

# Iron overload in multiply transfused patients who are HIV seropositive

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## Abstract

**Aims**—To assess histologically the amount of iron deposited in liver biopsy specimens from HIV positive patients; and to perform estimations of liver iron on tissue from patients with an increase in parenchymal stainable iron. To correlate the amount of blood transfused and the degree of iron overload.

**Methods**—Liver biopsy specimens (n = 120) from 109 HIV positive patients, 74 of whom had AIDS, were examined retrospectively and the amount of iron, as visualised with Perls's stain, was graded. Fibrosis was assessed using connective tissue stains. Estimations of liver iron were performed on tissue retrieved from paraffin wax blocks in cases with histological grade 3 or 4 iron overload. The amount of blood transfused before liver biopsy was determined from the notes for each patient.

**Results**—Fifteen of the 120 liver biopsy specimens had significantly increased amounts of iron in their hepatocytes, as assessed histologically, and this was confirmed in seven cases by measurement of liver iron. There was a close correlation between the amount of blood transfused and the degree of iron overload. In the initial biopsy specimens only one case showed portal tract expansion. Three of the five patients who had repeat biopsies, however, showed progressive fibrosis.

**Conclusion**—Multiply transfused HIV positive patients may develop clinically important iron overload and are at risk of developing progressive fibrosis. Superimposed liver disease, especially viral hepatitis, in these high risk patients may exacerbate the effects of the iron overload.

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Liver biopsy specimens from patients infected with HIV may show a wide range of abnormalities, ranging from minor non-specific changes to opportunistic infections and tumours.<sup>1-4</sup> In the course of reviewing liver biopsy specimens from HIV infected patients it was noted that a number of them contained an excess of stainable iron. Many of these patients had clinical histories of receiving zidovudine (AZT) or having had multiple blood transfusions. Up to 50% of patients (dependent on disease stage) infected with

HIV and treated with zidovudine develop anaemia requiring blood transfusion. In a preliminary study a strong correlation between the amount of blood transfused and the degree of iron overload was found.<sup>5</sup> We have extended these observations to include more patients and to provide follow up information.

## Methods

Liver biopsy specimens (n = 120) from 109 HIV positive patients were examined retrospectively. All the patients were male and aged between 20 and 69 years. Seventy four had AIDS according to the Centers for Disease Control definition at the time of liver biopsy. They either underwent liver biopsy at St Mary's Hospital Medical School, London, or had liver biopsies performed elsewhere and reviewed at St Mary's between January 1984 and July 1989. Abnormal liver function tests were the commonest indication for biopsy.

The specimens were fixed in 10% buffered formol-saline overnight and routinely processed and wax embedded. Serial sections were cut and stained with haematoxylin and eosin, Perls's, haematoxylin van Gieson, diastase periodic acid Schiff, orcein, Masson's trichrome, reticulin, and Ziehl-Neelsen stains. The slides were reviewed blind by two different pathologists and in the case of any discrepancy agreement was reached by discussion.

The amount of iron, as visualised with Perls's stain, was graded according to the criteria of Scheuer *et al.*<sup>6</sup> Grade 0 corresponds to the absence of stainable iron in liver cells and grades 1 to 4 to progressively increasing amounts of parenchymal iron. For the purposes of grading, iron within Kupffer cells and portal tracts was ignored (although it was recorded). Portal tract expansion was assessed using the connective tissue stains listed above and it was divided into: normal; expansion; linking with or without bridging; and cirrhosis.

Estimations of liver iron were performed on tissue retrieved from paraffin wax blocks. Sufficient material was available to do this on seven of the biopsy specimens from patients with an increase in parenchymal stainable iron. This was carried out as described by Barry.<sup>7</sup> Information as to the amount of blood transfused was obtained from the records of the Department of Haematology, with the help of Dr Helen Dodsworth, and by examination of the patients' notes.

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Siderosis score	Transfusion history		Total
	< 20 units	> 20 units	
0	84	0	84
1-2	16	5	21
3-4	0	15	15
Total	100	20	120

Grade of parenchymal iron as assessed histologically and amount of blood transfused ( $p < 0.001$ ,  $\chi^2$  test)

### Results

Of the 120 biopsy specimens, 84 showed no increase in stainable iron in hepatocytes, although 17 showed some degree of iron deposition within Kupffer cells. Sixteen of these 84 patients had been transfused but only three had received more than 10 units. Twenty one biopsy specimens were assessed as showing either grade 1 or 2 hepatocyte iron deposition which does not correlate with clinically important overload.<sup>6</sup> Thirteen of these patients had been transfused and they had received between 4 and 41 units.

