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Orthotopic Liver Transplantation in US Veterans under Primary Tacrolimus (Prograf™) Immunosuppression

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Introduction

The evolution and refinement of surgical techniques, perioperative patient care and immunosuppression has established orthotopic liver transplantation (OLTX) as a highly successful therapeutic modality for patients with end-stage liver disease[1-7]. In February of 1989, Tacrolimus (Prograf®, formerly FK 506) was first used successfully at the University of Pittsburgh Medical Center to treat patients with rejection refractory to cyclosporine based immunosuppression[8]. Clinical trails utilizing Tacrolimus in solid organ transplantation followed and in April of 1994 it was approved for use by the Food and Drug Administration [9-11].

In 1989 the Veterans Administration (VA) recognized the need for a national center for liver transplantation for U.S. veterans in need of such care. With the guidance and efforts of Dr. Thomas Starzl and others, one center was established in Pittsburgh, affiliated with the University of Pittsburgh (Pittsburgh Transplantation Institute). Since October of 1989 a consecutive series of veterans have been transplanted under primary Tacrolimus based immunosuppression [12-14]. In this chapter, we wish to present our overall results for OLTX in this unique, high risk patient population. In addition, we have found an inordinately high incidence of native portal vein thrombosis in our veterans undergoing transplantation and will discuss the perceived risk factors and surgical approach to these patients [14]. Lastly, end-stage liver disease due to hepatitis C has been documented in 53% of patients undergoing transplantation at the Pittsburgh VA between 1989 and 1995 [15]. Herein we will also discuss the implication of disease recurrence and current approach to therapy for recurrent disease.

Five Year Experience with Tacrolimus in U.S. Veterans

Undergoing Liver Transplantation

United States veterans represent a unique patient population compared to the demographics reported from other transplant centers here in the United States as well as in Europe [15-18]. This predominantly male, older population has a high prevalence of end-stage liver disease secondary to hepatitis (B and/or C) and toxin induced liver disease or combinations thereof. This combined with other factors presents a significant therapeutic challenge which we believe adds further supportive testimony to the efficacy of liver transplantation in high risk patients [19,20].

Patients and Methods

Between October 1989 to October 1995 140 OLTX were performed in 130 consecutive U.S. veterans under a primary Tacrolimus based immunosuppressive protocol. There were 128 (98%) males and 2 (2%) females with a mean age of 47.3 years (range 22-70). There were 15 patients (11.5%) over the age of 60 (all male). The etiology of underlying liver disease is shown in Table 1. The mean follow-up of this patient series is 36.6 months with a range of 3 to 74 months. Other pre-transplant patient factors and morbidity are shown in Table 2. It is notable that the number of patients with abnormal renal function (creatinine $\geq$ 2.0) was somewhat high and the overall patient profile reflects a high risk group.

The immunosuppressive protocol utilized was identical to that of the University of Pittsburgh with much higher initial doses of Tacrolimus in the early group. At present we initiate Tacrolimus intravenously at a dose of 0.025 - 0.05 mg/kg over 24 hours after reprefusion along with a bolus of 1 gm of Methylprednisolone. The amount

of Tacrolimus administered was largely dependent upon pre-operative renal function and/or urinary output in the first 24 hours after graft reprefusion. Prednisone was given at a dose of 20 mg per day with gradual reduction of dosage over three to 12 weeks in absence of any proven rejection. Imuran was used in 14 patients (short-term) with nephrotoxicity or perceived neurotoxicity and 3 patients were left on long-term therapy. At present 58% of patients are free of steroids. Rejection episodes were routinely treated with increasing maintenance Tacrolimus therapy and a 1 gm bolus of Methylprednisolone. Less commonly, patients not responding to this received Methylprednisolone re-cycles (tapering dose steroid therapy starting at 200 mg intravenous daily to 20 mg per day over 5 days). OKT3 (Orthoclone®) was used for steroid resistant rejection.

