POINTER

Porto Internacional

in Liver Transplantation and Hepatocellular Carcinoma

6-7 MAY 2011
Biblioteca Almeida Garrett, Porto, Portugal

For more information please go to: **www.ibmc.up.pt/pointer**

Sponsors:

Design: Office for Science Communication - IBMC•INEB

Program

POINTERS in Liver Transplantation and Hepatocellular Carcinoma 2011

May 6-7, 2011

FRIDAY, 6 MAY

9.30-10.00: Introduction | ***Bissell, D. M.*** | *San Francisco*

Pointers in Liver Transplantation: Hepatitis C

10.00-10.45: Immune response to Hep C virus | ***Klenerman*** | *Oxford*

10.45-11.30: Retransplantation for recurrent hepatitis C in the transplant recipient: a contraindication? | ***Berenguer, M.*** | *Valencia*

11.30-12.00: Coffee Break

12.00-12.45: HCV disrupts BMP signaling and suppresses hepcidin. Why? | ***Drakesmith, H.*** | *Oxford*

12.45-13.30: Organ allocation: should the decision be guided by disease severity or by transplant benefit? | ***Biggins, S.*** | *Denver*

13.30-14:30: Lunch

Pointers in Liver Transplantation: reperfusion injury, immunity and surgery aspects

14.30-15.15: Novel targets for hepatic ischemia and reperfusion injury: Integrins and matrix metalloproteinases | ***A. Coito*** | *Los Angeles*

15:15-16:00: Allograft tolerance; minimizing immunosuppression | ***A. Sanchez-Fueyo*** | *Barcelona*

16:00-16:30: Coffee Break

16.30-17.00: The Portuguese experience in familial amyloidosis: patient and domino results | ***Linhares-Furtado*** | *University of Coimbra*

17.00-17.30: Liver allograft pathology | ***Cipriano, M. A.***| *HUC, Coimbra*

17.30- 18.00: Hepatic transplantation in S. Antonio Hospital – Porto | ***Daniel, J.*** | *CHP, Porto*

18.00-18.30: General discussion

SATURDAY, 7 MAY

Hepatocellular carcinoma

9.30-10.15: Reptin and Pontin in human hepatocellular carcinoma. Expression, role and therapeutic targeting | ***Rosenbaum, J.*** | *INSERM, Bordeaux*

10.15-11.00: Hepatocellular carcinoma, Resection or Liver Transplantation? Our experience | ***Barroso, E.*** | *Lisbon*

11.00-11.30: Coffee break

11.30-12.15: HCC: Natural history, and expansion of the Milan criteria | ***Durand, F.*** | *INSERM U773, Université Paris VII, Paris*

12.15-13.00: Concluding Remarks

List of Abstracts:

1. Barroso, E.
2. Berenguer, M.
3. Biggins, S.
4. Cipriano, M. A.
5. Coito, A.
6. Daniel, J.
7. Drakesmith, H.
8. Durand, F.
9. Klenerman, P.
10. Linhares-Furtado
11. Rosenbaum, J.
12. Sanchez-Fueyo

1.

Hepatocellular carcinoma, Resection or Liver Transplantation? Our experience

***Barroso, E.*[1]**

[1] Hospital Curry Cabral, Lisbon, Portugal; Faculty of Medicin, Universidade Nova de Lisboa

Background: The surgical management of hepatocellular carcinoma in patients with well-compensated cirrhosis is controversial. The purpose of the current study was to compare the outcome of patients with well-compensated cirrhosis and early-stage hepatocellular carcinoma treated with initial hepatic resection versus transplantation.

Methods: Between 1985 and 2008, 245 patients underwent hepatic resection and 134 patients underwent liver transplantation for early-stage hepatocellular carcinoma. All patients had well-compensated cirrhosis. Prognostic factors were evaluated using univariate and multivariate analyses; survival was calculated using the Kaplan-Meier method.

Results: Transplantation was associated with better 5-year disease-free and overall survival compared with resection. Hepatitis status, presence of microscopic vascular invasion, and tumor size were predictors for recurrence, while the presence of microscopic vascular invasion, and tumor size conferred an increased risk of death. The disease-free survival advantage with transplantation was more pronounced in hepatitis C patients compared with non-hepatitis and hepatitis B patients. The overall survival advantage with transplantation persisted in cases of solitary lesions £ 3cm, but was attenuated in patients with a MELD score £ 8.

Conclusion: In well-compensated cirrhotic patients with early-stage hepatocellular carcinoma, transplantation is associated with longer disease-free and overall survival. Patients best suited for initial resection for the treatment of hepatocellular carcinoma are those with a MELD £ 8 without evidence of hepatitis.

2.

Re-Transplantation in HCV

Berenguer, M.[1]

[1]Hepatology-Liver Transplantation Unit, Digestive Medicine Service, and CIBEREHD, National Network Center for Hepatology and Gastroenterology Research, Instituto de Salud Carlos III, Spain; Ciberehd is funded by the Instituto de Salud Carlos III.

