

## **SUMMARY**

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Pessôa MG. *Severe recurrent cholestatic hepatitis after liver transplantation: virological, histological and immuno-histochemical evaluation*. São Paulo, 2003. 100p. Tese (Doutorado) – Faculdade de Medicina, Universidade de São Paulo

Following liver transplantation the HCV-related disease severity is highly variable, with a minority of cases progressing to an extremely severe form of cholestatic hepatitis, that only recently had the diagnostic criteria established. The mechanisms by which severe cholestatic hepatitis develops after liver transplantation are not fully understood and, although HCV immunostaining has been suggested for early diagnosis of this form of recurrent hepatitis C in liver grafts, reports on this matter are scarce. Also, the role of HCV diversity as a mechanism of disease progression both in immunocompetent chronic HCV-infected patients as well as in the liver transplant population has been suggested, but findings are controversial. HYPOTHESIS: We propose that quasispecies evolution of HCV is modified by the presence of immunosuppression and therefore, contributes to severity of recurrence post-OLT either through changes in viral complexity (number of quasispecies) or through changes in viral divergence (emergence/selection of new species). We also looked at HCV viremia and core antigen immunohistochemical-reactivity as predictors of disease severity. AIMS: (i) To measure changes in viral complexity and/or divergence in immunosuppressed transplant patients compared to chronic HCV-infected immunocompetent controls; (ii) to compare these changes in patients developing severe cholestatic and mild post-transplant HCV recurrence; (iii) to measure HCV core antigen immunohistochemical-reactivity in patients developing severe or mild post-transplant HCV recurrence. METHODS: 12 patients with recurrent HCV infection were studied (6 with severe and 6 with mild disease). Patients were matched for HCV genotype (all type 1), type of immunosuppression and length of follow-up. Five HCV-infected immunocompetent patients were used as controls for quasispecies diversity with comparable follow-up. HCV quasispecies were characterized by heteroduplex mobility assay of the hypervariable region. Ten to twenty clones at each time point were examined and confirmed by sequence and

phylogenetic analysis. Rb246 pab anti-core yielded specific, granular cytoplasmic staining in hepatocytes of FFPE liver tissue. HCV-core Ag was semi-quantified from 0 to 3+. Serum and liver samples were analyzed at three time points, one pre- and two post-transplantation for each transplant patient. HCV-RNA was also measured on the different time-points by branched DNA. RESULTS: A strong correlation between mobility ratio and nucleotide substitutions was observed ( $r = 0.92$ ). Complexity did not change with time either in immunocompetent or immunocompromised patients. Divergence was greater in immune compromised patients, particularly in those with severe cholestatic disease, in whom nucleotide mutations occurred earlier following transplantation, when compared to patients with mild HCV recurrence (median of 38 and 19, respectively;  $p < 0.001$ ). On the explant samples, HCV immunostaining was strongly positive in patients who further developed severe cholestatic hepatitis, and mildly positive in patients who further developed mild recurrence. Post-transplantation, HCV core antigen immunoreactivity was only mildly correlated to severity of recurrent hepatitis. HCV-RNA was significantly higher in the severe cholestatic group, early post-transplantation. HCV-core Ag was not directly associated to HCV-RNA on the different time-points. CONCLUSIONS: (i) In the absence of immunosuppression, there is little change in viral divergence; (ii) In severe recurrent cholestatic hepatitis, the majority of pre-transplant variants become extinct. This is also true in patients with mild recurrence, but the changes occur later in time. These data support the hypothesis that severe cholestatic hepatitis following transplantation is associated with emergence/selection of viral species of enhanced pathogenic potential; (iii) Our results also suggest that strong HCV immunostaining in the explant is predictive of more severe disease recurrence.