Liver transplantation

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Foreword by Professor Sir Roy Calne

Although we qualified the same year from London University, I did not meet Roger Williams until I was a surgical registrar at the Royal Free Hospital and he was one of Professor Sheila Sherlock's brilliant young lieutenants, arriving from the Hammersmith to inject academic verve into the traditional old teaching hospital.

Our paths next crossed in 1968 after we had done our first human liver grafts in Cambridge. I recognised the weakness of trying to develop a liver transplant programme without help from a physician. Unfortunately, most hepatologists regarded liver transplantation as a crazy procedure and resented surgeons moving into the field. Roger Williams was quite different and responded to my suggestion of collaboration with the characteristic enthusiasm he applies to all his activities. The Cambridge/King's College Hospital programme was initiated and for more than 20 years has been an effective endeavour.

Roger and I became close friends as well as colleagues. We tend to think alike because Roger has a positive 'surgical' approach to new procedures. In the early days there were many

disappointments, often heart breaking, when a patient seemed to be doing well only to be suddenly struck down by an unexpected complication. Roger Williams never even contemplated giving up and slowly many errors have been recognised and are now avoided.

Roger is not only an excellent clinical scientist, he is an outstanding medical opinion. He never refused any request for his help with a difficult case and would motor up to Cambridge after a hard day's work in London and usually put his finger on the trouble and suggested the right course of treatment.

As the years went by, liver transplantation became an established treatment and the results gave hope to many patients who previously we would attempt to make comfortable as their disease progressed to a fatal outcome. Roger Williams's contribution to this progress has been enormous. He is the voice of authority from the medical point of view in all matters relating to liver transplantation and it must, for him, be a source of great happiness to see the major revolution in hepatology that now has a good form of treatment for what we previously considered hopeless cases.

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Historical background to liver transplantation at King's College Hospital

The first British liver transplant programme (and the second successful venture worldwide) developed, as described above, under the direction of the powerful combination of Roy Calne and Roger Williams. By the time they joined forces in 1968, Calne had already carried out one heterotopic and one orthotopic liver transplant in Cambridge. Between then and the end of 1974, a further 34 orthotopic liver transplants were carried out, with 16 of these operations being performed at King's College Hospital. The logistics of liver transplantation at that time were considerably complicated by the necessity to await cardiac asystole before retrieving the donor organ and enormous flexibility on the part of both the donor and recipient care teams was required. Thereafter, however, developments led to the ability to concentrate surgical and anaesthetic resources at Addenbrooke's Hospital where most of the transplants in the joint programme were performed until the end of 1986. The postoperative care of all the patients there was supplemented by the infamous 'Cambridge rota' for clinical research fellows at King's College Hospital. The bulk of the preoperative assessment, medium and longterm follow up continued to be based at King's College Hospital, where, in addition, a special service for transplanting patients with chronic hepatitis B infection was maintained between 1975–86.

The phenomenal expansion of liver transplantation in the middle of the last decade, coupled with the need to integrate liver grafting into the overall management plan of patients with acute liver failure, prompted the rejuvenation of surgical activity at King's College Hospital. In 1987–8, 55 patients received their grafts at King's, accounting for almost 25% of the cases in the joint programme during that period. In October 1989, when over 600 patients had been transplanted, King's College Hospital was accorded official recognition by the Department of Health as an independent liver transplant centre and subsequently expanded its patient profile beyond the specialist interests of hepatitis B and acute liver failure to reflect the global role of liver transplantation in the management of liver disease. The number of patients transplanted under the surgical direction of Mr K C Tan, consultant surgeon at King's College Hospital, increased from 37 in 1989 to 74 in 1990, and the two year actuarial survival rate for this combined cohort is 80%. Thus the enormous pioneering efforts of Roger Williams in the field of liver transplantation have, finally, been duly rewarded by the establishment of a comprehensive and successful transplant programme at King's College Hospital. Through his efforts, the physical infrastructure supporting transplantation at King's has been extended with the opening of King's House in 1989 as a 'half-way house' and the new liver intensive care unit in April 1991.