Introduction:

Hepatitis C virus (HCV) alone or in association with alcohol is the most common cause of cirrhosis and hepatocelular carcinoma (HCC) in the Western world. In turn, HCV cirrhosis ± HCC represents the leading indication for liver transplantation (LT) in most registries (www.ont.es; www.eltr.org). HCV recurrence occurs in all patients and results in histologic HCV-disease with different patterns of presentation. Overall, fibrosis progression, cirrhosis and clinical decompensation occur more rapidly in HCV-transplant recipients than in immunocompetent patients, with a median interval from graft re-infection to cirrhosis of only 9.5 years. One third of transplant recipients develop cirrhosis within the first 5-10 years from transplantation (1), a reality that is likely to worsen with the increasing use of poor quality organs. Once cirrhosis is established, the risk of clinical decompensation is high in the short term, prompting consideration for hepatic retransplantation (RT) (2). While antiviral therapy with peginterferon-ribavirin has shown that it can positively modify the natural history of recurrent hepatitis C, its applicability is still low in most transplant programs and only 30% to 40% achieve a sustained viral response (SVR)(3). Based on all these facts, some authors estimated that the future burden from HCV recurrence could surpass the number of available grafts used for all indications. In an early UNOS series based on 1539 adults undergoing RT, the prevalence of HCV infection increased significantly from 6.5% in 1990 to 38.4% in 1995 (4). Data from more recent registries report a stability in the number of patients undergoing RT due to recurrent hepatitis C (5,6), possibly reflecting improvements in the management of HCV-recipients following the first transplant as well as an increased reluctance to list these patients due to the awareness of poor results together with organ shortage (11).

HCV-graft cirrhosis accounting for 3.6%-35% of all RT indications (4--11). Although results have improved in recent years (5), overall, patient and graft survival rates are inferior to those after primary LT (5-14) and are associated with a greater cost (12). Indications for RT include those regarded as urgent, such as primary non-function (PNF) and hepatic artery thrombosis (HAT) and those considered elective, particularly allograft failure due to recurrent disease. Indications of RT in the urgent forms are well established and universally accepted. In contrast, the use of elective RT, and more specifically RT in HCV- recurrent cirrhosis is still a matter of controversy due to the poor results reported in some studies, the fear that re-recurrent hepatitis C will result in the loss of a third liver, the lack of highly effective antiviral therapies, the organ shortage and the personal relationships developed over the years between the transplant team and the patient at need of RT. In summary, in the current era of critical organ shortage, whether RT, historically associated with increased resource utilization and diminished survival, should be offered to a patient whose first allograft is failing from HCV recurrence, has become a pressing question.

Results of RT in indications different from HCV-graft cirrhosis

RT remains the only therapeutic option for irreversible liver graft failure and represents between 5% to 23% of all LT. Recurrence of the primary disease as opposed to chronic rejection has become the main cause of RT (5).

Overall RT is associated with lesser survival and greater cost when compared with primary LT and the timing of RT and its specific etiology have important technical, prognostic, and ethical implications (5,11-17). Indeed, although survival rates have improved in the last two decades likely due to improvements in technical aspects, immunosuppression, organ allocation and a more careful selection of patients, there are still in general 10%-20% below primary transplants. The main causes of death are infectious complications and multiorgan failure. Some authors have attempted to create prognostic scores to be used as screening tools in the decision to consider RT (18-23). Factors that have been reported to predict worse results include age over 50 years, mechanical ventilation, renal insufficiency, a high Model for End-Stage Liver Disease (Meld) score, prolonged ischemia time and old donor age (5,6,12-23). In addition, in most series higher mortality rates have been described in elective RT where hepatitis C recurrence is the main cause for RT compared to outcomes in the urgent cases (4-6).

Specific results of RT in HCV-cirrhosis

(i) Graft and patient survival: Survival rates after RT for HCV-related graft failure are inferior to those achieved after the first LT procedure.. Controversy exists though as to whether the outcome of RT in HCV-infected patients is worse than that of uninfected RT patients, that is whether HCV itself increases mortality in a group of patients already predisposed to an inferior outcome. Another question is whether among HCV-infected patients undergoing RT there are differences between those retransplanted for HCV recurrence as opposed to those retransplanted for reasons other than HCV. Early single center studies showed that survival following RT was particularly poor in patients with recurrent HCV, even in those with concurrent causes of graft failure (15, 24-28). Studies using larger databases comparing the outcome of RT for HCV as opposed to other indications have yielded conflicting results. In general, HCV was found to be an independent factor associated with increased mortality in earlier studies. In 1999, Rosen reported diminished survival of HCV-serologically positive patients versus HCV-negative patients (57%, 55% and 54% vs 65%, 63% and 61% at 1, 3 and 5 years, respectively) (4). In 2003, Yoo and col published one of the largest series on RT using the UNOS database (n=4000 RT performed between 1988 and 2001) and found that HCV infection was one of the 7 risk factors associated with increased mortality together with PNF, recipient and donor age, creatinine levels and Afro-american race (29). In 2003, Royaie and col reported on 42 patients with allograft failure due to HCV-recurrence undergoing RT more than 3 months after the first transplant and compared their evolution to that of 55 non-HCV patients undergoing RT for other causes (30). Survival at 1 and 3 years was significantly lower in the HCV group (52% and 38%, vs 83% and 68%, respectively) and RT for recurrent HCV was independently associated with shorter survival on multivariate analysis. Among the HCV-positive RT patients, predictive factors of survival in the multivariate analysis were donor age and prothrombin time. As in many other series, the main causes of death were sepsis in the early post-RT (less than six months) and recurrence of HCV after 6 months. Pelletier and col. analysed 1,718 RT patients from 1997 to 2002 in the SRTR database, 27% of which were HCV-positive. HCV- recipients had a 30% higher risk of mortality than those without HCV (HR:1.30; 95CI:1.10-1.54; p=0.002). Most deaths occurred between 3 and 12 months after RT. HCV infection was found to be independently associated with increased mortality together with donor and recipient age, serum creatinine levels and the presence in the intensive care unit (31).

