

Liver iron concentration and distribution in chronic hepatitis C before and after interferon treatment

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Abstract

Background—Recent studies have suggested that, in patients with chronic hepatitis C, elevated iron stores are predictive of a poor response to interferon.

Aims—To assess liver iron concentration and distribution before and after interferon treatment in patients with hepatitis C in order to evaluate further the role of iron in the pathogenesis of hepatitis C.

Patients—Fifty five patients with hepatitis C treated with alpha interferon for six months.

Methods—Patients were evaluated for liver iron concentration (normal value <36 $\mu\text{mol/g}$), and liver iron distribution before and six months after therapy.

Results—At entry: liver iron concentration was elevated in 16/55 patients (29%); iron staining (Perls' staining) was found in 31/55 patients (56%) mainly within Kupffer and endothelial cells. Iron load was significantly higher in patients with the most histological inflammatory activity. Following treatment: liver iron concentration decreased significantly (40 (24) to 30 (17) $\mu\text{mol/g}$, $p=0.001$); this was related to iron depletion in mesenchymal cells. Iron depletion occurred regardless of the response to therapy. Elevated liver iron concentration was not found to be a predictive factor of failure of interferon.

Conclusion—Although liver iron stores were usually normal or only slightly elevated in patients with chronic hepatitis C, biochemical and histological liver iron content decreased following treatment due to the diminution in mesenchymal iron deposits. Iron depletion was interpreted as both a consequence of the anti-inflammatory effect of treatment and a factor of improvement in liver histology.

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Keywords: chronic active C hepatitis; interferon; iron overload; response to interferon treatment

Recent studies have suggested that, in patients with chronic hepatitis C, elevated iron stores assessed by either serum ferritin¹⁻³ and/or liver iron concentration³⁻⁵ are predictive of a poor response to interferon (IFN) treatment. Despite hepatic iron concentrations which are often within the normal range,⁴⁻⁶ studies of iron depletion by venesection have been performed

to try to improve the response rate to interferon treatment.⁷⁻⁹ The increase in liver iron could be related to the activity of the inflammatory process as suggested by the correlation between: serum ferritin and the degree of hepatic injury (measured by the serum transaminases activity)⁶; and the presence of iron deposits within Kupffer cells and portal macrophages.¹⁰ If hepatic iron deposition is a secondary response to chronic inflammation, IFN treatment should result in a decrease in hepatic iron. In this study, the effect of antiviral treatment on liver iron concentration and distribution was studied before and after treatment to evaluate further the role of iron in the pathogenesis of chronic hepatitis C.

Methods

SELECTION OF PATIENTS

Fifty five patients (36 males and 19 females) with chronic hepatitis C were studied retrospectively. All had been enrolled into a randomised trial comparing IFN for six months (5 million units for the first month (m1), 3 million units for the second month (m2), and 3 or 5 million units for the last four months according to whether serum alanine aminotransferase (ALT) levels had been normalised or not) ($n=28$) versus INF from m1 to m6 plus ursodeoxycholic acid (UDCA, 10 mg/kg/day) from m1 to m9 ($n=27$).¹¹ Patients were selected on the basis of the availability of a liver biopsy specimen both before (m0) and after (m12) treatment; this was not the case for the 25 remaining patients included in the therapeutic trial. As the IFN and IFN+UDCA treated groups were identical with respect to: clinical, biochemical, and virological data; response to therapy; and iron studies (table 1), the group of 55 patients was studied as a whole.

DIAGNOSTIC CRITERIA

The diagnosis of chronic hepatitis C was based on abnormal serum ALT for more than six months, positive hepatitis C virus (HCV) serology (second generation tests - ELISA and RIBA, Ortho Diagnostic Systems, Raritan, NJ, USA), and positive viraemia when patients entered the study. HCV viraemia was assessed by reverse transcription-polymerase chain reaction (RT-PCR) using primers from the

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TABLE 1 General, biochemical, and histological characteristics of the 55 patients in the whole population and according to treatment regimen

