CLINICAL INVESTIGATION

Efficacy of hepatic computed tomography to detect iron overload in chronic hemodialysis

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Efficacy of hepatic computed tomography to detect iron overload in chronic hemodialysis. The diagnostic efficacy of hepatic computed tomography density (HCTD) in comparison with serum ferritin for the detection of iron overload was investigated in uremic patients on maintenance hemodialysis (HD) and in patients with idiopathic hemochromatosis (IHC). Ten IHC patients, 38 HD patients and 40 healthy subjects underwent the CT scanning of the liver and determination of percent saturation of transferrin, serum ferritin concentration and HLA typing. Liver iron content was determined by histochemical grading and direct measurement of liver iron concentration either in IHC patients or in HD patients. Nineteen HD patients were considered to have iron overload on the basis of liver iron concentration exceeding 3.6 μmol/100 mg dry weight. The mean ± SD values of HCTD in healthy subjects, IHC patients, HD patients with iron overload and without iron overload were 60.2 ± 5.6, 79 ± 5.6, 71.4 ± 3.6, 58 ± 3.8 Hounsfield units, respectively. HCTD showed positive correlations with liver iron concentration and serum ferritin either in IHC patients or in HD patients. The analysis of the diagnostic efficacy of HCTD in comparison with serum ferritin for the detection of excessive hepatic iron in HD patients demonstrated that HCTD had higher sensitivity, specificity, positive and negative predictive values. Cut-off points were arbitrarily fixed to 66 Hounsfield units for HCTD, 400 μg/liter for serum ferritin and 3.6 μmol/100 mg dry weight for liver iron concentration. Seventeen HD patients who possessed the histocompatibility antigens associated with IHC, namely HLA-A_3 and/or HLA-B_7 and/or HLA-B_{14}, had liver iron concentration, serum ferritin and HCTD values higher than those of the HD patients without these "hemochromatosis alleles". The diagnostic approach to the disorders of excessive iron storage of the liver may evolve to a new level of sophistication with the introduction of the CT scanning. HCTD may be an accurate, non-invasive, alternative to liver biopsy for the detection of hepatic iron overload in HD patients and in IHC patients. In HD patients HCTD is more efficient than serum ferritin in diagnosing iron overload. However, liver biopsy, which permits a definition of the presence of fibrosis and cirrhosis, maintains its importance from a standpoint of prognosis and follow-up. In HD patients the presence of any of the histocompatibility antigens of IHC is associated with an increased risk of iron overload and excessive hepatic iron storage.

Idiopathic hemochromatosis and maintenance hemodialysis are two of the most frequent causes for iron overload. In both conditions iron overload may be underrecognized for years,

until tissue damage of the organs involved becomes clinically manifest. Idiopathic hemochromatosis is a hereditary defect in the regulation of intestinal iron absorption which leads to a progressive toxic accumulation of iron in the body resulting in tissue damage and functional insufficiency of the organs involved [1–3]. Idiopathic hemochromatosis is associated with an abnormal iron-loading gene tightly linked to the A locus of the HLA complex on chromosome 6 [4–6]. Histologically iron is found in increased amounts in many organs, particularly in the liver and pancreas, and to a lesser extent, in the endocrine glands and the heart [7]. In patients with end-stage kidney disease on maintenance hemodialysis blood transfusions and inappropriate iron supplementation are usually responsible for iron overload [8–11]. Although the ultimate nature of the defect leading to iron overload in hemodialysis patients remains in part unknown, recent studies have demonstrated a genetic linkage between the risk of iron overload and certain histocompatibility antigens, notably HLA-A_3 and/or HLA-B_7 and/or HLA-B_{14} [12–16]. These histocompatibility antigens have been often found associated with idiopathic hemochromatosis and are called "hemochromatosis alleles" [4–6]. Iron overload is a serious problem for some dialysis patients and may be manifest by hepatosplenic siderosis, cardiomyopathy, proximal myopathy, impairment of phagocytic activity of polymorphonuclear leukocytes with increased incidence of bacterial infections, and several endocrine disturbances [8].

Taken together these observations indicate that iron overload, when pronounced, should be considered as a harmful clinical condition. It is known that early diagnosis followed by periodic phlebotomy can prevent most, if not all, the complications of idiopathic hemochromatosis [1, 3, 7]. In addition, several investigators reported the efficacy of the long-term chelation therapy with desferrioxamine in dialysis patients with iron overload [17, 18]. Thus, both in hemochromatosis and in dialysis patients every effort should be made to allow early diagnosis and to avoid the deleterious consequences of iron overload.

Useful methods available for the demonstration of iron overload include measurement of serum iron, determination of percent saturation of transferrin, estimation of chelatable iron stores using desferrioxamine, and measurement of serum ferritin concentration. However, at this time, the definitive test for the diagnosis of excessive parenchymal iron remains the biopsy

which permits histochemical examination of tissue iron, measurement of iron concentration and assessment of the extent of tissue damage. Bone marrow examination and liver biopsy are considered a reliable guide for iron stores in hemodialysis patients [19, 20]. Recently, it has been demonstrated that iron deposition in the liver increases hepatic density determined by computed tomography (CT) [21]. In idiopathic hemochromatosis hepatic CT density has been proposed as an alternative to liver biopsy for measuring hepatic iron concentration [22–24].

In the present study we investigated the efficacy of hepatic CT density in comparison with serum ferritin for the detection of iron overload in patients on maintenance hemodialysis and in patients with idiopathic hemochromatosis. The purpose of the study was to know the answers to the following questions: 1) Does hepatic CT density have any advantage compared with serum ferritin? 2) Does hepatic CT density provide an accurate, noninvasive, alternative to liver biopsy to the direct evaluation of hepatic iron content? 3) May the diagnostic approach to the patient with suspected iron overload evolve to a new level of sophistication with the introduction of the CT scanning?

