

HEPATIC IRON CONCENTRATION AND TOTAL BODY IRON STORES  
IN THALASSEMIA MAJOREMANUELE ANGELUCCI, M.D., GARY M. BRITTENHAM, M.D., CHRISTINE E. MCLAREN, PH.D., MARTA RIPALTI, PH.D.,  
DONATELLA BARONCIANI, M.D., CLAUDIO GIARDINI, M.D., MARIA GALIMBERTI, M.D., PAOLA POLCHI, M.D.,  
AND GUIDO LUCARELLI, M.D.**ABSTRACT**

**Background and Methods** We tested the usefulness of measuring the hepatic iron concentration to evaluate total body iron stores in patients who had been cured of thalassemia major by bone marrow transplantation and who were undergoing phlebotomy treatment to remove excess iron.

**Results** We began treatment with phlebotomy a mean ( $\pm$ SD) of  $4.3 \pm 2.7$  years after transplantation in 48 patients without hepatic cirrhosis. In the group of 25 patients with liver-biopsy samples that were at least 1.0 mg in dry weight, there was a significant correlation between the decrease in the hepatic iron concentration and total body iron stores ( $r=0.98$ ,  $P<0.001$ ). Assuming that the hepatic iron concentration is reduced to zero with complete removal of body iron stores during phlebotomy, the amount of total body iron stores (in milligrams per kilogram of body weight) is equivalent to 10.6 times the hepatic iron concentration (in milligrams per gram of liver, dry weight). With the use of this equation, we could reliably estimate total body iron stores as high as 250 mg per kilogram of body weight, with a standard error of less than 7.9.

**Conclusions** The hepatic iron concentration is a reliable indicator of total body iron stores in patients with thalassemia major. In patients with transfusion-related iron overload, repeated determinations of the hepatic iron concentration can provide a quantitative means of measuring the long-term iron balance. (N Engl J Med 2000;343:327-31.)

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**Q**UANTITATIVE studies of iron balance in patients with thalassemia major have been limited by the lack of a method for determining the total amount of stored iron in the body. The plasma ferritin concentration and the amount of urinary iron excreted after the administration of an iron-chelating agent are only qualitative indexes of iron loading and are influenced by infection, inflammation, liver disease, ascorbate deficiency, and other factors.<sup>1</sup> Phlebotomy with careful measurement of the amount of iron in the blood removed is the most accurate means of measuring total body iron stores<sup>2</sup> but it cannot be used in transfusion-dependent patients. Measurement of the iron concentration in a liver-biopsy specimen is the reference method for assessing body iron stores,<sup>3</sup> but the relation between the hepatic iron concentration and the total amount of stored iron in the body has

been a missing link in our understanding of transfusion-related iron overload.

In patients with thalassemia major who have been cured by allogeneic bone marrow transplantation, the engrafted marrow mounts a hyperplastic erythropoietic response to phlebotomy.<sup>4</sup> Because humans lack an effective means of excreting excess iron, such patients retain the iron that accumulated before or in association with the transplantation.<sup>5</sup> Therapeutic phlebotomy is a safe and effective means of reducing body iron stores to normal levels and avoiding the problems associated with persistent iron overload in patients who have undergone successful bone marrow transplantation for thalassemia major.<sup>4</sup> To determine the relation between the hepatic iron concentration and total body iron stores, we conducted quantitative studies in patients undergoing therapeutic phlebotomy and repeated liver biopsy after successful marrow transplantation for thalassemia major.

**METHODS****Patients**

We studied patients who had undergone successful allogeneic bone marrow transplantation for thalassemia major and were enrolled in our program of regular phlebotomy for the treatment of persistent iron overload.<sup>4</sup> Patients with mixed hematopoietic chimerism or evidence of active chronic graft-versus-host disease were excluded from the study because they did not undergo phlebotomy. Written informed consent was obtained from all patients, their parents, or both after the investigative procedures and risks had been explained in detail. Protocols for transplantation, liver biopsy, and phlebotomy therapy were approved by the institutional review board of the Azienda Ospedale di Pesaro in Pesaro, Italy.

