DISEASE MANAGEMENT

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Chronic Rejection of the Liver

The Role of Immunosuppression

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Contents

Abstract . . . 283
1. Causes of Graft Failure . . . 284
2. Recurrent Disease . . . 285
   2.1 Hepatitis B Infection . . . 285
   2.2 Hepatitis C Infection . . . 285
   2.3 Primary Biliary Cirrhosis . . . 286
   2.4 Primary Sclerosing Cholangitis . . . 287
   2.5 Autoimmune Hepatitis . . . 287
3. Chronic Rejection . . . 287
   3.1 Risk Factors . . . 287
   3.2 Diagnosis . . . 288
4. Immunosuppression and Chronic Rejection . . . 288
   4.1 Cyclosporin . . . 288
   4.2 Tacrolimus . . . 289
   4.3 Mycophenolate Mofetil . . . 292
   4.4 Sirolimus . . . 293
5. Quality of Life . . . 293
6. Adverse Events . . . 293
   6.1 Drug-Related Events . . . 293
   6.2 Cytomegalovirus Infection . . . 294
   6.3 *De Novo* Tumours . . . 294
7. Conclusions . . . 294

Abstract

Liver transplantation is now widely recognised as an effective treatment option for patients with advanced liver disease. Many units now achieve greater than 85% survival at 1 year, with the majority of patients having a high quality of life.

The maintenance of a high quality of life requires careful clinical management to ensure that the continued maintenance of excellent liver graft function is not achieved at the expense of immunosuppressive drug complications or morbidity.

Acute liver rejection will occur in between 30 to 45% of patients, although with modern immunosuppressive protocols, usually combining one of the calcineurin agents, either cyclosporin or tacrolimus, with both azathioprine and corticosteroids (prednisolone) ensures that relatively few grafts are lost from severe acute rejection.

While the incidence and severity of acute rejection may be one factor in raising the risk of chronic rejection, it may not be the principal one in many patients. It is important to recognise that the frequency of rejection also varies with the

primary underlying liver disease, with patients with hepatitis B or alcoholic liver disease having relatively low rejection rates, compared with patients with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC), which range between 20 to 70%.

Chronic rejection will account for some 5% of grafts lost in the first 3 to 5 years. Indeed, there is some evidence that the incidence of chronic rejection is actually declining over the past few years. While the reason for this apparent decline is uncertain, and it could relate to better immunosuppression management, or more likely to the growing recognition that chronic graft dysfunction may be due to recurrent liver disease, such as autoimmune hepatitis, PBC, PSC, or recurrent hepatitis C. The differentiation of recurrent primary liver disease from chronic rejection can prove to be very difficult in clinical practice. Thus, the clinician must carefully monitor liver and graft function, evaluate any biochemical changes, and try to reach a clear diagnosis before considering any modification of immunosuppressive schedules.

Liver transplantation is now widely recognised as an effective treatment option for patients with advanced liver disease. In the last 20 years it has evolved from an experimental undertaking to a relatively routine procedure in many institutions. Across Europe more than 100 centres perform more than 3000 liver transplants a year, with a similar situation in the US. Many units now achieve a >85% patient survival at 1 year, with the majority of patients having a high quality of life.

1. Causes of Graft Failure

Liver graft failure in the early weeks after transplantation is unusual, but does occur in a small proportion of patients, largely related to primary graft nonfunction or hepatic vascular thrombosis. Acute liver rejection occurs in between 30 and 45% of patients. However, modern immunosuppressive protocols – usually combining one of the calcineurin inhibitors, either cyclosporin microemulsion formulation or tacrolimus, with both azathioprine and corticosteroids (prednisolone) – ensure that relatively few grafts are lost from severe acute rejection.

This early success is matched by long term outcomes following liver transplantation, with 75% of patients being well 5 years after transplantation. Clearly the maintenance of a high quality of life requires careful clinical management to ensure that continued maintenance of excellent liver graft function is not achieved at the expense of immunosuppressive drug complications or morbidity.

A deterioration in transplant graft function due to chronic rejection is common in kidney, lung or heart transplants, occurring in more than 50% of patients by 5 years. In contrast, in liver transplantation, although chronic rejection of the liver does occur and accounts for some 5% of grafts lost in the first 3 to 5 years, it may be difficult to differentiate from recurrent primary liver disease, such as autoimmune hepatitis or recurrent hepatitis C. Thus, determination of the cause of chronic liver graft dysfunction becomes crucial.

Although the incidence and severity of acute rejection may be one factor that increases the risk of chronic rejection, it may not be the principal one in many patients. It is important to recognise that the frequency of rejection also varies according to the primary underlying liver disease. Thus, patients with hepatitis B or alcoholic liver disease have relatively low rejection rates compared with patients with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC), whose rates range between 20 and 70%.

