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Does a visual analogue colour chart carried by the retrieval team help in assessment of the fat content of donor livers?

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Introduction

Improved surgical techniques and better organ preservation [2, 7] have resulted in a reduction in the incidence of primary nonfunction (PNF) following orthotopic liver transplantation [3]. However, the occurrence of this devastating complication remains considerably higher in livers that exhibit histopathological evidence of macrovesicular steatosis (fatty liver). The recipients of such livers have an increase in morbidity and mortality [3, 8]. Unfortunately, because of the continuing shortage of donor organs, there is now a prolonged recipient "waiting time" and a growing waiting list mortality rate. As a result, several transplant centres have resorted to using "fatty livers" for elective transplantation. Because the function of these "marginal livers" is difficult to predict prior to transplantation, various methods have been evaluated to assess their suitability for transplantation [6]. Donor risk factors, such as prolonged ICU stay, excessive use of vasopressor agents, increased bilirubin and excessive cold ischaemic times can all be predictive of initial poor function or PNF.

All those factors apart, we still rely on visual inspection of the liver (often by a relatively junior donor surgeon) to assess the degree of fatty infiltration. This places the retrieval team under considerable pressure when a decision has to be made on whether an organ is transplantable and has resulted in a number of livers being used or discarded inappropriately.

We attempted to standardise the assessment of the donor liver at harvesting with the aid of a visual analogue colour chart carried by the retrieval team. We prospectively evaluated 50 liver donors to assess the reliability of this approach. The visual analogue score recorded by the retrieving surgeon was compared both with the clinical outcome following implantation and with the histological assessment of the liver biopsy obtained before and after perfusion of the graft. This was examined using image analysis methodology.

The Newcastle Ethical Committee has been approached for ethical approval, and consent to the study was obtained from all United Kingdom liver transplant centres.

Materials and methods

Harvesting technique

During 50 multi-organ retrievals performed at our centre between June 1995 and March 1996, 50 livers were harvested. The standard retrieval technique involved in situ aortic perfusion with 2 l of Mar-

Table 1 Scores on visual analogue colour chart for donor organs harvested with frequency, amount of fat present, whether or not transplanted, and outcome (*PNF* primary nonfunction)

Score	Number of organs	Amount of fat (area %)	Transplanted (yes/no)	Outcome
1–5	23	0–5.257	Y	Good
6–8	10	5.23–17.593	Y	Good
9	1	25.26	Y	PNF
10	1	22.001	N	Discarded
11	1	22.122	N	Discarded
12	1	28.539	N	Discarded

shal's solution and in situ portal vein perfusion with 2 l of UW (University of Wisconsin) solution, via the inferior mesenteric vein. An additional 1 l of UW was perfused via the portal vein on the back table.

The visual analogue colour chart

The visual analogue system was constructed using colour charts from paint companies. In consultation with senior members of the transplant team, 12 colours were chosen. The colours were felt to relate best to the degrees of fatty infiltration – normal, mild < 30%, moderate 30–60% and severe > 60%. Each colour received a number from 1 to 12, where 1 was normal and 12 was "lardatious" (Table 1). A duplicate chart was held by the consultant surgeon on call. If he was not involved with the retrieval operation but was asked for advice by telephone, he could refer to the colour chart and was able to offer an informed opinion.

The retrieving surgeon scored the donor liver visually for its overall fat content. This scoring was always performed after perfusion. If the score equated to normal or mild < 30% (1–5), no conferring with the on call consultant was required. Any score of 6 or greater required consultation with the senior surgeon, who could refer to his chart and help to make an informed decision. A "biopty-gun" needle biopsy or wedge biopsy of the retrieved liver was then obtained for histological quantification of the overall fat content. The subjective assessment using the visual analogue chart was then correlated with the clinical outcome at 1 week, 3 months and 6 months in all livers transplanted, and also with the histological appearances.

Donor data

These included, age, gender, length of intensive care stay, serum bilirubin, alanine transaminase, alkaline phosphatase, prothrombin time and cold ischaemic time.

