Liver disease in chelated transfusion-dependent thalassemics: the role of iron overload and chronic hepatitis C

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ABSTRACT

Iron overload and hepatitis virus C infection cause liver fibrosis in thalassemics. In a monocentric retrospective analysis of liver disease in a cohort of 191 transfusion-dependent thalassemics, in 126 patients who had undergone liver biopsy (mean age 17.2 years; 58 hepatitis virus C-RNA positive and 68 hepatitis virus C-RNA negative) the liver iron concentration (median 2.4 mg/gr dry liver weight) was closely related to serum ferritin levels (R = 0.58; $p<0.0001$). Male gender (OR 4.12) and serum hepatitis virus C-RNA positivity (OR 11.04) were independent risk factors for advanced liver fibrosis. The majority of hepatitis virus C-RNA negative patients with low iron load did not develop liver fibrosis, while hepatitis virus C-RNA positive patients infected with genotype 1 or 4 and iron overload more frequently developed advanced fibrosis. Hepatitis virus C infection is the main risk factor for liver fibrosis in transfusion-dependent thalassemics. Adequate chelation therapy usually prevents the development of liver fibrosis in thalassemics free of hepatitis virus C-infection and reduces the risk of developing severe fibrosis in thalassemics with chronic hepatitis C.

Key words: thalassemia, liver iron concentration, ferritin, LIC, hepatitis C virus, serum HCV RNA, liver fibrosis, cirrhosis.

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Introduction

Thalassemics can develop liver fibrosis because of iron liver overload[1] and hepatitis virus C (HCV) infection.[2] Multicenter cross-sectional studies have reported that the development and the severity of liver fibrosis are strongly related to the extent of liver iron overload and to the presence of chronic HCV infection.[3-7] Long-term observation of thalassemics who had undergone bone marrow transplantation showed that severe iron overload and chronic HCV infection were independent risk factors for liver fibrosis progression.[8] In the last 20 years, intensive and adequate iron chelation therapy has reduced the iron overload in transfusion-dependent thalassemics,[9] and the screening of blood donors for HCV markers has minimized the risk of *de novo* HCV infection.[10]

As a result, younger thalassemics should have a lower risk of developing liver fibrosis, and the role of HCV infection should be prominent in adult patients.

We analyzed biochemical, virological and histological features of a cohort of transfusion-dependent thalassemics who had undergone chelation therapy and who had had a 15 year follow-up.

Design and Methods

Patients

We performed a retrospective analysis of a prospective cohort of 191 transfusion-dependent thalassemics followed since 1990 at the Center for Thalassemia at the Paediatric Hospital in Palermo, Italy. All patients underwent regular blood transfusions maintaining pre-transfusion hemoglobin values at 9-9.5 g/dL and were treated with iron chelation therapy. The children started deferoxamine at a dose of 20 mg/kg infused subcutaneously 5–6 days per week, and doses were increased during infancy until reaching 40–60 mg/kg/day in adults. Deferiprone was administrated at a dose of 75 mg/kg/day. The iron chelation therapy was defined as adequate if the serum ferritin values, checked every six months, were always lower than 2,500 ng/mL.[11] Patients underwent splenectomy if the volume of blood transfused was more than 250 mL/kg/year. The study was performed in accordance with the principles of Good Clinical Practice and was approved by the hospital's Ethics Committee. All adult patients, or parents of pediatric patients, gave their consent to the collection of all clinical data in a database. All patients were followed until December 2007 or death.

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Biochemical, virological and histological data

Serum ALT was measured at each transfusion, and serum ferritin every six months. Anti-HCV antibodies were tested by enzyme-immunoassay (EIA-2 and EIA-3 by Ortho Diagnostic Systems, Raritan, NJ, USA) at baseline. Anti-HCV positive patients were tested for qualitative serum HCV-RNA by polymerase chain reaction (Amplicor HCV, Roche Molecular Systems, Basel, Switzerland) at baseline, at the time of liver biopsy and every year thereafter. HCV genotypes were determined by a line probe assay (Innolipa, Innogenetics, Belgium).