Indeed, there is some evidence that the incidence of chronic rejection is actually declining, with many centres reporting current rates of 4 to 8%,[1] whereas many early series reported rates of 15 to 20%. The reason for this apparent decline is

uncertain; it could relate to better immunosuppression management or, more likely, to the growing recognition that chronic graft dysfunction may be due to recurrent liver disease.

Thus, the clinician must carefully monitor liver and graft function, evaluate any biochemical changes, and try to reach a clear diagnosis before considering any modification of immunosuppressive schedules.

2. Recurrent Disease

Differentiation of recurrent primary liver disease from chronic rejection can prove to be very difficult in clinical practice.

In addition to standard haematology and liver chemistry, a review of autoantibodies, serum immunoglobulins and viral hepatitis status and degree of viral load may be needed. However, a liver biopsy is also usually required, although even after liver biopsy in some patients the situation may not be entirely clear. Hubscher et al.[2] found, on protocol biopsies in well patients with good liver chemistry, that there were histological changes in as many as 40% of cases at 1 year, with an uncertain and nonspecific mild inflammatory process. In addition, in 28% of patients, features compatible with the development of chronic hepatitis were present (signs of recurrent hepatitis B in 3%), with 3% of patients showing features of chronic rejection. It is important to emphasise that these histological features were relatively mild in annual protocol biopsies in asymptomatic patients with normal liver function, but clearly indicate the importance of frequent serial histological assessment.

2.1 Hepatitis B Infection

The early experience of transplantation in hepatitis B patients was disappointing, with significant reinfection of grafts and graft loss. The concomitant use of high dose immunosuppression (prednisolone or muromonab CD3) appeared to accelerate viral replication and an aggressive graft reinfection. The introduction of hyperimmune globulin (HBIG) at 10 000IU during the anhepatic phase still resulted in recurrent disease within a year in >75% of patients. Lauchart et al.[3] in Hanover then used more extended HBIG therapy in the early post-transplant period, maintaining surface antibody levels of >100 IU/L, and achieved a patient survival rate of 83.6%.[3] Samuel et al.[4] reported an overall 2-year patient survival of 85% with long term therapy. However, cessation of short term therapy is associated with reinfection, and a number of groups now use HBIG in a longer term effort to prevent reinfection, maintaining titres at >200 IU/L; some centres recommend >500 IU/L[5] in patients at particular risk with high serum viral titres at the time of transplantation.[6]

In initial studies, the introduction of lamivudine, a cytosine nucleoside analogue, proved effective in control of viral infection and has been shown to effectively suppress hepatitis B virus replication *in vitro* and *in vivo*. The emergence of lamivudine-resistant hepatitis B virus has, however, led to reinfection, particularly in those with high pretransplant viral titres.[7,8] Reinfection may occur in 10 to 40% of patients, usually after 8 to 10 months of therapy.[8] Many units currently use lamivudine in combination with HBIG in an effort to prevent reinfection, with encouraging initial results.[9]

In summary, the transplant team need to balance underimmunosuppression, with the attendant risks of developing rejection, against overimmunosuppression, with the risk of causing aggressive graft reinfection with the hepatitis B virus. HBIG in combination with lamivudine may help to prevent recurrent disease. Monitoring should include viral titres and hepatitis B surface antigen titres as well as biopsies to evaluate graft function.

2.2 Hepatitis C Infection

Hepatitis C is now one of the most common indications for transplantation worldwide, accounting for nearly half of all transplants in many units. Considerable uncertainty remains over the long term outcome of these transplants, with graft reinfection universal and at least 50% of patients developing chronic hepatitis in the short term (table I). Preliminary reports suggest that repeated acute rejection episodes are associated with a

Table I. Role of post-transplant hepatitis C virus infection in liver graft rejection

Study	No. of patients	Follow-up (mo)	Immunosuppression	Chronic hepatitis (% of patients)	Cirrhosis (% of patients)
Feray et al.[10]	79	12	Cyclosporin + azathioprine + prednisolone	41	1
		36	Tacrolimus + prednisolone	ND	ND
Chazouilleres et al.[11]	78	12	Cyclosporin or tacrolimus + azathioprine + prednisolone	50	0
Gretch et al.[12]	18	12	Cyclosporin + azathioprine + prednisolone	78	0
Gane et al.[13]	82	12	Cyclosporin + azathioprine + prednisolone	91	0
	39	60	Tacrolimus + prednisolone	74	21
Gordon et al.[14]	42	38	Cyclosporin + azathioprine + prednisolone	38	19
Shuhart et al.[15]	50	40	OKT3 or ATG, then cyclosporin + azathioprine + prednisolone	60	6
Berenguer et al.[16]	63	12	Cyclosporin + azathioprine + prednisolone	63	2
	40	24		75	0
Herrero et al.[17]	23	12	Cyclosporin + azathioprine + prednisolone	78	ND

ATG = antilymphocyte globulin; **ND** = no data; **OKT3** = muromonab CD3 (anti-CD3 monoclonal antibody).

