Clinical course and risk factors of hepatitis C virus related liver disease in the general population: report from the Dionysos study

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Abstract

Background—The severity, clinical course, and risk of hepatitis C virus (HCV) related chronic liver disease are still rather poorly defined.

Aims—To investigate the prevalence, risk factors, and severity of HCV related liver disease in the general population, and investigate whether infection with a specific genotype is associated with an increased risk of cirrhosis or hepatocellular carcinoma.

Methods—HCV RNA determination by polymerase chain reaction (PCR) and HCV genotyping were performed in all anti-HCV positive subjects belonging to the Dionysos study (6917 subjects). Diagnosis of cirrhosis and hepatocellular carcinoma was established by liver biopsy in all cases. All the data were analysed by univariate and multivariate statistics in all the cohort. To investigate the natural history of HCV infection, anti-HCV positive subjects were followed up every six months for three years with liver function tests and ultrasonograms.

Results—The overall prevalence of HCV RNA positivity was 2.3%. Positivity increased progressively with age, and was higher in women (ratio of men to women = 0.7). Genotypes 1b and 2a were the most frequent (42 and 24% of HCV RNA positive patients), with a prevalence of 1 and 0.6% respectively. Intravenous drug use, blood transfusions received before 1990, history of previous hepatitis among the cohabiting, and history of animal (mainly dogs) bites were significantly (p<0.05) associated with HCV infection, independently of age and sex. Multivariate analysis showed that, independently of age, sex, and alcohol intake, genotype 1b infection, with or without coinfection with other genotypes, is the major risk factor associated with the presence of cirrhosis and/or hepatocellular carcinoma. During the three years of follow up, 57 (35%) of the HCV RNA positive subjects had consistently normal alanine aminotransferase and γ-glutamyltransferase values. Two of the 22 HCV RNA positive cirrhotic patients, all drinking more than 90 g of alcohol a day, developed hepatocellular carcinoma (incidence rate = 3.0% per year).

Conclusions—In the general population of Northern Italy, HCV infection is widespread, but only less than 50% of the anti-HCV positive subjects, particularly those infected with genotype 1b, are associated with a more severe liver disease. Alcohol consumption greater that 30 g a day significantly aggravates the natural course of the disease.

(*Gut* 1999;**44**:874–880)

Several genotypes of the hepatitis C virus (HCV) show a great variability in genomic sequence.[1–5] A controversial issue is whether HCV genotype 1b infection carries a worse prognosis for chronic liver disease[6–15] and whether it is more resistant to interferon treatment than other genotypes.[16 17] Most published data are based on selected series of patients referred to liver centres, and data on the prevalence of different HCV genotypes and the severity of associated chronic liver disease in the general population are few.[6] Seven years ago, our group started a cohort study, named Dionysos,[18 19] in order to document the true prevalence of chronic liver disease in a geographically defined population in Northern Italy.

To address controversial issues about the prevalence of HCV infection and its genotype distribution, pattern of transmission, role of alcohol consumption, and the severity of associated chronic liver disease in the general population, we determined the prevalence of different HCV genotypes and the risk factors for HCV transmission. In all the subjects enrolled in the continuing Dionysos cohort study, the presence of HCV related cirrhosis or hepatocellular carcinoma (HCC) was also evaluated.

Materials and methods

ENROLMENT OF PATIENTS AND COMPLIANCE

The details of the overall design of the Dionysos study have already been published.[18–20] Briefly, all the 10 151 inhabitants between 12 and 65 years of age of two North-

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Abbreviations used in this paper: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; PCR, polymerase chain reaction; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; ELISA, enzyme linked immunosorbent assay; OR, odds ratio.

ern Italian towns received a written invitation to participate in the study. Of these, 6917 were enrolled during the two years of the study. This number represents 69% of the eligible population—that is, a fraction adequate to validate this type of study.[20]

INITIAL EVALUATION

As described more completely elsewhere,[18] the following were performed.

(1) A medical history was taken including family history of hepatitis in cohabiting people, presence of gallstone disease, history of past surgical operations, blood transfusions before 1990, drug abuse, dental procedures, dog or other domestic animal bites, homosexuality, acupuncture. The use of drugs was also registered.