Methods

Study protocol

Ten patients with idiopathic hemochromatosis, 38 patients with end-stage kidney disease on maintenance hemodialysis, and 40 healthy subjects were included in the study after giving informed consent. All patients and healthy subjects underwent the CT scanning of the liver and measurement of serum iron, percent saturation of transferrin and serum ferritin concentration. All patients with idiopathic hemochromatosis had had a percutaneous needle biopsy of the liver within four weeks of the CT scanning and no patient had been venesected between the time of CT scanning and biopsy. In dialysis patients serum iron, percent saturation of transferrin and serum ferritin concentration were measured at three month intervals for a total of at least four estimations over a period of one year. The mean values are reported. At the end of the period of the study the patients underwent the CT scanning of the liver and hepatic iron determination. HLA typing of dialysis and hemochromatosis patients were carried out to identify specific alleles known to be associated with hemochromatosis.

Patients with idiopathic hemochromatosis

Ten patients with idiopathic hemochromatosis were included in the study. They ranged in age from 40 to 56 years (mean ± SD 48 ± 5 years). All patients were men. The diagnosis of idiopathic hemochromatosis was based on the following criteria [1–4]: evidence of heavy parenchymal iron overload established by liver biopsy, evidence of tissue damage consistent with iron effects, absence of other causes of iron overload, and HLA typing.

Hemodialysis patients

Thirty-eight patients from the chronic dialysis program were included in the study. They ranged in age from 27 to 72 years (mean ± SD 52.6 ± 10.9 years). Seventeen patients were men and 21 women. The mean duration of dialysis was 88 months (range 12 to 120 months). Each patient received regular hemodialysis and was dialyzed four to five hours three times weekly,

against a fluid containing 1 g glucose/liter. Etiology of the underlying renal disease included chronic pyelonephritis, chronic glomerulonephritis and polycystic kidney disease. Patients were excluded from the present study if suffering from diabetes mellitus, liver disease, malignancies or chronic inflammatory disease. None of them had history of alcohol abuse. None was obese (>20% increase in relative weight). None of the patients received iron supplementation during the course of the study, and the extent of such a supplementation prior to the study was negligible. None received blood transfusions in the three months preceding the determination of hepatic CT density and liver iron content.

Healthy subjects

The control group consisted of 40 healthy subjects (19 men and 21 women). They ranged in age from 31 to 73 years (mean ± SD 51.4 ± 17.1 years). None of them had evidence of diabetes mellitus, liver disease, infection or inflammation disease. None had history of alcohol abuse. All were well nourished, none was obese. Hemoglobin, serum iron, percent saturation of transferrin, serum ferritin as well as the biochemical and hematological markers of alcohol intake and liver disease (mean corpuscular volume, serum concentrations of γ-glutamyl-transpeptidase, glutamic-pyruvic and glutamic-oxalacetic transaminase) were within the normal limits.

Iron studies

Serum iron and total iron binding capacity were determined using standard techniques. Serum ferritin was assayed by a radioimmunoassay method [25] using a commercial kit (Bio Rad, Milan, Italy). Normal limits were 18 to 200 μg/liter for women and 38 to 350 μg/liter for men. In uremic patients blood samples were carried out immediately before dialysis. Liver iron content was determined either by histochemical grading or by direct measurement of liver iron concentration. Liver sections were stained using the Prussian blue method [26]. Liver iron content was graded by the method of Scheuer, Williams and Muir [27]. Liver iron content was graded by an observer who had no knowledge of the hepatic CT density or clinical information. Liver iron concentration was measured according to the Barry's and Sherlock's method [28]. There was a good correlation between the results of chemical and histochemical iron grading. Patients were considered to have iron overload if liver iron concentration exceeded 3.6 μmol/100 mg dry liver weight, as previously indicated by Brissot and coworkers [29]. Most values exceeding this limit corresponded to grades 3 and 4 of histochemical grading.

Hepatic computed tomography density

The CT scan of all patients and healthy subjects were obtained with a GE CT/T 8000 scanner with 4.5-second scan time according to the usual protocols using 8 mm thick slices. No intravenous contrast material was given. Five 1 cm^2 areas were selected at the periphery of the liver slice, approximately 1 cm from the liver edge, avoiding obvious bile ducts, fissures and infiltrations. The CT densities of these areas were averaged to obtain the hepatic CT density.

Table 1. Iron studies, liver biopsy data and hepatic CT attenuation values in patients with idiopathic hemochromatosis

Patients	Sex	Age *yr*	Transferrin saturation *%*	Serum ferritin *μg/liter*	Hepatic CT attentuation value *HU*	Histology	Iron-stain grade	Liver iron concentration *μmol/100 mg dry wt*
1	M	40	90	2120	90	cirrhosis	4	25
2	M	56	81	1480	79	cirrhosis	4	15
3	M	53	72	1110	75	cirrhosis	4	14
4	M	47	90	1780	81	cirrhosis	4	15
5	M	42	76	1070	74	cirrhosis	4	12
6	M	52	82	1650	79	cirrhosis	4	16
7	M	44	84	1910	87.5	cirrhosis	4	19
8	M	45	80	1380	76	cirrhosis	4	15
9	M	51	74	1040	74	cirrhosis	4	12
10	M	50	70	1170	75	cirrhosis	4	14
Mean ± SD		48 ± 5	80 ± 7	1471 ± 382	79 ± 5.6			15.7 ± 3.8

Other biochemical and hematological parameters

Hemoglobin and mean corpuscular volume were determined using standard techniques (Hemalog 8, Technicon Instruments Co. Basingstoke, UK). The serum concentrations of γ-glutamyl-transpeptidase, glutamic-pyruvic and glutamic-oxalacetic transaminase were measured using a Sequential Multiple Analyzer with computer system (SMAC, Technicon Instruments Co. Basingstoke, UK). HLA typing was carried out by a standard microlymphocytotoxicity technique as previously described [30].