higher rate of recurrence of hepatitis C, as is the use of high doses of corticosteroids or muromonab CD3.[18] However, the role of other immunosuppressive agents in the acceleration of viral replication seems uncertain. Early reports of the use of tacrolimus raised concern that it might also accelerate recurrent viral disease,[19,20] but the situation remains uncertain. In the US multicentre study of cyclosporin and tacrolimus in patients undergoing liver transplantation, there was no significant difference at 3 years in patient or graft survival in 113 randomised hepatitis C patients.[21] More recently, a comparison between cyclosporin (81% of 119 hepatitis C patients alive and well at 2 years) and tacrolimus (85% of 132 patients alive and well) showed no difference between the 2 agents.[22] The 2-year graft survival was also similar (cyclosporin 68.5% and tacrolimus 64%). This study again demonstrated that retransplantation is more frequently needed for recurrent hepatitis C (3.4%) than for chronic rejection (2.1%).

Diagnosis of recurrence should be based on measurement of viral titres (polymerase chain reaction is better than serological tests) and liver biopsy (lobular hepatitis is associated with high serum viral titre). Only a small proportion of patients appear to respond satisfactorily to the use of interferon-α-2b in preventing recurrent hepatitis C. It may prove beneficial when combined with other antiviral agents,[23] such as ribavirin, although toxicity may limit this.[24,25]

2.3 Primary Biliary Cirrhosis

PBC remains a common indication for liver transplantation, particularly in Northern Europe, and many centres report 5-year survival >80%. However, a number of studies have shown that patients undergoing transplant for PBC are at greater risk of developing both acute and chronic rejection.[26,27] In our own series between 1994 and 1997, acute early rejection (<28 days) occurred in 43% of PBC transplant patients, compared with only 20% of those receiving grafts for alcoholic liver disease and 12% in hepatitis B disease.[28] One hypothesis suggests that the genetic susceptibility that predisposes patients to develop PBC might be the same one that allows rejection, with the biliary epithelial cells being the immune target in patients already sensitised to biliary antigens.[29]

If that is the case, then the level of immunosuppressive therapy could affect the pattern of recurrence and chronic rejection.

2.4 Primary Sclerosing Cholangitis

Recurrent PSC has been suggested in up to 15% of transplant recipients,[30,31] although it may be difficult to differentiate from other causes of hepatic allograft dysfunction resulting in biliary injury, stricture and graft dysfunction,[32] including chronic rejection.

Graziadei et al.[33] reported a series of 150 PSC patients who received transplants and noted acute rejection in 68.7% and chronic rejection in 8%.

2.5 Autoimmune Hepatitis

Patients who have undergone liver transplantation for autoimmune hepatitis are often difficult to wean off corticosteroids without elevation of their liver transaminases, and the risk of recurrent autoimmune hepatitis has been noted in up to 27% of patients.[34] However, Trouillot et al.[35] showed no difference in the development of chronic disease, or chronic rejection, when corticosteroids were withdrawn from patients with autoimmune hepatitis.

Hayashi et al.[36] showed high incidences (81%) of acute rejection in 33 patients with autoimmune hepatitis compared with 46.8% in 47 patients with alcoholic liver disease. In addition, high incidences of chronic rejection have been noted in autoimmune hepatitis (11.1%) compared with alcoholic liver disease (2.1%).[35-37] Recurrent autoimmune hepatitis has been described in HLA DR3-positive recipients of HLA DR3-negative grafts.

In summary, therefore, pre-existing autoimmune hepatitis confers a higher risk of acute rejection and recurrence of disease after liver transplantation. Careful long term management of these patients is required, and prolonged treatment with corticosteroids may be required.

3. Chronic Rejection

3.1 Risk Factors

Although, understandably, much attention has been directed to the role of immunosuppression in the development of chronic rejection in liver transplantation, other factors may also be of key importance (table II). Although mild early acute rejection probably has little effect on the risk of developing chronic rejection, delayed or severe late acute rejection has much greater significance.[38] Anand et al.[38] showed that severe acute rejection occurring after 6 weeks following transplantation was associated with a high risk of development of chronic ductopenic rejection in 27% of cases.

A major review by Candinas et al.,[37] using a multivariate analysis of multiple risk factors, indicated that the risk of chronic rejection was significantly increased by cytomegalovirus infection, female donor to male recipient, advanced recipient age and severe acute rejection, as well as the absence of azathioprine for 3 months following transplantation. In patients receiving transplants for both PBC and autoimmune hepatitis, there appeared to be an enhanced risk of developing chronic rejection.[1]

Patients undergoing retransplantation where the initial graft has failed from chronic ductopenic reduction are at significantly increased risk of chronic rejection. In adults undergoing regrafting for technical reasons, the overall incidence of chronic rejection in our own programme was only

Table II. Risk factors for increase in chronic rejection

Underlying liver disease: primary biliary sclerosis, primary sclerosing cholangitis, autoimmune hepatitis
Cytomegalovirus infection
Low levels of immunosuppression
HLA donor-recipient matching
Positive lymphocyte cross-match
Recipient age
Donor-recipient ethnic origin
Male donor into female recipient
Number of acute rejection episodes
Histological severity of acute episodes
Retransplantation for chronic rejection

6.5%, but when the first graft had been lost because of chronic rejection the incidence rose to 17%, and in the third graft it was 25%.[39]

3.2 Diagnosis

Clinically, chronic rejection is characterised by progressive jaundice accompanied by cholestatic liver biochemistry. Usually these findings are nonspecific and the diagnosis therefore requires histological confirmation.