In contrast to these early reports, more recent studies have reported improved RT results, and in general, HCV infection is no longer identified as a factor independently associated with greater mortality. The adoption of measures to improve the results such as the use of RT models to select appropriate candidates and decide the optimal timing for RT (5,14, 17,23) or the preferential use of young donors in HCV-recipients undergoing RT (16) have possibly contributed to these changes. In the multicenter US study (11), 272 patients were divided in 3 groups: Group 1 comprised 43 HCV-positive patients undergoing RT for an HCV-related indication; group 2, 73 non-HCV patients undergoing RT for chronic rejection (36%), hepatic artery thrombosis (31%) and recurrent

primary sclerosing cholangitis (17%), and group 3, a group of 156 HCV-infected patients with HCV-related allograft failure not retransplanted. MELD scores were similar between groups 1 and 2. They found no-differences in survival between the RT groups (group 1 vs 2 at 1 yr: 69% vs 73%; at 3 years: 49% vs 55%). One important observation was that among patients belonging to group 3 (recurrent HCV not undergoing RT), 30% had been evaluated for RT but only 15% were listed and the 3-yr survival was only 47%. The most common reasons for not listing for RT were recurrent HCV within 6 months (22%), fibrosing cholestatic hepatitis (19%), and renal dysfunction (9%). RT patients were considered to be highly selected with 67% to 81% being calculated as low risk measured by the Rosen and Markmann RT survival models. Interestingly, 1-year survival was 0% in those retransplanted because of cholestatic hepatitis and time from first LT to RT < 1 year was associated with less survival than RT>1 year (11). Using the UNOS database, Ghabril evaluated the results of RT in 1,034 HCV and 1,249 non-HCV candidates who underwent RT at least 90 days or more after the first LT. Patient and graft survival were significantly lower in the HCV group compared to the non-HCV. However, on multivariate analysis, the only independent variables predictive of mortality were recipient age >60 years, MELD>25, RT during the first year after the first LT, donor age >60 and warm ischemia time>75 minutes (17). Finally, Marti and col recently reported on the potential benefit of adopting the Rosen model to improve results (5). In order to study the evolution of RT over time, their entire series of 108 patients RT for non-urgent causes was divided between a first period where indications were in general more liberal (1988 to 1997, n=53) to a second period where stricter criteria were adopted (1997-2006, n=55). In particular the Rosen model was used since 2003. Only patients who developed cirrhosis after 3 or more years from the first transplant were listed for RT. No significant differences in survival after RT at 1, 5 and 10 years were observed between patients with HCV recurrence and those RT for other causes (70%,57% and 57% vs 72%,50% and 45% respectively). In contrast, when comparing the 2 periods, outcomes were shown to have significantly improved (1, 5 and 10 yr survival: 66%, 45% and 40% vs 76%, 69% and 69%, respectively; p=0.014) despite the fact that the Donor Risk Index was greater in the second period (9). According to the UNOS Rosen risk score, patients in the low-risk group showed greater survival than patients in the high-risk group (5 yr survival of 75% vs 59%, p<0.01).

Recurrent HCV in the second allograft.

The development of aggressive re-recurrent disease in the second allograft is one of the fears that transplant physicians face when considering the option of RT in a patient who has already lost 2 livers to HCV-disease. The most common causes of death in the HCV-positive RT patients though are sepsis and multiorgan failure. These causes of death are similar to those reported for any other indication of RT, and refer mostly to early deaths occurring within 6 months of RT. After 6 months, a significant proportion of deaths are related to hepatitis C recurrence (25,26,30). The natural history of re-recurrent HCV disease and factors that may influence the fibrosis progression in the second LT have not been adequately explored.

Predictive models of patient survival:

Mathematical models have been developed to identify the most adequate RT candidates, and particularly to define the best timing for RT. These models though have often been based on urgent and elective indications (18-22). Because hepatitis C is usually an indication for elective RT, some of these models may not prove to be useful in candidates with hepatitis C graft cirrhosis. Variables that most frequently are included in the models because of their greater impact on survival are creatinine and bilirrubin levels, recipient and donor age, poor conditioning and interval to re-LT. In turn, the scores more often used in HCV groups include the Rosen score (18,21), the Meld score (11,16,17,32), the "donor-risk index" (19) or the "Markmann" model (20).