Statistical analysis

The significance of difference among mean values was calculated using the Student's two tailed *t*-test for unpaired data. Correlations were assessed by linear regression according to the method of least squares. We also determined the sensitivity (how the test is in positive cases), specificity (how the test is in negative cases), positive predictive value (how accurate the test is when it gives a positive result) and negative predictive value (how the test is when it gives a negative result) of hepatic CT density and serum ferritin concentration in diagnosing iron overload in dialysis patients, by applying the following equations:

$$\text{Sensitivity} = \frac{\text{true positive (tp)}}{\text{true positive + false negative (fn)}} \times 100$$

$$\text{Specificity} = \frac{\text{true negative (tn)}}{\text{true negative + false positive (fp)}} \times 100$$

$$\text{Positive predictive value} = \frac{\text{tp}}{\text{tp + fp}} \times 100$$

$$\text{Negative predictive value} = \frac{\text{tn}}{\text{tn + fn}} \times 100$$

Results

Healthy subjects

The mean ± SD values of percent saturation of transferrin and serum ferritin concentration in healthy subjects were 25 ± 12% and 139 ± 116 μg/liter. The prevalence of the histocompatibility antigens associated with idiopathic hemochromatosis, namely HLA-A$_3$, HLA-B$_7$ and HLA-B$_{14}$ was 25%, 10% and 8%,

respectively. The mean ± SD value of hepatic CT density was 60.2 ± 5.6 Hounsfield units with a range of 50 to 70 Hounsfield units. There was no correlation between hepatic CT density and age. The upper limit of normal for hepatic CT density to evaluate the diagnostic efficacy of the technique for the determination of hepatic iron excess was arbitrarily defined as 66 Hounsfield units. This value corresponded approximately to the mean ± 1 SD for the whole group of healthy subjects.

Patients with idiopathic hemochromatosis

The values of percent saturation of transferrin, serum ferritin concentration and hepatic CT density as well as liver biopsy data (histology, iron-stain grade and measured iron concentration) are summarized in Table 1. All patients had serum ferritin concentration above 1000 μg/liter and percent saturation of transferrin above 50%. The mean ± SD values of hemoglobin and mean corpuscular volume were 14.9 ± 1.4 g/dl and 96 ± 5 fmol/liter, and they did not differ from those of healthy subjects (14.6 ± 1.3 g/dl and 91 ± 7 fmol/liter). The histocompatibility antigens HLA-A$_3$ and/or HLA-B$_7$ and/or HLA-B$_{14}$ were found in all patients. Their frequencies were 75%, 25% and 25%, respectively. Liver iron concentration ranged from 12 to 25 μmol/100 mg dry weight. All patients showed a grade 4 of histochemical liver iron content. The mean ± SD value of hepatic CT density was 79 ± 5.6 Hounsfield units with a range of 72 to 90 Hounsfield units. All the CT values were well above the defined normal range obtained in healthy subjects. In the whole group of patients hepatic CT density correlated either with liver iron concentration ($r = 0.93$, $P < 0.0001$) or with serum ferritin ($r = 0.87$, $P < 0.001$).

Hemodialysis patients

The values of percent saturation of transferrin, serum ferritin concentration, lifetime estimated transfusional iron load and hepatic CT density as well as liver histology, iron-stain grade and measured iron concentration in hemodialysis patients are shown in Table 2. Nineteen patients had iron overload as indicated by liver iron concentration exceeding 3.6 μmol/100 mg dry weight. Comparison of some clinical, biochemical and hematological parameters for the two groups of patients with iron overload and without iron overload, respectively, is given in Table 3. Liver iron concentration ranged from 0.5 to 3.2 μmol/100 mg dry weight in patients without iron overload and

Table 2. Data regarding hepatic CT attenuation value and some clinical, biochemical and histological parameters in hemodialysis patients

Patients	Sex	Age yr	Transferrin saturation %	Serum ferritin µg/liter	Lifetime estimated transfusional iron load mg	Hepatic CT attenuation value HU	Liver histology	Iron-stain grade	Liver iron concentration µmol/100 mg dry wt
1	M	41	26	198	2500	62	—	2	2.6
2	M	72	31	436	750	64	fibrosis	2	3.2
3	F	49	17	48	—	54	—	1	0.6
4	F	50	27	197	750	62	—	2	2.7
5	F	46	27	205	2500	59	—	2	2.3
6	M	56	25	148	750	59	—	1	1.8
7	M	56	19	57	—	58	—	1	1.8
8	F	68	19	53	—	55	—	1	1.6
9	M	47	17	40	—	54	—	1	0.7
10	M	30	21	115	750	59	—	2	2.3
11	M	50	22	157	750	60	—	2	2.3
12	F	60	18	51	—	51	—	1	0.5
13	M	49	26	189	—	62	—	2	2.5
14	M	60	29	411	1000	64	fibrosis	2	3.2
15	F	66	18	40	—	53	—	1	1.2
16	F	50	23	173	—	57	—	1	1.2
17	F	40	22	112	—	56	—	1	1.1
18	F	41	18	51	—	54	—	1	0.7
19	F	44	28	229	1000	59	—	2	3.1
20	F	67	44	1600	1000	76	fibrosis	4	15.3
21	M	42	31	900	750	70.5	fibrosis	3	9.5
22	F	58	32	950	—	73	fibrosis	4	10.2
23	M	72	28	840	750	69.5	—	3	8.3
24	F	59	38	980	—	72	fibrosis	4	11.2
25	F	72	25	490	1000	69	—	3	9.5
26	F	54	38	990	—	74	fibrosis	4	11.8
27	F	62	15	315	—	65	—	2	5.8
28	M	42	26	537	2500	76.5	fibrosis	4	13.5
29	F	60	45	1605	6000	75	fibrosis	4	17
30	F	49	24	417	1500	68	—	3	7.1
31	F	27	26	605	—	69	fibrosis	3	8.7
32	M	56	16	331	750	67	—	2	6.8
33	M	54	18	360	750	68	—	3	7.6
34	F	60	24	470	4000	70	fibrosis	4	11.8
35	F	45	43	1250	3000	75	fibrosis	4	13.8
36	M	48	42	1170	3000	76	fibrosis	4	12
37	M	43	35	980	1000	75	fibrosis	4	12.9
38	M	53	29	518	—	68	—	3	6.8
Mean ± SD		52.6 ± 10.9	26.6 ± 8.2	479 ± 442	967 ± 1330	64.7 ± 7.7			6.2 ± 4.9

