ‘Progetto Europa’, 1994).
Even if no significant correlation between γGT plasma levels and alcohol was found when the group was considered as a whole, in cirrhotics and in males, γGT levels increased with the increase of ethanol intake. This indicates that HCV infection per se will elevate plasma γGT, but gender and alcohol may also be contributory factors. This is in keeping with the findings of others (Pol et al., 1998) who showed a significant correlation between γGT and alcohol only in excessive drinkers.

The γGT enzyme regulates the transport of glutathione into the cells and is therefore an important defence against free-radical-mediated damage (Lieberman et al., 1995). Liver fibrosis is also an expression of this type of damage (Brent and Rumak, 1993) and both these findings may be influenced by ethanol misuse (Tate and Meister, 1981; Nordmann et al., 1992). In patients with HCV-related chronic hepatitis, Pessione et al. (1998) reported a significant correlation between ethanol consumption and liver fibrosis, not confirmed by others (Ostapowicz et al., 1998). Our results documented a significant increase of γGT and fibrosis at higher daily doses of ethanol only in male subjects. In fact, women had a significantly higher fibrosis score than men, even if abstainers or with <40 g/daily of ethanol, but their γGT levels did progressively increase as their intake increased. This suggests that women may have less defence against an oxidative insult and consequently a more marked fibrosis. This is in keeping with the fact that women are more susceptible to alcohol-induced liver damage (Watson, 1991).

Generally, people who have been diagnosed as having liver disease and undergo IFN therapy are advised to abstain from alcohol, and also our patients, at the moment of the diagnosis, were advised to totally abstain from alcohol use. However, our data show that a considerable number of these subjects continued drinking, probably because they thought that the dose they were consuming was not dangerous. In these patients, a direct relationship between alcohol use and response to IFN therapy was found. In fact, the number of sustained responders decreased as ethanol consumption increased. IFN therapy is less effective in habitual drinkers than in infrequent drinkers (Okazaki et al., 1994) and the adverse effects of drinking on the efficacy of IFN might be reversed by abstinence for long periods prior to treatment (Ohnishi et al., 1996). Alcohol intake also increases HCV RNA plasma levels and reduces the rate of HCV RNA clearance during IFN therapy (Oshita et al., 1994; Cromie et al., 1996; Pessione et al., 1998). Alcohol might promote HCV replication (Okazaki et al., 1994), reduce host immune response contributing to the lysis of virus-infected hepatocytes (Balart et al., 1993) or induce mutational alterations by selecting quasi-species (Ohnishi et al., 1996). Even if the significance of HCV RNA as a factor capable of regulating IFN response is controversial (Saracco and Rizzetto, 1996), we found that IFN response was influenced both by ethanol intake and by HCV RNA levels, with significant differences between abstainers and drinkers, between >80 g ethanol daily and other groups, and in responders and non-responders.

In conclusion, our data indicate that more attention must be given to alcohol consumption. An educational programme on alcohol use in chronic liver disease should be provided, even if induced by viruses, according to recommendations given by recent consensus conferences in France and the USA (Conference de Consensus, 1997; NIH Consensus Conference, 1997). Furthermore, our results suggest the need for further evaluation of the interaction between HCV and other cofactors, particularly ethanol, in the pathogenesis and progression of liver damage, and in response to therapy.

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REFERENCES


