American Journal of Gastroenterology
© 2008 by Am. Coll. of Gastroenterology
Published by Blackwell Publishing

ISSN 0002-9270
doi: 10.1111/j.1572-0241.2008.01948.x

Natural Course of Chronic HCV and HBV Infection and Role of Alcohol in the General Population: The Dionysos Study

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BACKGROUND: Population-based studies of the natural course of chronic viral liver disease that consider comorbidity factors are lacking. Using data from the Dionysos Study, we quantified the burden of chronic viral liver disease and the role of alcohol intake to morbidity and mortality in a representative sample of subjects from the general population of two communities of Northern Italy.

METHODS AND FINDINGS: We followed up 139 subjects with chronic hepatitis C virus (HCV) infection and 61 with chronic hepatitis B virus (HBV) infection for a median (IQR) time of 8.4 (1.0) and 8.3 (0.9) yr, respectively. Ethanol intake was evaluated using a food-frequency questionnaire, fatty liver (FL) was diagnosed by ultrasonography, and liver cirrhosis (LC) and hepatocarcinoma (HCC) were diagnosed by liver biopsy. Exact multivariable Poisson regression was performed to identify predictors of death. The incidence and remission rates of FL were 9.0 and 29.7 in the HCV cohort and 4.0 and 30.4 per 1,000 person-years (PY) in the HBV cohort. Progression to LC and HCC was more common in the HCV than in the HBV cohort (4.5 *vs* 2.0 and 2.7 *vs* 2.0 per 1,000 PY, respectively). Ethanol intake was an independent predictor of LC in the HCV cohort [rate ratio (RR) = 4.15 (95% CI 1.02–41.2) for every increase of 30 g/day of ethanol intake at baseline] and of death rate in both cohorts [RR = 8.53 (95% CI 1.40–24.61) and 3.56 (1.34 to 26.50) for every increase of 30 g/day of ethanol intake at baseline].

CONCLUSIONS: The morbidity and mortality rate of HBV and HCV infection in the general population is lower than that reported in secondary-care populations, blood donors, or clinical series. Ethanol intake is an independent predictor of LC in subjects with chronic HCV infection and an independent predictor of death in subjects with either HCV or HBV infection.

(Am J Gastroenterol 2008;103:2248–2253)

INTRODUCTION

Knowledge of the burden of disease in the general population is essential to the development and improvement of health strategies (1, 2).

Few data are available on the natural course of chronic liver disease in the general population and the available estimates of morbidity and mortality for chronic hepatitis C virus (HCV) infection were obtained mainly from studies of individuals infected through blood transfusions or immunoglobulin preparations and from military recruits (3–7). Importantly, a recent study conducted in a nationally representative sample of secondary-care patients suggests a substantially worse prognosis of HCV infection as compared to earlier studies (8). Likewise, the available estimates of morbidity and mortality for chronic hepatitis B virus (HBV) infection were obtained from clinical series and studies of blood donors and are not a measure of the burden of disease at the population level (9–11).

Evidence obtained mainly from cross-sectional studies of tertiary-care patients shows that alcohol abuse is associated with liver fibrosis, liver cirrhosis (LC), and hepatocarcinoma (HCC) in HCV-infected subjects (12, 13). Although fewer studies are available on the ethanol–HBV relationship, alcohol intake is discouraged in viral infected subjects (14). However, prospective studies on the role of ethanol in the progression of chronic viral liver disease are not available for the general population.

Nonorgan specific autoantibodies (NOSA) are common in patients with HCV infection (15). In the Dionysos Study, nearly one of every four HCV subjects had NOSA, and association was observed between NOSA and the biochemical and histological severity of liver disease (16). Even if NOSA do not appear to modify the response to treatment in HCV-infected subjects, their long-term prognostic significance remains unknown (15, 17).

Using the Dionysos cohort (18, 19), we quantified the burden of disease and evaluated the contribution of alcohol intake, NOSA, and other risk factors to morbidity and mortality in a representative sample of HBV and HCV subjects from the general population of two communities of Northern Italy.

MATERIALS AND METHODS

Study Design

The aim of the Dionysos Study was to assess the prevalence, incidence, and natural course of liver disease in the general population of two communities of Northern Italy (18–21).