Conclusions: strategies to improve RT outcome in HCV-LT infected patients

Recent reports have demonstrated that results of RT for HCV-graft failure can be similar to those obtained in other indications. In order to achieve reasonable outcomes though, certain conditions are required, particularly a selection of patients who fulfil minimum criteria based on RT survival models. In 2003, the International Liver Transplantation Society Expert Panel already established that patients with bilirubin levels ≥10mg/dl, creatinine ≥ 2mg/dl, recipient age>55 years, donor age>40 years and early HCV recurrence were variables associated with poor outcome after RT. The use of these strict criteria (low patient age, low Meld score, lack of renal failure and hyperbilirrubinemia) have consistently been associated with improved survival. Unfortunately, it is exactly the patients who do not meet these criteria (jaundiced patients with renal failure and high Meld score) who gain more points, and hence priority, in the current allocation system. The question in an era of organ shortage and limited antiviral treatment efficacy is whether patients with allograft failure due to recurrent HCV should be upgraded so as to be transplanted when they may best benefit from transplantation, as it is already done for other indications such as HCC. This could potentially be considered in an HCV-patient in whom specific conditions, such as "poor quality donor", prolonged ischaemia time or complicated surgery likely contributed to the first LT failure, particularly if there is no history of antiviral failure. In the absence of such modifications in the allocation system, RT candidates will either die in the waiting list or will be retransplanted with very low chances of reasonable post-transplant outcome. Currently many patients are not listed for RT. The most common causes for not listing inlcude a short interval between the first transplant and recurrent HCV (< 6 months), fibrosing cholestatic hepatitis, renal dysfunction or age > 60 years. In addition, of those listed, most (79%) die while awaiting RT (15). Rosen et al analysed the impact of using different allocation policies on allograft utilization and patient outcome; limiting the use of donor organs for RT for patients at low risk (bilirrubin < 5 mg/dl) would result in an acceptable ratio of lives saved to allografts used (33). In conclusion, there are criteria to both support and not support RT in HCV patients. As for primary LT candidates though, the development of models based on donor and recipient factors, that allow for the identification of the futile RT is essential in an era of organ shortage.

References

(1) Samuel D, Forns X, Berenguer M,et al. Report of the monothematic EASL conference on liver transplantation for viral hepatitis. J Hepatol 2006; 45:127-143.

(2) Berenguer M, Prieto M, Raoyon JM, Mora J, Pastor M, Ortiz V, et al. Natural history of clinically compensated hepatitis C virus-related cirrhosis after liver transplantation. Hepatology 2000;32:852-858.

(3) Berenguer M. Systematic review of the treatment of established recurrent hepatitis C with pegylated interferon in combination with ribavirin. J hepatol 2008;49:274-287.

(4) Rosen HR, Martin P. Hepatitis C infection in patients undergoing liver retransplantation. Transplantation 1999; 66:1612-1616

(5) Martí J, Charco R, Ferrer J, Calatayud D, Rimola A, Navasa M, Fondevila C et al. Optimization of liver grafts in liver retransplantation: A European single-center experience. Surgery 2008;144:762-769.

(6) Magee JC, Barr ML, Basadonna GP, Johnson MR, Mahadevan S, McBride MA, et al. Repeat organ transplantation in the US,1996-2005. Am J Transpl 2007;7:1424-33.

(7) Burton JR Jr, Rosen HR. Liver retransplantation for hepatitis C virus recurrence: a survey of liver transplant programs in the United States. Clin Gastroenterol Hepatol. 2005;3(7):700-4 .

(8) Onaka M, Levy MF, Ueno T et al. An outcome comparison between primary liver transplantation and retransplantation based on the pretransplant MELD score. Transplant Int 2006;19;637-640.

(9) Moya A, Torres-Quevedo R, San Juan F, Montalvá E, López-Andujar R, Pareja E, de Juan M et al. Indications for and results of liver retransplantation. Clinical Transplants 2009;171-178

(10) Landaverde C, Berenguer M, Aguilera V, San Juan F, Prieto M, Berenguer J. Liver retransplantation: outcome analysis in 50 patients. Med Clin (Barc). 2005;124:721-5.

(11) McCashland T, Watt K, Lyden E, Adams L, Charlton M, Smith A et al. Retransplantation for hepatitis C: Results of a US Multicenter Retransplant study. Liver Transpl 2007;13:1246-1253

(12) Markmann JF, Markowitz JS, Yersiz H, Morrisey M, Farmer DG, Farmer DA et al. Long term survival after retransplantation of the liver. Ann Surg 1997;226:408-18

(13) Feng S, Goodrich NP, Bragg-Gresham JL, Dystra DM, Punch JD, Debroy MA et al. Characteristics associated with liver graft failure: the concept of a donor risk index. Am J Transplant 2006;6:783-790.

(14) Thuluvath PJ, Guidinger MK, Fung JJ, Johnson LB, Rayhill SC, Pelletier SJ. Liver transplantation in the United States, 1999-2008. Am J Transpl 2010;10:1003-19

(15) Rosen H, O´reilly P, Shackleton C, McDiamid S, Holt C, Bussutil RW et al. Graft loss following liver transplantation with chronic hepatitis C. Transplantation 1996;62:1773-1776.

(16) Ghabril M, Dickson R, Machicao V, Aranda-Michel J, Keaveny A, Rosser B, Bonatti H et al. Liver retransplantation of patients with hepatitis C infection is associated with acceptable patient and graft survival. Liver Transpl 2007;13:1727-1727.