Results

The actuarial patient survival rates were 90%, 87%, 85%, and 80% at 6, 12, 24 and 60 months respectively (Figure 1). Graft survival was 86%, 83%, 76% and 70% at 6, 12, 24 and 60 months respectively. Ten patients (8%) were re-transplanted with a long-term actuarial survival rate of 50%.

Additional immunosuppression was required in the first six months post-transplant in 53 patients (41%). Forty of these patients had biopsy proven rejection episodes wereas the remainder were clinical diagnoses. Forty-five patients (35%) received additional steroid boluses (1gr Methylprednisolone) while an additional 26 patients (20%) required steroid re-cycles. Only one patient (1%) received OKT3 for steroid resistant rejection within the first six months post-OLTX. OKT3 was required for two episodes of late rejection (at approximately 1 and 2 years post-OLTX)

attributable to malabsorption of FK 506 in one patient with inflammatory bowel disease and an inadequate maintenance dose in the remaining patient. Both of these patients recovered from their rejection episodes and are currently alive and well with normal allograft function. These results compare favorably with recently reported multi-center trials and with those at the University of Pittsburgh Medical Center [11, 18,19].

Although FK 506 is a potent immunosuppressive agent it does not appear to be associated with a higher incidence of infectious complications compared to previous studies with cyclosporine-based immunosuppressive protocols [22-27]. The range of reported infectious complications in these studies is 47 - 83% with the incidence of major infections being reported as ranging between 57% and 67%. In our updated series we have observed at least one major infection in 58 (45%) of our patients.

Major bacterial infections accounted for the majority of significant post-transplant infectious morbidity with 45 patients (34%) having at least one major bacterial infection (Table 3). The median time of onset for these episodes was 35.5 days with a relatively low incidence of intra-abdominal sources.

Pulmonary infections have been reported in 13-32% of the liver transplant recipients in the previous reports and account for 16 to 49% of major infections these patients pneumonias occurred in 15% of the patients; 27% of the episodes of major infections in our patients were due to pneumonia. None of the patients had cytomegalovirus or Pneumocystis carinii pneumonia. Thirty-seven percent (7/19) episodes of pneumonia were due to fungi ie. Aspergillus and Cryptococcoccus, Legionella species accounted for 27% of the bacterial pneumonias.

Major fungal infections were seen in 14 (11%) of our patients (median onset 188 days post-OLTX). This compares favorably with what is generally reported in the literature of 16 to 24% [28-29]. Paya et al. observed a low incidence (4%) of fungal disease in their patients and this was partly attributed to a policy of peri-operative selective enteral decontamination [22]. We utilize a similar regimen however it is given after OLTX and only until the patient is no longer ventilator dependent. We have noted in our updated series that Candida and Aspergillus no longer represent the most common pathogens as previously noted. This is perhaps related to lower overall need for steroids with Tacrolimus and possibly to our routine use of early post-operative selective enteral decontamination. [30] It was also observed that elevated serum creatinine and need for pre-transplant dialysis were significant predictive factors for post-transplant invasive fungal infectious morbidity. [30]

Cytomegalovirus (CMV) infection developed in 32% (41/130) of our patients of which 25 were asymptomatic. Only 12% (16/130) developed symptomatic CMV disease at a median of 45 days post-transplant. Types of symptomatic CMV disease included viral syndrome 44% (7/16), CMV hepatitis 25% (4/16), CMV enteritis 13% (2/16), disseminated CMV 13% (2/16) and CMV retinitis 6% (1/16). A relatively low incidence of symptomatic disease may be attributable to our high prevalence of seropositive recipients (inherently low risk for CMV with tacrolimus containing regimens) and our use of short course pre-emptive ganciclovir therapy for the prevention of CMV disease.[31] Other viral infections included cutaneous herpes simplex infections 8% (10/130) and herpes zoster infections 4% (5/130), all of which responded to standard anti-viral therapy. In addition, we observed a unique clinical

syndrome of fever, severe thrombocytopenia, encephalopathy and vasculitis skin rash associated with Variant B Human Herpes Virus-6. [32] This virus has been implicated as a pathogen in patients after bone marrow transplantation, patients with HIV and lymphoproliferative diseases. [33-35] We have further hypothesized that this virus may represent an early post-transplant pathogen that may be responsible for unexplained cytopenias or encephalitic phenomena that is often interpreted as medication related neurotoxicity.

