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Recurrent liver disease after liver transplantation

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A conference was organised addressing a number of questions on viral re-infection of the liver graft and their possible answers. Presentations focused on the controversial indications for liver transplantation in hepatitis B virus (HBV)-related disorders due to the high rate of hepatitis B recurrence in the graft, to the selection of patients with hepatocellular carcinoma, to the prognosis of transplantation in hepatitis C virus (HCV) disorders and the role of HCV genotypes in determining the outcome of surgery, to the relatively favourable outcome of hepatitis D virus (HDV) transplants and of transplants with double HBV-HCV infection compared to HBV or HCV alone, to the role of immunosuppression in influencing the course of recurrent viral hepatitis.

Dr. Umberto Cillo (Cattedra Malattie Apparato Digerente, Clinica Chirurgica I, Patologia Medica I, University of Padova) reported on behalf of the Padova liver transplant group (Patrizia Burra, Stefano Fagiuoli, Rosa Maria Iemmolo, Remo Naccarato) their experience on liver transplantation in HBV- and HCV- related cirrhosis and hepatocellular carcinoma.

HBV-related cirrhosis

As long-term passive immunoprophylaxis with anti-HBs immunoglobulins diminishes the rate of HBV recurrence in transplanted HBsAg +/HBV-DNA positive patients, candidates undergoing evaluation for liver transplantation in Padua were selected on the basis of undetectable serum HBV-DNA as assessed by polymerase chain reaction (PCR); at the time of liver transplantation, during the anhepatic phase, passive immunoprophylaxis with 10.000 IU anti-HBs IG was given i.v. and the same dose was administered daily during the first 7 post-operative days and weekly for 4 weeks after transplantation. The maintainance dose was 1500 IU i.m. weekly to keep anti-HBs titre above 400 IU/l.

At the beginning of the transplant program, five patients were transplanted before the selection/immunoprophylaxis protocol was started; 4/5 developed HBV-related liver disease, 2/4 died of hepatic failure, confirming that morbidity and mortality are high in non-selected patients.

In the first series of 17 patients enrolled in the study, 6 died within three months and the remaining 11 (all HBsAg+/HBV-DNA- by PCR prior to liver transplantation and all receiving passive immunoprophylaxis) were re-evaluated at 3, 6, 12, 24, 36 months after transplantation.

HBV-DNA was undetectable in serum in all patients at months 3 and at month 6 it was undetectable both in serum and tissue; it became positive in one patient who also developed anti-HCV ("*de novo*" HCV infection post-transplantation).

DISCUSSION

At month 36 of follow up all patients but one were HBsAg- and HBV-DNAnegative by PCR in serum and tissue, confirming that a strict selection based on undetectable HBV-DNA and long term immunoprophylaxis are needed to mantain good graft function and patient survival after transplantation.

HCV-related cirrhosis

The recurrence of HCV disease is recognised as a potential factor limiting both graft and recipient survival in patients undergoing liver transplantation for HCV-related cirrhosis.
In Padua HCV-related cirrhosis was the indication for liver transplantation in 36 patients, equal to 29.8% of the total of 127 patients transplanted (29 males, 7 females, age 31-63 years).
Anti-HCV, serum HCV-RNA activity and Knodell score on liver biopsy were assessed at 3, 6 and 12 months after liver transplantation.
Hepatitis C recurred in 19 patients (52.7%) between 3 and 6 months after liver transplantation; chronic active hepatitis developed within 12 months in 9 patients and 1 patient died of liver failure 24 months after transplantation. HCV-RNA was found in 14/27 tested patients before transplantation; it was confirmed at each interval time after transplantation.
Anti-HCV remained positive in all patients after the operation.
In the HCV-RNA positive subgroup of patients, the mean Knodell score in liver biopsies was 9.7 ± 3.5. By contrast, a recurrence of HCV disease occurred in 5 of the 13 patients who were HCV-RNA negative at transplantation; chronic active hepatitis at 12 months was seen in 2 who had become HCV-RNA positive 3 months after surgery. The Knodell score in liver biopsies in these patients was significantly lower compared to HCV-RNA+ patients (3.0 ± 1.4, $p<0.001$).
The persistence of active viral replication is therefore associated with a more aggressive course of the recurrent hepatitis. In the medium term, graft loss due to HCV recurrence is uncommon but morbidity seems to increase with longer follow-ups.