**Liver Biopsy**

Before phlebotomy treatment was started, samples for iron measurements and pathological studies were obtained by percutaneous biopsy of the center of the right lobe of the liver under ultrasound guidance.<sup>4</sup> A final biopsy was performed when the serum ferritin concentration was less than 250 ng per milliliter and the transferrin saturation was less than 50 percent. In some patients, interim liver samples were obtained to monitor phlebotomy therapy, the progression of fibrosis, or the activity of hepatitis. We used atomic absorption spectrophotometry to measure the hepatic iron concentration. The methods used for these measurements and for histologic

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assessment of fibrosis and cirrhosis have been described previously.<sup>6-8</sup> Atomic absorption spectrophotometry measures the total (heme and nonheme) content of iron, but for the purpose of our study, the contribution of nonheme iron was considered negligible.<sup>9</sup>

**Phlebotomy and Calculation of Total Body Iron Stores**

Patients underwent regular phlebotomy, with the removal of 6 ml of blood per kilogram of body weight every 14 days.<sup>4</sup> The amount of body iron removed by phlebotomy was calculated according to the amount of blood removed by phlebotomy, with adjustments for the amount of iron lost by menstruating women and the amount in the increased volume of blood in patients who were growing. The amount of blood removed during each phlebotomy session was recorded, the hemoglobin content measured, and the amount of iron calculated, assuming that each gram of hemoglobin contains 3.4 mg of iron.<sup>10</sup> For women with regular menses, we assumed an iron loss of 0.5 mg per day during each month of menstruation.<sup>11</sup> For patients whose body weight increased during the study, the amount of iron in the corresponding increased volume of blood was calculated, assuming a blood volume of 61.9 ml per kilogram for female patients and 62.4 ml per kilogram for male patients.<sup>10</sup> No adjustment was made for an increase in gastrointestinal iron absorption during phlebotomy.

**Statistical Analysis**

For continuous variables with a symmetric distribution, the results are expressed as means ±SD, and for those with a skewed distribution, the results are expressed as medians and interquartile ranges (25th to 75th percentile). Linear regression analysis<sup>12</sup> was used to determine the relation between the hepatic iron concentration and total body iron stores and to establish a linear equation for estimating total iron stores according to the reduction in the liver iron concentration that resulted from phlebotomy. The re-

gression model was fitted without an intercept term, on the assumption that complete removal of iron stores by phlebotomy would reduce the hepatic iron concentration to nearly zero. The coefficient of determination was calculated to estimate the proportion of variation in total body iron stores that could be accounted for by variation in hepatic iron stores. Regression analysis was also used to determine standard errors and 95 percent confidence intervals for the predicted total body iron stores, given the hepatic iron concentration. We used a cross-validation technique to predict residuals over the full range of values for the reduction in the liver iron concentration and total iron stores,<sup>13,14</sup> as recommended to obtain an estimate of the true average error in prediction, assuming that the subjects in this study are representative of the population of patients with thalassemia major who have been cured by allogeneic bone marrow transplantation.<sup>15</sup> S-Plus, version 4.5 (MathSoft), and BMDP (Statistical Solutions) were used for statistical tests. All tests were two-tailed, and a significance level of 0.05 was used.

**RESULTS**

We studied 54 patients (19 female and 35 male patients; age range, 8 to 30 years) who had undergone successful allogeneic bone marrow transplantation for thalassemia major. The marrow donors for 34 of the patients were heterozygous for β-thalassemia, and the donors for the other 20 patients were normal. Fifty-one patients (94 percent) had antibodies against hepatitis C. Histologic examination of liver-biopsy samples showed that six patients had cirrhosis; these six patients were excluded from further analysis. The characteristics of the other 48 patients are shown in Table 1. All but two of the patients had

**TABLE 1. CHARACTERISTICS OF AND FINDINGS IN THE 48 PATIENTS WITHOUT CIRRHOSIS.\***

VARIABLE	TOTAL (N=48)	LIVER SPECIMEN <1.0 mg (N=23)	LIVER SPECIMEN ≥1.0 mg (N=25)
Age (yr)	17±4	16±4	17±3
Female sex (no. of patients)	17	8	9
Spleen present (no. of patients)	37	18	19
Hepatic iron (mg/g, dry weight)†			
Before phlebotomy	10.8±6.3	12.6±6.4	9.0±5.8
After phlebotomy	1.1±0.4	1.1±0.4	1.1±0.4
Scrum ferritin (ng/ml)‡			
Before phlebotomy			
Median	1498	1818	1061
Interquartile range	842-2344	1027-2570	726-2045
After phlebotomy			
Median	110	110	105
Interquartile range	59-147	61-147	54-141
Transferrin saturation (%)§			
Before phlebotomy			
Median	87	92	84
Interquartile range	65-96	68-97	59-96
After phlebotomy			
Median	23	21	27
Interquartile range	18-34	16-29	18-39
Total body iron stores (mg/kg of body weight)¶	103±66	122±66	84±61

\*Plus-minus values are means ±SD.

†The upper limit of the normal range is 1.6 mg per gram, dry weight.

‡The normal range is 12 to 300 ng per milliliter.