	IFN (n=28)		IFN+UDCA (n=12)		Total (n=55)	
General data						
Age (y) mean (SD)	37 (14)		39 (14)		43 (14)	
Sex ratio (female/male) (%)	8 (28)/20 (72)		11 (40)/16 (60)		19 (34)/36 (66)	
Liver function tests						
ALT (×ULN) (mean (SD))	<i>m0</i>	3.4 (3.7)	2.9 (1.8)		3.1 (2.9)	
	<i>m6</i>	0.8 (0.5)	0.8 (0.7)		0.9 (0.7)	
	<i>m12</i>	2 (2)	1.9 (1.7)		1.9 (1.8)	
Virology						
HCV-RNA (+) (mean (%))	<i>m0</i>	28 (100)	<i>m12</i>	20 (74)	<i>m0</i>	54 (98)
					<i>m12</i>	41 (77)
Response to treatment (HCV-RNA negative)						
No response (mean (%))	13 (38)		9 (34)		22 (41)	
Response and relapse (mean (%))	7 (26)		12 (45)		19 (36)	
Sustained response (mean (%))	7 (26)		5 (23)		12 (23)	
Liver biopsy						
Cirrhosis (mean (%))	<i>m0</i>	3 (10)	<i>m12</i>	3 (10)	<i>m0</i>	6 (11)
					<i>m12</i>	6 (11)
Knodell score (mean (SD))	<i>m0</i>	7.8 (4)	<i>m12</i>	7.5 (4)	<i>m0</i>	8.4 (3.6)
					<i>m12</i>	7.6 (4.3)
METAVIR score (mean (%))	<i>m0</i>		<i>m12</i>		<i>m0</i>	
A0=no activity		1 (0.3)		1 (0.3)		1 (0.2)
A1=mild activity		9 (32)		14 (50)		26 (47)
A2=moderate activity		10 (35)		7 (40)		11 (20)
A3=severe activity		6 (21)		4 (14)		11 (20)
Iron studies (mean (SD))						
LIC (normal <36 μmol/g)	<i>m0</i>	33.0 (14.0)	48.0 (30.0)		40.0 (24.0)	
	<i>m12</i>	26.0 (11.0)	35.0 (22.0)		30.0 (17.0)	
Total iron score (0-60)	<i>m0</i>	2.0 (2.8)	3.9 (4.2)		2.9 (3.7)	
	<i>m12</i>	0.8 (1.7)	1.9 (2.1)		1.3 (2.1)	
Mesenchymal iron (0-24)	<i>m0</i>	1.4 (2.1)	2.5 (2.7)		1.8 (2.8)	
	<i>m12</i>	0.5 (0.2)	1.3 (1.5)		0.9 (1.6)	
Hepatocytic iron (0-36)	<i>m0</i>	0.6 (1.6)	0.9 (1.9)		0.9 (1.8)	
	<i>m12</i>	0.3 (0.9)	0.6 (1.1)		0.5 (0.9)	

No significant difference was found between the two treatment groups for all data. IFN, interferon; UDCA, ursodeoxycholic acid; ULN, upper limit of normal; LIC, liver iron concentration.

5' non-coding region (sense primer RC1 (5'GTCTAGCCATGGCGTTAGTA3') and antisense primer RC2 (5'TCTCGCGGGG-CACGCCCAA3')), which corresponded respectively to nucleotides 246 to 265 and 96 to 115 on the HCV1 sequence.¹² Quantitative HCV-RNA and genotype were not assessed in this study. Response to treatment was assessed according to HCV-RNA: "response" was defined as negative viraemia at the end of IFN treatment; "non-response" as positive viraemia at the end of IFN treatment; "response and relapse" as negative viraemia at the end of IFN treatment and positive viraemia after six months of follow up; and "sustained response" as negative viraemia after six months of follow up.

TABLE 2 Histological iron grading

Hepatocytic iron score	Zone 1 ^a	+ Zone 2	+ Zone 3	=HIS (0-36)
Absence of iron	0	0	0	
Faint staining	3	3	3	
Non-confluent granules	6	6	6	
Confluent deposits	9	9	9	
Large masses	12	12	12	+
Sinusoidal iron score^b				
	Zone 1	+ Zone 2	+ Zone 3	=SIS (0-12)
Absence of iron	0	0	0	
Faint staining	1	1	1	
Small granules	2	2	2	
Large deposits	3	3	3	+
Clusters of overloaded cells	4	4	4	
Portal tract iron score				
	Phagocytes	+ Biliary cells	+ Vascular walls	=PIS (0-24)
Absence of iron	0	0	0	
Deposits in <1/3 portal tracts	1	1	1	
Deposits in 1/3 portal tracts	2	2	2	
Deposits in 2/3 portal tracts	3	3	3	
Deposits in all portal tracts	4	4	4	
Total iron score				=TIS (0-60)

^aZone refers to Rappaport's acinus. ^bSinusoidal refers to Kupffer, endothelial and stellate cells. Mesenchymal iron score (MIS)=sinusoidal iron score (SIS)+portal iron score (PIS). TIS=total iron score=MIS+hepatocytic iron score (HIS).