Biopsy material

The tissue was fixed in 10% formalin overnight. It was then placed in a working solution of equal parts of 5% potassium dichromate and 1% osmium teroxide, where it remained for 6 h. This produced a combination of dense black staining of the fat droplets and low-level background staining [10]. This was followed by a 2-h wash in running water, and the tissue was then routinely processed through graded alcohols and cleared in xylene before being embedded in paraffin wax. Sections (4 mm) were cut, mounted on glass slides and dried for 20 min prior to staining. These sections were rehydrated through graded alcohols to running water and further stained using picro-sirius red solution for 30 min [11]. The sections were briefly rinsed in running water, dehydrated through graded alcohols, cleared in xylene and mounted in DPX for subsequent analysis with an image analyser. The distribution and type of fat (macrovesicular, microvesicular or mixed) within the hepatocytes and the size and grade of fatty deposits were assessed by a single observer using light microscopy.

The volume of fat was then quantified by means of an automatic image analysis system (Leica Q500MC). Using a standard objective (× 25) an image (512 × 512 pixels) was recorded for ten consecutive fields from each biopsy. Using the colour difference between the fat, which stained intensely black, and the remaining parenchyma, the absolute area, the area fraction and the percentage of fat within the hepatocytes were calculated. This method was chosen because it was felt to be more objective than any other. Although the percentage of fat appeared to be much lower than would normally be expected especially in the very obviously fatty livers, this was a function of the way in which the computer interpreted the stain compared with surrounding liver: 30% determined by the image analysis method related to 60% or greater.

Recipient data

All clinical data were extracted from clinical observation, laboratory values and relevant clinical history. All relevant clinical information on patients not transplanted in our own centre was obtained by post from the relevant centre.

Allograft function

Immediate function and primary nonfunction were recorded, as were the results of liver function tests as described above, at 1 week, 3 months and 6 months.

Results

Of the 50 donors, 31 were male and 19 were female, with a mean age of 57 years. None of the donors was in intensive care for > 72 h prior to harvesting. None of the donors in the study was morbidly obese, and 8 were known to be diabetic. All 50 donors had pre-retrieval bilirubin and alanine transaminase levels that were less than 1.5 times normal. In 67% the cause of death was an intracerebral bleed.

Unfortunately, only 37 livers were available for study, and 33 of the 37 livers were felt on observation by the retrieving surgeon to be transplantable. The outcome was successful in each of these cases. Twenty-three livers scored between 1 and 5 on the visual analogue chart, and the retrieving surgeon felt confident of the outcome. Ten livers were scored as 6–8, and after consultation with the consultant's chart the liver was felt to be transplantable. The mean fat area of the 33 livers was 6.29%, with a range of 0.65–17.59%. The results of the livrer function tests are shown in Fig. 1. Four of the 37