Our overall results are comparable to those recently reported and lend further evidence of less rejection, less overall immunosuppression and excellent patient and graft survival rates utilizing a primary Tacrolimus and low dose steroid immunosuppressive protocol. In addition, comparable rates of infectious morbidity were observed as compared to cyclosporine-based protocols.

Native Portal Vein Thrombosis in U.S. Veterans Undergoing Liver Transplantation

It is well recognized that native portal vein thrombosis (PVT) is a significant risk factor for liver transplantation and that it is no longer considered a contraindication to the procedure. [36-41] PVT has been reported in 2.1 to 13.8% of liver transplant recipients. [36-42] We recently reported our observations of an inordinately high incidence of portal vein thrombosis in U.S. veterans undergoing transplantation. [14] We looked at a number of previously reported risk factors for PVT to try and elucidate the reason for the high incidence in our patient population. Patients reported to be a higher risk for PVT included males, patients with post-necrotic cirrhosis (due to ethanol or viral hepatitis) hypercogulable states, Budd-Chiari syndrome, etc. [35-41] In addition to reporting these results we will also discuss our surgical approach.

Patients and Methods

From October 1989 to February 1994, 88 U.S. veterans (87 male, 1 female) received 99 OLTX under primary Tacrolimus-based immunosuppression. Of these patients, 23 (26%) were found to have PVT either at the time of OLTX or as evident by pretransplant imaging studies. The classification system for grading the extent of PVT is shown in Table 4 the clinical parameters examined as possible risk factors for PVT included, age, Child's-Pugh score, underlying liver disease, liver volume, history of prior abdominal surgery and complications of portal hypertension.

Results

With a mean follow-up of 39 months (range 13 - 64 months) overall actuarial patient survival rates were 88, 85 and 79% at 1, 2, and 4 years respectively. The presence of native PVT did not influence patient survival (83% vs 88% no PVT 1 year; 83% vs 84% 2 years, and 77% vs 81%, four years)(Figure 2). However, poorer graft survival was observed in patients with PVT (Figure 2)(85% vs. 55%, 1 year; 81% vs 55%, 2 years; 81% vs 61%, 4 years; p=0.03). None of the graft losses were consequence of problems with portal in-flow and four of six patients requiring re-transplantation are currently alive and well.

The majority of patients in both groups were either hospitalized (UNOS 3†, 57% with PVT 63% without PVT) or in the intensive care unit (UNOS 4†, 43% with PVT, 32% without PVT). The average (mean) Child's-Pugh score for both groups was 12. The incidence of previous abdominal surgery was essentially identical (39% PVT, 38% no PVT) and liver size and age were also equivalent. When we examined the incidence of various consequences of portal hypertension (ascites, spontaneous

bacterial peritonitis, encephalopathy, variceal bleeding +/- sclero therapy) we found no significant difference between those patients with and without PVT. When dealing with the notoriously difficult intra-operative course of patients with PVT we employed a patient flexible approach to portal revascularization. We know that failure to establish adequate portal venous inflow is a sure recipe for patient mortality. [42] This was recognized early in hepatic transplantation as is evident by the routine procurement of vascular homografts from donors and their use for vascular reconstruction. [43] These grafting techniques have been well described and were utilized in this series of patients. [43-51] Unexpected PVT found at the time of surgery was not unusual and we frequently perform intraoperative portograms to better define the anatomy of the splanchnic inflow. Dissection of the portal vein towards the confluence also helps to define the extent of thrombosis however this can be quite difficult at times due to extensive collateralization. It is only when the extent of thrombosis is established (either pre-operatively or intra-operatively) that a decision regarding reconstruction can be made. Incomplete (grade 1) thrombosis (n=6) was usually detected intra-operatively, (pre-operative Doppler ultrasound invariable showed residual flow) and thrombectomy with end-to-end anastomotic reconstruction was performed in each case. Thrombectomy with end-to-end anastomosis was also utilized for four patients with complete thrombosis of the portal vein not extending to