from 5.8 to 17 µmol/100 mg dry weight in patients with iron overload. The mean value of hepatic CT density in dialysis patients with iron overload was significantly higher than those of healthy subjects (71.4 ± 3.6 vs. 60.2 ± 5.6 Hounsfield units, $P < 0.0001$) and dialysis patients without iron overload (71.4 ± 3.6 vs. 58 ± 3.8 Hounsfield units, $P < 0.0001$). In turn, the mean value of hepatic CT density in dialysis patients without iron overload did not differ from that of healthy subjects (58 ± 3.8 vs. 60.2 ± 5.6 Hounsfield units). In the whole group of patients on maintenance hemodialysis hepatic CT density closely correlated either with liver iron concentration ($r = 0.95$, $P < 0.0001$) or with serum ferritin ($r = 0.9$, $P < 0.0001$). The analysis of the diagnostic efficacy of hepatic CT density in comparison with serum ferritin for the detection of excessive hepatic iron is shown in Table 4. The cut-off point for serum ferritin to evaluate the diagnostic efficacy of the test in the determination of iron overload was arbitrarily defined as 400 µg/liter. Hepatic CT density had higher sensitivity, specificity, positive and

Table 3. Some clinical, biochemical and hematological parameters in the 2 groups of hemodialysis patients respectively with iron overload and without iron overload

	Patients without iron overload N = 19	Patients with iron overload N = 19
Sex M/F	9/10	8/11
Age *years*	51 ± 10	53 ± 11
Time on dialysis *months*	80 ± 43	84 ± 42
Lifetime estimated transfusional iron load *mg*	565 ± 780	1368 ± 163?
Hemoglobin *g/dl*	9.1 ± 1.8	9.1 ± 1.7
Mean corpuscular volume *fmol/liter*	97 ± 7	99 ± 7
Percent saturation of transferrin	22.8 ± 4.5	31 ± 9
Serum ferritin *µg/liter*	153 ± 115	806 ± 404
Liver iron concentration *µmol/100 mg dry weight*	1.9 ± 0.9	10.5 ± 3

Table 4. Analysis of the diagnostic efficacy of hepatic CT density in comparison with serum ferritin concentration for the detection of excessive hepatic iron in hemodialysis patients ($N = 38$)

	Serum ferritin	Hepatic CT density
Sensitivity (TP/TP + FN × 100)	84.2	94.7
Specificity (TN/TN + FP × 100)	89.4	100
Positive predictive value (TP/TP + FP × 100)	88.8	100
Negative predictive value (TN/TN + FN × 100)	85	95

Cut-off points are: serum ferritin concentration, 400 μg/liter; hepatic CT density, 66 Hounsfield units; liver iron concentration, 3.6 μmol/100 mg dry weight. Abbreviations are: TP, true positives; TN, true negatives: FP, false positives; FN, false negatives.

Table 5. Comparison of hepatic CT density, liver iron concentration, and some clinical, biochemical and hematological parameters for hemodialysis patients with (IHC-HLA$^+$) and without (IHC-HLA$^-$) any of the histocompatibility antigens associated with idiopathic hemochromatosis (HLA-A$_3$, HLA-B$_7$, HLA-B$_{14}$)

	IHC-HLA$^+$ patients	IHC-HLA$^-$ patients	Significance P values
Number	17	21	
Sex M/F	8/9	9/12	
Age $years$	50 ± 11	52 ± 13	NS
Time on hemodialysis $months$	90 ± 59	84 ± 43	NS
Lifetime estimated transfusional iron load mg	1350 ± 1500	710 ± 1470	NS
Hemoglobin g/dl	9.2 ± 1.6	9.5 ± 1.6	NS
Mean corpuscular volume $fmol/liter$	98 ± 7	99 ± 6	NS
Percent saturation of transferrin	28 ± 9	24 ± 7	NS
Serum ferritin $\mu g/liter$	768 ± 393	362 ± 451	0.04
Liver iron concentration $\mu mol/100$ mg dry weight	8.8 ± 4.8	3.5 ± 3.3	0.01
Hepatic CT density $Hounsfield units$	70.5 ± 5.2	61.9 ± 8.9	0.01

negative predictive values. The analysis of HLA typing demonstrated that one or more of the histocompatibility antigens of hemochromatosis, namely HLA-A$_3$, HLA-B$_7$, HLA-B$_{14}$, were present in 17 dialysis patients. The mean values of serum ferritin, hepatic CT density and liver iron concentration in the group of patients presenting any of these antigens were significantly higher than those of the group without these histocompatibility antigens. Conversely, the two groups did not differ with respect to age, duration of hemodialysis, hemoglobin and mean corpuscular volume. Lifetime estimated transfusional iron load was slightly, but not significantly, higher in patients who possessed any of the histocompatibility antigens associated with idiopathic hemochromatosis (Table 5).