The 2 main abnormalities in chronic rejection are loss of bile ducts and an obliterative arteriopathy affecting large and medium sized arteries. Less specific are changes in the liver parenchyma.[40-42]

Bile duct lesions are characterised by lymphocytic infiltration in early stages and morphological abnormalities in the form of nuclear pleomorphism, disordered polarity and focal attenuation and/or disruption of biliary epithelium producing a 'dysplastic' or atrophic appearance.[43] At late stages, there is a loss of bile ducts, typically associated with a diminishing cellular infiltrate, eventually producing a characteristic 'burned-out' appearance in end-stage liver disease. It is generally accepted that bile duct loss should be present in more than 50% of portal tracts.

Vascular lesions are observed in large and medium sized arteries and are typically manifest as intimal aggregates of lipid-laden foamy macrophages, although other layers of the arterial wall can also be affected. Smooth muscle cells and/or myofibroblasts may also be seen, with varying degrees of fibrosis. Vascular lesions generally do not affect small arterial branches and are thus seldom detected in middle biopsy specimens.

Perivenular (zone 3) cholestasis and necrosis are prominent findings as parenchymal lesions. Chronic hepatitis–like changes consist of portal/periportal inflammatory cell infiltration associated with increasing amounts of fibrosis.

4. Immunosuppression and Chronic Rejection

In the 1960s and 1970s, the mainstays of immunosuppressive management after liver transplantation were azathioprine and prednisolone, but chronic rejection occurred in more than 10% of patients, and only a third of patients achieved good quality medium term survival.[44-47] The principal difficulty with this schedule was the need for high doses of corticosteroids in the early postoperative phase in patients often critically ill at the time of transplantation. The introduction of cyclosporin to clinical practice in the early 1980s fundamentally changed the situation of immunosuppressive management, and for more than a decade it was the principal agent in immunosuppressive protocols.[48,49]

4.1 Cyclosporin

Cyclosporin acts early in the immune process by forming a cyclosporin-cyclophilin complex with calcineurin, and inhibits T cell activation. It is highly lipid soluble and partitions in fat, with approximately 90% being protein bound. The findings of early international kidney trials that cyclosporin reduced the incidence of acute cellular rejection[50] were confirmed in liver transplantation studies.[51]

However, its adverse effects of neurotoxicity and nephrotoxicity proved to be particularly troublesome, and most programmes therefore incorporated cyclosporin as part of a triple therapy schedule (cyclosporin, azathioprine and corticosteroids), endeavouring to achieve prevention and control of rejection with lower dosages of cyclosporin without significant adverse effects.

The introduction of cyclosporin into clinical practice was associated with a fall in chronic rejection from between 15 and 20% to less than 5%.[40] With wider recognition of the possibility of recurrent liver disease as the cause of graft dysfunction rather than chronic rejection, lower schedules of immunosuppression have been introduced. In liver transplantation, the variable bioavailability of the original standard formulation of cyclosporin (Sandimmun®),[1] because of its need for bile for adequate absorption, caused concern, and some

1 Use of trade names is for identification purposes only, and does not imply endorsement.

Table III. Comparison between the original (standard formulation; S) and microemulsion (cyclosporin-modified-Novartis; N) formulations of cyclosporin in liver transplantation

Study	No. of patients		Total acute rejection (%)		Early rejection[a](%)		Adverse effects
	N	S	N	S	N	S	
Grant et al.[52]b	95	93	45.9	49.2	41.2	39.3	Similar
Miraz et al.[53]c	94	64	29.8**	65.6	25*	56	-
MILTON Study [54]d	98	92	49.9	46.5	30.1	43.7	Similar

a ≤2 weeks after transplantation.
b 16 weeks after transplantation.
c 12 weeks after transplantation.
d 52 weeks after transplantation.
* p = 0.001; ** p < 0.005

patients appear to develop chronic liver rejection with low concentrations of cyclosporin.

The introduction of the microemulsion formulation of cyclosporin (cyclosporin-modified-Novartis; Neoral®)[1] is associated with better bioavailability and more consistent absorption. However, optimal drug concentration monitoring remains uncertain, as measurements of trough concentrations correlate poorly with the area under the plasma concentration-time curve and clinical events, and measurement of peak cyclosporin concentrations (C_{max}) or concentrations at 2 hours after administration (C_{max} + 2) provide better indicators.[52] Mirza et al.[53] showed a reduction in acute rejection in liver transplantation when cyclosporin-modified-Novartis was compared with the standard formulation, and the MILTON international study group confirmed these observations, with 44.9% of patients treated for acute rejection with the standard formulation compared with only 30.2% of the cyclosporin-modified-Novartis group (table III).[54] Although detailed long term studies of chronic rejection were not incorporated into these studies, our own observations suggest that, in practice, the introduction of cyclosporin-modified-Novartis causes little difference in the already quite low levels of chronic rejection.