(2) A semiquantitative colour illustrated food questionnaire[21–23] was administered which included detailed questions on the use of alcoholic beverages (wine, beer, alcoholic aperitifs, hard liquor) and the dose, duration of use and the time of drinking. Daily alcohol intake (in g) was computed by multiplying the frequency of consumption of each unit of beverage by the alcohol content of the specified portions, according to standard procedures.[24 25]

(3) A detailed physical examination was carried out aimed at detecting physical signs related to chronic liver disease.

(4) A blood sample was taken for determination of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), mean cell volume, platelet, erythrocyte and leucocyte count, hepatitis B surface antigen, hepatitis B surface antibody (Abbott Diagnostic Kits, North Chicago, Illinois, USA) and hepatitis C virus antibody (anti-HCV) (enzyme linked immunosorbent assay (ELISA), third generation; Ortho Diagnostic Systems, Raritan, New Jersey, USA).

The diagnosis of cirrhosis was clinically suspected when at least two of the following features were present: (*a*) spider nevi, scleral icterus, palmar erythema, ascites, flapping tremor, hepatic or splenic enlargement; (*b*) platelet count less than 140 000/mm³; (*c*) portal vein diameter greater than 12 mm or irregular margins of the liver parenchyma at ultrasonography.

VIROLOGICAL STUDIES

In all patients the presence of anti-HCV antibodies was assayed by a second generation (four antigen) immunoenzymatic screening test (Ortho-HCV; Ortho Diagnostic Systems). This assay detects specific reactivity to four HCV antigens, including three non-structural (C100-3, 5-1-1, C-33c) and one structural (C22). In positive samples the presence of anti-HCV antibodies was confirmed by a third generation immunoenzymatic test (Ortho Diagnostic Systems) and by a confirmatory test (RIBA; Chiron Corp, Emeryville, California, USA). Sera showing two or more positive bands were considered "positive", whereas those with only one band (usually C22) were defined as "indeterminate" and those without HCV antigen bands as "negative".

HCV RNA DETECTION

The presence of HCV RNA in the serum was assessed by polymerase chain reaction (PCR) amplification of the 5' untranslated region of HCV, which is considered to be the most conserved region of the virus. The test was performed in all subjects testing positive for the presence of anti-HCV antibodies (screening test). The amplification was performed in two steps, so called nested PCR.

After extraction, cDNA synthesis was performed with an antisense external primer according to standard methods.[26]

HCV RNA detection was performed in the serum samples collected at presentation. If viral RNA was found, no additional determination was carried out; if HCV RNA was undetectable, the test was repeated on fresh serum samples collected during follow up.

HCV GENOTYPING

The HCV genotype was determined by PCR amplification of the core region as described by Okamoto,[26 27] and classified according to the international Simmonds nomenclature.[28] Each HCV type was characterised by a different nucleotide length: 49 for type 1a, 144 for type 1b, 174 for type 2a, and 123 for type 2b. When a double infection was suspected because of the presence of several bands, a second PCR was repeated separately with each of four type-specific antisense primers. Primers were also added to enable detection of type 3a HCV.[27 29]

FOLLOW UP OF ANTI-HCV POSITIVE SUBJECTS

Each participant initially positive for anti-HCV atibody (and confirmed to be positive by third generation ELISA and the RIBA test) and each subject for whom the diagnosis of cirrhosis was suspected clinically (see above) underwent the following additional procedures every six months for at least three years: (*a*) repetition of baseline blood tests plus, as well as serum alkaline phosphatase, bilirubin, albumin, γ-globulin, prothrombin time, and α-fetoprotein, blood assay of glucose, cholesterol, and triglycerides; (*b*) ultrasonography of the liver, biliary system, pancreas, and spleen with the measurement of portal vein and retropancreatic splenic vein diameters (all sonograms were performed by one operator).

All of the 162 HCV RNA positive subjects with either ALT more than 1.5 times the upper limit of normal in at least two after one month interval check (n = 55) or with clinically suspected cirrhosis according to the criteria reported above (n = 25) were asked to undergo percutaneous liver biopsy. Of these, 79 met these criteria, and 77 had biopsies. Two patients with ALT more than 1.5 times the upper limit of normal, but without clinically suspected cirrhosis, declined to have a biopsy.

