A case–control histological study on the effects of phlebotomy in patients with chronic hepatitis C

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Objective **The aim of this study was to assess the actual effectiveness of long-term phlebotomy by comparing histological improvement (HI) in 69 Caucasian HCV-RNA-positive CHC patients undergoing phlebotomy or receiving an interferon-based therapy without virological response [nonresponders to interferon therapy(IBT-NR)].**

Methods **HI was defined by at least one point reduction of the staging score or, in the case of unchanged stage, by at least two points reduction of the grading score (Knodel's Activity Index) and was retrospectively evaluated by comparing two consecutive (56 ± 28 months apart) liver biopsies from 30 phlebotomized and 39 IBT-NR patients.**

Results **HI was observed in 15 of 30 (50%) patients treated with phlebotomy and in six of 39 (15%) IBT-NR subjects ($P = 0.002$). Furthermore, AST, ALT, and GGT serum levels were significantly reduced only in phlebotomized patients ($P \leq 0.003$) at the time of the second biopsy. Univariate and multivariate analysis showed that histological grading score before therapy ($P = 0.001$) and phlebotomy ($P = 0.002$) were independently predictors of HI.**

Conclusion **HI induced by long-term phlebotomy effectively exceeds that spontaneously occurring in patients IBT-NR confirming the efficacy of iron depletion in attenuating CHC progression when other therapies have failed.** ***Eur J Gastroenterol Hepatol*** **23:1178–1184** © **2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

European Journal of Gastroenterology & Hepatology 2011, **23:**1178–1184

Keywords: hepatitis C, histology, interferon, iron, oxidative stress, phlebotomy

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Received 1 April 2011 **Accepted** 5 June 2011

Introduction

Hepatitis C virus (HCV) infection represents a major cause of chronic hepatic inflammation worldwide [1]. In about 20% of these patients the disease evolves to liver cirrhosis and/or hepatocellular carcinoma [1]. Anti-HCV therapies based on the association of interferon and ribavirin are effective in about half of the treated patients by inducing sustained virological response [2,3]. Nonetheless, there is an urgent need of other therapeutic options capable to prevent the progression of the disease for those patients who do not respond to the antiviral therapy.

The progression of chronic hepatitis C (CHC) is not linear and is greatly influenced by a variety of factors including male sex, age at the infection, obesity, coinfection with hepatitis B or HIV viruses, alcohol consumption, and iron overload [4,5]. Mild hepatic iron accumulation is common in CHC patients and is associated with higher aminotransferase levels and more severe fibrosis [6,7].

In recent years, several groups have reported on the efficacy of iron reduction therapies based on phlebotomy in combination with or without an iron-poor diet in ameliorating aminotransferase levels in CHC patients who were nonresponders to the interferon treatment [8–11]. Moreover, few reports have also addressed the long-term effects of phlebotomy showing its effectiveness in promoting CHC histological improvement (HI) [12,13]. However, one bias of these latter studies is that improvements in liver histology may occur spontaneously during the natural history of CHC [14,15]. In particular, the recent Hepatitis C Antiviral Long-term Treatment Against Cirrhosis trial has shown improvements in Ishak's fibrosis score in about 30% of untreated patients who were nonresponders to peginterferon–ribavirin therapy [15]. Iron depletion might be a simple and inexpensive treatment for those patients not responding to the antiviral therapy or having contraindications to its use. A previous study from our center [13] has addressed the role of phlebotomy in patients with CHC demonstrating that males with mild iron overload may have particular benefit from this procedure. Nonetheless, it is important to verify whether the HIs induced by phlebotomy effectively exceed those occurring spontaneously. To this aim, we have performed a case–control study comparing, retrospectively, the histological outcomes of a group of Caucasian CHC patients treated with long-term phlebotomy (LTP) with those of patients receiving an interferon-based therapy

0954-691X DOI: 10.1097/MEG.0b013e328349923c

without virological response who underwent a second liver biopsy within 2–5 years from the diagnosis.