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† UNOS classification system prior to April 1, 1995

the confluence (grade 2). More extensive thrombosis to or below the confluence of the superior mesenteric vein (SMV) and splenic veins (grade 3 and 4) was encountered in the other 13 patients. Reconstruction in these patients involved thrombectomy in two patients (end-to-end anastomosis), interposition vein graft (1) and mesoportal jump grafts (transmesocolic allogeneic iliac vein grafts from the SMV) in 11 patients. Patients with PVT had a significantly greater median blood loss (21 units with PVT vs 14 units without PVT; p=0.04) and this was not unexpected. We were able to use the standard veno-venous bypass in 10 thrombectomized patients while single (axillo-femoral) bypass was used for the rest.

The observed 26% incidence of PVT in our patient population is the highest reported in the literature to date. We concluded that our patient population had a high prevalence of risk factors for PVT that had been previously reported in the literature and that these high risk patients can be transplanted with acceptable rates of survival and morbidity.

Liver Transplantation for Hepatitis C in U.S. Veterans

End-stage liver disease secondary to hepatitis C viral infection (HVC) is becoming one of the leading indicators for OLTX. [52-54] HCV has been detected in 20 - 42% of the patients undergoing OLTX and it appears that persistent post-transplant viremia is nearly ubiquitous. While positive HCV serology is commonly found in patients with alcoholic cirrhosis (37-51%), hepatitis B (12-53%) and cryptogenic cirrhosis (50%), it is rarely present in patients with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC). [55-56] The incidence of HCV (+/- associated history of ethanol abuse) induced end-stage liver disease is quite high (53%) in our

patient population and we are understandably concerned about recurrent disease and it's consequences.[15] Although we are still learning about the natural history of post-OLTX recurrent HCV infection, it appears histopathologic recurrence occurs in 30-70% of patients within the first year of post-transplantation. [52-54, 57] Recent reports have observed progression of recurrent disease to cirrhosis and death in 15-20% one to three years after transplantation. [52, 54, 57] In spite of increased morbidity observed in those with recurrent disease the survival rates have been acceptable. [57] In our experience, patients transplanted for HCV have the poorest outcome at five years compared to those transplanted for other diseases, however, the differences are not statistically significant. In addition, patients with histopathologic recurrence not unexpectedly have a lower survival rate compared to those without recurrence (Figs. 3,4). Regardless of these observations, the 70-75% five year survival rates for these patients are quite acceptable.

The persistence of HCV after transplantation and the pathogenesis of hepatocyte injury are two phenomena that we are still trying to understand. Is hepatocyte injury an immunomediated cytotoxicity or does the virus have a direct cytopathotic effect? Evidence supporting a direct cytopathic effect is the observation that significant pre or early post-transplant viremia is associated with rapid re-infection in the new allograft. [54,58] Cytotoxic T-lymphocytes specific for HCV have been observed in patients with chronic active HCV hepatitis. The role of these cells and post-transplant rejection and hepatocyte injury is not clear at this time. [59]

The factors contributing to the rate and severity of recurrent HCV are yet to be

fully elucidated, however, some have observed that the level of pre-transplant viremia, virus genotype and degree of immunosuppression appear to be important. [57,60,61] Aggressive HCV is more commonly associated with HCV type I-a genotype. [60] Allograft rejection and augmented immunosuppression appeared to lead to a higher incidence and earlier onset of recurrent HCV hepatitis. [57, 61] Herein, we would like to report our experience with OLTX for HCV in our first 100 patients and our observation of increased infections in patients with recurrent HCV. [62] In addition, we will discuss current treatment strategies in the setting of recurrent disease.