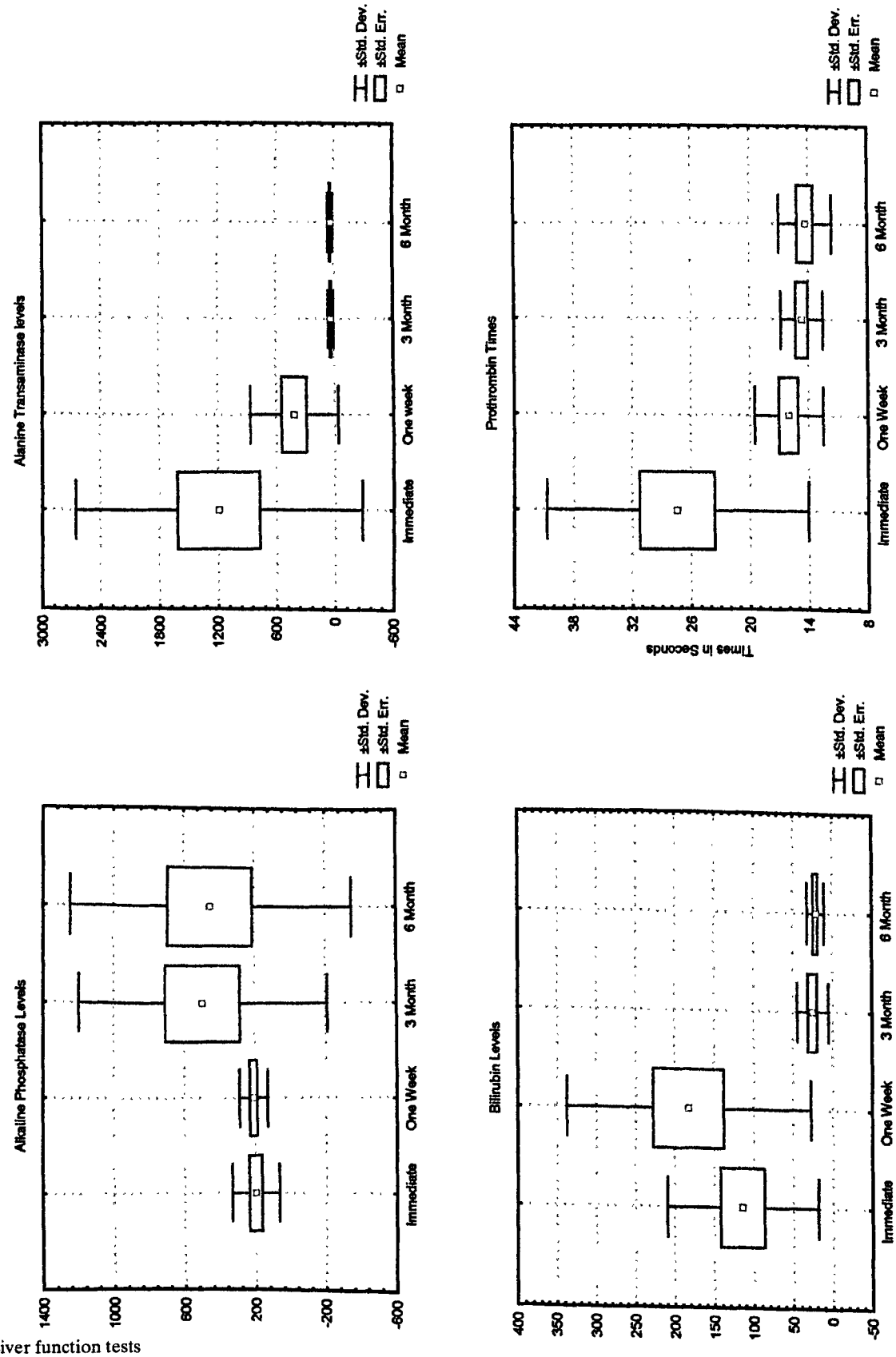

Fig. 1 Liver function tests

livers were considered by the retrieval surgeon to be too fatty. All 4 of these livers scored 9 or greater on the analogue score chart. After consultation with the consultant on call, and with the advantage of the visual analogue chart, 3 of the 4 organs were discarded. One of the "marginal" livers, with a visual analogue score of 9 and a fat area, according to the image analyser, of 25.26%, was transplanted because of the urgency of the recipient's condition. This decision was taken against the advice of the retrieval team. This graft failed owing to PNF, and the patient underwent retransplantation with a good outcome. The mean fat area of the 4 "marginal" livers was 25.3%, with a range of 22.1–28.5%.

Discussion

It is well documented that the risk of PNF following liver transplantation increases exponentially with the degree of fatty infiltration [3]. Similarly, graft function is influenced by the type of steatosis, either micro- or macrovesicular, with worse outcome following the latter. Moreover, a recent report from Birmingham suggests that livers exhibiting severe microvesicular steatosis are associated with poor outcome in patients undergoing retransplantation [4]. Therefore, liver grafts with severe steatosis are no longer recommended for implantation, despite anecdotal reports suggesting that donor livers should not be excluded on the basis of the degree of fatty infiltration alone [1]. The reason that steatotic livers fail to function properly has been attributed to the increased susceptibility of fatty livers to cold preservation even after short periods of time, with deterioration of liver mitochondrial function leading to microcirculatory disturbances [9]. The histological examination of the liver biopsy specimen obtained during the retrieval procedure and examined by a standard frozen section technique has led to a decrease in the incidence of PNF [5]. However, this requires the availability of a cryostat facility and of an expert pathologist or donor surgeon to interpret the result before retrieval of the liver, which can be difficult in a remote donor hospital. In our study the visual colour analogue charts held by the donor team and by a more experienced consultant surgeon offered an easy, quick, and reliable method of evaluating the degree of steatosis. It allowed the donor surgeon to consult with senior colleagues during the retrieval, thus facilitating the decision on whether to use or abandon the liver. However, this technique is not a substitute for a prospective liver biopsy as it does not predict other pathology, such as balloon degeneration, centrilobular necrosis, and chronic hepatitis, that would adversely affect the graft outcome.

In conclusion, this study has shown that a visual analogue colour chart carried by the retrieval team, especially less experienced members, can be used as an additional tool to aid the donor surgeon in assessing the degree of fat content within the donor liver. A duplicate chart held by the senior member of the team helps to facilitate a decision if the retrieving surgeon is unsure.

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