Between 1991 and 1992, we evaluated 6,917 (68%) of 10,151 individuals aged 12–65 yr living in Campogalliano (Modena, Emilia Romagna) and Cormons (Gorizia, Friuli Venezia Giulia). Eighty-seven subjects were positive to hepatitis B surface antigen (HBV cohort) and 226 had anti-HCV antibodies (HCV cohort) at the baseline visit (16, 19, 22).

We re-evaluated these two cohorts in 2001–2002 to define the natural course of chronic viral liver disease in the general population. All subjects underwent liver ultrasonography (US) and were evaluated at the Liver Research Center every 6–12 months for a median time of 8.4 yr (HCV cohort) and 8.3 yr (HBV cohort). The Mortality Registers of Emilia Romagna and Friuli Venezia Giulia were used to determine the number and causes of deaths during the study period. The study protocol was approved and supervised by the Scientific Committee of the Fondo per lo Studio delle Malattie del Fegato-ONLUS (Trieste, Italy). All subjects gave their written consent to participate in the study.

Methods

The methods employed by the Dionysos Study were described in detail elsewhere (18–23). Briefly, each subject underwent a clinical and laboratory evaluation, a liver US, and a food-frequency questionnaire for the assessment of food and ethanol intake. The laboratory evaluation also included an assessment of HBV-DNA, HCV-RNA, and NOSA (16, 19, 22). Fatty liver (FL) was diagnosed by US using standardized criteria (23, 24). LC and hepatocellular carcinoma (HCC) were diagnosed by liver biopsy. The same trained physicians in Campogalliano and Cormons performed US at the baseline and follow-up visits. Body mass index (BMI) was calculated as weight (kg)/stature $(m)^2$.

Statistical Analysis

Continuous variables are given as medians and interquartile ranges (IQR) because of skewed distributions. Between-group comparisons of continuous variables were performed with the Mann-Whitney U-test and those of categorical variables with the Fisher's exact test. Incident FL, LC, HCC, HBV-DNA (positivization), HCV-RNA (positivization), and NOSA (positivization) were defined as FL, LC, HCC, HBV-DNA, HCV-RNA, and NOSA absent at baseline and present at follow-up; remitting FL, HBV-DNA (negativization), HCV-RNA (negativization), and NOSA (negativization) as FL, HBV-DNA, HCV-RNA, and NOSA present at baseline and absent at follow-up; death as death occurring between March 18, 1991 (first baseline visit of Dionysos Study Phase 1) and June 30, 2002 (last follow-up visit of Dionysos Study Phase 2). Rates are expressed as number of cases per 1,000 person-years (PY). For the HCV cohort, a prespecified multivariable exact Poisson regression model was used to evaluate the relationship between death rate, ethanol intake at baseline, and NOSA at baseline after taking into account the effects of gender, age at baseline, HCV-RNA at baseline, and altered liver enzymes at baseline (25, 26). These latter were defined as alanine transaminase (ALT) $\geq$ 40 U/L or aspartate transaminase (AST) $\geq$ 37 U/L or gamma-glutamyl-transferase (GGT) $\geq$ 50 U/L (18, 19). For the HBV cohort, we considered only ethanol at baseline, age at baseline, and gender as predictors because of the low number of deaths. Age and ethanol were coded as continuous while the other predictors were coded as binary (0 = No; 1 = Yes). Age was divided by 10 in order to model the effect of one decade of age. Ethanol was divided by 30 because 30 g/day was the lowest value of ethanol intake associated with liver disease in the Dionysos Study and because it was the lowest reported intake at which ethanol and HCV act synergistically (12, 20). The standardized mortality ratio (SMR) was calculated using age- and gender-specific mortality rates for Northern Italy (18). Statistical significance was set to a two-tailed P-value <0.05 for all tests. Statistical analysis was performed using STATA 10.0 (STATA Corporation, College Station, TX) and LogXact 8.0 (Cytel, Cambridge, MA).