HISTOLOGICAL STUDIES

Paraffin wax embedded blocks were cut for Perls' staining. Iron deposits were assessed according to both their amount and their cellular and lobular location in Rappaport's acinus,¹³ using the grading of Deugnier *et al*¹⁴ modified in order to take into account the heterogeneity of iron distribution (table 2). This grading led to three different iron scores: hepatocytic (HIS, range: 0-36), sinusoidal (SIS, range: 0-12), and portal (PIS, range: 0-12). The sum of these three scores defined the total iron score (TIS, range: 0-60) and the sum of the sinusoidal and portal iron scores defined the mesenchymal iron score (MIS, range: 0-24). In order to quantitate the degree of inflammation, liver biopsy specimens were blindly reviewed by a single pathologist (YD) and classified as having mild, moderate, and severe activity according to METAVIR classification. This is a simple and validated method for categorising chronic viral hepatitis.^{15 16}

LIVER IRON CONCENTRATION

The liver iron concentration (LIC) was determined according to Barry and Sherlock¹⁷ on biopsy samples taken from paraffin wax embedded blocks (normal concentration <36 μmol/g dry weight).¹⁸

STATISTICAL ANALYSIS

Values were expressed as mean or percentage (SD). Data were compared using Wilcoxon's test or ANOVA for paired data with Bonferroni correction, Mann-Whitney U test, and χ^2 test. All p values were two-sided; p values

≤ 0.05 were considered statistically significant.

Results

PRETREATMENT DATA

Liver iron concentration was elevated ($>36 \mu\text{mol/g}$) in 16/55 patients only. Positive iron staining was found in 31/55 (56%) patients. Cellular iron distribution was mainly located in non-hepatocyte cells, as shown by the histological semi-quantitative analysis (table 1). Indeed, iron was found within Kupffer and endothelial cells (fig 1A) and, to a lesser extent, within portal macrophages.