Hepatocellular carcinoma

The role of liver transplantation in the treatment of hepatocellular carcinoma is controversial because the risk of tumour recurrence after the operation has not been clearly established.
The data of 221 patients who underwent liver transplantation between 1990 and 1996 were reviewed. Hepatocellular carcinoma was present in 27 patients (12.2%); in 13, the tumour was found incidentally at the time of transplantation.
The liver disease was HCV-related in 18 patients, HBV-related in 4, alcoholic in 2, primary sclerosing cholangitis-related in 2. Two patients underwent liver transplantation for primitive hepatocellular carcinoma.
All 14 patients with hepatocellular carcinoma diagnosed before surgery received transarterial chemoembolisation (TACE) 6 to 3 months before transplantation. Sixteen patients (11 of 14 with diagnosed hepatocellular carcinoma and 5 of 13 with incidental hepatocellular carcinoma) underwent post-transplant chemoprophylaxis with 5-fluorouracil (5-FU) and carboplatin. Only one patient developed severe leucopenia requiring withdrawal of treatment.
After a mean 27.9 months follow-up, the cumulative survival rate was 92.5%; two patients died within 4 weeks after transplantation for surgical complications, one patient died three years later for recurrence of HBV cirrhosis with no recurrence of hepatocellular carcinoma. Only one patient who did not receive adjuvant chemotherapy developed hepatocellular carcinoma in the graft.
Thus liver transplantation may be considered an option in well-selected patients with hepatocellular carcinoma; neoadjuvant TACE and post-transplantation chemoprophylaxis may help in limiting tumours after liver transplantation.
Dr. T. Starzl argued that the stage of HCC at the time of transplantation is important, with 3 cm as the critical size of the HCC

nodule permitting "safe" transplantation; even more critical is a timely operation, since many such patients will be screening themselves out simply because they have developed metastasis if surgery is delayed.
An exception are fibrolamellar hepatomas. These tumours may be separate and distinct; tumour size may not matter. Even with extra-hepatic metastases some patients have survived more then ten years, with 5 years survival at nearly 70%. **Dr. Starzl** stressed that transplantation may also offer a consistent salvage to patients with large or widespread tumours that by any current criteria would be considered hopeless. Thus ultimately it becomes a matter of applied ethics to withhold a therapy which would provide some kind of help even to the badly selected liver patient. Along this line **Dr. D'Amico** from Padua stressed that marginal livers are currently thrown away by the hundreds and that many more of them could be used for neoplastic patients, providing a larger therapeutic window for this unfortunate group. One example is duct cell carcinoma; although transplantation survival is only 30% at 5 years, this proportion of patients would have been doomed to death without operation. On this issue, **Dr. Starzl** commented that as a non-neoplastic patient he would rather have a whole liver from a 60 year-old man than a split liver from a 20 year-old donor, but as a neoplastic patient he would take any type of liver.
Of course the use of marginal livers implies a commitment to re-transplantation if things go wrong; if not, no responsible surgeon and no patient would accept a marginal liver when they had a chance to wait for a good one from a healthy donor.
Dr. Starzl reiterated that when the doctor operates he commits himself to that patient and not to some statistic and that patient abandonment is never possible.
Dr. Ignazio Marino in collaboration with Dr. T. Gayowski and N. Singh (National Liver Transplant Center, Dept. Veterans Affairs, Pittsburgh, PA and the T. E. Starzl Transplantation Institute; Dept. of Surgery, University of Pittsburgh, PA) addressed the issue of the role of HCV genotypes in influencing the recurrence of hepatitis C after orthotopic liver transplant (OLT).