RESULTS

HCV Cohort

Of the 226 members of the HCV cohort, 139 were re-evaluated, including 22 who died. These 139 (62%) subjects had the same gender, mean corpuscular volume (MCV), ethanol intake, and BMI of those not available at follow-up but were older and had higher values of ALT, AST, and GGT, and lower values of platelets (Table 1). Sixty-five percent of HCV subjects who tested positive for NOSA, 73% of those with FL, 65% of those with LC, 80% of those with HCC, and 71% of those HCV-RNA+ at baseline were available at follow-up (Table 1). HCV-RNA+ subjects with normal liver enzymes were not treated. Twelve HCV-RNA+ subjects were

Table 1. Baseline characteristics of subjects available and not available at follow-up

	HCV Cohort (N = 226)			HBV Cohort (N = 87)		
	Available (N = 139)	Not Available (N = 87)	*P*-value*	Available (N = 61)	Not Available (N = 26)	*P*-value*
Gender (M/F)	58/81	41/46	0.4910	39/22	16/10	1.0000
Age (years)	58 (12)	50 (23)	0.0006	43 (17)	40 (20)	0.0735
ALT (U/L)	32 (36)	23 (27)	0.0064	23 (17)	22 (12)	0.0486
AST (U/L)	27 (21)	22 (14)	0.0038	24 (9)	22 (9)	0.2691
GGT (U/L)	26 (42)	20 (25)	0.0093	21 (18)	14 (11)	0.0247
MCV (fL)	89 (5)	88 (6)	0.1311	89 (8)	88 (8)	0.9482
Platelets (U^*10^9/L)	209 (80)	232 (80)	0.0031	206 (45)	206 (69)	0.9557
Ethanol intake (g/day)	16 (32)	9 (21)	0.1037	19 (40)	14 (19)	0.2951
BMI (kg/m^2)	25 (5)	25 (5)	0.8294	25 (5)	24 (3)	0.1727
NOSA (+ve/–ve)	37/102	20/67	0.6370	3/58	0/26	0.5510
Fatty liver (yes/no)	61/78	22/65	0.0050	23/38	3/23	0.0200
Liver cirrhosis (yes/no)	17/122	9/78	0.8310	4/57	3/23	0.4220
HCC (yes/no)	4/135	1/86	0.6510	0/61	0/26	–
HBV-DNA (+ve/–ve)	–	–	–	27/34	9/17	0.4800
HCV-RNA (+ve/–ve)	113/26	46/41	<0.0001	–	–	–

*Mann-Whitney U-test for continuous variables and Fisher's exact test for categorical variables.
Values of continuous variables are given as median (interquartile range) and those of categorical variables as the number of subjects with the characteristic of interest.
HCV = hepatitis C virus; HBV = hepatitis B virus; ALT = alanine transaminase; AST = aspartate transaminase; GGT = gamma-glutamyl-transferase; MCV = mean corpuscular volume; BMI = body mass index; NOSA = nonorgan specific autoantibodies; HCC = hepatocarcinoma; DNA = deoxyribonucleic acid; RNA = ribonucleic acid.

treated with interferon alpha at doses of 3 MU thrice weekly for 6–12 months.

The median (IQR) follow-up time of the HCV cohort was 8.4 (0.9) yr and the total follow-up time was 1,111 PY. Eleven HCV subjects developed FL (rate = 9.0 ∗ 1,000 PY) and 33 had remission of FL (rate = 29.7 ∗ 1,000 PY) (Table 2). Four HCV women aged 55–63 yr and drinking from 0–24 g/day of ethanol and one man aged 41 yr and drinking 240 g/day of ethanol at baseline developed LC (rate = 4.5 ∗ 1,000 PY for n = 5) (Table 2). Ethanol intake at baseline was a predictor of LC [rate ratio (RR) = 4.15 (exact 95% CI 1.02–41.2, exact *P*-value = 0.0430) every 30 g/day increase] independently from age (exact *P*-value = 0.9151) and gender (exact *P*-value = 0.2780). Two women aged 48 and 63 yr and not drinking ethanol at baseline and one man aged 62 yr and drinking 40 g/day of ethanol at baseline developed HCC (Table 2). All subjects who developed HCC had LC at baseline (rate = 2.7 ∗ 1,000 PY for n = 3).