STATISTICAL ANALYSIS

Statistical analysis was performed with an SPSS/PC statistical package (SPSS Inc, Chi-

Table 1 Anti-hepatitis C virus (HCV) positivity and HCV genotype distribution according to age, as well as overall prevalence (%) of the different HCV genotypes in the general population (n=6917) and the age distribution in the population of reference (total Dionysos cohort)

HCV genotype	12–25 years	26–35 years	36–45 years	46–55 years	56–65 years	Total ≤45 years	Total >45 years	Overall
Total Dionysos population	1464 (21.2)	1347 (19.5)	1354 (19.6)	1368 (19.8)	1384 (20.0)	4165 (60.2)	2752 (39.8)	6917
Anti-HCV positive	9 (4.0)	20 (8.8)	26 (11.5)	56 (24.8)	115 (50.9)	55 (24.3)	171 (75.7)★	226 (3.2)
HCV-RNA positive	5 (3.1)	14 (8.6)	12 (7.4)	43 (26.5)	88 (54.3)	31 (19.1)	131 (80.9)★	162 (2.3)
Type 1a	0 (0.0)	2 (28.6)	1 (14.3)	2 (28.6)	2 (28.6)	3 (42.9)	4 (57.1)	7 (0.1)
Type 1b	1 (1.5)	5 (7.4)	5 (7.4)	23 (33.8)	34 (50.0)	11 (16.2)	57 (83.8)★	68 (1.0)
Type 2a	1 (2.6)	1 (2.6)	4 (10.3)	12 (30.8)	21 (53.8)	6 (15.4)	33 (84.6)★	39 (0.6)
Type 2b	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	1 (100)	1 (0.01)
Type 2c	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	2 (50.0)	0 (0.0)	4 (100)	4 (0.06)
Type 3a	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)	1 (33.3)	2 (66.7)	1 (33.3)	3 (0.04)
Co2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	3 (100)	3 (0.04)
Co3	0 (0.0)	3 (27.3)	1 (9.1)	1 (9.1)	6 (54.5)	4 (36.4)	7 (63.6)	11 (0.16)
UT	3 (11.5)	1 (3.8)	1 (3.8)	3 (11.5)	18 (69.2)†	5 (19.2)	21 (80.8)★	26 (0.38)

Values in parentheses are percentages.
Co2, coinfections by two HCV genotypes (two patients infected by genotypes 1b and 2b and one by genotype 1b and 2 a); Co3, coinfections by three HCV genotypes (five of the 11 patients infected by genotypes 1a, 1 b and 2a and the other six by genotypes 1b, 2a and 2b); UT, untypable genotypes.
★p<0.002 *v* total≤ 45; †p<0.01 *v* all the other age ranges.

cago, Illinois, USA). All p values reported are two tailed. Statistical comparisons between means were made by one way analysis of variance, and, when the variances were not homogeneous, by the Kruskal-Wallis one way analysis of variance.[30]

At the univariate analysis within the general Dionysos population, the following covariates were considered as possibly related to HCV infection: age, sex, daily alcohol intake, history of hepatitis among the cohabiting people, past surgical procedure, past dental procedures, intravenous drug use, acupuncture, blood transfusion before 1990, animal bites, and homosexuality. The association between the presence of cirrhosis/HCC and different HCV genotypes, after correction for age, sex, and daily alcohol intake, was also considered both within the general Dionysos population and the HCV infected population.

The factors selected were judged eligible to enter the multivariate model on the basis of the following tests[31]: (*a*) significance test on Pearson correlation coefficient under the null hypothesis of $\rho = 0$; (*b*) two-sample *t* test (analysis of variance) on mean values with correction for unequal variances when detected; (*c*) likelihood ratio χ^2 statistic for testing the significance of explanatory variables, one at a time, from a linear logistic regression model. A significance level lower than 0.05 was considered the criterion for entry into the initial multivariate model.