Discussion

Limited information is available on hepatic CT density in healthy subjects, and reported values vary considerably due to differences in CT scanners, scanning energies and patient size [31]. Our data obtained in healthy subjects with normal parameters of iron metabolism indicate that the mean value of hepatic CT density is 60.2 Hounsfield units with an upper limit of 66 Hounsfield units. Liver disease may produce a variety of changes detectable by CT scanning [32] but, unlike fatty infiltration which lowers CT number, the presence of fibrosis, cirrhosis or increased copper content does not influence this parameter [33, 34]. Conversely, since iron has a high atomic number, hepatic density determined by CT is elevated in the presence of excessive liver iron content.

In the present study we evaluated the diagnostic efficacy of hepatic CT density in comparison with serum ferritin in the detection of iron overload in uremic patients on maintenance hemodialysis and in patients with idiopathic hemochromatosis. Serum ferritin provides a good indirect index of body iron stores in hemochromatosis [35], although it may be normal in the early stage of the disease and there have been some families reported with normal immunoreactive serum ferritin levels [36, 37]. Howard and coworkers [23] demonstrated that in hemochromatosis patients hepatic CT density provides an index of exchangeable iron stores but is less sensitive than serum ferritin. In our study either serum ferritin or hepatic CT density had an elevated sensitivity in diagnosing iron overload. All hemochromatosis patients had serum ferritin levels above 1000

μg/liter and hepatic CT density values well above the upper limit of normal. Such an elevated sensitivity of hepatic CT density is due to the fact that in none of our patients iron overload coexisted with fatty infiltration of the liver, which may occur when hemochromatosis is associated with diabetes mellitus, alcoholism or obesity.

Serum ferritin is commonly considered a reliable guide for iron stores in patients with end-stage kidney disease on maintenance hemodialysis [18–20, 38–41]. This, however, may be questioned at least in a number of pathological conditions, not rarely associated with maintenance hemodialysis, such as acute or chronic liver disease, especially when associated with hepatocellular necrosis, lymphoma, lymphocytic leukemia and other malignancies, rheumatoid arthritis and various inflammatory and infectious states. In these conditions serum ferritin may be elevated in the absence of iron overload [40]. Furthermore, normal values for serum ferritin have been proposed in dialysis patients that, for unexplained reasons, are higher than those of healthy subjects [42, 43], and that vary considerably from one author to another. Since the bone marrow and the liver are the main storage organs in healthy people, each accounting for about one third of the total body iron stores [44], iron measurements in these organs have been proposed to estimate the iron overload in patients on maintenance hemodialysis [42, 43, 45, 52].

In the present study liver iron concentration closely correlated either with serum ferritin or with hepatic CT density. However, the comparison of the efficacy in diagnosing excessive hepatic iron demonstrated that in dialysis patients hepatic CT density was a more sensitive and specific test than was serum ferritin. The predictive value of an elevated CT density was higher than that for serum ferritin. In fact, a high hepatic CT density provided iron excess in 100% of cases compared

with approximately 89% for serum ferritin. In addition, the predictive value of a normal hepatic CT density was higher than that for serum ferritin. A normal hepatic CT density predicted normal iron concentration in 95% of cases as compared with 85% for serum ferritin. In practical terms, these data in hemodialysis patients suggest two considerations: 1) patients with elevated hepatic CT density have a high probability of having iron overload; and 2) patients with false positive elevations of serum ferritin could be correctly identified by performing a CT scan of the liver. Because of its high sensitivity, the simpler, less expensive, serum ferritin test must be considered as the first step in the diagnostic approach to the dialysis patient with presumed iron overload. Because of its high specificity, hepatic CT density will identify patients with true iron overload from those with false elevation of serum ferritin concentration. Furthermore, since abdominal CT scans are being increasingly used for diagnostic purposes in hemodialysis patients, radiologists should be on the alert for the possibility of hepatic iron overload when the hepatic CT density, measured before the administration of intravenous contrast material, is above 66 Hounsfield units.

What is the role of the CT scanning of the liver in the approach to the patient with iron overload? Our data have proven that an accurate diagnosis of iron overload can be made by noninvasive means, thus confirming previous studies [21–24]. Certainly, from a diagnostic standpoint in patients with suspected iron overload CT scanning can be used to assess hepatic iron levels, providing an efficient alternative to liver biopsy. However, from a standpoint of prognosis and follow-up it is useful to define the histologic pattern and the degree of hepatic injury. More specifically, it is important to know the presence or absence of fibrosis-cirrhosis on biopsy, since the presence of such dramatically increases one's risk of hepatic malignancy, whereas its absence rules that out [31]. Thus, although the diagnostic approach to the patient with suspected iron overload has evolved to a new level of sophistication with the introduction of the CT scanning, liver biopsy, unless controindicated, should still be considered in these patients.