(17) Ghabril M, Dickson R, Wiesner R. Improving outcomes of liver retransplantation: An analysis of trends and the impact of Hepatitis C infection. Am J Transplant 2008; 8: 404–411.

(18) Rosen HR, Madden JP, Martin P. A model to predict survival following hepatic retransplantation. Hepatology 1999;29:365-70

(19) Northup PG, Pruett TL, Kashmer DM, Argo CK, Berg CL and Schimtt TM. Donor factors predicting recipient survival after liver retransplantation: The retransplant donor risk index. Am J of Transplantation 007;7:1984-1988.

(20) Markmann JF, Gornbein J, Markowitz JS, Levy MF, Klintmalm GB, Yersiz H et al. A simple model to estimate survival after retransplantation of the liver. Transplantation 1999;67:422-430

(21) Rosen HR, Prieto M, Casanovas-Taltavull T, Cuervas-Mons V, Guckelberger O, Muiesan P et al. Validation and refinement of survival models for liver retransplantation. Hepatology 2003;38:460-9.

(22) Linhares MM, Azoulay D, Matos D, Castelo-Filho A, Trivino T, Goldberg A, et al. Liver retransplantation: a model to predict survival.Transplantation 2006;81:1016-1021.

(23) Carrión JA, Navasa M, Forns X. Retransplantation in patients with hepatitis C recurrence after liver transplantation. J Hepatol. 2010;53(5):962-70

(24) Schluder L, Sheiner P, Thung S, Lau JY, Min A, Wolf DC et al. Severe recurrent cholestatic hepatitis following orthotopic liver transplantatin. Hepatology 1996;23:971-6.

(25) Sheiner P, Schluger LK, Emre S, Thung S, La JYN, Guy SR et a. Retransplantation for recurrent Hepatitis C. Liver Transpl and Surg 1997;3:130-136

(26) Berenguer M, Prieto M, Palau A, Rayón JM, Carrasco D, San Juan F et al. Severe Recurrent hepatitis C alter liver retransplantation for hepatitis C virus-related graft cirrhosis. Liver Transpl 2003;9:228-235.

(27) Carmiel-Haggai M, Fiel I, Gaddipati H, Abittan C, Hossain S, Roayaie M et al. Recurrent hepatitis C after retransplantation: factors affecting graft and patient outcome. Liver Transpl 2005;11:1567-1573.

(28) Ercolani G, Grazi GL, Ravaioli M, Del Gaudio M, Cescon M , Lauro A et al. Histological recurent hepatitis C after liver transplantation: outcome and role of retransplantation. Liver Transpl 2006;12:1104-1111.

(29) Yoo HY, Maheshwari A, Thuluvath PJ. Retransplantation of liver: primary graft nonfunction and hepatitis C virus are associated with worse outcome. Liver Transpl. 2003;9:897-904.

(30) Roayaie S, Schiano T, Thung S, Emre S, Fishbein M, Miller M et al. Results of Retransplantation for recurrent hepatitis C. Hepatology 2003;38:1428-1436

(31) Pelletier S, Schaubel D, Punch J, Wolfe R, Port F, Merion R. Hepatitis C is a risk factor for death after liver retransplantation. Liver Transpl 2005;434-440.

(32) Watt DS, Lyden E, McCashland. Poor survival after retransplantation. Is hepatitis C to blame? Liver Transpl 2003;9:1019-1024

(33) Rosen H. Retransplantation for hepatitis C: implications of different policies. Liver Transpl 2000: 6:S41-46.

3.

Organ Allocation for Liver Transplantation: Urgency, Utility and Net-Benefit

***Biggins, S. W.*[1]**

[1] MD, MAS University of Colorado, Denver, CO, USA

Donor livers are a scarce, life saving resource. For patients whose lives depend upon liver transplantation, the policies defining priority for donor livers are of ultimate importance. Fair and just utilization of available livers requires that policy makers understand how to balance the needs and interests of each of the stakeholders. There are at least three possible bases for organ allocation: medical urgency, utility and net-benefit. Under an urgency based allocation system, patients with the highest mortality without a liver transplantation are given the greatest priority. Conversely, a utility based system grants highest priority in accordance with lowest expected post-transplant mortality. A net-benefit system considers both waiting list and post-transplant outcomes. Without appropriate safeguards, urgency based systems can risk futile transplantation at the peril of not one, but two patients; the recipient and the other potential donor liver recipient. Utility systems optimize post-transplant survival yet require minimum transplant criteria to avert unnecessary transplantation. Net-benefit allocation systems aim to maximize the total life years gained in the population as a whole.

Both utility and net-benefit allocation systems rely heavily on prognostic models that are less accurate and less precise than the models available for use in urgency based allocation. Several validated models to predict mortality while waiting for liver transplantation exist which accurately rank the likelihood of death in the vast majority of patients with liver disease. The success of urgency based prognostic models is based on several identified objective, reliable, and reproducible surrogates of liver disease severity. However, prediction of post-transplant outcomes, though affected by liver disease severity, has many more influences including the quality of the donor liver, matching of donor and recipient characteristics, as well as the experience of the transplant center and surgeons. Additionally, random events in the peri-operative period, which are by definition not predictable, can reduce the ability to confidently estimate post transplant outcome.