Materials and methods

Patient selection

The clinical and histological data of all consecutive HCV-RNA-positive Caucasian patients who underwent a second liver biopsy for CHC between 2000 and 2006 at two Italian centers (Ospedale Maggiore della Carità in Novara and Spedali Civili in Brescia) were reviewed. Criteria of exclusion from the study were HBs Ag and/or HIV-Ab positivity, personal or familial history of hemochromatosis, hemoglobin less than 13 g/dl for men and less than 11 g/dl for women at the time of their first liver biopsy, antiviral or immunosuppressive therapies during the 6 months that preceded the first liver biospy, active drug addiction, mean daily alcohol intake greater than 80 g/day (evaluated by trained medical staff according to a standardized questionnaire, presented as part of a survey on life habits), the presence of hepatocellular carcinoma at imaging studies, and the lack of a treatment (interferon-based or by phlebotomy) between the two liver biopsies. Tests for hemochromatosis gene mutations were not performed. However, the hepatic iron concentration (HIC) and iron index excluded phenotypic hemochromatosis in all the recruited patients.

The process identified 72 patients, of whom three were excluded because the first liver biopsy specimen was no longer available (one case) or was inadequate (two cases).

Among the 69 recruited patients (51 male and 18 female; mean age 53.1 ± 10.6), 30 patients (22 men, eight women; mean age 58.4 ± 8 years) who were either previously nonresponders ($n = 14$) or had contraindications to antiviral therapy ($n = 16$) were treated exclusively with iron reduction by phlebotomy between the two liver biopsies (group 1). These patients underwent an initial period of bimonthly or monthly phlebotomy of 250 ml of blood, until serum ferritin levels of 35 ng/ml or less were reached. Thereafter, maintenance phlebotomies were performed every 1 to 3 months during a minimum 2-year period to maintain a serum ferritin at 70 ng/ml or less. No side-effects related to the mild iron depletion were reported. Phlebotomized patients underwent a second liver biopsy after 1442 ± 502 days. In these patients, who had given informed consent to be treated exclusively by phlebotomy, the second biopsy was performed as a part of a study approved by the local ethical committee to evaluate on an individual basis whether iron depletion by phlebotomy was effective and worthwhile to be continued.

The remaining 39 patients (29 male and 10 female; mean age 48.9 ± 10.5 years) showed a nonresponse to the antiviral treatment, which they had received after the first liver biopsy and underwent a second liver biopsy after 1963 ± 880 days, to decide whether antiviral treatment should be reoffered. These patients (group 2) had received antiviral therapy between the two liver biopsies, consisting of regular interferon α at standard doses and duration according to HCV genotype in 38 patients, 32 of whom were treated with monotherapy for either 24 weeks (12 cases) or 48 weeks (20 cases) and six with interferon α plus ribavirin. One patient was treated with peginterferon α and ribavirin at standard doses for 24 weeks. Of them, 32 were primary nonresponders, whereas the remainder experimented on a breakthrough after an initial virological response (three cases) or relapse after treatment (four cases). The demographic, clinical, and laboratory characteristics of the

Table 1 Characteristics of 69 patients with chronic hepatitis C at the time of their first liver biopsy

Variable	Number	Mean ± SD	Range
Age (years)		53.1 ± 10.6	25–73
Sex (males/females)	51/18		
Drug addiction (yes/no)	4/65		
Previous transfusion (yes/no)	24/45		
Alcohol intake (g/day)		12.3 ± 15	0–60
BMI (kg/m^2)		25 ± 3.2	19–38
HCV genotype (1/non-1)	42/27		
Previous antiviral treatment (yes/no)	18/51		
Aspartate aminotransferase (U/l; 0–40)		93 ± 54	24–275
Alanine aminotransferase (U/l; 0–40)		126 ± 65	52–390
γ-Glutamyl transpeptidase (U/l; 0–50)		77 ± 63	13–395
Hemoglobin (g/100 ml; 13.7–17)		14.7 ± 1.4	10.5–17.6
Serum iron (μmol/l; 11–32)		23 ± 7	5.7–43
Transferrin saturation (%; 20–50)		41 ± 13	19.3–91.5
Serum ferritin (ng/ml; 5–365)		308 ± 215	34–1051
Histological grade (Knodell's score)		5[a]	1–15
Histological stage (Knodell's score 0/1/3/4)	1/37/26/5		
Steatosis grade (0/1/2/3)[b]	17/37/12/1		
Advanced fibrosis or cirrhosis (Knodell's staging score 3–4) (yes/no)	31/38		
Hepatic iron grade (0/1/2/3/4 according to Searle's score)	29/31/7/0/2		
Hepatic iron concentration (μmol/g dry tissue; <25)[c]		21 ± 9.4	5.4–53
Iron index (hepatic iron concentration/age; <1.9)[c]		0.41 ± 0.2	0.1–1.18