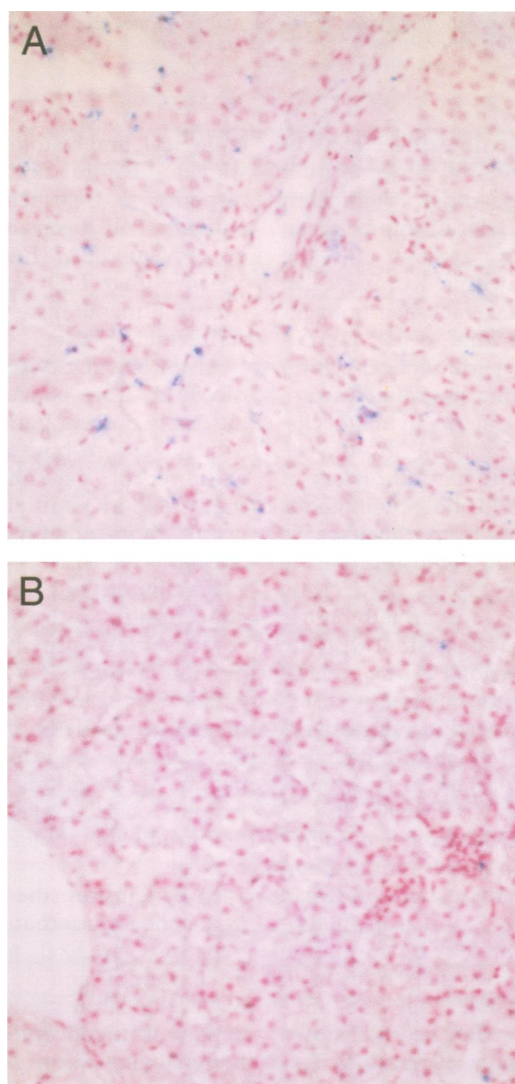


Figure 1: (A) Pretreatment liver biopsy specimen from a 26 year old woman, with post-transfusional hepatitis C ($ALT=2.2 \times$ upper limit of normal; liver iron concentration= $40 \mu\text{mol/g}$; total iron score=6; histological activity=moderate (A2)). Iron is mainly located within macrophages in the portal region. (B) Post-treatment liver biopsy specimen of the same patient who was a sustained responder to INF treatment ($ALT=0.3 \times$ upper limit of normal; liver iron concentration= $20 \mu\text{mol/g}$; total iron score=1; mesenchymal iron score=0; histological activity=mild). There was a reduction in the number of iron-loaded macrophages. Perls' method for iron $\times 180$.

EVOLUTION OF IRON STATUS UNDER THERAPY

Evolution of liver biochemical iron

As fig 2 shows, a significant decrease in LIC was observed following treatment (40 (24) to 30 (17) $\mu\text{mol/g}$, $p=0.001$).

Evolution of histological iron

A significant decrease in total iron score (2.9 (3.7) to 1.3 (2) $\mu\text{mol/g}$, $p=0.001$) was also found following treatment. Iron distribution was modified in the following way: there was a significantly lower mesenchymal iron score in the second liver biopsy series (1.8 (2.8) to 0.9 (1.6) $\mu\text{mol/g}$, $p=0.01$), while the hepatocytic iron score remained unmodified (figs 1B, 2); the decrease in total iron score was therefore mainly related to the removal of iron from mesenchymal cells.

Evolution of iron status according to histological activity (METAVIR score)

The total and mesenchymal iron scores were significantly higher in patients with the most active disease, and a decrease in total and mesenchymal iron scores was found following treatment, irrespective of the initial degree of disease activity (fig 3). The hepatocytic iron score did not differ according to disease activity (data not shown).

RESPONSE TO TREATMENT ACCORDING TO IRON STATUS

At the end of IFN therapy (m6), patients were distributed into responders ($n=33$, 60%) and non-responders ($n=22$, 40%). Six months after cessation of IFN treatment (m12), 19/33 responders (57%) had relapsed, 12 (22%) presented a sustained response, and two were not assessable.

Predictive value of iron status with regard to response to treatment

Pretreatment liver iron concentration, and mesenchymal and hepatocytic histological iron scores were not significantly increased in non-responders compared with responders, and thus were not predictive of failure of interferon therapy.

Evolution of iron status according to response to treatment

As fig 4 shows, liver iron concentration, and total and mesenchymal iron scores decreased significantly during treatment in non-responders and patients with relapse. In sustained responders, liver iron concentration and deposition decreased but this did not reach statistical significance.

Discussion

Previous studies of iron in hepatitis C have described iron deposition only in the initial liver biopsy sample.²⁻⁶ In this study, we