A liver biopsy was proposed to all patients who underwent splenectomy, to all HCV-RNA positive patients before the antiviral therapy with interferon, and to patients who were to be switched to new iron chelation drugs. We evaluated the first biopsy of each patient, and none of the HCV-RNA positive patients were treated with antiviral therapy before undergoing liver biopsy. The degree of inflammation and the staging of fibrosis were evaluated with the Scheuer score[12] by a single pathologist. The liver iron concentration (LIC) was measured on fresh tissue cores that weighed more than 4 mg to reduce the variability of the measurement.[13] Measurements were performed by atomic absorption spectrometry using the Spectra 880 (Varian, Australia). Results were expressed as mg of iron per gram of liver, dry weight, and 1.8 mg/gr was considered the normal limit.

Statistical methods

All data were entered into a database and analyzed using SPSS 13.0 for Windows software (SPSS Inc., Chicago, IL, USA). The differences between continuous data were analyzed by parametric test (t-test) for variables with normal distribution, and by non-parametric test (Mann-Whitney) for iron overload indices. χ^2 analysis was used for dichotomous or categorical variables. Multiple logistic regression models were used to assess the relationships between demographic data, biochemical features, virological characteristics, liver iron overload of the patients, and liver fibrosis. Variables found to be significant on univariate analysis ($p<0.05$) were included in multivariate logistic regression models.

Results and Discussion

From 1990 we followed 191 transfusion-dependent thalassemics (167 were born before 1990, and 24 between 1991 and 1998). Sixty patients (31.4%) were anti-HCV negative, 63 patients (32.9%) were anti-HCV positive but persistently HCV-RNA negative, and 68 patients (35.7%) had chronic active hepatitis C because they were anti-HCV positive and persistently HCV-RNA positive. One hundred and twenty-six patients underwent liver biopsy during the 15 years of observation (Figure 1). At the time of biopsy, the mean age of these patients was 17.3 years; the median serum ferritin was 1,884 ng/mL, and 96 patients (76%) had values of serum ferritin persistently lower than 2,500 ng/mL; 58 patients (46%) were HCV-RNA positive, and 68 (54%) were HCV-RNA negative.

Liver iron concentration was measured on 100 available fresh tissue cores that weighed more than 4 mg (51 HCV-RNA negative patients, and 49 HCV-RNA positive patients). Overall, median LIC was 2.4 mg/gr dry liver weight (range 0.3-22 mg), 15 patients (14%) had LIC values higher than 7 mg/gr and only 2 patients values higher than 15 mg/gr dry liver weight. There were no differences in LIC between intra-operative liver biopsy performed during splenectomy and needle liver biopsy. LIC was closely related to serum ferritin levels (R=0.58; $p<0.0001$ 95% CI 0.442-0.695). There were no significant differences in iron overload between thalassemics with and without HCV infection, as shown by median values of serum ferritin and LIC, whereas HCV-RNA positive patients had a higher mean age, higher serum ALT levels, more severe liver inflammation, and more frequently had severe liver fibrosis or cirrhosis (Table 1). Male gender, high serum ALT values and HCV-RNA-positivity, but not serum ferritin levels or LIC values, were significantly associated with severe fibrosis or cirrhosis in univariate analysis (Table 2). Multivariate analysis showed that male gender (Adjusted Odds Ratio 4.12; 95% CI 1.32-12.84) and serum HCV-RNA-positivity (Adjusted Odds Ratio 11.04; 95% CI 2.99-40.79) were independent and significant risk factors for severe fibrosis or cirrhosis. A sub-analysis of 68 HCV-RNA negative patients showed that 21 out of 23 patients without liver fibrosis, but only 19 out of 45 patients with each grade of liver fibrosis, had serum ferritin values persistently lower than 2,500 ng/mL ($p=0.02$). As regards LIC, 8 out of 16 patients without liver fibrosis and 5 out of 35 patients with each grade of liver fibrosis had values lower than 1.8 mg/gr dry liver weight ($p=0.01$). The mean age of HCV-RNA negative patients without iron overload was 16.6 years. The 3 HCV-RNA negative patients with severe fibrosis or cirrhosis had a median LIC of 4.9 mg/gr dry liver weight, and a mean age of 14.3 years. Among HCV-RNA positive patients, 34 out of 39 with absent, mild or moderate fibrosis had serum ferritin values persistently lower than 2,500 ng/mL compared to 12 out of 19 patients with severe fibrosis or cirrhosis ($p=0.04$). As regards the liver iron concentration of this group of patients, 19 out of 32 with absent, mild or mod-