Indications for transplantation and patient selection

The original descriptive report by Calne and Williams in 1968¹ is notable in that it described liver transplantation for alcoholic liver disease and biliary atresia in a 10 month child, both daring concepts in those early days of transplantation. The conclusion of this paper based on short term results was that primary hepatic or biliary malignancies and biliary atresia were the most suitable cases for transplantation. The former belief has now been considerably modified on the basis of the results obtained in 93 consecutive cases with malignant disease which indicated a distressingly high mortality from tumour recurrence after otherwise successful liver transplants.² Although the latter patients ultimately died, the often excellent liver graft function documented for up to periods of five years appear to have sustained the efforts of those involved in transplantation through some difficult and critical times. Currently, however, liver transplantation for malignant disease is performed only when there is a good chance of a cure being obtained or in exceptional cases when worthwhile or prolonged palliation can be anticipated. The main indication in the curative category is solitary primary hepatocellular carcinomas, arising in cirrhotic livers, and not exceeding 6 cm in diameter. Ten such cases are currently alive and tumour free for periods up to 15 years. Extended palliation is often achieved in patients with the carcinoid syndrome,³ and with the fibrolamellar variant of hepatocellular carcinoma.²

The indications for transplantation in chronic liver disease have come a long way from the original recommendation by Calne and Williams of patients who '... are too ill for an independent existence outside hospital'.¹ The present philosophy at King's College Hospital is to consider transplantation as part of the longterm strategy and to intervene when the disease has become endstage and is unresponsive to other available treatments, but not too late for the patient to have the maximum chance of surviving the procedure.⁴ Consideration of individual cases involves a balance of three aspects – the prognosis without transplantation, the complications present and the quality of life reported by the patient. Primary biliary cirrhosis is the most predictable of the chronic liver diseases, and a number of good prognostic models have been developed which allow a fairly accurate determination of anticipated survival. The model developed by James Neuberger, when he was working at King's College Hospital, and his colleagues involved in a multicentre study of azathioprine in primary biliary cirrhosis incorporates age, serum bilirubin and albumin, histological characteristics and previous therapy with azathioprine.⁵ The alternative model developed by the Mayo Clinic replace the latter two components with prothrombin time and oedema.⁶ Although liver transplantation is usually planned when the anticipated median survival is around 12 months, earlier intervention may be triggered by severe complications, especially uncontrolled variceal haemorrhage or osteodystrophy, or even by an unsatisfactory quality of life because of

severe pruritus or lethargy. Prognostic models have recently been described for primary sclerosing cholangitis and the one currently being developed by Mark Farrant and colleagues at King's College Hospital incorporates hepatomegaly, splenomegaly, serum alkaline phosphatase, histological state, and age.⁷ The development of cholangiocarcinoma is a complicating factor, and it is of concern that this diagnosis was established in 23% of patients undergoing liver transplantation,⁷ usually with a poor outcome because of tumour recurrence.²

The selection of patients for transplantation is less well defined for patients with cirrhosis secondary to chronic viral hepatitis, autoimmune chronic active hepatitis, cryptogenic and alcoholic cirrhosis. Most patients undergoing transplantation are Child's category B or C, but the chances of a successful transplant can be dramatically reduced by delayed intervention as shown by the data of Shaw *et al* from the University of Nebraska who found one year actuarial survival rates of only 44.5% in 22 high risk patients, as compared with 85.2% and 90.5% for 27 intermediate and 52 low risk patients, respectively.⁸ The lack of precision in prognostication in these patients has led to the evaluation of other functional tests – for example, MEGX test – but the role of such measurements awaits definition.

The unrivalled experience of the Liver Unit in the management of acute liver failure resulted in the availability of a large database from which to determine prognostic factors and subsequently develop models for the selection of patients for transplantation in this condition.⁹ There are separate models for paracetamol induced fulminant hepatic failure and acute liver failure of other aetiologies. After paracetamol overdose, an arterial pH <7.30 or, in patients not having this degree of acidosis, the coexistence of grade 3–4 encephalopathy, prothrombin time >100 sec and serum creatinine >300 µmol/l indicate a poor prognosis. In all other cases a poor prognosis is indicated by a prothrombin time >100 sec or INR >6.5 or, in patients not having such prolongations of prothrombin time, the presence of at least three of the following five features – aetiology being non-A, non-B (NANB) hepatitis, halothane hepatitis or idiosyncratic drug reaction, age >10 or >40 years, duration of jaundice before the onset of encephalopathy >7 days, prothrombin time >50 sec or INR >3.5 and serum bilirubin >300 µmol/l. These models have performed well on prospective assessment at King's College Hospital and await validation in other centres and countries. The feasibility of liver transplantation in acute liver failure is now well established,¹⁰ although the advantage of relative easier surgery is offset by anaesthetic and medical problems caused by haemodynamic instability, cerebral oedema, and sepsis. Consequently the results are not quite as good as for elective transplantation and our current two year actuarial survival rate is 61%.