The multivariate analysis was carried out using a linear logistic regression model,[32] with a stepwise variable selection procedure (level for entry = 0.1, level for stay = 0.5). The models explain either the probability of HCV infection according to the different exposure risk factors or the probability of having cirrhosis/HCC according to the different HCV genotypes among both the total Dionysos cohort and the HCV infected population followed up for three years. As coinfection with two or three HCV genotypes was always associated with genotype 1b, results for patients infected with multiple genotypes were pooled with those for patients infected with HCV genotype 1b in the multivariate analysis. Independently of the used selection criteria, age and sex factors were always considered in the multivariate analysis in order to improve model fitting. During

analysis for the association between the presence of cirrhosis/HCC and the presence of different HCV genotypes, daily alcohol intake was also always forced into the model. Odds ratios (OR) and 95% confidence intervals were also calculated.[32]

Results

PREVALENCE OF HCV GENOTYPE INFECTION ACCORDING TO AGE AND SEX

The anti-HCV screening test was positive in 226 cases (3.2%). RIBA gave indeterminate results in 20 cases (9%), negative in 27 (12%), and positive in the remaining 179 cases (79%). Viral RNA was found in 154 of 179 RIBA positive cases (86%) and in six RIBA "indeterminate" cases (30%). Interestingly, two of the 27 RIBA negative cases (7%) were found to be HCV RNA positive. HCV RNA was detected in the serum in 162 cases (2.3%). The prevalence (P) of anti-HCV and HCV RNA positivity increased progressively with age. As shown in table 1, the prevalence was low in the age range 12–25 years, and more than two thirds of the positive subjects were older than 45.

Of the 162 HCV RNA positive subjects (table 1 and fig 1), seven (4.3%) were type 1a (P = 0.1%), 68 (42%) type 1b (P = 1.0%), 39 (24%) type 2a (P = 0.6%), one (0.6%) type 2b (P = 0.01%), four (2.5%) type 2c (P = 0.06%), three (1.8%) type 3a (P = 0.04%), three (1.8%) were coinfected with two genotypes (P = 0.04%), and 11 (6.8%) with three genotypes (P = 0.16%). Coinfection was always associated with the presence of genotype 1b. In 26 cases the core amplification was positive, but multiple or unexpected bands were found in the agarose and polyacrylamide gels; as PCR amplification was performed three times with the same results, these cases were considered as "untypable" (P = 0.38%).

The HCV genotype varied according to age (table 1). The percentage of subjects infected with all the HCV genotypes, except for genotype 3a, was significantly higher over 45 years (p<0.002) than under. The percentage of "untypable" genotype was significantly (p<0.05) higher in the 56–65 age range than in the others (table 1). The mean age of patients infected by the various HCV genotypes does not differ significantly. The prevalence of both
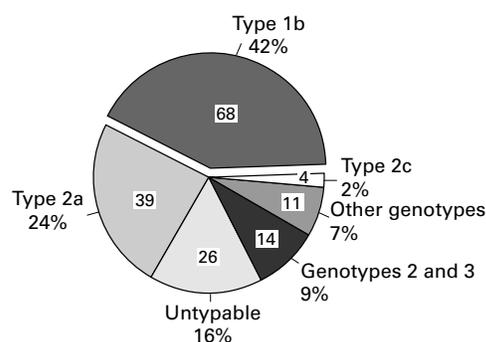
*Figure 1 Distribution of different hepatitis C virus
(HCV) genotypes in the HCV RNA positive subjects of the
Dionysos cohort (n = 162).*

anti-HCV and HCV RNA positivity was
greater in women than men (ratio of men to
women = 0.7) in all the age ranges considered.

RISK FACTORS FOR HCV INFECTION IN THE
DIONYSOS COHORT

Tables 2 and 3 give the risk factors for exposure
to HCV that were significantly (p<0.05)
associated with positivity for HCV RNA and
for different HCV genotypes both in the
univariate and multivariate analysis. The uni-
variate analysis (table 2) identified the presence
of chronic hepatitis among the cohabiting, both
surgical and dental procedures, intravenous
drug use, blood transfusions before 1990, and
animal (mainly dog) bites as possibly related
risk factors for the presence of HCV infection.
When these factors were entered into the logis-
tic multiple regression analysis (table 3), after
correction for sex and age, only history of
intravenous drug use, blood transfusions,
previous hepatitis among the cohabiting, and
animal bites were significantly (p<0.05) associ-
ated with the presence of HCV infection.