§The normal range is 16 to 50 percent.

¶The calculation of total body iron stores is described in the Methods section.

hepatic fibrosis, which ranged from slight to severe. There were no complications of liver biopsy.

#### Hepatic Iron Concentration

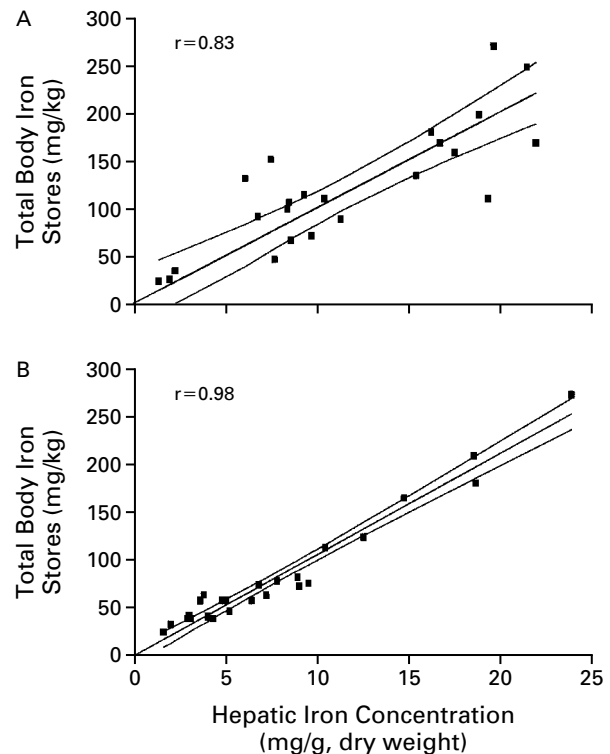
We began phlebotomy a mean ( $\pm$ SD) of  $4.3 \pm 2.7$  years after transplantation in the 48 patients without hepatic cirrhosis. The mean hemoglobin concentration before phlebotomy was  $121 \pm 14$  g per liter, and the mean duration of phlebotomy was  $26 \pm 16$  months (range, 9 to 66). After a mean of  $37 \pm 27$  phlebotomy sessions (range, 12 to 141), the mean hepatic iron concentration was reduced from an initial value of  $10.8 \pm 6.3$  mg of iron per gram of liver, dry weight, to  $1.1 \pm 0.4$  mg (normal value,  $<1.6$  mg). With phlebotomy, both the plasma ferritin concentration and the transferrin saturation fell from elevated levels to values within the normal range (Table 1), and no hemosiderin granules were seen on histologic examination of the samples from the final liver biopsy.

#### Correlation and Linear Regression Analyses

In the group of 48 patients without cirrhosis, there was a significant correlation between the decrease in the hepatic iron concentration as a result of phlebotomy and total body iron stores ( $r=0.91$ ,  $P<0.001$ ). The correlation between the initial plasma ferritin concentration and total body iron stores was weaker ( $r=0.67$ ).

The data suggested that the variance in the relation between the decrease in the hepatic iron concentration due to phlebotomy and total body iron stores was influenced by the weight of the liver sample obtained at biopsy. The variance of the residual values from the regression model for samples that had a dry weight of less than 1.0 mg was more than eight times the variance for samples that had a dry weight of 1.0 mg or more ( $F=8.54$ , 22 and 24 df, respectively;  $P<0.001$ ), indicating a better fit of the model to the data obtained with the larger liver samples (Fig. 1). Accordingly, we restricted further analyses to data from the 25 patients with liver samples that were at least 1.0 mg in dry weight.

Figure 1B shows the relation between total body iron stores and the decrease in the hepatic iron concentration in the group of 25 patients with liver samples that had a dry weight of at least 1.0 mg ( $r=0.98$ ,  $P<0.001$ ). The estimated slope was significantly different from zero ( $t=21.9$ , 23 df;  $P<0.001$ ), but the estimated intercept was not significantly different from zero ( $t=0.5$ , 23 df;  $P=0.63$ ). A second regression analysis, in which we assumed that the hepatic iron stores were reduced to zero (i.e., body iron stores were completely removed),<sup>1</sup> showed that the variation in the hepatic iron concentration accounted for 98 percent of the variation in total iron stores. The measured total iron stores fell within the 95 percent confidence intervals for the predicted values in all 25 patients. The presence or absence of the spleen and the



**Figure 1.** Correlation between the Hepatic Iron Concentration and Total Body Iron Stores in 48 Patients without Cirrhosis.