Liver transplantation may be indicated in patients with metabolic diseases which may or may not have the liver as a target organ. Patients with Wilson's disease may present with acute liver failure or endstage chronic liver disease

necessitating transplantation, but severe neurological deficits can also be completely reversed by successful transplantation.¹¹ Primary hyperoxaluria is an example of a condition where an otherwise normal liver is replaced to correct the underlying metabolic defect, while another organ (in this instance the kidney) is transplanted to treat the clinical condition. This approach has been shown to be successful in depleting the oxalate pool and preserving renal function.¹² The outcome is not always as successful, and our first case of erythropoietic protoporphyria treated by transplantation¹³ remained well for over three years before developing recurrent disease.

Disease recurrence

The contribution of the Liver Unit over the years has been especially active in this important area of post-transplant hepatology. Many of the indications for transplantation have the potential to recur, albeit with substantial modification of the disease process. The most obvious example is malignant disease and, apart from small hepatocellular carcinomas arising in cirrhotic livers

as discussed above, the risk is substantial.² Although the morphology of the tumour remains unchanged, the growth rate appears to be increased by a factor of up to five fold by immunosuppressive therapy, with our data suggesting that this effect is least marked in patients maintained on cyclosporine.¹⁴

Reinfection with the hepatitis B virus is a major problem effecting morbidity and mortality after liver transplantation. In recent studies the implications of hepatitis B virus reinfection was assessed by us in 29 patients followed for 1.7–15 years, of whom 20 had hepatitis B virus infection alone (nine were HBeAg and hepatitis B virus DNA seronegative, while 11 had evidence of hepatitis B virus replication as measured by HBeAg or hepatitis B virus DNA seropositivity), and nine had coexisting hepatitis B virus and delta virus infection.¹⁵ Five patients became HBsAg seronegative after transplantation (four immediately and one after an hepatic episode) and remain so for periods of two to 15 years despite the fact that passive immunoprophylaxis with hepatitis B virus immunoglobulin (HBIG) was only used for a short period in the one patient who formed the basis for first report of this approach to preventing hepatitis B virus recurrence.¹⁶ Of the 20 patients with hepatitis B virus infection alone, 17 showed evidence of viral replication after transplantation with markedly increased hepatitis B virus DNA levels. In comparison, the five patients with hepatitis delta virus infection who reinfected had significantly lower amounts of hepatitis B virus DNA in serum, and the overall results suggest that hepatitis delta virus infection conferred some medium term protection from graft loss through recurrent viral infection.¹⁵ Twenty five episodes of graft dysfunction were attributed to hepatitis B virus reinfection in 19 of these patients. Thirteen of these episodes (in 12 patients) were self-resolving acute hepatic illnesses. Six patients, however, had a rapidly progressive illness leading to graft loss within six weeks, with distinctive histological picture recently reported by Susan Davies, working with Bernard Portmann, under the term fibrosing cholestatic hepatitis.¹⁷ The essential features are extensive serpiginous periportal fibrosis and ductal transformation of hepatocytes extending far beyond the periportal areas, cholestasis, prominent cytoplasmic hepatitis B virus protein deposition and a comparatively mild inflammatory cell infiltrate (Fig 1). The liver function tests in these patients showed markedly abnormal serum bilirubin and prothrombin times, but only modest increases in serum transaminase concentrations. An additional six patients lost their graft as a consequence of hepatitis B virus recurrence through various pathogenetic mechanisms including possible (but unproven) fibrosing cholestatic hepatitis, chronic active hepatitis or late onset hepatic failure.¹⁵