Between October 1989 and October 1995, 140 othotopic liver transplantations were performed on 130 consecutive patients, under primary tacrolimus-based immunosuppression. Fifty-three percent (68/130) of these patients underwent transplantation for endstage liver disease due to hepatitis C. The authors investigated the influence of HCV genotypes on the incidence and timing of recurrent HCV hepatitis, survival, infectious morbidity, and response to interferon-α therapy in this unique patient population. HCV genotype was determined by direct sequencing of the NS5 region of HCV and with type specific primers. Genotype 1a (66%, 32/47) was the predominant genotype. Type 1b was found in 25% (12/47), and type 2b in 9% (4/47). Histopathologically, recurrent HCV hepatitis developed in 53% (25/47), of the patients after liver transplantation. These included 45% (14/31) of the patients with type 1a, 67% (8/12) with type 1b, and 25% (1/4) of the patients with type 2b ($p>0.5$). The time to recurrence and the severity of HCV recurrence as defined by aminotransferase levels or Knodell scores were not different between the 3 genotypes (Table 1).
No variable differed significantly among the three groups
There was a trend towards the higher incidence of major infections in patients with type 1b (75%) vs type 1a (48%), and type 2b (50%) ($p=0.11$) (Table 2) (In 2b there was only one recurrence).
The response to interferon-α therapy did not differ significantly between the genotypes. Mortality at 5 years was 16% (5/31) in patients with genotype 1a, 42% (5/12) in 1b, and 50% (2/4) in 2b ($p=0.06$). In conclusion, the incidence, time to recurrence, and response to interferon therapy did not differ between the various genotypes in liver transplant recipients. However, there was a trend towards higher morbidity and overall mortality in patients with genotype 1b after transplantation.

Dr. Giorgio Rossi, Institute of Experimental and Transplant Surgery, University of Milano, investigated the outcome of liver transplantation in HBV-HCV co-infected patients vs patients infected with HBV or HCV alone. Data were presented on viral

DISCUSSION

Table 1. Incidence, timing and severity of recurrent HCV in patients with 3 genotypes

Variable	1a (n=31)	1b (n=12)	2b (n=4)
Rate of Recurrence	52%	67%	25%
Median Time of Recurrence, in days (range)	301 (87-994)	198 (72-174)	111
Mean AST■	116	88	150
Mean ALT▲	124	87	224
Mean Knodell score	5.0	5.8	2

• *IU/L*; ■*AST, aspartate, aminotransferase*; ▲*ALT, alanine aminotransferase*

infection in 138 transplants; 48 of them had HBV disease with or without delta co-infection, 63 had HCV disease and 27 had double HCV and HBV disease (Figure 1). The HCV genotype (Table 3) was evaluated in a number of patients after the transplant. In patients with HCV infection genotype 1b was more frequent (n=23) compared to genotype 2 (n=13). Genotype 1b is widespread in Italy, is the most pathogenic and is the main indication for liver transplantation; type 2 is the second genotype in order of diffusion in Italy. However, in the groups with HBV-HCV co-infection type 2 was more frequent than type 1b (n=7 vs n=4). Thus there was a different distribution of genotypes in the 2 groups, although the difference was not statistically significant. There were 3 RNA negative adult patients among the 16 patients (19%) in the HBV-HCV group and 4 (9%) RNA negative pediatric patients in the HCV group. The results are reported in Table 4.

Survival in the HBV, HCV and HBV-HCV groups was respectively 65%, 54% and 74%. No re-transplantation was required in the HBV-HCV group.

The recurrence of the viral infection was diagnosed on the basis of histology, elevation of liver enzymes and the finding of the HBsAg.

In the group with HBV infection only, the rate of recurrence was 19%; in the HCV group it was 50%.