[a]Values are medians.
[b]Data were available for 67 of 69 patients. Steatosis grade: 0 = no steatosis, 1 ≤ 33% of hepatocytes, 234–66%; 3 ≥ 66%.
[c]Data were available for 66 of 69 patients.

Table 2 Epidemiological, clinical, and laboratory characteristics of 69 patients with chronic hepatitis C at the time of their first liver biopsy, grouped by treatment

Variable	Group 1[a]	Group 2[a]	*P*-value[b]
Age (years)	48.9 ± 10.5	58.4 ± 8	<0.001
Sex (males/females)	29/10	22/8	>0.99
BMI	25 ± 2.7	24 ± 3.8	0.54
Alcohol intake (g/day)	11 ± 13	14 ± 18	0.337
HCV genotype (1/non-1)	24/15	18/12	>0.99
Days between the two liver biopsies	1963 ± 880	1442 ± 502	0.005
Treatment before recruitment (interferon-based therapy /no treatment)	4/35	14/16	0.001
Hemoglobin (g/100 ml; 13.7–17)	15 ± 1.4	15 ± 1.5	0.894
Serum iron (μmol/l; 11–32)	22.2 ± 7	24.7 ± 7	0.136
Transferrin saturation (%; 20–50)	40 ± 14	43 ± 11	0.327
Serum ferritin (ng/ml; 5–365)	246 ± 175	390 ± 235	0.005
Hepatic iron concentration (μmol/g dry tissue; <25)[c]	18 ± 9.5	24 ± 8	0.014
Iron index (hepatic iron concentration/age; <1.9)[c]	0.40 ± 0.24	0.42 ± 0.15	0.753
Hepatic iron grading Searle's score system (0/1/2/3/4)	23/13/1/0/2	6/18/6/0/0	0.002
Steatosis grade (0/1/2/3)[d]	7/24/7/1	10/13/5/0	0.196
Aspartate aminotransferase (U/l; 0–40)	83 ± 52	106 ± 56	0.089
Alanine aminotransferase (U/l; 0–40)	123 ± 72	129 ± 57	0.725
γ-Glutamyl transpeptidase (U/l; 0–50)	73 ± 67	82 ± 59	0.539
Histological grading (Knodell score)[e]	5	5	0.877
Histological staging (Knodell score 0/1/3/4)	1/22/12/4	0/15/14/1	0.404
Advanced fibrosis (Knodell staging score 3–4) (yes/no)	16/23	15/15	0.476

Values are means ± SD, counts or medians when indicated.
[a]Group 1 (interferon-based therapy); group 2 (phlebotomy).
[b]Comparisons were performed with two sample *t*-test, the Fisher's exact test or the Wilcoxon rank–sum test.
[c]Data were available for 66 of 69 patients.
[d]Data were available for 67 of 69 patients. Steatosis grade: 0 = no steatosis, 1 ≤ 33% of hepatocytes, 234–66%, 3 ≥ 66%.
[e]Values are medians.

patients included in the study are summarized in Table 1. The study was planned according to the guidelines of the local ethical committee in conformity with the 1975 Declaration of Helsinki and all patients gave informed consent to retrospective data analysis.