Infectious Morbidity and Recurrent HCV Hepatitis

Patients and Methods

Between October 1989 and September 1994, 100 consecutive U.S. veterans underwent OLTX under primary Tacrolimus-based immunosuppression. Of these, 52% were transplanted for end-stage liver disease due to hepatitis C. Diagnosis was confirmed by the presence of anti-HCV antibodies (by EIA I prior to March 1992 and by EIA II after March 1992) and confirmed by a RIBA II assay. Recurrent hepatitis was diagnosed histopathologically using previously reported criteria. [55,63,64] Our immunosuppressive protocol was described earlier. Peri-operative antibiotic prophylaxis consisted on ampicillin and cefotaxime for 24 hours. Patients who had a known penicillin allergy received clindamycin or vancomycin and aztreonam for 24 hours. Bactrim (trimethoprim 80 mg and sulfamethoxazole 400 mg) once daily indefinitely was employed for Pneumocystis prophylaxis. This dose was later reduced to thrice weekly. Aerosolized pentamidine or dapsone was substituted for patients with sulfa allergies or those unable to tolerate Bactrim for other reasons. Of the 100

patients 47 had participated in a trial comparing high dose oral acyclovir vs 7 day pre-emptive ganciclovir (administered for CMV sheeding) for prophylaxis of CMV disease.[31] For the remaining patients acyclovir, 600 mg daily, was administered for one month post-operatively as herpes simplex prophylaxis.

Infections were defined using Centers for Disease Control (CDC) criteria. [65] The diagnosis of invasive fungal infections were established by positive blood culture or evidence of tissue invasion on biopsy or autopsy. Infections were characterized as either major or minor. Included in the major infection category were bacteremia, intra-abdominal abscess, wound infection peritonitis, invasive fungal infection, pneumonia, clostridium difficile colitis, cholangitis and all symptomatic CMV infections (CVM disease). Minor infections included mucocutaneous herpes simplex and herpes zoster viral infections, cystitis and asymptomatic CMV sheeding.

We obtained surveillance cultures for CMV (buffy coat and urine) every two weeks for two months then monthly for four months after transplantation. By definition CMV disease required the presence of symptomatology attributable to CMV in addition to laboratory evidence of infection. CMV disease was defined as previously reported.[31]

Results

The median follow-up for living patients was 1,083 days (range 201-1970 days) through March 1995. Recurrent HCV hepatitis developed in 42% (22/52) of patients transplanted for end-stage liver disease due to HCV or in 22% of all patients. The median time to recurrence was 246 days (range 72-994 days).

Incidence and Type of Infections

Major infections occurred in 64% (14/22) of the patients with recurrent HCV hepatitis vs 38% (30/78) of the other patients (p = .04). Patients with recurrent HCV had significantly more episodes of major infections per patient as compared with other patients (mean 1.45 episodes per patient vs mean .51 episodes per patient, p = .003). In addition, patients with recurrent HCV were significantly more likely to have recurrent episodes of infection. Forty-five percent of patients with recurrent HCV had more than one episode of major infection as compared to only 10% (8/78) of all other patients (p= .005).

Major Bacterial Infections

Although the incidence of major bacterial infection in patients with recurrent disease (41%, 9/22) was not significantly higher than in other patients (28%, 22/78, p=NS), patients with recurrent disease had more episodes of major bacterial infections per patient compared to the rest (mean .86 episodes per patient vs mean .33 episodes per patient, p = .09). Overall, there were 19 episodes of bacterial infection in the 9 patients with recurrent HCV hepatitis. These included six cases of pneumonia, bacteremia (n=6), intra-abdominal abscess or peritonitis (n=5), and C. difficile colitis (n=2). The 26 episodes of bacterial infections in the other patients were bacteremias (n=13), intra-abdominal abscesses or peritonitis (n=7), wound infection (n=2), colitis (n=2) and pneumonia (n=2).