Fifteen biopsy specimens were assessed as being grade 3 ( $n = 11$ ) or 4 ( $n = 4$ ) which does correlate with clinically important iron overload.<sup>7</sup> In addition to hepatocytes, iron was also present in Kupffer cells, bile duct epithelium, and endothelial cells. These 15 biopsy specimens came from 11 patients. All these patients had been treated with zidovudine and they had received between 25 and 63 units of blood. The amount of blood transfused is summarised in the table.

In the initial biopsy specimens of those with grade 3 or 4 iron deposition only one case showed portal tract expansion. But three of the four patients who had repeat biopsy specimens taken showed progressive fibrosis; two from normal to portal tract expansion, and one from portal tract expansion to portal tract linking. The iron content in the seven specimens in which this could be measured ranged from 906–2005  $\mu\text{g}/100$  mg dry weight with a mean of 1706  $\mu\text{g}/100$  mg (normal range 35–126). Only one of the patients with significantly increased iron had evidence of either infection or tumour. This was a patient with Leishmaniasis.

### Discussion

These results confirm our preliminary findings that multiply transfused HIV positive patients receiving zidovudine may develop clinically important iron overload. Furthermore, the grade of this overload correlates closely with the amount of blood transfused. All 15 biopsy specimens with grade 3 or 4 overload were from patients who had received more than 25 units of blood, whereas of the 105 patients with either no increased hepatocyte iron or only grade 1 to 2, only five had received more than 20 units of blood.

Several grading systems have been used to assess liver iron.<sup>6,8</sup> The system used here is simple, reproducible, and widely applicable. Furthermore, in the samples in which grade 3 or 4 iron overload was noted histologically, and in which there was sufficient material to

measure liver iron, the presence of a substantial degree of iron overload was confirmed by direct measurement (7–16 times the upper limit of normal). This agrees with previous work using the same grading system.<sup>6</sup> The most difficult part of the system is in distinguishing grade 2 from grade 3. A prospective study in which measurement of serum parameters of iron overload, such as serum ferritin, iron, and total iron binding capacity, are measured, would be of considerable interest.

The critical question is whether the iron overload seen is likely to produce progressive liver fibrosis and possibly the cirrhosis seen in genetic or primary haemochromatosis.<sup>9</sup> Bassett *et al* have shown that in the absence of associated alcoholic injury, patients with haemochromatosis do not develop fibrosis or cirrhosis until there is at least 2200  $\mu\text{g}$  iron/g dry weight in the liver. This value is just in excess of the highest values seen in this study but is consistent with the observation that three out of 11 of our patients had early fibrosis. This suggests that with further transfusion many of the patients may be on the borderline of developing progressive fibrosis which has already been seen in three of our patients.

Two additional points should be considered. First, the duration of the iron overload in our patients is relatively short compared with the duration of iron overload in patients with genetic haemochromatosis. It has been shown in the case of secondary haemochromatosis associated with thalassaemia that the severity of the fibrosis is a function of both the iron concentration in the liver and the duration of iron accumulation.<sup>10</sup> Secondly, patients with HIV infection are at increased risk of having superimposed liver disease, especially viral hepatitis.<sup>11</sup> The latter may, like alcohol in haemochromatosis, exacerbate the effects of the iron overload.

The major toxic effects of zidovudine recognised in clinical trials are granulocytopenia and anaemia which occur in up to 45% of recipients during relatively short term administration.<sup>12,13</sup> Anaemia is associated with hypoplasia or megaloblastic bone marrow changes and may occur as early as two to four weeks after treatment but is most common after the first six weeks.<sup>14</sup> Transfusion is required by about 30% of patients.

In a study of iron overload in non-thalassaemic adults with anaemia requiring blood transfusion it was found that in patients transfused with between 60 and 210 units of blood, the liver biopsy specimens contained seven to 26 times the normal amount of iron and showed focal portal fibrosis.<sup>15</sup> Although almost all these patients had received more blood than the group of HIV positive patients described here, there is considerable overlap in the actual amount of iron found in the livers of the two groups. There is no obvious explanation for this difference. In Schafer *et al*'s paper,<sup>15</sup> evidence of glucose intolerance and impaired left ventricular function was also found which they also ascribed to transfusional iron overload. It would be of interest

to carry out the same studies on heavily transfused HIV positive patients.

In conclusion, we have found that a clinically important degree of iron overload may be seen in HIV positive patients treated with zidovudine and multiply transfused. The degree of liver damage seen so far has been minor, but in view of the improved life expectancy of this group of patients and the likelihood that they will receive further blood transfusions and be exposed to hepatotoxic viruses and drugs, more clinically important liver damage may be seen in the future. This adds further impetus to attempts to develop alternative methods for treating anaemia induced by zidovudine, such as recombinant erythropoietin.<sup>16</sup>

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