Although it is difficult to find clear objective evidence to support triple therapy as opposed to cyclosporin monotherapy, the majority of liver groups find it difficult to minimise the adverse events associated with the high dosages of cyclosporin often needed to prevent rejection. Therefore, triple therapy with cyclosporin, azathioprine and corticosteroids is usually maintained for at least 3 months after liver transplantation. Padbury et al.[55] have confirmed that after 3 months, corticosteroids can be stopped in most patients, the exception being those with ongoing rejection or those with underlying inflammatory bowel disease such as ulcerative colitis in PSC patients. Over 85% of patients remain corticosteroid-free for up to 5 years. Chronic rejection rates remain low and are no different in corticosteroid-free patients from rates reported from other groups. Other groups have reported similar experience, and in children long term corticosteroids can be reduced or withdrawn.[56-58] There is some evidence that the removal of azathioprine in liver transplant patients who have already had corticosteroids withdrawn at 3 to 6 months, leaving them on monotherapy, may be associated with a higher incidence of chronic rejection.[59]

A note of caution has also been sounded by Fisher et al.,[60] who indicated that at least some of the nephrotoxic damage induced by cyclosporin was well established by 3 to 6 months after liver transplantation. Reduction of cyclosporin late on did not always result in an improvement in creatinine clearance or renal function, and low dosage schedules for cyclosporin designed to minimise toxicity may require lower dosages in the early post-transplant phase.

4.2 Tacrolimus

After the 1980s, the decade of cyclosporin triple therapy, the introduction of tacrolimus into clinical liver transplantation in the early 1990s had a fur-

ther significant impact on the options for control of rejection. Like cyclosporin, tacrolimus has inhibitory effects on T lymphocyte activation, binding to FK506 binding protein and inhibiting calcineurin. Tacrolimus may be given intravenously (with a higher incidence of adverse events) at 0.01 to 0.1 mg/kg/day (0.03 to 0.1 mg/kg/day in children) or orally at 0.1 to 0.3 mg/kg/day (0.15 to 0.3 mg/kg/day in children). It has confirmed efficacy as a primary or rescue therapy, with chronic rejection rates significantly lower than cyclosporin, but with graft and patient survival similar. The adverse effects of tacrolimus are dosage related. Tacrolimus is mainly metabolised in the liver and the main excretory pathway of metabolites is biliary.

Large European and US multicentre studies have directly compared tacrolimus and cyclosporin in liver transplantation (table IV). The major trials demonstrated less acute rejection in the tacrolimus groups than in the cyclosporin groups (45.4 *vs* 55.1%; $p < 0.06$). There was also less refractory acute rejection (1.5 *vs* 5.9%; $p = 0.004$). The incidence of histologically proven chronic rejection was also less with tacrolimus (2 *vs* 6.9%), in spite of an overall protocol-driven reduction in corticosteroids in the tacrolimus arm.[61] The rather complex schedules of the trials, however, meant that crossover and rescue was permitted, so differences in graft outcome or retransplantation between the groups were not seen. At 3 years, the European study showed a very low rate of chronic rejection in the tacrolimus group (1.5%), compared with 5.3% in the cyclosporin group ($p < 0.03$). These studies have now continued well beyond 7 years, with the same trend of reduced chronic rejection apparent.

Tacrolimus given at 0.1 mg/kg is now frequently used with monitoring of blood concentrations, to maintain concentrations between 5 and 12 μg/L, and such protocols have seen a significant reduction in adverse clinical events.[63]

Although Padbury et al.[55] had clearly shown that corticosteroids could be discontinued with cyclosporin-based triple therapy after 6 months, not all centres were confident to stop corticosteroids. The enhanced confidence produced by tacrolimus in controlling acute rejection with low corticosteroid schedules led to a more relaxed approach to the withdrawal of corticosteroids. The ability to reduce corticosteroids for the medium to long term has been demonstrated to be associated with a lower incidence of hypertension, from a level of 42% to less than 35% at 1 year. The effects on bone disease, cushingoid facies, ocular problems and skin changes are all clearly evident in practice.