Assessment of liver histology

Liver biopsies were performed using a modified Menghini procedure. Five-micron-thick sections were stained with haematoxylin/eosin, Masson's trichrome, and periodic acid-Schiff after diastase digestion as well as with the Gomori's method for reticulin and the Perls's method for iron staining. Only specimens with a minimum length of 15 mm and at least six portal tracts were considered adequate for histological assessment. Liver biopsy specimens were scored in a blind fashion by two expert pathologists (R.B., C.B.) unaware of the clinical data. In the case of discordant opinions, the two examiners analyzed together the discrepancies to reach a consensus.

The original histology activity index proposed by Knodell [16] was used for grading inflammation/necrosis and for staging fibrosis. By this scoring system inflammation/necrosis score ranges from 0 to 18 (0–10 periportal ± bridging necrosis, 0–4 intralobular degeneration and focal necrosis, 0–4 portal inflammation), whereas fibrosis stage only includes four steps: 0, no fibrosis; 1, fibrous portal expansion; 3, bridging fibrosis; 4, cirrhosis. Steatosis was scored semiquantitatively as: 0 = no steatosis, 1 = steatosis 33% or less of hepatocytes, 2 = steatosis in 34–66% of hepatocytes, 3 = steatosis in at least 66% of hepatocytes. Intrahepatic iron deposition was evaluated in specimens stained by the Perls' method and evaluated using the Searle's semiquantitative score [17]. As in previous studies, patients were defined as histologically improved when they showed at least one point reduction of staging score or, in the case of unchanged staging score, an at least two point reduction of the grading score [18,19].

Measurement of hepatic iron content

HIC was measured by atomic absorption spectroscopy on a portion of each liver biopsy, as previously reported [20], and the values were expressed as μmol/g dry weight. Normal cut-off values for HIC were taken at 25 μmol/g. Hepatic iron index was calculated to rule out phenotypic hemochromatosis.

Statistical analysis

Stata Statistical Software (release 10.0 College Station, Stata Corporation, Texas, USA) was used in all of the statistical analyses. Each variable predictive or associated with the presence of histological response was analyzed with univariate logistic regression. The variables selected by every univariate analysis were entered into logistic regression models with the use of a forward stepwise elimination algorithm (terms with $P > 0.05$ were eligible for removal). The characteristics of the patients at the time of their first liver biopsy, grouped by treatment, were compared with the two-sample *t*-test, the Fisher's exact test, or the Wilcoxon rank-sum test. Clinical and laboratory changes according to the treatment between the two liver biopsies were compared with the paired Student's *t*-test or the Wilcoxon matched-pairs signed-

Table 3 Clinical and laboratory changes according to the treatment between the two liver biopsies