RISK FACTORS FOR CIRRHOSIS/HCC IN THE
DIONYSOS COHORT

The diagnosis of liver cirrhosis and/or HCC
was confirmed by liver biopsy in all 78 cases in
which the diagnosis was suspected clinically. As
reported elsewhere,[18] [19] the main causes of
cirrhosis/HCC were HCV and alcohol abuse.
Of the 162 HCV RNA positive subjects, 20
(12%) had cirrhosis and five (3%) had HCC
(four with cirrhosis as well). Diagnosis of
cirrhosis was made at the initial evaluation.
Fourteen of the 20 patients with cirrhosis
(70%) were infected with genotype 1b, three
(15%) with genotype 2a, and the remaining
three with genotype 1a, "untypable" genotype,
and multiple genotypes (types 1b, 2a, and 2b).
Two of the five patients with HCC, infected
with genotype 1b, had that diagnosis made at
the initial evaluation, while the other three (one
without cirrhosis, infected with genotype 1b
and 2a, the other two with cirrhosis, infected
with an untypable genotype) developed HCC
during the three years of follow up, with an
incidence rate of two per 22 patients with HCV
induced cirrhosis per three years, or 3% per
year. All five patients with HCC were alcohol
abusers (mean (SD) daily alcohol intake was
122 (46) g; p<0.01 *v* other patients with
cirrhosis).

Of the remaining 52 HCV RNA positive
patients who underwent liver biopsy during the
three years of follow up, 18 had minimal
inflammation (11%) and 34 (21%) had
chronic active hepatitis.

Fifty six HCV RNA positive patients (34%)
showed no abnormality of ALT, AST, or GGT,
and 29 (18%) had only a slight increase in
either ALT or GGT.

Finally, of the 64 subjects positive for
anti-HCV but negative for HCV RNA, 53
(83%) showed no clinical, biochemical, or
ultrasonographic alterations during the three
years follow up. In the remaining 10 cases
(15.5%) a transient mild alteration in either
GGT or ALT occurred. Of these 10 patients,
one was obese, two had gallstones, and seven
were alcohol abusers (60 g ethanol a day). Only
one of these HCV RNA negative patients met
the criteria for biopsy. He was found to have
cirrhosis, but he was an alcohol abuser (>120
g ethanol a day).

In order to discriminate which is the most
important factor correlated with the presence
of cirrhosis and/or HCC between different
HCV genotypes, age, sex, and daily alcohol
intake in the Dionysos cohort, we performed a
multiple regression logistic analysis. As re-
ported in table 4, after correction for age, sex,
and daily alcohol intake, the odds for a patient
infected by both HCV genotype 1b and multi-
ple genotypes of having cirrhosis and/or HCC
was 31 times higher (range 16–59) than for
those patients not infected by HCV
(p<0.0001). The risk for a subject infected by
all the other HCV genotypes of having cirrho-
sis and/or HCC was lower (OR = 11.2, range
4.4–28.6, p<0.0001) but still highly significant
compared with HCV RNA negative subjects.

When the multivariate analysis was per-
formed considering only the HCV RNA
positive population—that is, 162 subjects—
after correction for sex, age, and daily alcohol
intake, the only HCV genotype significantly
associated (p = 0.002) with the risk of having
cirrhosis and/or HCC was genotype 1b, either
alone or combined with other genotypes (OR =
4.0, range 1.6–9.8).

Similarly, the prevalence of cirrhosis and/or
HCC in HCV RNA positive patients was
significantly higher (p<0.01) in patients in-
fected by genotype 1 than in patients infected
by all the other genotypes. In detail, 72% (18/
25) of the liver cirrhosis with or without HCC
was associated with genotype 1b. Conversely,
only 25% of patients infected with genotype 1b
showed consistently normal levels of ALT or
GGT during the three years of follow up.