However, in a number of studies hyperglycaemia and new-onset diabetes mellitus occurred significantly more frequently with tacrolimus than with cyclosporin (19 *vs* 11%),[63-66] although it was reversible in most patients (40 to 50%). Predictors of the development of diabetes mellitus in renal

Table IV. Efficacy of tacrolimus-based regimens as primary immunosuppression in liver allografts

Study	No. of patients	Regimen	Graft survival (%)	Patient survival (%)	Acute rejection (%)	Refractory rejection (%)	Chronic rejection (%)
Pichlmayr et al.[61] (European multicentre; 3 years)	264	Tacrolimus	70.6	77.0	45.4	1.2	2.0
	265	Cyclosporin	65.2	69.7	55.1	5.9	6.9
Fung et al.[62]	79	Tacrolimus	ND	84	Free from rejection 35	ND	ND
	75	Cyclosporin	ND	84	Free from rejection 15	ND	ND
Wiesner[69] (US multicentre; 3 years)	263	Tacrolimus	77	72	17	2	4.9
	266	Cyclosporin	84	79	13	1	6.3

ND = no data.

transplant patients treated with tacrolimus included race (Black), increased corticosteroid dosage and elevated whole blood trough concentrations of tacrolimus.[67]

The ability for clinicians to convert from cyclosporin to tacrolimus, or indeed from tacrolimus to cyclosporin if adverse events occurred, offered a new range of options for medium term management.[68] Increasingly, good medium term results are now being reported in liver transplantation with tacrolimus, with one North American study[69] showing a 5-year survival of >79% of tacrolimus patients (n = 263), compared with 73.1% of patients receiving cyclosporin; corresponding retransplantation rates were 11 and 15%, respectively.

It was the initial observation by the Pittsburgh team[70] that grafts failing as a result of rejection in cyclosporin-treated patients could be rescued by conversion to tacrolimus that caused great clinical interest, and further studies have supported these initial observations. Recent studies by Sher et al.[71] suggest that many grafts could be rescued. Conversion from cyclosporin to tacrolimus for chronic rejection more than 3 months after transplant was successful in 70.3% of patients, although when conversion was delayed until bilirubin levels were higher than 10 mg/dl success was rarely achieved, because of continuous graft damage. The actuarial graft and patient survival for the total group after conversion to tacrolimus was 48.5% and 81.2% at 2 years.

The Pittsburgh team have recently updated their long term experience with tacrolimus in more than 1000 first liver transplants.[72] They have reported an overall 6-year patient survival of 68.1%, with 74.2% of patients maintained on tacrolimus monotherapy without additional agents. On monotherapy the rate of chronic rejection was noted as only 3% beyond 2 years. However, it should be noted that the most common cause of all deaths after transplantation was infection (34% of deaths), and the second most common cause was cardiopulmonary failure (16%). In addition, 7.2% of patients had become established on long term haemodialysis because of progressive nephrotoxicity, and 11% of patients had become diabetic and were taking insulin.

The very low rates of chronic rejection reported in this series and others, with relatively few grafts lost from chronic rejection in liver transplantation, must therefore be contrasted with the morbidity and mortality encountered on these schedules. This raises the possibility that we are significantly overimmunosuppressing our patients in the long term. Observations by the Pittsburgh group that some patients appear to have become tolerant to their graft, and that a further reduction in immunosuppression or actual cessation may be possible, suggest that greater efforts are needed in the medium term to avoid excessive immunosuppression.[73,74] At present, the major problem is to determine in which individuals immunosuppression could be progressively reduced.

A true comparison between tacrolimus and cyclosporin-modified-Novartis is difficult to make at this time. All the early studies compare tacrolimus with the standard cyclosporin formulation, and available data from comparisons of tacrolimus with the new microemulsion are limited. Recent studies comparing tacrolimus with cyclosporin-modified-Novartis in liver transplantation in Canadian patients, although not powered to demonstrate a difference in graft survival, have shown a continuing trend towards lower rates of acute rejection in tacrolimus-treated patients.[75,76]

Furthermore, the UK multicentre tacrolimus–cyclosporin-modified-Novartis liver transplant study, which is currently undergoing detailed evaluation and review, does suggest continuing evidence of low incidences of rejection with tacrolimus. Tacrolimus is increasingly being used as the basic agent in liver transplantation, usually in combination with azathioprine and corticosteroids for the first months. However, Tisone et al.[77-79] have now demonstrated that tacrolimus and azathioprine alone, in the absence of corticosteroids from day 1, are associated with good 3- and 6-month graft survival, although the incidence of acute rejection may be a little higher. Such a schedule might be of particular importance in patients re-

ceiving grafts for hepatitis C, in whom corticosteroids should ideally be avoided.

In addition, the use of tacrolimus with the antiproliferative agent sirolimus (rapamycin) is being explored, as well as its use with mycophenolate mofetil.

4.3 Mycophenolate Mofetil

Mycophenolate mofetil inhibits *de novo* purine synthesis by blocking inosine monophosphate dehydrogenase and inhibits lymphocyte division, probably at an early phase. Preliminary studies from the European Mycophenolate Mofetil Cooperative Study Group suggest a reduced frequency of acute rejection in renal transplantation compared with azathioprine.[80] Similar results were found in liver transplantation, with a lower incidence of acute rejection when comparing mycophenolate mofetil with azathioprine.[81,82] Fewer patients receiving mycophenolate mofetil 3 g/day as part of a triple therapy regimen with cyclosporin and corticosteroids had biopsy-proven rejection, although there was a greater frequency of gastrointestinal disturbance and leucopenia, as well as viral infection. Its influence on long term outcome and chronic rejection was not reported.