Major Fungal Infections

Major fungal infections were more commonly observed in patients with recurrent HCV hepatitis (18% vs 6%). The observed fungal infections in patients with recurrent disease comprised of cryptococcosis in two (one patient had concurrent candidemia),

invasive aspergillosis in one and chromoblastomycosis in one patient. In the remaining patients fungal infections included crytococosis in one, invasive aspergillosis in two (one with concurrent candidemia) and candidemia in one patient.

Major Viral Infections

There were no significant differences in the incidences of CMV infection in patients with or without recurrent HCV however, symptomatic CMV disease occurred significantly more frequently in patients with recurrent disease (32% vs 9% in other patients, p=.012).

Additional Observations

Infections occurring beyond six months post-transplant were also noted to be significantly higher in patients with recurrent HCV (27% vs 6% in other patients, p=.011). It is also notable that a higher incidence of infections was observed only in patients with HCV recurrence post-transplant and not in HCV positive patients who did not have clinical or histopathologic recurrent HCV hepatitis. Patients with HCV but without recurrent disease had significantly lower numbers of episodes of major infections, a lower incidence of major infections and a trend towards a lower incidence of fungal infections. There was also a trend towards less CMV disease than patients with clinically recurrent HCV hepatitis.

Risk Factors for Infections

In patients with recurrent HCV hepatitis compared to all other patients there was no observed significant difference in the severity of underlying liver disease at the time of transplantation as assessed by the UNOS score and Child's-Pugh score.

We did observe that biopsy proven rejection episodes within the first six months

were higher in patients with recurrent HCV hepatitis, however, the cumulative immunosuppression administered in both groups were not significantly different for patients with recurrent HCV versus all other patients. When we examined late rejection (greater than six months post-transplant) again there was no significant difference in the amount of immunosuppression.

The rate of re-transplantation in patients with recurrent HCV hepatitis (median 1083 days) was not different from that in all other patients (9% (1/22) vs 10%, 8/78). Survival at the latest follow-up was 82% (18/22) for patients with recurrent HCV as compared with 91% (71/78) for all other patients (p= NS).

Our data indicate that recurrent HCV hepatitis after OLTX is associated with a higher incidence of major infections particularly with pathogens associated with depressed cell mediated immunity.

Treatment Strategies for Recurrent HCV after Liver Transplantation

Recurrent HCV hepatitis post-OLTX indeed poses a dilemma given the largely unsatisfactory number and efficicacy of therapeutic options. Interferon-alpha (INF) suppresses HCV by a direct antiviral effect or through inhibition of viral replication. By increasing the MHC class I antigen expression on hepatocytes, INF may lead to an enhanced cytotoxic T-cell activity against infected hepatocytes. The non-transplant literature reports normalization of liver enzymes and loss of HCV RNA in approximately 50% of cases however nearly one half relapse upon cessation of therapy.

Very little data is available regarding the safety and efficacy of interferon in the post-transplant setting. Two studies have indicated that interferon therapy does not

appear to precipitate allograft rejection however, response to short term (6 months) of therapy, as defined by normalization of aminotransferases and/or diminution of HCV RNA levels, was disappointing (9% and 28%).[66-67] These effects also appeared to be transient. A recent report by Feray et. al has advocated caution and concern about the potential for the increased risk of rejection due to upregulation of HLA antigens by interferon.[68] They observed chronic rejection in five of 14 patients treated with INF therapy for recurrent disease and only a small percentage of complete responders. We assessed the safety and efficicacy of a six month course of interferon therapy in 18 consecutive OLTX recipients and report the long-term response with maintenance therapy.[69] To our knowledge no study in the post-transplant setting has evaluated the efficacy of long-term (greater than six months) INF therapy.

Patient and Methods

Recurrent HCV hepatitis developed in 42% (22/52) of patients over a five year period. Four patients received less than one month of interferon and were excluded from the study. Eighteen patients received ≥ six months of therapy and comprised the study sample. Recurrent HCV hepatitis was diagnosed histopathologically using previously defined criteria. [55,63,64] Diagnostic criteria for acute cellular rejection included portal inflammation with large activated lymphocytes, the presence of eosinophils, definite bile duct damage and destruction, endotheliitis, absence of lobular hepatitis and hepatocyte necrosis.