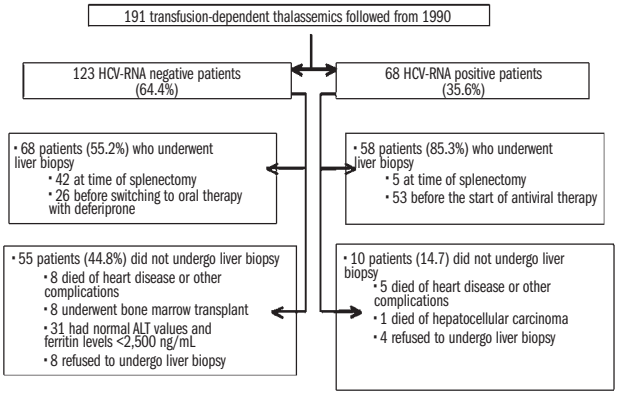

Figure 1. Flow chart of patients followed in the center, patients with and without hepatitis virus C infection, and patients who did or did not undergo liver biopsy.

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erate fibrosis versus 4 out of 13 with severe fibrosis or cirrhosis had values lower than 1.8 mg/gr dry liver weight (p=0.03). The median LIC in HCV-RNA positive patients with severe fibrosis or cirrhosis was 2.9 mg/gr dry liver weight. Finally, among patients with HCV hepatitis, infection with genotypes 1 or 4 was significantly associated with severe fibrosis or cirrhosis compared to genotypes 2 or 3 (19/49 *vs.* 0/9; p=0.02). Most adult thalassemics present iron overload and HCV infection, while young subjects, born in the era of regular transfusion regimens and intensive chelation therapy, have a mild iron overload and are frequently free of HCV infection. Several published studies have reported that the progression of liver fibrosis is strongly related to the extent of iron overload and to the presence of chronic HCV infection. Retrospective or cross-sectional datasets have shown some limitations and bias, including the high number of centers that selected patients in multicenter studies[3,5] or the low number of patients who underwent liver biopsies in the studies performed in single centers,[4,6,7] the selection of patients with biochemical and virological features of chronic hepatitis C[5] or with high iron overload,[14] the measurement of LIC by different laboratories[5,8] and the analysis of liver biopsy by several pathologists.[3] We evaluated liver damage in a cohort of 191 transfusion-dependent thalassemics treated with blood transfusion and adequate iron chelation drugs. The majority of thalassemics transfused before 1990 were infected by HCV in the first years of life. In fact, at baseline 131 patients (68%) were positive for anti-HCV antibodies, but only half of them had active chronic HCV infection, as shown by persistence of serum HCV-RNA. Patients with chronic hepatitis C were older, had higher ALT values and degrees of inflammation, and more frequently had a severe liver fibrosis or cirrhosis, while there were no differences in iron overload between the two groups, as shown by values of serum ferritin and LIC. The mean value of serum ferritin in all patients who underwent liver biopsy was lower than 2,000 ng/mL and the median value of LIC in 100 patients with adequate fresh tissue specimens was 2.4 mg/gr dry liver weight.

The indices of iron overload observed in our cohort were lower than those reported in other studies.[5-8] The high number of patients with values of serum ferritin persistently lower than 2,500 ng/mL suggests an adequate iron chelation treatment and good adherence to therapy. Some published studies have reported median values of serum ferritin lower than 2,000 ng/mL, but a median LIC higher than 6 mg/gr dry liver weight.[3,5,6] These data could be related to the methods used to measure LIC. We measured LIC in a single laboratory using the same method for the entire period of observation and used only fresh tissue cores that weighed more than 4 mg in order to reduce the variability of the results. The analysis showed no differences between LIC measured on liver biopsy performed during splenectomy and needle biopsy, and LIC was closely related to serum ferritin levels, as reported in other studies.[3,7]

The separate analysis of the HCV-RNA-negative group showed that one third of the patients had no liver fibrosis and that the majority of them had values of serum ferritin lower than 2,500 mg/dL and LIC lower than 1.8 mg/gr dry liver weight. The median LIC value in 3 HCV-RNA negative patients with severe fibrosis or cirrhosis was 4.9 mg/gr dry liver weight. There was no difference regarding age between HCV-RNA negative patients with or without liver fibrosis. These data confirm that patients free of HCV infection with a good adherence to chelation therapy usually do not develop liver fibrosis, and that only values higher than 5 mg/gr dry liver weight are associated with severe fibrosis.[8] HCV-RNA-positive patients had higher serum ALT levels, more severe liver inflammation and more frequently had severe liver fibrosis or cirrhosis. Multivariate analysis showed that male gender and serum HCV-RNA positivity were independent and significant risk factors for severe fibrosis or cirrhosis. Several studies suggest that HCV infection acquired early in life shows a slow pro-

Table 1. Clinical, biochemical, virological and histological features of 126 patients who underwent liver biopsy.

