Title: Lack of regeneration: a new concept of pathogenesis of non-anastomotic bile duct strictures

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Background: The aim of this study was to examine the development and molecular mechanisms of bile duct (BD) damage between organ retrieval and transplantation and its clinical relevance for patients.

Methods: Common BD samples after donor hepatectomy, cold storage and reperfusion were compared to healthy controls by H&E staining, immunohistochemistry (IH) for tight junction proteins (TJ), cytokeratin, e-cadherin, laminin, CD4, CD8 and CD14 and by global mRNA expression using Microarray technique. Bacterial infiltration was determined by FISH for bacterial antigens. Further, a BD damage score (BDDS) to quantify biliary epithelial injury was developed and correlated with recipient and donor data and patient outcome.

Results: Patients with major BD damage after cold storage, as quantified by the newly developed BDDS, had a significantly increased risk of biliary complications ($p<0.0001$) and graft loss ($p=0.0004$). After cold storage ($p=0.0119$) and even more after reperfusion ($P=0.0002$), epithelial damage categorized by the BDDS was markedly increased, and TJs were detected with inappropriate morphology. mRNA expression levels of adherens-junctions ($q=0.003$) and focal-adhesion-molecules ($q=0.04$) in damaged BDs without biliary complications were increased compared to damaged BD with biliary complications reflecting increased regenerative capacity of the biliary epithelium in the first group. Consecutively, IH showed significantly

increased cytokeratin, e-cadherin and laminin expression in this group. FISH analysis demonstrated equal distribution of bacterial infiltration of BDs, however, mRNA analysis detected induced antibacterial immune response (q=0.00084) and phagocytosis (q=0.04) in BDs with enhanced epithelial regenerative capacity corroborating with significantly increased $CD4^+$ and $CD8^+$ cell-mediated adaptive immune response.

Conclusions: In many cases, the common BD epithelium shows considerable damage after cold ischemia with further damage occurring after reperfusion. The extent of epithelial damage can be quantified by our newly developed BDDS and is a prognostic parameter for biliary complications and graft loss. Following bile duct damage during cold storage, we hypothesize from our data that the functional regenerative capacity of biliary epithelium and enhanced local adaptive immune cell infiltration are crucial for bile duct recovery. Such molecular immunological bile duct analyses therefore could help to predict biliary complications in cases of “major” epithelial damage after cold storage.