In conclusion, the rational and ethical application of prognostic models may allow for further optimization of liver allocation through a net-benefit allocation system yet improved accuracy of post transplantation prediction models is needed.

4.

Liver allograft pathology

Cipriano, M. A.[1]

[1]Department of Patology, University Hospital, Coimbra

Histological assessments continue to play an important role in the diagnosis and management of liver allograft dysfunction. In most cases of allograft biopsy interpretation, accurate diagnosis demands careful correlation of histological features with clinical, imaging and laboratory findings, and often comparison with previous sequential and follow-up biopsies.

The spectrum of diseases encountered in post-transplant liver pathology biopsies is broad. We will focus on problems concerning rejection, recurrent disease and *de novo* post-transplant abnormalities.

The changes occurring in acute and chronic rejection are well recognized and liver biopsy remains the 'gold standard' for their diagnosis. Late cellular rejection is different from early acute rejection and has features that overlap with *de novo* autoimmune hepatitis and idiopathic post-transplant chronic hepatitis.

Recurrent disease is the most common recognized cause of abnormal graft histology in late biopsies from adults, but is very uncommon in the paediatric population. The features of recurrent disease may be modified by the effects of immunosuppression and interaction with other graft complications, resulting in changes that are complex and difficult to interpret. The distinction between recurrent hepatitis C infection and rejection continues to be a problem in the assessment of liver allograft biopsies. In cases where graft dysfunction has more than one possible aetiological factor, liver histology is essential to identify the main cause of graft damage.

In late post-transplant biopsies some features such as perivenular cell dropout, chronic hepatitis or architectural anomalies may be difficult to ascribe to a single aetiology.

The role of protocol biopsies in identifying patients in whom immunosuppression can be safely reduced or withdrawn completely needs further investigation.

5.

Novel Targets for Hepatic Ischemia and Reperfusion Injury: Integrins and Matrix Metalloproteinases

Coito, A. J.[1]

[1] The Dumont-UCLA Transplant Center, Los Angeles, CA

Orthotopic liver transplantation (OLT) is considered the preferred therapy for a wide range of previously fatal chronic hepatic diseases. However, due to the shortage of liver donors, thousands of patients die every year while on the waiting list for a liver transplant. This gloomy scenario, together with the significant prevalence of obesity in the population, has led to an increased need of using suboptimal steatotic livers in transplantation at elevated risks of dysfunction based on their high susceptibility to ischemia and reperfusion injury IRI. While infiltrating leukocytes are implicated as major mediators of hepatic IRI, the mechanisms involved in their recruitment to sites of inflammatory stimulation in liver are still far from being understood. Leukocytes have to acquire strong adhesion interactions to the vessel wall to migrate across the vascular endothelium; this firm adhesion of the leukocytes to the endothelium is mediated primarily by integrins. Recent work from our laboratory has shown that blockade of two major leukocyte integrins a4b1 and a5b1 with fibronectin, a key ECM protein, significantly depressed leukocyte infiltration, improved both functional/histological preservation of steatotic rat liver grafts, and markedly increased recipient survival in steatotic OLTs. While adhesion molecules are essential to the successful promotion of leukocyte recruitment by providing leukocyte attachment to the vascular endothelium, matrix metalloproteinases (MMPs) are important for facilitating leukocyte transmigration across vascular barriers. Interestingly, the blockade of fibronectin-integrin interactions inhibited the expression/activation of MMP-9 (gelatinase A) by leukocytes in steatotic orthotopic liver transplants without significantly affecting the expression of MMP-2 (gelatinase A). Therefore, to examine whether lack of MMP-9 activity would confer protection against hepatic IRI, we used MMP-9 deficient mice, mice treated with a specific anti-MMP-9 neutralizing monoclonal antibody, and mice treated with a broad gelatinase inhibitor, which targets both MMP-2 and MMP-9, in a well-established model of partial warm liver IRI. This study demonstrated that specifically targeting MMP-9 profoundly ameliorated tissue damage after the liver I/R insult. Compared with wild-type mice, MMP-9-deficient mice and mice treated with a specific neutralizing anti-MMP-9 antibody showed significantly better liver preservation outcomes. This study also provided evidence that specifically targeting MMP-9 leads to more effective protection against liver IRI than simply using a broad gelatinase inhibitor. In conclusion, our studies support the view that cell attachment to ECM proteins and subsequent degradation are related events. They emphasize an important function for FN-integrin interactions in steatotic liver IRI. Further, they strongly support the rationale for identifying inhibitors that specifically target MMP-9 in vivo as a potential therapeutic approach in the pathogenesis of liver IRI.

6.

Daniel, J.

Abstract Online at: http://www.ibmc.up.pt/pointer

7.