Complete response to interferon therapy was defined as normalization of both aspartate and alanine aminotransferases (AST and ALT). Early responders were defined as patients having a complete response within the first six months of therapy.

Late responders were defined as patients with complete response after six months of therapy. Non-responders did not demonstrate a complete response either at six months or at the latest follow-up (median 24 months).

Complete blood counts, liver and renal function tests, after baseline studies and post-operative recovery, were obtained monthly. Leukopenia was defined as WBC count less than 2,500 and thrombocytopenia as a platelet count less than 75,000.

Results

The median follow-up period after the institution of INF was 24 months (range 7-54 months) and the mean age of the study patients was 48 years (range 32 - 60 years). All donors (except one) were seronegative for HCV. The median time to HCV recurrence was 192 days in a range between 30 and 1103 days. The seropositive donor organ was transplanted into an HCV seropositive recipient with clinical recurrence observed at 370 days post-transplant. Fifty-five percent (10/18) of patients received 3 million units thrice weekly; 28% (5/18) received 5 million units thrice weekly; and 16% (3/18) received 1.5 million units thrice weekly. Complete response at six months was observed in 28% (5/18) of patients resulting in a 62% (13/18) non-responder rate at six months. We encouraged both responders and non-responders to continue interferon therapy indefinitely. Seven patients did not receive continued maintenance therapy with five patients refusing to continue therapy and discontinuation of therapy due to CMV hepatitis (n=1), and leukopenia (n=1). Of the 11 patients continuing therapy, four were early responders and seven were non-responders at six months. The patients in the early responder group who continued therapy (4/4) all demonstrated sustained response at the latest follow-up. Of the 13 in

non-responders. late response was observed in 46% (6/13). Of these patients four had continued with maintenance therapy. While overall, only 43% (3/7) demonstrated a long-term sustained response after six months of therapy, 73% (8/11) patients who continue interferon therapy beyond six months experienced a sustained response (p=.33) The duration of maintenance therapy ranged between 11 and 36 months (median 21 months).

In the seven long-term non-responders, follow-up liver biopsy revealed no change in two patients and increased lobular inflammation in five patients. The early and late responders (n=11), when biopsied showed either a decrease in lobular inflammation (3/4) or no change in histology (1/4).

Side effects of interferon therapy included fatigue 39% (7/8), headaches and tremors 22% (4/18) (some of which also had fatigue) and cytopenias 28% (5/18) (leukopenia in four patients and thrombocytopenia in one patient). Rejection was observed in 6% (1/18) of patients receiving interferon. Given that in the majority of patients we did not initiate therapy until approximately six months post-transplant (median time to recurrent HCV) we compared the incidence of rejection occurring > six months post-transplant in the patients who did not receive interferon. The incidence of late > six months) rejection was 11% (8/70).

Responders and non-responders did not differ with respect with to age, total bilirubin, AST or ALT, re-transplantation rate or morbidity. Only one patient (non-responder) required re-transplantation and expired secondary to sepsis. There were no other mortalities. We did note a trend in non-responders to a higher baseline gammaglutamyl transferase (GGT) (p=0.05) and earlier recurrence (p=.12) compared

with responders. In addition, responders had a significant reduction from their baseline GGT at six months compared to non-responders. There was no correlation between dosage and response.

Conclusions

In the early 1980's the world of solid organ transplantation was revolutionized by the successful utilization of cyclosporine and since that time further technical refinements and improved organ preservation have allowed liver transplantation to evolve into an important and effective therapeutic modality for end-stage liver disease. In light of the recent evidence obtained from large multi-center trials in both the United States and Europe as well as the experience at the University of Pittsburgh it appears that we have stepped to a higher level of refinement in immunosuppression and we have further improved the overall survival and quality of life of liver transplant recipients. This is also readily evident with the results presented herein with a group of high risk patients requiring transplantation. The lower incidence of rejection, less reliance on long-term steroid use, and comparable infectious morbidity have contributed to excellent patient and graft survival rates in a notably high risk group.