Recurrence of viral infection is also potential risk after liver transplantation for hepatitis A acute liver failure and both acute and chronic liver disease after NANB hepatitis. In two of our patients, Elizabeth Fagan showed hepatitis A virus in liver tissue as early as seven days, and for up to seven months after liver transplantation

Figure 1: Fibrosing cholestatic hepatitis caused by hepatitis B virus recurrence in the liver graft. There is widespread pericellular fibrosis, parenchymal rarefaction and ductal plate transformation with mild overall inflammatory cell reaction (PE = portal tract). (a) H & E; (b) Reticulin.

using monoclonal antibody and *in situ* hybridisation techniques.¹⁸ This was associated in one instance with a mild self-resolving hepatitis, during which time hepatitis A virus was also detectable in faeces. The situation is more difficult with respect to possible NANB recurrence because of the absence of a reliable diagnostic serological test. The development of the new antibody tests for hepatitis C virus have not yet resolved this problem because of the low positivity rate both in the acute phase of the hepatic illness (only 15% of patients in one study¹⁹) and after transplantation.²⁰ There is, however, circumstantial evidence that a NANB virus infection may recur after transplantation. The cloned genome of the hepatitis C virus is similar to togaviridae or flaviviridae and Elizabeth Fagan, collaborating with Dr Ellis at the London School of Hygiene and Tropical Medicine, found toga like viral structures in both native and grafted liver tissue from patients with NANB acute liver failure.²¹ This has been linked to a syndrome of severe coagulative necrosis of the graft in the absence of features of primary graft non-function, major vascular problems or acute cellular rejection five to 10 days post-transplant, but a causal relationship has yet to be estab-

lished, especially as a similar picture has been reported in patients transplanted for other conditions.²² Probable recurrence of NANB hepatitis has also been seen in the first two years after transplantation, and has on occasions led to the need for retransplantation.

The issue of possible recurrence of primary biliary cirrhosis in the transplanted liver was first raised by the Unit in 1982,²³ but continues to be controversial. There is now general agreement that the immunological stigmata persist, especially antimitochondrial antibodies and raised IgM in serum. Furthermore, it is clear that in some patients associated autoimmune conditions progress or develop *de novo* after transplantation.²⁴ The most contentious issue concerns the histological changes, and whether or not these can adequately discriminate between recurrence of primary biliary cirrhosis and other pathologies after transplantation. The longest duration of follow up with histological assessment has been reported from this Unit, and evidence of primary biliary cirrhosis recurrence was found in nine of 10 liver biopsies taken more than one year after transplantation, including one instance of progression to death as a consequence thereof at 9.5 years.²⁴ One of these cases had granulomata and was found to concomitantly express the specific subtype (M2) of the antimitochondrial antibody in serum for the first time. There was also evidence of modification of the disease recurrence by immunosuppression with cyclosporine. Three other centres have failed to find supporting evidence,²⁵⁻²⁷ and longitudinal studies for 10-15 years in a larger number of patients will be needed to determine the clinical significance of recurrence of primary biliary cirrhosis. Auto-immune chronic active hepatitis has also been described as recurring after transplantation,²⁸ and while the recurrence of primary sclerosing cholangitis has been suggested by the Mayo Clinic group,²⁹ supporting evidence has not been seen in our experience.

The procoagulant state which is a feature of the Budd-Chiari syndrome can lead to rethrombosis of the hepatic veins, although involvement of the hepatic artery or portal vein is more likely after transplantation.³⁰

Immunology, tissue typing and immunosuppression

Immunological and other studies of the rejection processes were initiated at King's College Hospital right from the onset of the clinical transplant programme. In a communication to *Nature* in 1969, Adrian Eddleston and colleagues described a correlation between acute rejection and inhibition of leucocyte migration (a measure of sensitisation to donor antigens), as well as documenting improvement in, or normalisation of, this parameter with antilymphocyte globulin and steroids.³² This paper concluded that histocompatibility antigens probably initiated the rejection process, which was then associated with the induction of an immune response to organ specific antigens. A combined study with the University of Colorado Medical Center suggested that humoral immunity was relatively unimportant in the pathogenesis of liver graft

Figure 2: Liver histology of the first described case of chronic rejection with vanishing of the interlobular bile ducts. (a) Small portal tract devoid of any bile duct structure. Note the deposition of lipid laden macrophages within portal vessels and sinusoids. H & E. (b) Frozen section of the same liver stained for lipids (black). Oil red O.

failure in comparison with cell-mediated immunity and non-immunological factors.³³ This belief is still largely held, with the obvious exception of hyperacute or accelerated acute rejection which is unusual after liver transplantation, although a well documented case has been described by this Unit in a patient with preformed antibodies.³⁴ The role of such antibodies in the pathogenesis of chronic rejection is more contentious, and is discussed later.