Twenty-two HCV subjects died, giving a mortality rate of 19.8 ∗ 1,000 PY (Table 2) and corresponding to a SMR of 1.65 (95% CI 1.09–2.50). The causes of death were: nonliver cancer (N = 9), HCC (N = 7), cardio- or cerebro-vascular disease (N = 3), LC (N = 2), and respiratory insufficiency (N = 1). All seven deaths from HCC occurred in subjects with baseline LC (three cases of HCC developed during the study and four were present at baseline). At multivariable exact Poisson regression, ethanol intake (RR = 8.53 every 30 g/day increase, exact *P*-value = 0.0257) and age (RR = 1.60 every 10-yr increase, exact *P*-value = 0.0450) were predictors of death in the HCV cohort (Table 3). (We added a gender ∗ ethanol interaction to the regression model to account for the fact that only two women drank alcohol at

Table 2. Natural Course of Chronic HBV and HCV Infection

	HCV Cohort (N = 139)			HBV Cohort (N = 61)		
	N	%	Rate (95% CI)*	N	%	Rate (95% CI)*
FL incidence	11	7.9	9.0 (5.5–17.9)	2	3.3	4.0 (1.0–16.2)
FL remission	33	23.7	29.7 (21.1– 41.7)	15	24.6	30.4 (18.3–50.4)
LC incidence	5	3.6	4.5 (1.9–10.8)	1	1.6	2.0 (0.3–14.4)
HCC incidence	3	2.2	2.7 (0.9–8.4)	1	1.6	2.0 (0.3–14.4)
Death	22	16	19.8 (13.0–30.0)	3	4.9	6.1 (2.0–18.8)
HBV-DNA negativization	–	–	–	5	8.2	10.1 (4.2–24.3)
HBV-DNA positivization	–	–	–	2	3.3	4.0 (1.0–16.2)
HCV-RNA negativization	3	2.2	2.7 (0.9–8.4)	–	–	–
HCV-RNA positivization	3	2.2	2.7 (0.9–8.4)	–	–	–
NOSA positivization	13	9.4	11.7 (6.8–20.1)	0	0	–
NOSA negativization	25	18.0	22.5 (15.2–33.3)	1	1.6	2.0 (0.3–14.4)

*Rate is number of cases per 1,000 person-years.
FL = fatty liver; LC = liver cirrhosis; other abbreviations as in Table 1.

baseline and both in quantity ≤60 g/day while the intake for men ranged up to 240 g/day.)

Three HCV subjects had negativization of HCV-RNA (rate = 2.7 ∗ 1,000 PY); two of them had undergone treatment with interferon alpha for 12 months (Table 2). The positivization rate of HCV-RNA was equal to the negativization rate. Thirteen HCV subjects had positivization (rate = 11.7 ∗ 1,000 PY) and 25 had negativization of NOSA (rate = 22.5 ∗ 1,000 PY).

HBV Cohort

Of the 87 members of the HBV cohort, 61 were re-evaluated, including three who died. These 61 (70%) subjects had the same gender, age, AST, MCV, platelets, ethanol intake, and BMI of those not available at follow-up but had higher values of ALT and GGT (Table 1). One hundred percent of HBV subjects who tested positive for NOSA, 88% of those with FL, 57% of those with LC, and 75% of those HBV-DNA+ were available at follow-up. None of the HBV-DNA+ subjects was treated, because all were anti-HBeAg– or HBeAg+ with ALT within or close to the normal range.

The median (IQR) follow-up time of the HBV cohort was 8.3 (1.0) yr and the total follow-up time was 494 PY. Two HBV subjects developed FL (rate = 4.0 ∗ 1,000 PY) and 15 had remission of FL (rate = 30.4 ∗ 1,000 PY) (Table 2). An HBV woman aged 36 yr and not drinking alcohol at baseline developed LC (rate = 2.0 ∗ 1,000 PY). An HBV man aged 49 yr, drinking 19 g/day of ethanol and without LC at baseline, developed HCC (rate = 2.0 ∗ 1,000 PY), and was alive at the follow-up visit.

Three HBV subjects died, giving a mortality rate of 6.1 ∗ 1,000 PY (Table 2) and corresponding to a SMR of 1.08 (95% CI 0.35–3.34). All deaths occurred because of cardio- or cerebro-vascular disease. At multivariable exact Poisson regression, ethanol intake at baseline (RR = 3.56 every 30 g/day increase, $P = 0.0063$) was a predictor of death independently from age and gender (Table 3).

Five subjects (four men and one woman) had negativization (rate = 10.1 ∗ 1,000 PY) and two subjects (all men) had positivization of HBV-DNA (rate = 3.3 ∗ 1,000 PY). No subject had positivization and one had negativization of NOSA (rate = 2.0 ∗ 1,000 PY).