In the present study the "threshold" of increased hepatic iron concentration has been set at 3.6 μmol/100 mg (200 μg/100 mg) dry weight, as previously suggested by Brissot and coworkers [29]. Stated differently, this is 0.2 percent iron by tissue dry weight. It has been reported that the values of liver iron concentration in patients with hemochromatosis, including those with pre-cirrhotic liver injury are consistently in excess of 1000 μg/100 mg (that is, 1.0 percent) dry liver weight [29, 46]. As tissue injury is not expected until liver iron concentrations are well above 1.0 percent of dry weight, that is, at least fivefold greater than the threshold level employed in the present study, we feel that recognizing lesser degrees loading may be important so that preventive measures might be taken. In fact, in these patients with early iron overload state efforts aimed at removing the excess iron stores (by desferrioxamine therapy) and at reducing the worsening of the overload (with the use of erythropoietin in lieu of transfusions) might prevent significant tissue injury.

Although the nature of the inherited defect in patients with idiopathic hemochromatosis that leads to inappropriately high absorption of dietary iron is still unknown, great progress has been made in the identification of individuals who are at risk for developing the disease. Recent studies have confirmed a genetic linkage between idiopathic hemochromatosis and certain histocompatibility antigens, notably HLA-A$_3$, HLA-B$_7$, HLA-B$_{14}$ [4–6]. As with most HLA associations, however, the increased frequencies of the HLA aplotypes in idiopathic hemochromatosis are neither absolute nor diagnostic [31]. In our experience the frequency of HLA-A$_3$ in patients with idiopathic hemochromatosis and in healthy controls was 75% and 25%, respectively. These data are coincident with those of Edwards, Skolnick and Kushner [6], who reported that 75% of patients with idiopathic hemochromatosis had the HLA-A$_3$ antigen compared with approximately 30% in the normal population. Thus, although HLA typing provides information on gene frequencies and may be useful in evaluating families with the disease, it is of little clinical value as a marker of idiopathic hemochromatosis in individual subjects, unless it is established from family studies that the hemochromatosis gene is likely to be present.

Blood transfusions and parenteral iron are frequently given to patients on hemodialysis with the aim of correcting chronic anemia. However, it has been shown that an indiscriminated iron supplementation may lead to iron overload [8–11]. Bregman and coworkers [14] demonstrated that the dialysis patients who possessed any of the histocompatibility antigens associated with idiopathic hemochromatosis, such as, HLA-A$_3$ and/or HLA-B$_7$ and/or HLA-B$_{14}$, were at increased risk of iron overload. Recent studies [12, 13] have demonstrated that repeated blood transfusions and iron parenteral administration can induce higher serum ferritin level in hemodialysis patients with "hemochromatosis alleles" than in patients without these histocompatibility antigens, thus suggesting that iron overload in hemodialysis is a histocompatibility-linked disorder. However, in subjects at risk for idiopathic hemochromatosis the histocompatibility antigens appear only to act as a linked marker for the gene of idiopathic hemochromatosis, and the HLA loci are not thought to influence iron metabolism [47]. The gene for idiopathic hemochromatosis is uncommon in the general population [48], and in normal subjects the possession of HLA-A$_3$, HLA-B$_7$ and HLA-B$_{14}$ is not associated with increased levels of serum iron or other indices of increased iron stores [49]. Furthermore, recently Maher and Curtis [50] have reported that dialysis patients with HLA-A$_3$ and/or HLA-B$_7$ and/or HLA-B$_{14}$ do not appear to be at increased risk of iron overload.

In view of these apparent contradictions, and since hepatic CT density provides a direct index of tissue iron storage we decided to evaluate whether hepatic iron overload and increased CT values are associated with the presence of the "hemochromatosis alleles" in patients on maintenance hemodialysis. Hemosiderosis of the liver complicating parenteral iron therapy in patients on hemodialysis was first described by Curtis, Eastwood and Smith [51], but further reports of parenchymal damage are scanty [52]. In the present study the dialysis patients who possessed the HLA-A$_3$ and/or HLA-B$_7$ and/or HLA-B$_{14}$ had liver iron concentrations significantly higher than the patients without these HLA antigens. Furthermore, the mean values of hepatic CT density and serum ferritin in the patients with "hemochromatosis alleles" were significantly higher when compared either with patients without these HLA antigens or with healthy subjects. Since the lifetime estimated transfusional iron load in these patients was slightly, but not significantly, higher than patients without "hemochromatosis

alleles" our data suggest that in patients on maintenance hemodialysis the presence of any of the histocompatibility antigens of idiopathic hemochromatosis is associated with an increased risk of iron overload and hepatic iron storage. However, as in the group of dialysis patients with iron overload the frequency of HLA-A$_3$, HLA-B$_7$ and HLA-B$_{14}$ was 31.5%, 36.8% and 26.3%, respectively, the information given by HLA typing is of less value as a marker of iron overload in the individual patient.

In summary, although our data are strictly preliminary, the present study suggests a few considerations: 1) the diagnostic approach to the disorders of excessive iron storage of the liver may evolve to a new level of sophistication with the introduction of the CT scanning; 2) hepatic CT density may be an accurate, noninvasive, alternative to liver biopsy for the detection of hepatic iron overload in hemodialysis patients as well as in patients with idiopathic hemochromatosis; 3) however, liver biopsy, which permits a definition of the presence of fibrosis and cirrhosis, maintains its importance from a standpoint of prognosis and follow-up of these patients; 4) liver iron excess is highly probable if hepatic CT density exceeds 66 Hounsfield units; 5) in dialysis patients hepatic CT density is more efficient than serum ferritin in diagnosing iron overload; 6) the finding of an elevated serum ferritin concentration should indicate the CT scan of the liver for confirmation of presumable iron overload; 7) in dialysis patients the presence of any of the histocompatibility antigens of idiopathic hemochromatosis is associated with an increased risk of iron overload and excessive hepatic iron storage; and 8) hepatic CT density, which provides a direct evaluation of tissue iron storage, and serum ferritin, an indirect index of body iron stores, complete each other in the diagnostic approach to the patient with suspected iron overload.