Variable	First biopsy[a]	Second biopsy[a]	*P* value[d]
Alcohol intake (g/day): all patients	12.3 ± 15	5.65 ± 8	<0.0001
Treated with interferon-based therapy	11 ± 12.6	4.3 ± 6.4	0.003
Treated with long-term phlebotomy	14.3 ± 18	7.3 ± 9.4	0.003
BMI (kg/m^2): all patients	24.7 ± 3.2	24.8 ± 3.3	0.31
Treated with interferon-based therapy	24.9 ± 2.8	25.1 ± 2.9	0.24
Treated with long-term phlebotomy	24.4 ± 3.8	24.4 ± 3.7	0.94
Aspartate aminotransferase (U/l; 0–40): all patients	93 ± 55	73 ± 56	0.022
Treated with interferon-based therapy	83.5 ± 52	80.3 ± 62	0.706
Treated with long-term phlebotomy	106 ± 56	62.8 ± 47	<0.0001
Alanine aminotransferase (U/l; 0–40): all patients	126 ± 65	94 ± 63	0.0001
Treated with interferon-based therapy	123 ± 72	112 ± 71	0.35
Treated with long-term phlebotomy	106 ± 56	69 ± 38	0.001
γ-Glutamyl transpeptidase (U/l; 0–50): all patients	77 ± 63	69 ± 75	0.341
Treated with interferon-based therapy	72.8 ± 67	80.3.1 ± 86	0.55
Treated with long-term phlebotomy	82.3 ± 59	54 ± 55	0.003
Hemoglobin (g/100 ml; 13.7–17): all patients	14.7 ± 1.4	14.3 ± 1.5	0.001
Treated with interferon-based therapy	14.7 ± 1.4	14.7 ± 1.5	0.9
Treated with long-term phlebotomy	14.8 ± 1.5	13.9 ± 1.4	0.0001
Serum iron (μmol/l; 11–32): all patients	22 ± 7	19 ± 7	<0.0001
Treated with interferon-based therapy	22.2 ± 7	21 ± 6.5	0.19
Treated with long-term phlebotomy	24.7 ± 7	16.4 ± 6	<0.0001
Transferrin saturation (%; 20–50): all patients	41 ± 13	31 ± 13	<0.0001
Treated with interferon-based therapy	40 ± 14	36 ± 12	0.046
Treated with long-term phlebotomy	43 ± 11	24 ± 11	<0.0001
Serum ferritin (ng/ml; 5–365): all patients	308 ± 214	166 ± 187	<0.0001
Treated with interferon-based therapy	246 ± 175	260 ± 199	0.53
Treated with long-term phlebotomy	390 ± 235	44 ± 50	<0.0001
Histological grade (Knodell's score): all patients	*5*	*5*	0.88
Treated with interferon-based therapy	*5*	*6*	0.23
Treated with long-term phlebotomy	*5*	*4*	0.20
Histological stage (Knodell's score 0/1/3/4): all patients	1/37/26/5	3/28/30/8	0.13
Treated with interferon-based therapy	1/22/12/4	3/13/19/4	0.14
Treated with long-term phlebotomy	0/15/14/1	0/15/11/4	0.57
Steatosis grade (0/1/2/3)[b]: all patients	17/37/12/1	20/30/12/4	0.45
Treated with interferon-based therapy	7/24/7/1	8/18/10/3	0.12
Treated with long-term phlebotomy	10/13/5/0	12/12/3/1	0.41
Hepatic iron grade (0/1/2/3/4 according to Searle's score): all patients	29/31/7/0/2	49/14/3/1/2	0.004
Treated with interferon-based therapy	23/13/1/0/2	20/13/3/1/2	0.035
Treated with long-term phlebotomy	6/18/6/0/0	29/1/0/0/0	<0.0001
Hepatic iron concentration (μmol/g dry tissue; <25)[c]: all patients	21 ± 9.3	14 ± 11	<0.0001
Treated with interferon-based therapy	18 ± 9.3	19.5 ± 12	0.24
Treated with long-term phlebotomy	24 ± 8.4	8.2 ± 5.5	<0.0001

[a]Values are mean ± SD, counts or medians (italic characters).
[b]Data were available for 67 of 69 patients. Steatosis grade: 0 = no steatosis, 1 ≤ 33% of hepatocytes, 234–66%, 3 ≥ 66%.
[c]Data were available for 66 of 69 patients.
[d]Paired Student's *t*-test or Wilcoxon matched-pairs signed-ranks test.

ranks test. A *P* value of less than 0.05 was considered as statistically significant.

Results

The epidemiological, clinical, and laboratory characteristics of the 69 patients included in the study, recorded at the time of their first liver biopsy, are summarized in Table 2. The patients were grouped as receiving long-term phlebotomy (group 1) or interferon-based therapy (group 2). The patients receiving long-term phlebotomy were older and had higher serum ferritin, HIC, and hepatic iron grade at the time of their first liver biopsy than the nonresponders of the interferon-based therapy (IBT-NR) (Table 2). However, aminotransferase, γ-Glutamyl transpeptidase serum levels, grading, and staging scores at the time of the first liver biopsy did not significantly differ between the two groups (Table 2). The mean interval between the two liver biopsies was shorter in LTP patients (1442 ± 502 days) than in those treated with interferon-based therapy (1963 ± 880 days; $P = 0.005$).

At the time of the second liver biopsy AST ($P < 0.001$), ALT ($P = 0.001$), and GGT ($P = 0.003$) levels were significantly lowered only in the subjects receiving phlebotomy (Table 3). Overall, ALT levels decreased in all 30 LTP patients, whereas AST and GGT levels were lowered in 28 of 30 (93%) and 23 of 30 (77%), respectively. Moreover, eight of the 30 (27%) phlebotomized patients achieved persistently normal aminotransferase levels during the iron-depleting treatment. As expected hemoglobin, transferrin saturation, serum iron, serum ferritin, hepatic iron grade, and the HIC were significantly decreased only in phlebotomized patients. On the contrary, hepatic iron grade was slightly increased at the time of the second liver biopsy in IBT-NR patients (Table 3).

