

## New concepts in liver regeneration mechanisms in human severe alcoholic steatohepatitis

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### Introduction

Severe alcoholic steatohepatitis is a severe complication of alcoholic liver disease associated with high mortality. The prediction of patient's outcome remains challenging. Liver progenitor cells (LPC) are usually considered to be activated in case of impaired hepatocyte replication and hence markers of disease severity. However, their exact role as well as their interaction with hepatocytes and macrophages also implicated in liver regeneration remain poorly characterized in humans.

### Aim

The aim of this study is to characterize hepatocyte, LPC and macrophage populations in severe alcoholic steatohepatitis (sASH) and to link them with liver injury and patients' outcomes.

### Methods

The material used for this study is derived from the recent trial on enteral nutrition in severe biopsy proven alcoholic steatohepatitis, including initially 136 patients. Immunohistochemical and morphometric studies for total LPC (keratin 7 positive cells), macrophages (CD68 positive cells), proliferative hepatocytes (Ki67 positive hepatocytes) and proliferative LPC (double keratin 7 positive and Ki67 positive cells) were performed on the admission biopsies of patients with sASH recruited prospectively in several different centers in Belgium and France.

Patients were divided into improvers or non-improvers, according to MELD score change (a decrease of at least 3 points of MELD or more compared to baseline value defines improvers) and in responders or non-responders to corticosteroids, according to the Lille score at day 7.

### Results

Liver biopsies were available for 68 patients with sASH from 16 different centers. Eleven biopsies were excluded due to the poor quality of the remaining material. 57 cases were included for histological and morphometric assessment (mean age 50 years, mean Maddrey discriminant function 54, range 43.2-71.5).

No difference of total LPC, proliferative LPC, proliferative hepatocytes or macrophages was observed between improvers (n=26) and non-improvers (n=31) nor between the favorable (n=43) and unfavorable (n=14) Lille score groups. A greater degree of steatosis was the only histological parameter associated with a better prognosis based on MELD score evolution at 3 months (p=0.002).

The total amount of LPC was positively correlated to the severity of the disease evaluated by the MELD score (r=0.3416, p<0.01). A higher number of macrophages was associated with a higher proliferation of both hepatocytes and LPC (r=0.3012, p=0.02). Increased hepatocyte replication was also correlated to a higher proliferative LPC count (r=0.8112, p<0.0001).

### Conclusions

In biopsy proven severe alcoholic steatohepatitis, the proliferation of hepatocytes and LPC occurs in parallel, showing that LPC start to replicate even in the absence of massive hepatocyte senescence in humans, which contrasts with data coming from animal experiments. Liver macrophage expansion is correlated to the proliferation of both hepatocytes and LPC suggesting a potential role for driving the regenerative response.