In the group with HBV-HCV co-infection the rate of recurrence was 15% for HBV and

Table 2. Major infections in patients with recurrent HCV

	1a (n=16)	1b (n=8)
Major infection	50%	75%
Mean number of infections	1.0	1.67
Major bacterial infections	38%	62%
Major fungal infections	19%	0%
CMV infection	50%	62%
CMV disease	31%	25%

DISCUSSION

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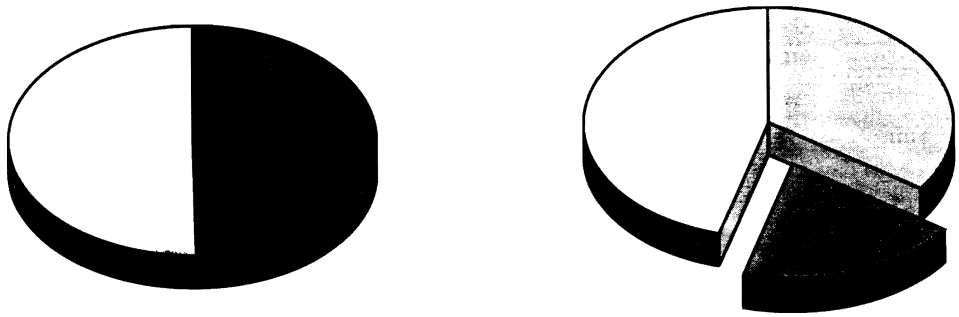

Figure 1. Liver transplantation for cirrhosis

40% for HCV; these figures are not significantly different from the recurrence rate of each single infection.
The fatality rate was 35%, 46% and 26% respectively, for the HBV, HCV and HBV-HCV groups; no death was caused by viral recurrence in the HBV-HCV group while viral recurrence was the cause of death in 11% of the HBV fatalities and 35% of the HCV fatalities. Thus no patient died for viral-related disease recrudescence in the HBV-HCV group although the rate of viral recurrence was not different from the single infections; this probably indicates that HBV-HCV re-infections run a milder clinical course than HBV or HCV infection alone.
Possibly HBV and HCV interact so that their virulence is diminished, the clinical balance being a decreased damage to the liver graft.

Dr. Mario Alessiani (Istituto di Patologia Chirurgica I, Università-IRCCS, Policlinico San Matteo, Pavia) elaborated on the role of immunosuppression; this is a factor that can enhance liver disease recurrence both in hepatitis and in tumour patients. If immunosuppression were to be lowered, or, ideally, avoided, both groups would stand a distinctly smaller risk of disease recurrence.
There are two main venues of research in this field:

i. the first is the improvement of pharmacologic immunosuppression with better and more specific immunosuppressive drugs;
ii. the second is the induction of better graft acceptance and the possibility to reach the final goal of graft tolerance without immunosuppression.

Table 3. ***HBV-HCV co-infection vs HCV infection.*** *Post-OLT HCV genotype infection*

HCV genotype	HBV-HCV (n=16)	HCV (n=44)
1a	1	2
1b	4	23
2	7	13
3	1	1
4	0	1
RNA negative	3	4

DISCUSSION

Table 4. Results of OLT in viral cirrhosis

Infection (#)	Alive	Re-OLT (%)	Recurrence (%)	Dead	Dead for recurrence (%)
HBV±HDV (48)	31 (65%)	1 (3%)	6 (19%)	17 (35%)	2 (11%)
HCV (63)	34 (54%)	1 (3%)	17 (50%)	29 (46%)	10 (35%)
HBV-HCV co-infection (27)	20 (74%)	0	3 HBV (15%) 8 HCV (40%)	7 (26%)	0

At present one can count on an immunosuppressive armamentarium which is four times bigger than 10 years ago; however, the majority of the new immunosuppressive drugs are still under investigation. In view of the wide choice of drugs, single and well defined immunosuppressive protocols should be used for all patients of transplant centre; these should be much more flexible using different baseline agents on the basis of clinical parameters such as:

- organ transplanted
- type of rejection
- primary or secondary disease
- tolerance to drugs
- age
- cross-match

An important parameter is the primary disease. Thus the first question is: which is at present the best primary baseline agent for hepatitis or tumour patients? In other words, is there a drug of choice that, without increasing the risk of rejection, can reduce the incidence and severity of disease recurrence? To date, the analysis can be performed only on two drugs considered as the main baseline agents: Cyclosporin A (CyA) and FK506. There is a lot of confusion on this subject in the literature, with claims in favour of CyA or in favour of FK506. There are reports in favour of CyA, but these studies contain biases, such as the small number of patients and the high dosage of FK506; in addition, a relationship between a higher incidence and severity of HCV recurrence and the use of FK506 has not been demonstrated. Instead, in a recent US multi-centre randomised trial, the evaluation of 113 patients demonstrates that there is a trend to better survival at 3 years in the FK506 arm.

Thus from reported data and from the personal experience, some conclusions can be made:

- FK506 is steroid sparing and decreases the need for OKT3
- When comparing FK506 and CyA for liver disease recurrence, both agents have equivalent outcome in HBV, HCV and tumour patients.
- The efficacy of new combinations, such as FK506 + MMF or Cyclosporin A + Rapa should be assessed.

The other main area of research is the development of new strategies to induce or facilitate donor-specific tolerance. One of these strategies is simultaneous BM infusion and OLT. Are there any effects of this procedure on HBV or HCV recurrence after the transplant? There are no data on early stages, but at a late stage a drug-free status can be achieved or, at least, a low immunosuppressive status; this lowers the risk of recurrence and, ultimately, the risk of re-transplantation or death.

After BMTx only, infused in HBV positive patients, there is no evidence of increased incidence of liver disease. Data from 3 major reports support the use of BM infusion simultaneously to OLT in hepatitis patients.

Dr. Antonina Smedile on behalf of the Turin liver transplant group (Dr. Marzano, De Bernardi, Ghisetti, Brunetto, Torrani, Salizzoni) addressed the issue of a safe level of pre-transplant viraemia and that of anti-viral prophylaxis in HBV transplants.

DISCUSSION

An important factor predicting the rate of HBV recurrences is HBV-DNA quantitation prior to OLT. Using a PCR-based assay for HBV-DNA detection in candidates undergoing OLT, it was demonstrated that the risk of re-infection was lower (<17%) in those testing PCR-negative before surgery. This evidence has prompted the use of available anti-viral therapy as a prophylactic measure, prior to OLT, to abate levels of viraemia below PCR sensitivity for the prevention of HBV recurrence. **Dr. Smedile** reported the experience in Turin between 1994-1997, in a subgroup of viraemic candidates with chronic HBV infection treated before OLT with gancyclovir, ARA-AMP or lamivudine followed by standard HBIg administration. Patients' features and schedule of treatment with nucleoside analogues are reported in Figures 2, 3.

In the period 1994-1996, 16 patients were treated with gancyclovir and ARA-AMP prior to OLT (12 gancyclovir, 4 ARA-AMP); 4 died after surgery and 12 are alive with a mean follow-up of 24 months (range 13-33 mo). In all treated patients the levels of viraemia before surgery were between 10^3 -10^7 genome/ml; serum HBV-DNA decreased after therapy by 1-2 log; at the time of transplantation 5 patients tested weakly positive by PCR for HBV-DNA, 6 were PCR-negative and in 5 viraemia had decreased but was still strongly positive by PCR.

HBV re-infection occurred in 3 patients (gancyclovir 2/8 25%; ARA-AMP 1/4 25%); the time of recurrence was between 2-4 months after OLT. All 3 re-infected patients had a symptomatic hepatitis which was treated with Lamivudine (100-300 mg); in all, HBV-DNA cleared in two months and the acute hepatitis evolved to chronic hepatitis.