Hepatitis C virus disrupts bone morphogenetic protein signalling and suppresses hepcidin

Lucy A. Eddowes[1], Narayan Ramamurthy†[2], John D. Ryan†[3], Sara Boninsegna[2,4], Andrew E. Armitage[1], Paolo Fabris[5], Maria Teresa Giordani[5], Diego Martines[4], Alain R.M. Townsend[1], John Crowe[3], Matthew W. Lawless†[3], Paul Klenerman†[2], Hal Drakesmith[1]*

[1]Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, UK; [2]Peter Medawar Building for Pathogen Research, University of Oxford, South Parks Road, Oxford, UK; [3]Centre for Liver Disease, Mater Misericordiae University Hospital, 55 Eccles Street, Dublin 7, Ireland; [4]Department of Surgical Gastroenterological Science, University of Padua, Padova, Italy; [5]Department of Infectious Diseases and Tropical Medicine, San Bortolo Hospital, Vicenza, Italy

Chronic inflammatory states are often associated with anemia, attributed to increased levels of the iron regulatory hormone hepcidin. An exception is chronic hepatitis C virus (HCV) infection, which is accompanied by reduced hepcidin and predisposes to hepatic iron accumulation that exacerbates disease. Hepcidin synthesis by hepatocytes is regulated by bone morphogenetic proteins (BMP) and the SMAD signalling cascade. We found altered expression of components of the BMP pathway in liver biopsies from HCV patients. Pre-treatment biopsies from patients non-responsive to antiviral therapy showed reduced levels of the BMP co-receptor HJV, reduced levels of the BMP target genes hepcidin and ID1, and relatively increased levels of SMAD6 and SMAD7, which mediate negative feedback onto BMP signalling. An *in vitro* virus replication model showed similar alterations in BMP pathway gene expression, and HCV infected cells exhibited a blunted hepcidin response to BMP6. BMP pathway inhibition was caused at least in part by virally-induced TNF-alpha. TNF-alpha suppressed the induction of hepcidin by BMPs and neutralizing anti-TNF-alpha antibodies restored the response to BMP6 by HCV infected cells. The identification of BMP signal inhibition by HCV that correlates with hepcidin suppression and treatment response suggests new options for antiviral therapy.

8.

HCC: Natural history, and expansion of the Milan criteria

François, D.[1]

[1]Pôle des Maladies de l'Appareil Digestif, Service d'Hepatologie, INSERM U773, Université Paris VII, Hôpital Beaujon, Clichy, France

In the long term, liver transplantation is the best option in patients with small hepatocellular carcinoma (HCC) as it cures both the tumor and the underlying liver disease (cirrhosis is most cases). Survival rates may be as high as 70-75% at 5 years. The main limitation of transplantation is organ shortage; the number of potential candidates exceeding by far the number of available organs. As a result (a) only patients with an excellent prognosis should be considered for transplantation and (b) acceptable alternatives to transplantation should always be considered. The Milan criteria are widely used to select candidates for transplantation but there is still a 10-15% tumor recurrence rate. Excluding patients within the Milan criteria but with too high AFP level, poor differentiation and/or cholangiocarcinoma components may help improve the results. At the opposite, selected patients who are beyond the Milan criteria proved to have a low risk of recurrence. This finding led to develop alternative selection criteria but there is no consensus on an acceptable upper limit. This is also the basis of the concept of down staging, which consists in reducing tumor size by an adjuvant therapy (transarterial chemoembolization is most cases) and bridging the patient to transplantation if the response to adjuvant therapy is good. An interval of 3 months or more between adjuvant therapy and transplant decision is an important step as it helps better assess the natural history of the tumor.

In patients with a small HCC, compensated cirrhosis and no significant portal hypertension, surgical resection is an attractive alternative to transplantation. Up to 5-years, the survival rates may be comparable. The majority of patients experience recurrence after transplantation but recurrence is generally confined to the liver. As a result, salvage transplantation may be considered at the time of recurrence, provided the tumor is within the Milan criteria and the age of the patient is compatible with transplantation. Detailed pathology of the resected tumor is an important tool for identifying the patients who could benefit from this approach.

Overall, organ allocation in patients with HCC depends upon both tumor status and the severity of the underlying cirrhosis. No "extra priority" is needed in patients with advanced cirrhosis and high MELD score. "Extra priority" is justified in patients with HCC and a low MELD score (provided no satisfactory alternative to transplantation, therapy can be considered). Between these two extremes, there is a continuum concerning the interaction between cirrhosis and HCC. Better understanding these interactions may help propose more effective algorithms in terms of organ allocation.

9.

Immune responses to Hepatitis C virus

Klenerman, P.[1]

[1] Wellcome Trust Research Fellow, Nuffield Department of Medicine, University of Oxford

Hepatitis C virus affects around 170 million people worldwide and is a major cause of liver failure and liver cancer. No current vaccine exists and although new treatments are emerging rapidly, these are complex and expensive. Although most people who are infected become chronic carriers, a fraction (around 20-25%) clear the virus from blood and liver and remain healthy. The innate and adaptive immune systems play a co-ordinate role in this control, and we have focused largely on the role of T cells in defining clinical outcomes. B cells also play a role in the disease although the complex hypervariable nature of the HCV envelope means this is a poor target for natural and vaccine-induced neutralising antibodies.

Control of virus after acute infection is characterised by sustained CD4+ and CD8+ T cell responses which are broadly directed against diverse antigens and retain functionality. In those who fail to clear the virus T cell responses decline rapidly although responses may remain detectable in the liver as a component of the chronic inflammatory infiltrate. Recent data has shed light on some unexpected features of this lymphocytic infiltrate based on the expression of the C Type lectin CD161 on T cells.