Table 4 Univariate and multivariate logistic regression analysis of the putative predictors of the histological improvement in 69 patients with chronic hepatitis C

	Odds ratio (95% CI)	*P* value
Univariate analysis		
Age (years)	1.01 (0.97–1.07)	0.53
Male sex (male vs. female)	2.5 (0.64–9.8)	0.190
Drug addiction (yes/no)	8.47 (0.8–87)	0.07
Previous transfusion (yes/no)	1.37 (0.47–4.03)	0.56
Alcohol intake (g/day)	1.02 (0.99–1.06)	0.11
BMI	0.94 (0.79–1.12)	0.483
HCV genotype (1 vs. non-1)	1.41 (0.49–4.05)	0.524
Previous antiviral treatment (yes/no)	1.32 (0.41–4.2)	0.637
Days between the two liver biopsies	1 (0.99–1.001)	0.66
Phlebotomy vs. Interferon therapy[b]	4.8 (1.56–14.86)	0.006
Aspartate aminotransferase (U/l)	1.01 (0.99–1.02)	0.156
Alanine aminotransferase (U/l)	1.00 (0.99–1.01)	0.198
γ-Glutamyl transpeptidase (U/l)	1.00 (0.99–1. 01)	0.415
Hemoglobin (g/100 ml)	1.32 (0.88–1.98)	0.182
Serum iron (μmol/l)	1.00 (0.98–1.01)	0.793
Transferrin saturation (%)	0.98 (0.94–1.02)	0.411
Serum ferritin (ng/ml)	1.002 (1.001–1.05)	0.066
Histological grade (Knodell's score)[b]	1.43 (1.13–1.82)	0.003
Histological stage (Knodell's score)	1.44 (0.93–2.24)	0.103
Steatosis grade (0/1/2/3)[c]	0.62 (0.28–1.37)	0.24
Hepatic iron concentration (μmol/g dry tissue)[a]	1.04 (0.98–1.1)	0.141
Iron index (hepatic iron concentration/age)[a]	3.87 (0.29–52)	0.306
Hepatic iron grading (Searle's score system)	1.16 (0.64–1.06)	0.61
Multivariate analysis		
Histological grade (Knodell's score)	1.62 (1.22–2.16)	0.001
Phlebotomy vs. Interferon therapy	10 (2.25–43.7)	0.002

[a]Data were available for 66 of 69 patients.
[b]These variables were selected to enter in the multivariate model.
[c]Data were available for 67 of 69 patients. Steatosis grade: 0 = no steatosis, 1 ≤ 33% of hepatocytes, 234–66%, 3 ≥ 66%.

During histological examination, it was found that the medians of the necroinflammatory grading and staging scores between the first and the second liver biopsy were not significantly different in the two groups (Table 3). HI was defined by at least one-point reduction of staging score or, in case of unchanged staging score, by at least two-point reduction of grading score, according to the Knodell's Hepatic Activity Index. HI was observed in 15 of 30 (50%) patients receiving phlebotomy and in six of 39 (15%) IBT-NR subjects ($P = 0.002$). Among the 21 patients showing HI, 10 showed improvement of both the grading and the staging scores, 10 of the grading score only, and one of the staging score only.

The univariate and multivariate analyses showed that histological grading score at the time of the first liver biopsy [odds ratio (OR): 1.62; confidence interval (CI) 1.22–2.16; $P = 0.001$], and the treatment with LTP (OR: 10; CI 2.25–43.7; $P = 0.002$) were the only factors predictive for HI (Table 4). These OR values were not substantially modified after correction for the difference in time laps between the two biopsies (histological grading at the first biopsy OR: 1.61; CI 1.23–2.19 and LTP OR: 10.8; CI 2.10–55.7).