DISCUSSION

The Dionysos Study is the first study on the natural course of chronic viral liver disease performed in a representative sample of the general population. It adds to previous studies performed on convenience, community, selected series, and clinical samples by quantifying the burden of HCV and HBV infection in the general population.

Eight percent of HCV and 3% of HBV subjects developed FL during 8.4 (0.9) and 8.3 (1.0) yr of follow-up, respectively. However, 24% of the former and 25% of the latter had remission of FL during the same period suggesting that, in the general population of subjects with HBV or HCV chronic liver disease, the remission of FL is more common than its incidence. We reported a similar finding for the Dionysos cohort with chronic liver disease without HBV and HCV infection (18). As we have noted before, it is possible that the lifestyle changes induced by the follow-up protocol of the study have contributed to the remission of FL (18).

Sixteen percent of our HCV subjects died during the 8.4-yr follow-up. The overall mortality rate of our HCV cohort (19.8 ∗ 1,000 PY) is slightly higher than the 10-yr mortality rate (13.9 ∗ 1,000 PY) reported for the Castellana Grotte HCV cohort from Southern Italy (27). The prevalence rate of HCV infection in the Castellana Grotte cohort is however much higher than in our cohort (40% in subjects aged ≥50). Likewise, our liver-related mortality rate (8.1 ∗ 1,000 PY) is only slightly higher than that reported for the Castellana Grotte cohort (5.5 ∗ 1,000 PY). Interestingly, the SMR of our HCV cohort (1.65) is lower than that (3.0) recently reported for a large secondary-care English cohort of HCV-infected patients followed up for 6.7 yr (8). Besides the different reference population (England *vs* Northern Italy), this difference is likely due to the fact that a general population and a secondary-care population are not comparable (1). Five percent of our anti-HBV subjects died during the 8.5-yr follow-up. This mortality rate (6.1 ∗ 1,000 PY) is lower than that reported

Table 3. Baseline Predictors of Death Rate in the HBV and HCV Cohorts

	HCV Cohort (N = 139; Deaths = 22)		HBV Cohort (N = 61; Deaths = 3)	
	IRR (Exact 95% CI)*	Exact-*P*	IRR (Exact 95% CI)*	Exact-*P*
Male gender	1.25 (0.40–3.86)	0.8433	0.69 (0.00–23.30)	0.7841
Age (yr/10)**	1.60 (1.01–2.84)	0.0450	5.70 (0.96 to ∞)	0.0550
Ethanol (g/day/30)**	8.53 (1.40–24.61)	0.0257	3.56 (1.34–26.50)	0.0063
Male ∗ Ethanol	0.14 (0.04–0.87)	0.0384	–	–
NOSA +ve	1.49 (0.52–3.98)	0.5222	–	–
HCV-RNA +ve	2.27 (0.49–21.46)	0.4507	–	–
Altered liver enzymes***	1.69 (0.56–5.75)	0.4450	–	–

* Exact multivariable Poisson regression.
**Continuous variable.
***ALT ≥ 40 U/L or AST ≥ 37 U/L or GGT ≥ 50 U/L
IRR = incidence rate ratio; other abbreviations as in Table 1.

by clinical series most likely because hospitalized subjects have more severe disease as compared to those in the general population (9).

LC was more incident in HCV (4.5 ∗ 1,000 PY) than in HBV subjects (2.0 ∗ 1,000 PY) and the same was true for HCC (2.7 *vs* 2.0 ∗ 1,000 PY). Ethanol intake at baseline was an independent predictor of LC incidence in the HCV cohort but the precision of the estimate was low (exact-RR from 1.02 to 41.2), because of the low number of positive outcomes. All HCV subjects who developed HCC had LC at baseline. The only case of cirrhosis in the HBV cohort occurred in a woman who did not drink alcohol at baseline. Moreover, the only case of HCC in the HBV cohort occurred in a man who did not drink alcohol and had not LC at baseline.