References

1. POWELL LW, ISSELBACHER KJ: Hemochromatosis, in *Harrison's Principles of Internal Medicine* (11th ed), New York, McGraw Hill Book Company, 1987, pp. 1632–1635
2. FINCH SC, FINCH CA: Idiopathic hemochromatosis, an iron storage disease. *Medicine* (Baltimore) 34:381–430, 1955
3. SIMON M, BOUREL M: Hémochromatose idiopathique. I. Aspects cliniques, biologiques et thérapeutiques. *Nouv Presse Med* 8: 855–859, 1979
4. SIMON M, BOUREL M, GENETET B, FAUCHET R: Idiopathic hemochromatosis. Demonstration of recessive transmission and early detection by family HLA typing. *N Engl J Med* 297:1017–1021, 1977
5. LIPINSKI M, HORS J, SALEUN JP, SADDI R, PASSA P, LAFAURIE S, FEIGOLD N, DAUSSET J: Idiopathic hemochromatosis: Linkage with HLA. *Tissue Antigens* 11:471–474, 1978
6. EDWARDS CQ, SKOLNICK MH, KUSHNER JP: Hereditary hemochromatosis: Contributions of genetic analyses. *Prog Hematol* 12:43–71, 1981
7. MILDER MS, COOK JD, STRAY S, FINCH CA: Idiopathic hemochromatosis, an interim report. *Medicine* 59:34–49, 1980
8. GOLDMAN M, VANHERWEGHEN JL: Multiple blood transfusions and iron overload in patients receiving haemodialysis. *Nephrol Dial Transplant* 2:205–207, 1987
9. ESCHBACH JW: Hematologic problems of dialysis patients; in Drukker, Parsons, Maher, *Replacement of renal function by dialysis* (2nd ed), Boston, Nijhoff, 1983, pp. 630–645
10. MARCO-FRANCO JE, ALARCON A, MOREY A, PIZÀ C, BESTARD J, MAIRATA S, GALMÉS A, DALMAU M: Serum ferritin in haemodialysis. *Nephron* 32:57–59, 1982
11. PITTS TO, BARBOUR GL: Hemosiderosis secondary to chronic parenteral iron therapy in maintenance hemodialysis patients. *Nephron* 22:316–321, 1978
12. GOMEZ E, ORTEGA F, MORALES JM, GAGO E, COMAS A, ALVAREZ J: Serum ferritin and HLA antigens in patients on maintenance hemodialysis. *Lancet* ii:836–837, 1981
13. TACCONE-GALLUCCI M, DI NUCCI G, MELONI C, MARIANI G, VALERI M, PIAZZA A, ELIA L, TORROMEO C, MANDELLI F, CASCIANI CU: Risk of iron overload and hemochromatosis allele(s) in patients on maintenance hemodialysis. *Am J Nephrol* 7:28–32, 1987
14. BREGMAN H, GELFAND MC, WINCHESTER JF, MANZ HJ, KNEPSHIELD JH: HLA-linked iron overload and myopathy in maintenance hemodialysis patients. Transactions. *Am Soc Intern Artif Organs* 26:366–368, 1980
15. QUEREDA C, TERNAL JL, LAMAS S, MARCÉN R, MATESANZ R, ORTUÑO J: HLA antigens and serum ferritin in hemodialysis patients. *Nephron* 45:104–110, 1987
16. MULLER-WEIFEL D, LENHARD V, SCHARER K: Body iron stores in children with chronic renal failure in relation with HLA phenotypes. *Proc Eur Dial Tranplant Assoc* 18:524–531, 1981
17. BAKER LRI, BARNETT MD, BROZOVIC B, CATTELL WR, ACKRILL P, McALLISTER J, NIMMON C: Hemosiderosis in a patient on regular dialysis: Treatment by desferrioxamine. *Clin Nephrol* 6: 326–328, 1976
18. KOVARICK J, IRSCHIK H, GRAF H, WOLOSZCZNK W, MEISINGER V, LINKERSCH W, STUMMVOLL HK: Iron removal by desferrioxamine in patients on chronic hemodialysis. Kinetic study and long-term results. *Contr Nephrol* 49:45–55, 1985
19. GOKAL R, MILLARD PR, WEATHERALL DJ, CALLENDER STE, LEDINGHAM JGG, OLIVER DO: Iron metabolism in hemodialysis patients. *Quart J Med* 191:369–391, 1979
20. LIPSCHITZ DA, COOK JD, FINCH CA: A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med* 290: 1213–1216, 1974
21. MILLS SR, DOPPMAN JL, NIENHUIS AW: Computed tomography in the diagnosis of disorders of excessive iron storage of the liver. *J Comput Assist Tomogr* 1:101–104, 1977
22. CHAPMAN RWG, WILLIAMS G, BYDDER G: Computed tomography for determining liver iron content in primary haemochromatosis. *Br Med J* 280:440–442, 1980
23. HOWARD JM, GHENT CH, CAREY LS, FLANAGAN PR, VALBERG LS: Diagnostic efficacy of hepatic computed tomography in the detection of body iron overload. *Gastroenterology* 84:209–215, 1983
24. ROUDOT-THORAVAL F, HALPHEN M, LARDÉ D, GALLIOT M, RYMER JC, GALACTÉROS F, DHUMEAUX D: Evaluation of liver iron content by computed tomography: Its value in the follow-up of treatment in patients with idiopathic hemochromatosis. *Hepatology* 3:974–979, 1983
25. BARNETT MD, GORDON YB, ANESS JAL, MOLLIN DL: Measurement of ferritin in serum by radioimmunoassay. *J Clin Pathol* 31:742–748, 1978
26. RATH CE, FINCH CA: Sternal marrow hemosiderin. A method for the determination of available iron stores in man. *J Lab Clin Med* 33:81–86, 1948
27. SCHEUER PJ, WILLIAMS R, MUIR AR: Hepatic pathology of relatives of patients with hemochromatosis. *J Pathol Bacteriol* 84: 53–64, 1962
28. BARRY M, SHERLOCK S: Measurement of liver iron concentration in needle-biopsy specimens. *Lancet* i:100–103, 1971
29. BRISSOT P, BOUREL M, HERRY D, VERGER JP, MESSNER M, BEAUMONT C, REGNOUARD F, FERRAND B, SIMON M: Assessment

of liver iron content in 271 patients: A reevaluation of direct and indirect methods. *Gastroenterology* 80:557–565, 1981