By the time 26 patients had undergone transplantation, a number of astute observations had already been made with respect to the histological features of acute and chronic graft rejection.³⁵ The important features in acute rejection were considered to be endothelialitis and a portal tract infiltrate, which while predominantly mononuclear in nature was recognised as containing neutrophils and eosinophils. The deposition of foamy macrophages in the intima of small and larger arteries within the liver graft was also correctly attributed to chronic rejection. These histological descriptions were lacking only in attention to the cholangiodestructive lesions seen during the rejection processes, which at the time were thought to reflect commonly associated ascending cholangitis. The idea of damage with progressive disappearance of the small intrahepatic being part of the rejection process was mooted after examining the autopsy material of one of our early patients (OL 51). He had died after a three month postoperative course of progressive jaundice. In addition to the classical arteriopathy of chronic rejection, the liver showed a virtual absence of interlobular bile ducts, a feature which had not previously been described. There was also severe cholestasis, but the previously observed portal tract infiltrate had largely disappeared (Fig 2). This case constituted the first description³⁶ of what later became known as the vanishing bile duct syndrome.

Interesting data in relation to the pathogenesis of the vanishing bile duct syndrome later emerged from the tissue typing and cytogenetics group at King's College Hospital. Evidence suggested that HLA antigens and cytomegalovirus infection were important interdependent cofactors in a series of 101 patients, including 16 with the vanishing bile duct syndrome.³⁷ The strongest association is with the presence of a class II match (relative risk=9.4) suggesting that class II restriction is important in the cytotoxic process. A weaker association was found for the absence of a class I match (relative risk=3.1). The relative risk associated with CMV infection was 7.5, and this increased to 10.1 when associated with a class II match.³⁷ Another study from King's College Hospital described high titres of donor specific antibodies to class I antigen as occurring exclusively in association with the vanishing bile duct syndrome, being present in 43% of cases, and developing after transplantation in all but one of these cases.³⁸

The observation that HLA matching was not necessary for prolonged graft survival was made at an early stage,³⁵ and the current understanding has not substantially changed. Apart from the specific role suggested for HLA matching in the pathogenesis of the vanishing bile duct syndrome, a dualistic role for HLA antigens

influencing global graft survival has been proposed by the Pittsburgh group.³⁹ This issue is being further explored in a collaborative European study, but at present HLA matching is rarely considered in clinical liver transplantation.

Prednisolone and azathioprine were initially used for maintenance immunosuppression, and an early study elegantly showed a reduction in the immunosuppressive activity of azathioprine in association with deteriorating graft function.⁴⁰ Cyclosporine became a pivotal component of the immunosuppressive regimen in 1980, and its efficacy was later demonstrated in a large series of patients.^{41,42} The toxicity profile of cyclosporine also received considerable attention, starting with the early observation that the acute nephrotoxicity was related to changes in haemoglobin concentration.⁴³ The ischaemic nature of the chronic nephrotoxicity related to cyclosporine was subsequently elucidated using radioisotope scanning and histological methods in a multidepartmental study at King's College Hospital and Dulwich Hospital.^{44,45} This particular complication, together with hypertension,⁴² resulted in the use of considerably lower doses of cyclosporine and its incorporation into the currently popular 'triple therapy' approach to maintenance immunosuppression regimens. Meanwhile pharmacokinetic studies were carried out which showed, amongst other things, the importance of internalised bile drainage for effective absorption of cyclosporine from the intestine.^{46,47} The stage has now moved to the evaluation of a potentially exciting new immunosuppressive agent, FK506, which may displace cyclosporine as the most valuable component of immunosuppression regimens. This drug was first used in the United Kingdom in a patient at King's College Hospital after Roger Williams was impressed by data presented to the European Transplant Association in October 1989 and arranged through the efforts of the Foreign Office and the British Embassy in Japan to obtain drug supplies. Unfortunately this patient with infection and chronic rejection after having a transplant for acute liver failure did not survive, but prospective controlled trials of FK506 are now in progress in association with seven other British and European centres.