In this talk I will focus on: 1. The evidence for the role of T cells in the control of virus.

2. Mechanisms of virus persistence including viral mutation, immune regulation and T cell exhaustion.

3. The specific phenotype and function of liver homing T cell populations.

4. Efforts to develop T cell based vaccines for the prevention and therapy of HCV.

10.
Linhares-Furtado

Abstract Online at: http://www.ibmc.up.pt/pointer

11.

Reptin and Pontin in human hepatocellular carcinoma. Expression, role and therapeutic targeting

Rosenbaum, J[1]

[1]INSERM, BORDEAUX10

Hepatocellular carcinoma (HCC) is the main type of primary human liver cancer and is associated with a poor prognosis. Looking for new targets, we performed a comparative proteomic analysis of human HCC and non-tumor liver from the same patients. This analysis allowed for the discovery of many deregulated proteins and especially the finding of an over-expression of Reptin in these tumors (1).

Reptin and its homolog protein, Pontin (that we later found also overexpressed in HCC), are members of the large AAA+ family (for ATPases associated with various cellular Activities) and are involved in chromatin remodeling, transcription regulation and DNA repair (2, 3). In a large series of patients, we found that a high level of Reptin or Pontin expression was associated with a poor prognosis as an independent factor. Using HCC cells in culture, we demonstrated that Reptin (4) and Pontin (5) are required for cell growth and viability. We further showed that Reptin and Pontin expression are co-regulated at a post-translational level with the consequence that silencing either one with RNAi leads to the simultaneous silencing of the other one (5). As a proof of concept that Reptin can be a therapeutic target in HCC, we inoculated mice with human HCC cells and showed that Reptin silencing *in vivo* led to tumor regression (6). Because gene silencing is not yet an option for cancer treatment, we aim at finding small molecules able to inhibit the ATPase function of Reptin and Pontin. To this end, we use molecular modeling and *in silico* screening followed by enzymatic assays *in vitro*, and have now identified several interesting candidates for Pontin ATPase inhibition.

1. Blanc *et al.*, Proteomics 2005,5: 3778-89; 2. Grigoletto *et al.*, Biochim Biophys Acta 2011,31: 91-103; 3. Huber *et al.*, Cancer Res 2008,68: 6873-6; 4. Rousseau *et al.,* Hepatology 2007,46: 1108-18; 5. Haurie *et al.*, Hepatology 2009,50: 1871-83; 6. Menard *et al.*, J Hepatol 2010,52: 681-9.

12.
The immune fingerprint of tolerance

***Alberto Sánchez-Fueyo*[1]**

[1] Unidad de Transplante Hepático, Hospital Clinic Barcelona, Barcelona, Spain

Attempts to intentionally induce tolerance in clinical organ transplantation have been unsuccessful other than in highly selected groups of recipients. In contrast, human transplant recipients occasionally develop spontaneous operational tolerance, a phenomenon in which recipients receiving no immunosuppressive therapy exhibit stable graft function for remakably long periods of time in the absence of harmful immune responses. This state of spontaneous operational tolerance is particularly prevalent in liver transplantation, where a sizable proportion of stable recipients could probably cease all immunosuppression without compromising the graft's viability. In recent years, considerable efforts have been devoted to the identification of non-invasive biomarkers of operational tolerance in kidney and liver transplantation. Most of these studies have employed blood cell immunophenotyping and gene expression profiling to search for immune parameters associated with tolerance. Methodological drawbacks common to all studies have been the small number of tolerant recipients available for study and their cross-sectional retrospective design.

We have investigated the immunological traits of operationally tolerant liver recipients by: 1) analyzing the differences in blood cell immunophenotypic and gene expression traits between tolerant and non-tolerant recipients; 2) identifying predictive biomarkers in conducting prospective immunosuppression withdrawal studies; and 3) comparing blood and liver tissue immune-related parameters.

Overall, our results indicate that tolerant liver recipients, but not kidney recipients, exhibit an over-enrichment in innate immune related transcripts in blood. This contrasts with the identification of a B-cell related transcriptional signature in the blood of tolerant kidney recipients, and is associated with an expansion of NK cells. In tolerant liver recipients these findings are stable over time, can be detected before immunosuppression is withdrawn, and could serve as a non-invasive means to identify tolerant patients before drug weaning is attempted. The mechanistic interpretation of these results, however, remains elusive. In contrast to these results, molecular profiling of liver tissue samples collected before immunosuppressive drugs are withdrawn reveals that tolerant and non-tolerant grafts mainly differ in genes involved in the regulation of iron metabolism. These findings correlate with differences in clinical iron parameters, and suggest that regulation of iron metabolism could constitute an unrecognized pathway involved in the control of intra-graft alloimmune responses.

Results from several unpublished reports indicate that the prevalence of operational tolerance in liver transplantation is much higher than previously estimated, particularly at late time points after transplantation. The observation that tolerant and non-tolerant recipients can be accurately differentiated employing either blood or liver tissue samples opens the door to the performance of large-scale immunosuppressive drug withdrawal trials.