IMPORTANCE OF IRON OVERLOAD AND STEATOSIS IN PATIENTS WITH CHRONIC LIVER DISEASE

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This thesis deals with the importance of hepatic iron and fat deposition in the context of chronic liver disease, with special focus on the role of the S65C mutation in the hemochromatosis (HFE) gene, the association between non-alcoholic fatty liver disease (NAFLD) and cryptogenic cirrhosis (CC) in patients evaluated for liver transplantation, the expressions of innate and adaptive immunity in non-alcoholic steatohepatitis (NASH), and the role of the iron regulatory hormone hepcidin in dysmetabolic iron overload (DIO).

NAFLD is the most common liver disease in the Western world. Some patients with NAFLD develop NASH and are then at risk for progressive liver disease, cirrhosis and hepatocellular carcinoma. NASH involves the innate immune system, but the role of the adaptive immune system in this context is less clear. A subgroup of patients with NAFLD develops DIO, the causes of which are unknown. Mutations of the HFE gene may be associated with DIO, but results are conflicting. The role of the most recently found HFE mutation, S65C, has not been known. Finally, in end-stage NASH cirrhosis, liver fat diminishes, and patients with CC may therefore have an underlying NAFLD.

In study I the HFE S65C gene mutation was retrospectively studied in 296 patients with suspected iron overload and 250 healthy controls in order to determine the HFE S65C frequency, and evaluate whether this mutation would result in a significant hepatic iron overload or not. We found that the S65C allele was enriched in patients with high serum ferritin compared with controls, and half of the carriers of this allele had mild or moderate hepatic iron overload, but no signs of significant fibrosis.

In study II, 39 patients with CC were compared with 431 patients having cirrhosis of other etiologies, to evaluate the presence of NAFLD in patients with CC and determine survival after liver transplantation. We found that 44% of the CC patients had an underlying NAFLD. CC patients had a higher frequency of diabetes, ascites, and hyponatremia compared with those having cirrhosis of other etiologies. Weight loss was significantly higher among patients with CC, but there was no difference in patient survival between the groups.

In study III, liver biopsies from 49 patients with suspected NAFLD were classified according to the NAFLD Activity Score (NAS) and liver fat was assessed with morphometry. Biopsies were stained with various markers of T-cells, macrophages, apoptosis and cell adhesion molecules (ICAM-1). We found an increased number of regulatory T-cells (Tregs) and CD68 cells in NASH, pointing at an involvement of both the adaptive and innate immune systems. ICAM-1-positive hepatocytes were only seen in NASH livers and localized in areas with microvesicular fat, and the ICAM-1 level in serum was increased in patients with NASH.

Study IV aimed to determine the association between hepcidin and iron parameters, lipid status and inflammatory markers in NAFLD in relation to other chronic liver diseases. Serum hepcidin was analyzed in 85 patients with chronic liver disease (38 of which had NAFLD) and 38 healthy controls. Liver biopsy was performed in 67 patients and hepcidin mRNA in liver was determined with real time-qPCR in 36 patients. We found that hepcidin regulation was similar in NAFLD compared to other chronic liver diseases with various degrees of hepatic iron overload. In NAFLD hepcidin correlated to serum ferritin and liver iron, but not to BMI, CRP, NAS or steatosis. Transferrin saturation, but not hepcidin, could be used to discriminate between hyperferritinemic NAFLD patients with or without iron overload.

In conclusion, we found that the HFE S65C mutation leads to mild to moderate hepatic iron overload, but neither to clinically manifest hemochromatosis, nor extensive liver fibrosis. Re-evaluation of patient data in cryptogenic cirrhosis discovered underlying NAFLD in 44% of patients evaluated for liver transplantation. There was no difference in patient survival between cryptogenic patients and those having cirrhosis of a known etiology. In NASH, an involvement of the innate adaptive immunity is seen, and immunohistochemical markers of inflammation are localized to areas of microvesicular steatosis. Serum hepcidin levels in patients with NAFLD correlate adequately to iron parameters, but not to BMI, NAS or inflammatory markers.