In the period 1996-1997, 21 patients in the waiting list have been treated with lamivudine 100-300 mg/day, obtaining a sustained suppression of viral replicaton; 10 patients (3 HBeAg positive) who became PCR HBV DNA negative under therapy, were transplanted. None had a recurrence of HBV after a medium follow-up of 6 months (range 6-12 mo). Lamivudine was well tolerated prior to OLT in decompensated cirrhosis and during the therapy of recurrent hepatitis. No adverse effects were noted in long-term treated transplants.

These data confirm the concept that HBV viraemia influences the recurrence of hepatits B in the graft; the risk is proportional to the level of viraemia and significantly diminishes with virus charges below 10^4 gen Eq/ml. Given pre-OLT, new nucleosides of recent development, such as lamivudine, are effective in decreasing the viral load and no re-infection was seen in the Turin series. However, larger studies in patients with chronic hepatitis B in the Far East treated with lamivudine, have reported a 10% of drug resistance with emergence of lamivudine-resistant HBV variants. Similar experience was reported in England in patients treated with lamivudine prior to OLT without HBIg prophylaxis after surgery. The use of prophylactic anti-viral therapy

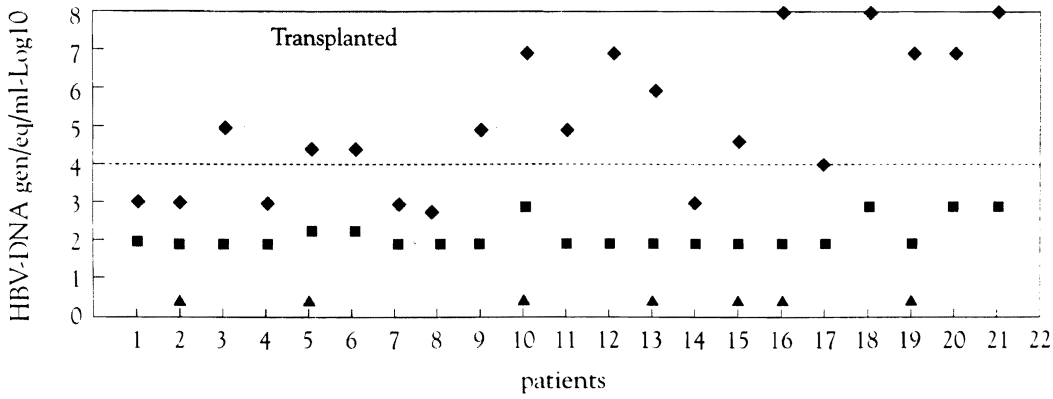

Figure 2. HBV-DNA before and after therapy with Lamivudine
◆ *HBV-DNA before therapy;* ■ *HBV-DNA after therapy;* ▲ *HBeAg*

DISCUSSION

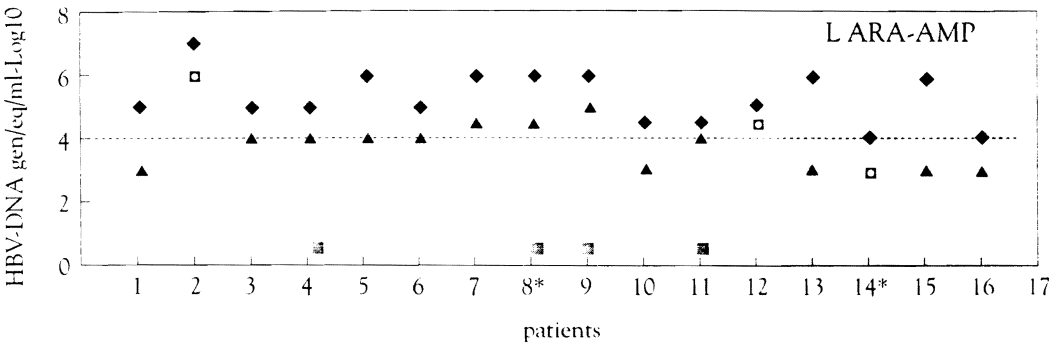

Figure 3. HBV-DNA before and after therapy with ganciclovir and lactosaminated ARA-AMP
◆ *HBV-DNA before therapy;* ▲ *HBV-DNA after therapy;* ■ *death;* ■ *recurrence;* ○ *HBeAg*