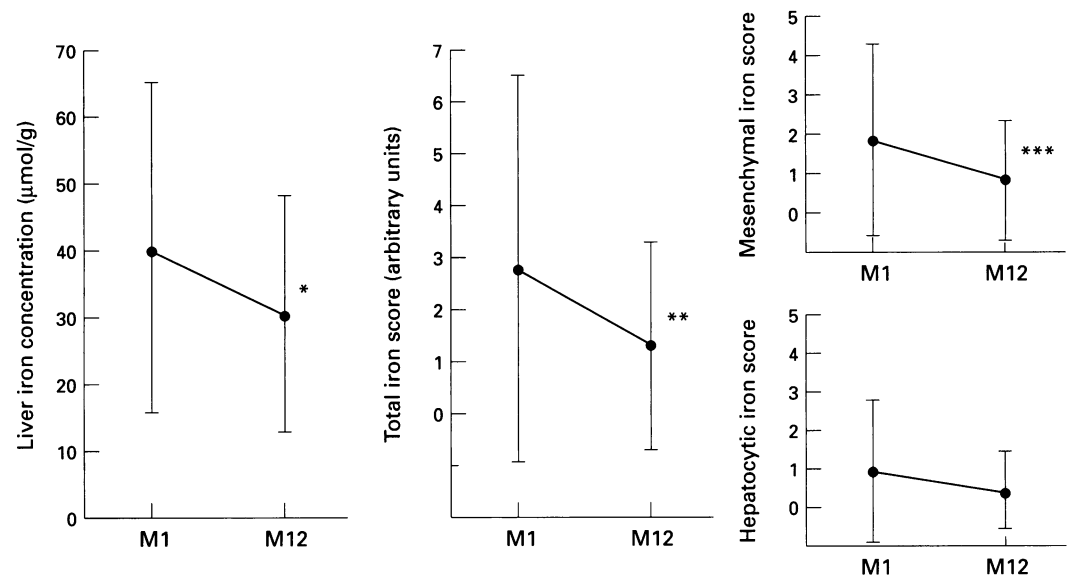


Figure 2: Evolution of liver iron concentration (mean (SD), normal <math><36 \mu\text{mol/g}</math>), and total, mesenchymal, and hepatocytic iron scores (mean (SD), arbitrary units) before and after treatment (* $p=0.001$; ** $p=0.001$; *** $p=0.004$).

demonstrated for the first time the effects of antiviral therapy on hepatic iron concentration and distribution. The most significant observations were: a clear relationship between hepatitis C inflammatory activity and the presence of iron deposits within Kupffer cells, endothelial cells, and portal tract macrophages (referred to as mesenchymal cells); and a significant decrease in liver iron concentration following treatment, primarily related to a removal of iron from mesenchymal cells. Although there was no control group, we believe it is unlikely that the natural history of chronic hepatitis C would be spontaneous progressive iron depletion from the liver. It is most likely that iron deposition is a consequence of the phagocytosis of necrotic hepatocytes secondary to the inflammatory process in the liver.¹⁹ This is consistent with the concept that mild iron overload is a sequela of chronic liver disease of various causes.²⁰

The iron depletion observed in our patients could be non-specifically related to a decrease in liver inflammation secondary to treatment. The relationship shown between disease activity and total and mesenchymal iron scores strongly supports this hypothesis. In addition there is a close relationship between inflammation, macrophages, and iron metabolism. Macrophages activated by inflammation have been shown to up regulate transferrin receptor²¹ and it is possible that the anti-inflammatory effect of IFN could down regulate transferrin receptor expression. Another explanation could be a shift of iron from the liver to extrahepatic sites, for example bone marrow. Indeed, IFN is known to induce a mild and transient bone marrow suppression followed, after cessation of therapy, by an increase in erythropoiesis.²² However, in this study, we did not notice any significant change in red blood cell count during or following therapy (data not shown).

A role of UDCA administration in iron depletion is unlikely, because all clinical,

biological, and histological data (including iron studies) before and after treatment, and response to treatment were similar whether patients had been treated with IFN alone or IFN+UDCA. Furthermore, it has been shown²³ that UDCA intake did not modify liver iron metabolism in both normal and iron-supplemented rats.

An interesting observation was the fact that the liver iron concentration decreased significantly even in patients that did not achieve a sustained response. This is in agreement with recent experimental studies showing that IFN, in addition to its antiviral action, exerts anti-inflammatory and antifibrotic effects.²⁴

Finally, in our study, elevated liver iron concentration was not predictive of a non-response to treatment. This is in contrast with previous studies³⁻⁵ which suggested that elevated liver iron concentration is a predictor of failure of IFN. This discrepancy could be related to differences between the patient populations analysed (number of patients, age, prevalence of cirrhosis), and definitions of clinical and biochemical outcomes.

In conclusion, the changes seen in hepatic iron concentration and distribution in chronic hepatitis C are likely to be secondary to chronic inflammation and do not represent a primary disorder of iron metabolism. The decrease in hepatic iron concentration and the removal of mesenchymal iron found after therapy are consistent with an anti-inflammatory effect of interferon and probably participate in the limitation of hepatic lesions by reducing iron-related damage. However, the place of adjuvant venesection in the treatment of patients with chronic hepatitis C remains to be determined.