The correlation is shown for two groups of patients: 23 patients with liver samples that had a dry weight of less than 1.0 mg (Panel A), and 25 with samples that had a dry weight of 1.0 mg or more (Panel B). The regression line (middle line) and 95 percent confidence limits (upper and lower lines) are shown in each panel.

presence or absence and severity of hepatic fibrosis did not significantly influence the relation between the hepatic iron concentration and body iron stores.

Assuming that the hepatic iron concentration was reduced to zero during phlebotomy, with complete removal of body iron stores, the relation between these two variables can be expressed as follows:

total body iron stores = 10.6 (hepatic iron concentration), with total body iron stores expressed in milligrams of iron per kilogram of body weight and the hepatic iron concentration expressed in milligrams of iron per gram of liver, dry weight. On the basis of this equation, we could reliably estimate total body iron stores as high as 250 mg per kilogram of body weight, with a standard error of less than 7.9.

#### DISCUSSION

In our study of patients who had undergone successful bone marrow transplantation for thalassemia major, there was a significant correlation between the

amount of iron that was mobilized from body iron stores and the reduction in the hepatic iron concentration as a result of treatment with phlebotomy. Our analyses showed that the variation in the hepatic iron concentration accounted for 98 percent of the variation in total iron stores, suggesting that other factors, such as gastrointestinal iron absorption, menstrual-blood loss, splenectomy and its timing, and hepatic fibrosis, had little influence.

Two conditions had to be met to obtain an accurate estimate of the correlation between total body iron stores and the hepatic iron concentration. First, liver biopsy had to show that cirrhosis and focal lesions were absent. In the absence of cirrhosis and focal lesions, iron is uniformly distributed within the liver, so that the iron concentration in a sample is representative of that in the whole liver. Second, the liver sample had to have a dry weight of at least 1.0 mg for reliable results.

Our results suggest that the distribution of transfused iron between the liver and extrahepatic sites is remarkably uniform in patients with thalassemia major, despite differences in age, the amounts of blood transfused, the total amount of deferoxamine given for iron chelation, and other factors. Over the range of total body iron stores in our patients, the relation between hepatic and total iron stores was linear, suggesting that a constant proportion of transfused iron is sequestered within the liver. Studies performed before the use of deferoxamine for iron chelation suggested that with body iron stores that were substantially greater than those in our patients, the relation between these stores and the hepatic iron concentration was exponential.<sup>16</sup> In patients with hereditary hemochromatosis, there is a different relation between hepatic iron and total iron stores,<sup>17</sup> in part because iron is preferentially stored in the liver, especially early in the course of iron accumulation.<sup>18</sup>

The link between the hepatic iron concentration and total body iron stores can be used in long-term studies of iron balance to evaluate iron-chelating regimens and agents in patients who require transfusion therapy.<sup>19</sup> Currently, studies of iron balance require hospitalization in a specialized facility for several weeks, use of a diet with a measured amount of iron, and collection of all urine and feces for measurement of iron content. With the use of the close relation between the hepatic iron concentration and total body iron stores reported here, the iron balance can be assessed over a period of months or years, with initial and final measurements of iron in liver-biopsy specimens. Such studies should make it possible to individualize treatment with deferoxamine or other iron chelators by determining the dose required to reduce body iron stores to a selected level.

The discomfort and risk of liver biopsy have prompted a search for noninvasive means of measuring hepatic iron.<sup>20</sup> Use of a superconducting quan-

tum-interference-device (SQUID) susceptometer to quantify paramagnetic ferritin and hemosiderin directly in patients with iron overload is the only method we know of that has been shown to yield results that are quantitatively equivalent to and that can be used interchangeably with those obtained by chemical analysis of biopsy tissue.<sup>20-23</sup> Although much more widely accessible, both computed tomography<sup>24</sup> and conventional magnetic resonance imaging<sup>25,26</sup> yield estimates of hepatic iron that are too variable to be clinically useful. New approaches being developed to measure hepatic iron on the basis of magnetic susceptibility, including the use of high-transition-temperature SQUID susceptometers,<sup>20</sup> room-temperature magneto-resistive magnetometers,<sup>27</sup> and magnetic resonance susceptometers,<sup>28</sup> may make magnetic susceptometry more widely available.

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**CORRECTION**

**Hepatic Iron Concentration and Total Body Iron Stores in Thalassemia Major**

Hepatic Iron Concentration and Total Body Iron Stores in Thalassemia Major . On page 328, the sentence that begins on the second line of the left-hand column should have read, "Atomic absorption spectrophotometry measures the total (heme and nonheme) content of iron, but for the purpose of our study, the contribution of *heme* iron was considered negligible," not "the contribution of *nonheme* iron was considered negligible," as printed.