Finally, by taking 30 g daily alcohol intake as
the risk threshold for alcohol induced liver
damage, as we previously showed in the same
Dionysos cohort (21), 36 of the HCV RNA
positive subjects (22%) could be considered as
drinkers at risk, while the remaining 126 were
not. Thirteen of the 126 HCV RNA positive
subjects not at risk (10%) had cirrhosis or
HCC, while 12 of the 37 drinkers at risk (32%)
had cirrhosis (p<0.05). The risk of developing
cirrhosis for HCV RNA positive drinkers was
three times higher than that for drinkers not at

Table 2   Univariate analysis of the potential risk factors for exposure to hepatitis C virus (HCV) infection in the Dionysos cohort (n = 6917 subjects)

Risk factors	Anti-HCV negative (n = 6691)	Anti-HCV positive (n = 226)	Pearson correlation coefficient p value
Male sex	3158 (47)	95 (42)	0.2524
Alcohol intake (>30 g/day)	1449 (22)	52 (23)	0.0704
Hepatitis among the cohabiting	1024 (15)	60 (27)	0.0001
Surgical procedure	4878 (73)	183 (81)	0.0056
Dental procedures	3794 (57)	151 (67)	0.0295
Intravenous drug use	22 (0.3)	4 (2)	0.0001
Acupuncture	355 (5)	18 (8)	0.1395
Blood transfusion	401 (6)	35 (15)	0.0001
Animal bites	743 (11)	36 (16)	0.0391
Homosexuality	7 (0.1)	0 (0)	0.7556

Values in parentheses are percentages.

Table 3   Multivariate analysis of the potential risk factors for exposure to hepatitis C virus (HCV) infection in the Dionysos cohort (n = 6917 subjects). Analysis of maximum likelihood estimates

Risk factors	χ^2 (p value)	Odds ratio	95% CI
Sex (forced)	0.2568	0.82	0.6–1.1
Age (forced)	0.0001	1.1	1.0–1.1
Hepatitis among the cohabiting	0.0003	2.0	1.4–2.8
Intravenous drug use	0.0001	18.4	5.3–64.0
Animal bites	0.0444	1.6	1.0–2.5
Blood transfusion	0.0006	2.2	1.4–3.4

CI, confidence interval.

Table 4   Multivariate analysis of the risk factors for the presence of cirrhosis and/or hepatocellular carcinoma in the Dionysos cohort (n = 6917 subjects). Analysis of maximum likelihood estimates

Risk factors	χ^2 (p value)	Odds ratio	95% CI
Sex (forced)	0.06	1.7	1.0–3.0
Age (forced)	0.002	1.0	1.0–1.1
Alcohol intake (g/day) (forced)	0.0001	1.0	1.0–1.1
HCV genotype 1b (including multiple genotypes)	0.0001	30.9	16.2–58.9
HCV genotypes other than 1b	0.0001	11.2	4.4–28.6

CI, confidence interval.

risk (95% confidence interval = 1.2–7.4, p<0.01). Two of the 12 HCV RNA positive cirrhotic patients who drank more than 30 g alcohol a day (17%) were younger than 45 compared with only one of 13 cirrhotic non-drinkers (7.7%, p<0.05).

Discussion

Over the last few years, several studies have reported on the relation between different HCV genotype infections, and the increased risk of developing either cirrhosis or HCC.[8 11 14–16] However, most of these clinical studies are limited by a number of confounding factors, by fragmentary sampling, or by other risk factors such as alcohol intake. The Dionysos study avoids many of these problems because the cohort of subjects studied is more representative of the general population than that of most other studies, and the data on prevalence of chronic liver disease as well as of risk factors for liver disease were collected according to a well defined protocol. In the present work, we report on the prevalence and genotype composition of HCV infection in the general population in relation to sex, age, daily alcohol intake, and risk factors for exposure to HCV infection. In addition, a three year prospective study was performed to examine the natural history of HCV infection and the influence of cofacors on the natural history of cirrhosis and its evolution to HCC.

Compared with previous reports, this study showed a higher prevalence of anti-HCV antibodies. Prevalence of anti-HCV positivity in our population was 3.2%, three times that reported in several series studying blood donors,[33–35] but lower than that recently reported in a large random US population (7%).[36] Recent data[37–39] from Central and Southern Italy also indicate that HCV infection is more frequent (8.4 and 12.6% respectively) than in our study. It is clear that prevalence of anti-HCV positivity is very sensitive to population and geography. Prevalence of anti-HCV positivity was higher in women than in men, and in older (>45 years old) than younger people, in keeping with other studies.[14–16 36–40]