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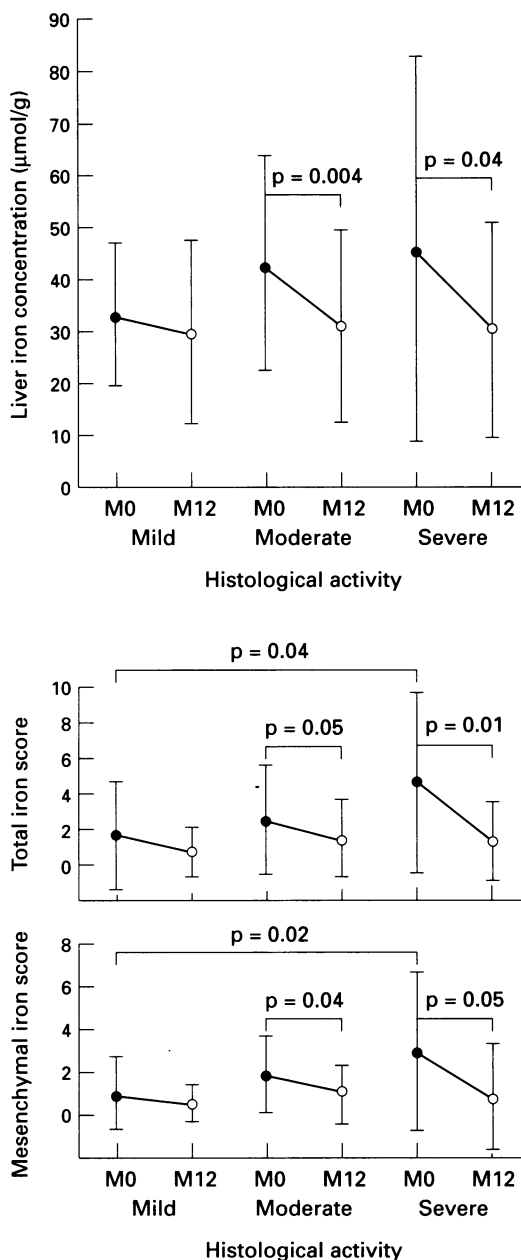


Figure 3: Evolution of liver iron concentration (mean (SD), normal <36 μmol/g), and total and mesenchymal iron scores (mean (SD), arbitrary units), before and after treatment related to histological disease activity according to METAVIR score.

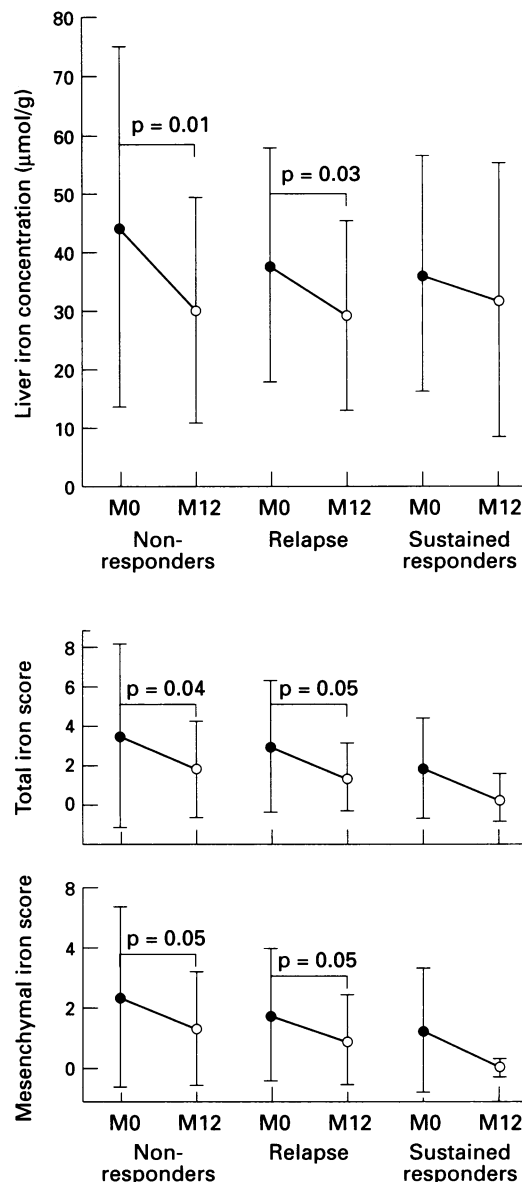


Figure 4: Evolution of liver iron concentration (mean (SD), normal <36 μmol/g), and total and mesenchymal iron score (mean (SD), arbitrary units), according to response to treatment.

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