Importantly, baseline ethanol intake was a predictor of death rate in both cohorts. In HCV subjects, the lower 95% CI of the RR for ethanol intake was 1.40, which is not negligible also considering that the alcohol intake at baseline was an independent predictor of incident LC. None of the HBV subjects died however because of liver-related disease, but all died because of cardio- or cerebro-vascular disease. Ethanol is a "double-edged sword" as cardiovascular health is concerned, offering some protection at intakes <30 g/day and substantial offense at higher intakes (28). The HBV woman who died consumed a modest amount of ethanol (8 g/day) but the two HBV men who died consumed a very high amount of ethanol (113 and 190 g/day) at baseline. Thus, it is possible that ethanol was a comortality factor in our HBV subjects because of its known cardio-toxic effect at high intakes. Even if the precision of the estimate of the effect size was low because of the low number of positive outcomes, the lower 95% CI of the RR was 1.34, which is not negligible also owing to the fact that our study subjects are from the general population.

In the first phase of the Dionysos Study, NOSA activity was associated with histological and biochemical indexes of liver disease in HCV subjects (16). In the present analysis, however, we found no association between baseline NOSA and 8.4-yr mortality. A possible reason for this observation may be related with the NOSA pattern detected in our series: smooth-muscle antibodies (SMA)-V and anti-nuclear antibodies (ANA)-speckled were the reactivities most frequently found while patterns more closely associated to liver damage and in particular to autoimmune liver disease were rare (three cases of anti-LKM (liver-kidney microsomal), two of SMA T/G, and none of ANA homogeneous) (15). Equally interesting is the fact that the negativization rate of NOSA was about two times its positivization rate, showing that the most common course of nonorgan specific autoreactivity in HCV infection is remission.

Even if the Dionysos Study is the first study on the natural course of chronic viral disease performed in a representative sample of the general population, it has some limitations. The first and most important limitation is that 38% of the members of the HCV cohort and 30% of those of the anti-HBV cohort were not available at follow-up. The subjects available at follow-up were older and had higher values of liver enzymes and lower values of platelet counts as compared to those not available at follow-up. If these parameters are taken as surrogate measures of liver disease severity, it appears that the subjects with more severe disease were more likely to have the follow-up visit. Thus, the natural course of chronic viral liver diseases described may overestimate both morbidity and mortality. Despite this, our estimates of morbidity and mortality are substantially lower than those reported in secondary- and tertiary-care patients. The second limitation is that our general population is not representative of the whole Italian or European general population. A third limitation is that the most serious complications of chronic viral liver disease may take more than our median follow-up time to develop. A fourth limitation is the use of US for the diagnosis of FL, which is not accurate as liver biopsy and cannot evaluate the degree of inflammation and fibrosis (18).

In conclusion, the morbidity and mortality rates of HBV and HCV infection in the general population are lower than those reported for secondary-care populations, blood donors, or clinical series. Ethanol intake is a predictor of LC in subjects with chronic HCV infection and a predictor of death in subjects with chronic HCV or HBV infection.

STUDY HIGHLIGHTS

What Is Current Knowledge

- The available knowledge on the natural course of chronic viral liver disease is based on studies performed on blood donors, military recruits, and secondary or tertiary care series.

What Is New Here

- We report on the history of chronic HCV and HBV infection in the general population of two communities of Northern Italy. We also assess the contribution of ethanol intake and nonorgan specific autoantibodies (NOSA) to the course of chronic viral disease.

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Received January 20, 2008; accepted March 19, 2008.

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CONFLICT OF INTEREST

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Specific author contribution: Giorgio Bedogni analyzed the data and wrote the first draft of the manuscript; Stefano Bellentani and Claudio Tiribelli designed the Dionysos Study and contributed to the final version of the manuscript; Lucia Miglioli, Flora Masutti, and Lory Saveria Crocè performed the medical evaluation of subjects; Silvia Ferri, Marco Lenzi, and Alessandro Granito performed measurements of nonorgan-specific autoantibodies. All authors read and approved the manuscript.

Financial support: The Dionysos Study was supported by grants from Fondazione Cassa di Risparmio di Modena, Fondazione Cassa di Risparmio di Gorizia, Banca Popolare dell' Emilia Romagna, Comune di Campogalliano, Azienda USL di Modena, Assessorato alla Sanità della Regione Emilia Romagna, Assessorato alla Sanità della Regione Friuli Venezia Giulia, Associazione Amici del Fegato-ONLUS, and Fondo per lo Studio delle Malattie del Fegato-ONLUS.

Potential competing interests: The authors declare not to have any conflict of interest.