Complications after liver transplantation

Infection remains one of the most serious complications after liver transplantation, and it is interesting that the spectrum of bacterial, viral and fungal infection was already evident in the first nine patients to survive the immediate postoperative period.⁴⁸ It is particularly noteworthy that cytomegalovirus infection was recognised in six of these nine cases, as current work in association with Sheena Sutherland indicates that this is the commonest clinically significant infection after liver transplantation. Cytomegalovirus appears to preferentially target the transplanted organ, and this is certainly true in liver transplant patients. The diagnosis of cytomegalovirus hepatitis can be difficult to establish using conventional methods, and the

successful adaptation of an *in situ* hybridisation technique by Nikolai Naoumov (now a professor in Bulgaria) while a visiting fellow at King's College Hospital was a valuable contribution to research and clinical practice.⁴⁹

Biliary sepsis was especially severe in the early years, and complications of biliary drainage were the most common cause of death during this period.⁴⁸ The mortality related to biliary complications was reduced by the use of the gall bladder conduit technique,⁵⁰ although a later review of 76 patients by Dr Evans indicated that 63 had radiological evidence of biliary complications (strictures 50, inspissated bile 23, bile leak 14).⁵¹ Factors contributing to the observed inspissation of bile included infection, supersaturation with cholesterol and mucosal damage.⁵² The incidence of biliary complications appears to have been reduced by reverting to end-to-end bile-duct anastomoses as is currently practised in most patients.

The incidence of malignant disease arising *de novo* after transplantation appears to be increased, and a study by Polson *et al* found seven cases in a series of 122 liver transplant cases.⁵³ The increased risk is especially true for lymphomas which appear to relate to Epstein-Barr virus infection. An early fascinating report of one such case occurring in the transplanted graft at five months used sex determination to establish that the tumour was of host (female) rather than donor (male) origin.⁵⁴ At the same time, the interesting observation was made that the Küpffer cell population of the liver had already been replaced by cells of host origin.

Interesting observations and studies

An experience in excess of 20 years of clinical liver transplantation, not unexpectedly, leads to many interesting observations and anecdotes. An excellent example is the study of parathyroid-hormone activity in association with an intra-hepatic cholangiocarcinoma which resulted in hypercalcaemia.⁵⁵ This was one of the early King's College Hospital patients and Knill-Jones and others were able to obtain blood from the major vessels at the time of surgery and convincingly localise the source of the hormone production, as well as documenting a fall in serum and urinary calcium after transplantation in this unusual association.

The electroencephalographic changes secondary to acute and chronic encephalopathy were shown to be reversible after successful liver transplantation by Iain Murray-Lyon in collaboration with Professor Parkes of the department of neurology.⁵⁶ The improvement in a young patient with acute liver failure was dramatic with disappearance of the triphasic pattern within 24 hours, while in chronic encephalopathy the recovery was much slower and the EEG pattern did not become normal until the second month.

An interesting study, also by Iain Murray-Lyon on protein synthesis using two dimensional immunoelectrophoresis after liver transplantation documented two distinct patterns.⁵⁷ Acute phase proteins (haptoglobin, alpha-1-antitrypsin, caeruloplasmin etc) were increased during the postoperative period and during

episodes of acute and chronic rejection, while at other times normal levels were maintained. In contrast the production of other proteins like albumin, transferrin and haemopexin was selectively decreased, particularly after the 18th day.

Perhaps the most remarkable of the medical and surgical joint ventures was the first patient to undergo combined liver and heart-lung transplantation as described in 1987.⁵⁸ The recipient was a long standing patient at King's College Hospital with primary biliary cirrhosis who developed primary pulmonary hypertension and right ventricular failure, a most unusual association. The operation was carried out at Papworth by John Walwork and Roy Calne, while Roger Williams personally supervised the medical management. The patient is currently well over four years later. The implications of this breakthrough are especially important for patients with cystic fibrosis who are being increasingly managed in this way.

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