On the basis of RIBA and PCR HCV RNA assays, our results showed that only 72% of the anti-HCV positive patients were HCV RNA positive, and that there was a 12% false positive rate for anti-HCV assays. Therefore, of the 28% of the subjects who were HCV RNA negative, 12% probably never had the disease, and 16% had the disease but probably cleared the virus. This figure is similar to the 9–10% false positive anti-HCV assays previously reported, and it is identical with the 16% of RIBA positive healthy blood donors who are HCV RNA negative reported in the United States.[36]

Distribution of HCV genotypes was different from that reported in several recent studies. For example, prevalence of genotype 1b was less (42%, or 51% if including multiple genotypes) than that in other reports from the same geographic area (65–70%).[9] Among patients infected with HCV, genotype distribution shows wide geographic differences. The prevalence of genotype 1b is high in Japan (78%),[4 41] intermediate in Italy (68%),[11 42] and low in the United States (21%).[17] The risk factors for transmission of HCV were similar to those reported by others,[43–45] with the exception of animal bites (mainly dog bites) which has not been reported before. We have no explanation of this finding, which must in any case be regarded with caution, because of other confounding factors such as the social condition of the subject (in our cohort most of the people that own a dog are farmers).

We also found that 35% of the HCV RNA positive subjects had consistently normal ALT and GGT values during the three years of follow up, a figure similar to other series.[10 16] As other authors recently reported the presence of mild to severe chronic hepatitis in HCV RNA positive patients with normal ALT,[46 47] we may have missed some cases of active hepatitis when we set biopsy criteria at ALT more than 1.5 times normal. Therefore, in order to understand which factors among age, sex, alcohol intake, and HCV genotypes in our cohort are important in the evolution of chronic liver disease, we selected histologically proven cirrhosis and/or HCC as the final outcome in the multivariate analysis. In line with other series,[11 14 40] our data indicate that, independently of HCV genotype, male sex and alcohol abuse were closely associated with the presence of both cirrhosis and HCC. As in our cohort we cannot

establish the exact time when the HCV infection was contracted, we were not able to correct our data for the duration of infection. However, multivariate analysis after correction for age, sex, and alcohol intake indicated that the relative risk of having cirrhosis and/or HCC is significantly more closely associated with the presence of HCV genotype 1b (OR = 31) than the presence of other genotypes (OR = 11.2, p<0.0001). As the mean age of the patients infected by different HCV genotypes is similar and the design of our study—that is, population based—avoids the bias of a patient oriented selection, these data suggest that the greater severity of HCV type 1b chronic liver disease is not only due to an age "cohort effect". They also indicate that the risk of progression to cirrhosis depends primarily on HCV infection, mainly type 1b infection, rather than the age of the patient at the time of infection. Our findings are in conflict with those of other studies, which showed no impact of HCV genotype on the severity of chronic liver disease,[14 15] but are in line with other reports,[10 11 41] including a recent one from the same geographic area,[16] which showed a high prevalence of genotype 1b infection in patients with cirrhosis and/or HCC. This is probably due to the fact that important cofactors in the progression of chronic liver disease, such as excessive alcohol intake, have not been taken into account by other authors. In addition, earlier studies are mainly based on selected populations, while the latter concordant studies[10 11 16] are all either case-control or cross-sectional studies of a well defined homogeneous population.

In conclusion, this cohort study provides reliable information on HCV genotype and chronic liver disease in an open population. From these data we can conclude that: (*a*) the prevalence of HCV related infection is higher than reported in selected series (in women more than in men, and age dependent); (*b*) genotype 1b is the most prevalent genotype, but it is less widespread than previously reported in selected series of patients affected by HCV induced chronic liver disease; (*c*) main risk factors for HCV infection in the Dionysos open population are similar to those found in selected series, with the exception of animal bites; (*d*) more than 50% of the HCV RNA positive subjects showed no clinical and biochemical signs of liver damage or had only a slight increase in either ALT or GGT during the three years of follow up; (*e*) the association between HCV infection, especially genotype 1b infection, and alcohol abuse dramatically enhances the liver damage and the progression of cirrhosis to HCC; (*f*) independently of age, sex, and alcohol intake, HCV infection, particularly with genotype 1b, is the major risk factor associated with the presence of cirrhosis and/or HCC in the general population, at least of Northern Italy. However, further studies providing prospective data on the real duration and natural history of HCV infection are necessary to confirm this latter finding.

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