	HCV-RNA negative (68 patients)	*HCV-RNA positive (58 patients)*	*p*
Age (mean,SD)	13.9±(8.85)	21.2± (8.87)	<0.001
Gender (M/F)	35/33	32/26	0.681
ALT (median, range)	27 (9-200)	82 (17-547)	<0.001
Ferritin (median, range)	1,892 (141-5,952)	1,750 (188- 5,503)	0.590
LIC (median, range)	3.3 (0.3-22)	2.3 (0.4-11.8)	0.064
Histological inflammation (grading)			
Absent	23 (34%)	0	
Mild/moderate	45 (66%)	56 (96%)	<0.001
Severe	0	2 (4%)	
Histological fibrosis			
Absent	23 (34%)	2 (4%)	
Mild/moderate	42 (62%)	37 (64%)	<0.001
Severe/cirrhosis	3 (4%)	19 (32%)	

Scheuer score (12). Histological inflammation (grading): absent (grade 0), mild (grade 1), moderate (grade 2), severe (grade 3). Histological fibrosis (staging): no fibrosis (stage 0), periportal fibrosis (stage 1), porto-portal septa (stage 2), porto-central septa (stage 3), cirrhosis (stage 4).

Table 2. Univariate analysis of biochemical, virological and histological features associated with severe fibrosis (Stage 3 and 4 by Scheuer score) versus milder or no fibrosis (Stages 0-2) in 126 patients who underwent liver biopsy.

	Fibrosis stage 0-1-2 (104 patients)	*Fibrosis stage 3- 4 (22 patients)*	*p*
Age (mean,SD)	16.8±8.7	19.7±9.2	0.2
Gender (M/F)	50/54	17/5	0.01
ALT (mean, SD)	69.1±80.1	112.5±61.2	< 0.001
Ferritin (median, range)	1,583 (141-5,952)	2,115 (188- 5,503)	0.3
HCV-RNA positive	39 (37.5%)	19 (86.4%)	< 0.001
LIC (median, range)	2.3 (0.3-22)	2.9 (0.4-11.8)	0.3
Histological inflammation (grading)			
Grade 0	23 (22%)	0	
Grade 1/Grade 2	79 (76%)	22 (100%)	
Grade 3	2 (2%)	0	0.2

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gression during the first 20 - 30 years of life,[15-17] but other studies report that despite a young age at the time of HCV infection, the progression of liver disease in children is comparable to that seen in adults.[18] Among our 58 patients with active chronic hepatitis C, 14 patients (24%) had cirrhosis. Patients with mild or moderate fibrosis frequently had low values of serum ferritin and normal LIC, while patients with severe liver fibrosis or cirrhosis more frequently had values of serum ferritin higher than 2,500 ng/mL and a median LIC value 2.9 mg/gr dry liver weight. These findings confirm that HCV-RNA-positive patients with normal liver iron concentration usually do not develop severe liver fibrosis during the first 20-30 years of life, whereas patients with active HCV replication and moderate iron overload more frequently develop severe fibrosis or cirrhosis.[8] Finally, in thalassemics with active HCV infection, another determinant of liver disease progression may be infection with genotypes 1 or 4, as reported in a long-term observational cohort of adult subjects with chronic hepatitis C.[19]

In conclusion, HCV infection is the main risk factor for liver fibrosis in transfusion-dependent thalassemics and infection with genotypes 1 or 4 increases the risk of developing severe fibrosis. Adherence to adequate chelation therapy usually prevents the development of liver fibrosis in thalassemics free of HCV-infection and reduces the risks of developing severe fibrosis in thalassemics with chronic hepatitis C.

Authorship and Disclosures

VDM: co-odinator of the study,conception and design, analysis and interpretation of data; drafting the article, revisiting critically the article and approval the final version; MC coordinator of the centre,analysis and interpretation of data, conception and design, revisiting critically the article and approval the final version; FG, ZB, DC, FB, DF, LC, GBR, FB: acquisition of data, revisiting critically the article and approval of the final version; RDS, PLA: analysis and interpretation of data; drafting the article, revisiting critically the article and approval the final version; AC: co-ordinator of study group, revisiting critically the article and approval the final version.

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