With the advent of the Chiron assay in 1989 for HCV detection and evolution of more sophisticated detection and quantation methodology, HCV hepatitis is being recognized as a leading cause of end-stage liver disease and indication for OLTX.[70-72] It is now well known that HCV persists after transplantation and 95% (or more) patients remain viremic. It would seem that clinical and histologic recurrent disease is inevitable, however, thus far it has only been observed in 30 - 70% of patients. The natural history of this disease after transplantation will become clearer with time and

further investigation. Viruses such as CMV and hepatitis B have been described as immunomodulatory in that they appear to exert a direct immunosuppressive effect and facilitate super infections by other opportunistic pathogens. [73] This phenomena is well described with CMV [73,74] and a higher incidence of septic complications has been observed in patients undergoing transplantation for hepatitis B. In one report 51 HBsAg positive patients there was an overall 45% mortality with one half of these secondary to sepsis. Data regarding the potential immunomodulatory effect of HCV are sparse. One study predating HCV testing in renal transplant patients reported a higher incidence of life-threatening infections in patients with "non-A, non-B hepatitis"(NANB).[75] In addition, allograft survival in patients with NANB hepatitis was superior suggesting again that hepatitis itself had an immunosuppressive effect. Unfortunately, the nature of infectious complications and other confounding variables such as immunosuppression were not reported.[75] Our data show that recurrent HCV hepatitis after transplantation is associated with a higher incidence of major infections and their attendant morbidity.[15] While major bacterial infections did not differ between patients with and without recurrent HCV (41% vs 28%), fungal disease (18% vs 6%), and CVM disease (32% vs 9%) were distinctly more common. The increase susceptibility to these pathogens associated with depressed cell mediated immunity suggests that HCV infection has an immunomodulatory effect on the recipient. It is notable that this effect was apparent only in patients with histopathologically proven recurrent disease. Although our patients with recurrent HCV had a higher incidence of early rejection the cumulative immunosuppressive was not significantly different from those without disease.[15,61]

There are only a few clues at present that point to a possible immunodefect associated with recurred HCV hepatitis. Adler et. al. reported a higher incidence of death in patients with parenchymal disease compared with patients with cholestatic disease.[76] Although HCV testing was not available at the time, 67% of the patients with parenchymal disease had cryptogenic cirrhosis. We now know the majority of patients with cryptogenic cirrhosis are found to have HCV.[77] Adler et. al. observed significantly lower CD4 cells and CD4/8 ratios in patients with parenchymal disease. It is conceivable that a significant number of these patients had HCV.[76] Lower CD4 levels and increased CD8 T lymphocytes were also observed in renal transplant patients with NANB hepatitis.[75]

Our data indicate that HCV is not only a significant cause of liver disease after transplantation but it is associated with a higher incidence of infections due to pathogens associated with depressed cell mediated immunity. Further investigation is needed to elucidate the effect of recurrent HCV on host defenses. In addition, since the propensity to increased infections appears to be a problem in patients with recurrent disease and not in those without clinical recurrence, efforts to prevent recurrence should be explored.

Therapeutic options for recurrent HCV hepatitis after OLTX remain largely unsatisfactory. In contrast to previous reports describing a poor and non-sustained response to IFN we observed a sustained response rate of 73% with long-term (> 6 months) interferon therapy. We did not encounter problems with acute or chronic rejection as did Feray et. al. and this may be attributable to different immunosuppressive protocols (tacrolimus-based vs cyclosporine-based). While our

data were uncontrolled and our sample size was small it is still one of the largest reported to date in the post-transplant setting. Our data also supports a suppressive rather than a curative role of interferon therapy for HCV recurrence.

In our experience, INF in liver transplant recipients was well tolerated with relatively benign side-effects. Future trials should evaluate the longer courses of therapy for HCV recurrence until more effective anti-viral therapies become available.

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