Mycophenolate mofetil has also been used in rescue therapy in renal transplantation and has been reported to result in a reduction in graft loss at 6 months,[83] as well as reduced use of antilymphocyte therapy.

Studies in liver transplantation are now under way, with use of mycophenolate mofetil monotherapy in stable liver transplant patients with severe adverse effects due to cyclosporin. Herrero et al.[84] were able to use the benefits of this relatively powerful non-nephrotoxic immunosuppressive agent to initially reduce the dosage of cyclosporin and then withdraw it; there was improvement in renal function in 7 of 11 patients, with improved creatinine clearance, and a reduction of hypertension as well. Other groups were also able to reduce the dose of cyclosporin and improve renal function by conversion from azathioprine to mycophenolate mofetil.[85]

International phase III multicentre trials of liver transplantation with mycophenolate mofetil as part of triple therapy schedules were undertaken in 1998 to 1999, and preliminary results and analysis will be available shortly.

Other studies with mycophenolate mofetil as part of a triple schedule in children are reporting encouraging results. In one study,[86] 26 children aged between 1 month and 16 years underwent liver transplantation, and at the end of 1 year 77% of patients remained on mycophenolate mofetil, although 5 patients had been converted to tacrolimus because of the main adverse effect of gastrointestinal intolerance.

Mycophenolate mofetil treatment has also been reported in corticosteroid-refractory rejection and chronic rejection when used in graft rescue.[87] In one study of 19 patients whose grafts were failing on cyclosporin-based conventional treatment, conversion from azathioprine to mycophenolate mofetil allowed complete histological resolution of rejection in 12 patients, with 2 partial resolutions, and only in 3 patients was there worsening of rejection. In 13 of the 19 patients there was significant biochemical improvement and response. The major adverse event was again that of gastrointestinal disturbance and nausea, with diarrhoea in 8 patients, and gastritis, duodenitis and oesophagitis in 4 patients. In 9 patients there was a degree of bone marrow suppression.

It seems increasingly clear that mycophenolate mofetil may routinely replace azathioprine as part of combination therapies, and it may allow much lower dosages of the calcineurin agents from the outset, or actual cessation in those who develop adverse events with cyclosporin or tacrolimus.[88] The development of serious adverse events with cyclosporin or tacrolimus and the need for reduced dosages have often in the past been followed by progressive rejection. By reducing this risk, mycophenolate mofetil could prove to have an important role in the reduction of chronic rejection.

4.4 Sirolimus

Sirolimus is a secondary metabolite derived from *Streptomyces hygroscopicus* that appears to inhibit growth factor–dependent proliferation of haemopoietic and nonhaemopoietic cells in the late stage of the cell cycle through a calcium-independent signal. There are laboratory studies that suggest it may inhibit fibrosis *in vitro*.[89] It also appears to inhibit the proliferation of cultured hepatic stellate cells, a crucial component of the hepatic fibrogenesis process. Its main adverse effects are hypercholesterolaemia, hypertriglyceridaemia and thrombocytopenia.

Sirolimus has been approved in the US for use in renal transplantation, and a derivative, everolimus (SDZ-RAD), is currently being evaluated in both renal and liver transplantation studies. The initial experience suggests that sirolimus is not nephrotoxic and appears to be synergistic in combination with cyclosporin.

Pilot studies in liver transplantation using tacrolimus with sirolimus have shown a remarkably low incidence of acute rejection in liver transplantation.[90] The dosage of tacrolimus was reduced in this combined protocol without any significant evidence of rejection. Wider studies are under way, but sirolimus could prove to be a valuable agent when used in combination with low dosages of calcineurin agents, and its antifibrogenesis properties may reduce the risk of chronic vascular damage seen in chronic rejection.

5. Quality of Life

Patients undergoing liver transplantation often do so in an advanced stage of liver disease, with many in intensive care before transplantation. For most, a return to full activities can be expected, with high quality of life. A reduction in incapacitating symptoms such as fatigue, pruritus and osteopenia is combined with general improvement in the sense of well-being and nutrition in most patients. Gross et al.,[91] analysing 157 cholestatic patients, showed statistically significant improvement by 1 year in quality of life after liver grafting. Patients reported fewer health problems or limitations in mobility and enhanced social and sexual life, and more objective rating evaluations showed a reduction in poor quality of life and Karnofsky scoring. The majority of patients rated their quality of life and health status as good or excellent, with only 7% reporting significant limitation in life activities because of health problems.[91] Although some lifestyle limitations and reductions in quality of life, such as hypertension, bodyweight gain and obesity, headaches and other comorbidity, relate to the immunosuppressive drugs,[92] many of the underlying limitations represent the long term effects of advanced liver disease present before transplantation.