30. DE MARCHI S, CECCHIN E, BASILE A, PROTO G, VILLALTA D, SANTINI G, TESIO F: Close genetic linkage between HLA and renal glycosuria. *Am J Neprhol* 4:280–286, 1984

31. GOLLAN J: Diagnosis of hemochromatosis. *Gastroenterology* 84: 418–421, 1983

32. FAWCITT RA, FORBES W ST C, ISHERWOOD I, MORRIS AI, MARSH MN, TURNBERG LA: Computed tomographic scanning in liver disease. *Clin Radiol* 29:251–254, 1978

33. STEPHENS DH, SHEEDY PF, HATTERY RR, MacCARTY RL: Computed tomography of the liver. *Am J Roentgen* 128:579–590, 1977

34. SMAVIK B, RITLAND S, NILSEN T, JOHANSEN O: Liver attenuation values at computed tomography related to liver copper content. *Scand J Gastroenterol* 17:461–463, 1982

35. POWELL LW, HALLIDAY JW, COWLISHAW JL: Relationship between serum ferritin and total body iron stores in idiopathic hemochromatosis. *Gut* 19:538–542, 1978

36. WANDS JR, ROWE JA, MEZEY SE: Normal serum ferritin concentrations in pre-cirrhotic hemochromatosis. *N Engl J Med* 294: 302–305, 1976

37. FELLER ER, PONT A, WANDS JR: Familial hemochromatosis: Physiological studies in the precirrhotic stage of the disease. *N Engl J Med* 296:1422–1426, 1977

38. MULLER MAG, SCHNEIDER H, HOVELBORN V, STREICHER E: Ferritin: A reliable indicator of iron supplementation in patients on chronic hemodialysis/hemofiltration treatment? *Artif Org* 5:168–174, 1981

39. ALJAMA P, WARD MK, PIERIDES AM, EASTHAM EJ, ELLIS HA, FEEST TG, CONCEICAO S, KERR DNS: Serum ferritin concentration: a reliable guide to iron overload in uremic and hemodialyzed patients. *Clin Nephrol* 10:101–104, 1978

40. VAN DE VYVER FL, VANHEULE AA, MAJELYNE WM, D'HAESE P, BLOCKX PP, BE KAERT AB, BUYSSENS N, DE KEERSMAECKER W, DE BROE ME: Serum ferritin as a guide for iron stores in chronic hemodialysis patients. *Kidney Int* 26:451–458, 1984

41. MIRAHMADI MK, VAZIRI ND: Value of serum ferritin determination in hemodialysis patients. *Int J Artif Org* 3:318–319, 1980

42. BELL JD, KINCAID WR, MORGAN RG, BUNCE H, ALPERIN JB, SARLES HE, REMMERS AR: Serum ferritin assay and bone marrow iron stores in patients on maintenance hemodialysis. *Kidney Int* 17:237–241, 1981

43. MILMAN N, CHRISTENSEN TE, PEDERSEN NS, VISFELDT J: Serum ferritin and bone marrow iron in non-dialysis, peritoneal dialysis and hemodialysis patients with chronic renal failure. *Acta Med Scand* 207:201–205, 1980

44. BOTHWELL TH, CHARLTON RW, COOK JD, FINCH CA: *Iron Metabolism in Man*. Blackwell Scientific Publications, Oxford, 1979

45. WALKER RJ, MILLER JPG, DYMOCK IW, SHILKIN KB, WILLIAMS E: Relationship of hepatic iron concentration to histochemical grading and to total chelatable body iron in conditions associated with iron overload. *Gut* 12:1011–1014, 1971

46. HALLIDAY JW, COWLISHAW JL, RUSSO AM, POWELL LW: Serum ferritin in diagnosis of haemochromatosis. *Lancet* II: 621–623, 1977

47. KIDD KK: Genetic linkage and haemochromatosis. *N Engl J Med* 301:209–210, 1984

48. EDWARDS CQ, GRIFFEN LM, GOLDGAR D, DRUMMOND C, SKOLNICK MH, KUSHNER JP: Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med* 318: 1355–1362, 1988

49. STEVENS FM, FALLON MG, McCARTHY CF, GRIMES H: HLA-A_3 and serum iron. *Tissue Antigen* 21:402–404, 1983

50. MAHER ER, CURTIS JR: Serum ferritin in haemodialysis patients: Is there a relationship to "heamochromatosis alleles" HLA-A_3, B_7, B_{14}? *Nephron* 43:43–44, 1986

51. CURTIS JR, EASTWOOD JB, SMITH EKM: Maintenance haemodialysis. *Quart J Med* 38:49–89, 1969

52. ALI M, FAYEMI AO, RIGOLOSI R, FRASCINO J, MARSDEN T, MALCOLM D: Hemosiderosis in haemodialysis patients. An autopsy study of 50 cases. *JAMA* 244:343–345, 1980