Discussion

CHC patients unsuitable for antiviral treatments or not achieving sustained virological response to interferon–ribavirin therapy are at high risk for progression to cirrhosis, decompensated liver disease, and hepatocarcinoma [1,5]. Therefore, there is a high interest in developing alternative treatments that can attenuate the disease evolution. Mild iron accumulation is a common feature in liver biopsies from CHC patients and is associated with higher serum aminotransferase levels and worsening of liver fibrosis [6]. Consistently, several studies have demonstrated that lowering hepatic iron by phlebotomy alone or in combination with an iron-poor diet ameliorates serum aminotransferase levels in CHC patients who failed a sustained virological response by interferon-based treatments [8–11]. Furthermore, we have reported that menstruating women experienced a milder CHC than men of the same age in relation to the lower HIC due to blood losses [21]. Although phlebotomy does not interfere with HCV RNA levels [22], Yano *et al.* [12] have shown that in CHC patients nonresponders to interferon, a 5 year iron-reducing treatment significantly lowers necroinflammatory grading and prevents the worsening of fibrosis in two sequential liver biopsies. In addition, Kato *et al.* [23] have reported that phlebotomy, in combination with an iron-poor diet, significantly reduces the development of hepatocarcinomas associated to CHC.

Our present study not only confirms the capacity of phlebotomy to decrease aminotransferase release in Caucasian CHC patients [22], but also shows that 27% of the phlebotomized subjects achieve persistently normal aminotransferase levels during the iron-depleting treatment. Furthermore, half of our patients treated with phlebotomy alone showed hepatic histological

improvement (as defined by one-point reduction of the individual staging score or, in the case of unchanged staging score, as a two-point reduction of grading score) after a mean period of 4 years since the beginning of treatment. Recent reports have shown that histological improvement occurs spontaneously in a variable proportion of CHC patients in whom antiviral therapy failed to eradicate the virus [14,15]. Our data demonstrate that the frequency of histological improvements among phlebotomized patients is significantly higher ($P = 0.002$) than that occurring spontaneously in nonphlebotomized subjects. Furthermore, following the multivariate analysis phlebotomy results as a predictor of histological improvement in those patients. To our knowledge, this is the first attempt to evaluate the effectiveness of phlebotomy in CHC patients by a case–control analysis. However, we are well aware of the limitations of this study connected with its retrospective nature and the fact that most of the patients receiving the interferon-based therapy were recruited at the time when pegylated interferon and the combination therapy were not yet available.

So far the mechanisms responsible for the beneficial effects of phlebotomy in preventing the evolution of CHC have not been characterized in detail. Growing evidence indicates the oxidative stress as one of the mechanisms by which HCV causes hepatic injury [24]. In this context the capacity of the iron to exacerbate oxidative stress suggests that iron accumulation might amplify the processes leading to both hepatocellular damage and fibrosis [25–27]. Consistently, the measurement of 8-hydroxy-2′-deoxyguanosine, a well-recognized marker of oxidative stress, in liver biopsies from CHC patients is significantly associated with hepatic iron content and necroinflammation indices [28]. Moreover, iron depletion by phlebotomy greatly reduces the liver 8-hydroxy-2′-deoxyguanosine content in CHC patients [29], suggesting that the efficacy of phlebotomy in preventing the CHC progression might relay on the reduction of oxidative damage promoted by the combination of HCV infection and iron accumulation.

In conclusion, our results indicate that in Caucasian CHC patients long-term phlebotomy is effective in promoting histological improvement above that spontaneously occurring in patients without virological response to interferon-based therapy, supporting the usefulness of this treatment in attenuating CHC progression when other therapies have failed.

Acknowledgements

Author contributions: M. Sartori collected clinical data, and contributed to the study design and the writing of the manuscript; S. Andorno contributed to the study design and performed statistical analysis; A. Rossini and S. Carmagnola collected clinical data and contributed to the study design; R. Boldorini and C. Bozzola performed histological analysis; M. del Piano contributed to the study design; E. Albano supervised the work.

Conflicts of interest

There are no conflicts of interest.

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