6. Adverse Events

6.1 Drug-Related Events

Adverse events associated with the commonly used immunosuppressive agents are listed in table V.

Table V. Drug-related adverse events associated with immunosuppression after liver transplantation

Corticosteroids
Cushing's syndrome, growth retardation, diabetes mellitus, hypertension, aseptic osteonecrosis, osteoporosis, skin fragility, hypokalaemic syndrome, nausea and vomiting, pancreatitis, peptic ulcer, intestinal perforation, cosmetic changes, manic attacks, posterior capsular cataract, mental depression, paranoia, disorientation, euphoria, hallucinations
Cyclosporin and tacrolimus
Headache, tremor, paraesthesia, neurotoxicity, nephrotoxicity, hypertension, hyperkalaemia, hypomagnesaemia, hyperuricaemia, hyperglycaemia, anorexia, nausea, vomiting, insomnia, diarrhoea, constipation, anaemia, thrombocytopenia, abdominal pain, asthenia, back pain, hepatotoxicity, dyslipidaemia, haemolytic-uraemic syndrome, facial dysmorphism, pleural effusions, atelectasis, dyspnoea, pruritus, rash, peripheral oedema, ascites, fever
Cyclosporin
Hypertrichosis, gingival hypertrophy
Azathioprine
Myelotoxicity, hepatotoxicity, allergic reactions
Sirolimus
Dyslipidaemia, myelotoxicity, bone toxicity
Mycophenolate mofetil
Digestive toxicity (nausea, vomiting, diarrhoea, abdominal pain), myelotoxicity, invasive cytomegalovirus infections

6.2 Cytomegalovirus Infection

Cytomegalovirus (CMV) infection is one of the suggested risk factors for chronic allograft rejection.[93] Clinical and experimental studies have shown that CMV may be implicated in rejection mechanisms and in the generation of graft arteriosclerosis characteristic of chronic rejection.

In liver transplantation there is evidence of an association between CMV and vanishing bile duct syndrome, which occurs in chronic liver allograft rejection. The mechanism of the process leading to vanishing bile duct syndrome is either direct, in which the ducts are thought to be the target of immunological mechanisms, or indirect, resulting from vascular damage and disturbance of nutrition of the ducts.[94] As the bile ducts are destroyed during the slow process of chronic rejection, it could be suggested that persistence of the virus in ductal epithelial cells is involved in the pathogenesis. In general, vascular changes and intimal thickening are characteristics of chronic allograft rejection, which appears as graft arteriosclerosis. A possible link between allograft arteriosclerosis and CMV has been suggested to be the CMV-induced subendothelial inflammation (endothelitis) in the vascular wall.[95]

Initial protocols of immunosuppression should be directed to control acute rejection without an increased incidence of CMV infection. The amount of immunosuppression to reduce acute rejection rates to <20% may be associated with an increased risk of significant infection problems. It is important to remember that infection is the most common cause of death following liver transplantation. A low rejection rate but increased morbidity or death is clearly unacceptable.

6.3 *De Novo* Tumours

An increased incidence of de novo malignancies in immunosuppressed organ transplant patients was first predicted by Dr Thomas Starzl in 1968.[43,44] Since then, the frequency of *de novo* malignancies in this population has been estimated to range from 4.1 to 16%.[96] Predominant among these tumours are post-transplant lymphoproliferative disorders (PTLD), squamous cell carcinomas of skin and Kaposi's sarcoma.

A recent collaborative study quantified the risk of PTLD after kidney and heart transplantation to be, respectively, 20 and 120 times higher than the rate in the general population.[97] In liver allograft recipients the overall incidence of PTLD has been assessed to range from 3.6 to 7.3%.[98] However, the risk of developing neoplastic disease is known to vary with the kind of graft transplanted and is related to the aggressiveness of immunosuppression therapy. Besides, a wealth of evidence suggests that lymphoid cells carrying Epstein-Barr virus may have a special role in the pathogenesis of PTLD in children.

Jain et al.[99] described the risk over time of developing *de novo* malignancies after liver transplantation in 1000 patients taking tacrolimus: risk was increased 7.6-fold for developing oropharyngeal cancer and 1.7-fold for lung cancer. The most common cancer was skin cancer, which occurred in 2.2% of all patients and accounted for 33.3% of all cancers diagnosed. Interestingly, rates of gynaecological cancer (breast, ovary, uterus and cervix) were 1.9 times lower than in the general population, matched for age, gender and length of follow-up. This may reflect a diligent policy of pre- and post-transplant gynaecological evaluation.

7. Conclusions

Advances in selection of patients and techniques with improved immunosuppression protocols have significantly reduced the risks of liver transplantation. The development of chronic rejection of the liver is not now the most common cause of chronic graft dysfunction; recurrent disease, especially hepatitis C, is the most common. Lower dosages of immunosuppression may now be possible, with perhaps a reduced adverse effect profile, if the initial studies with the newer immunosuppressive agents are borne out.

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