combined with standard HBIg protection after OLT remains an open question since there is no clear evidence that HBV recurrence can be prevented with antiviral drugs alone. **Dr. Starzl** commented that there is indeed a tremendous penalty for HBV patients. In these patients, therapy with interferon may increase the risk of rejection. This, however, may be compensated by increasing immunosuppression; interestingly, the overall risk of liver rejection under IFN therapy in liver transplants is much less consistent than in kidney transplants.

Transplantation for chronic Delta Hepatitis

Liver transplantation is a valid therapeutic measure for the therapy of decompensated delta cirrhosis. Delta hepatitis is an uncommon cause of liver transplantation in the United States but represents a large proportion of transplants candidates in Italy of 27 patients who received short-term HBIg prophylaxis against HBV or no prophylaxis at all, 22 (81%) became re-infected with HDV, but only 11 (14%) were also re-infected with HBV and had a HDV relapse. Treatment of recurrent delta hepatitis has been unsatisfactory. Various therapies targeted to HBV have been tried without success. However, the recurrence of hepatitis after transplantation for chronic delta hepatitis is less frequent than for chronic hepatitis B, the reason being that delta hepatitis cannot recur unless hepatitis B also recurs. As a consequence prevention of HBV recurrence using passive immunoprophylaxis has also been efficacious in the prevention of HDV infection. The re-infection rate, after the introduction of standard HBIg prophylaxis against HBV, has diminished from 67 to 12-9% in two larger series studied in Italy and France. Thus, the long-term prophylaxis with immunoglobulins against HBsAg is highly effective. In the Turin liver transplant program 45 patients were transplanted for HDV cirrhosis.

The characteristics of the patients are reported in Table 5. The clinical analysis demonstrated that there was in most reinfected patients an early HDV reinfection persisting at low levels and causing no hepatic damage. When HBV infection recurred, HDV increased exponentially and caused clinical disease. These evidences were recently revisited using sensitive PCR-assays to study the subsequent appearance of HDV/HBV nucleic acids in serum and liver of re-infected transplants. This analysis clearly demonstrated that there is a strict and interfering influence of HBV in the final appearance of liver damage in the graft.

Table 5. OLT HDV patients in Turin

Male/female	27/13
Mean age (yrs)	41 (23-56)
Median follow-up	43 (6-106)
Post operative deaths	4
HDV recurrence (n° pts)	6

Moreover, the presence of nucleic acids (HDV RNA in 3; HDV-RNA/HBV-DNA in 2) in no re-infected patients under permanent immunoprophylaxis raises doubts on the hypothesis to stop HBIg in this category of patients.

There were 4 patients who developed chronic active hepatitis (CAH) and 2 who developed an histologic cirrhosis. The 8 years survival curve according to Kaplan Mayer was excellent; 98% of patients are alive and well.

In conclusion, liver transplantation for HDV hepatitis is one of the better indications for viral cirrhosis. Considering the maximum peak of HDV disease expression in the early 70-80s in North Italy and the persistence of HDV infection in Southern regions of the country, it is likely that more cases of HDV cirrhosis will require OLT in the next future. The long-term outcome of liver transplantation for HDV cirrhosis, since the introduction of permanent HBIg prophylaxis looks very good. Results are also promising even for those few candidates with concomitant HBV replication; they are likely to benefit from anti-viral therapy with nucleoside analogues to inhibit HBV replication prior to surgery.