tested 2 propositions: 1) That early stimulation of vasculogenesis is key for the prevention of liver failure after extended hepatectomy. 2) That hepatocytes massively undergo Epithelial to Mesenchymal Transition which is favorable to proliferation but compromises hepatocyte function, leading to liver failure.
Aim: In this study, we want to assess if administration of G-CSF in order to manage vascular damage induced by a SFSS-setting hepatectomy can rescue survival. We also want to assess if hepatocytes engage in an EMT process after an extended hepatectomy and evaluate whether this would jeopardize liver function for proliferation in the liver remnant.
Methods: G-CSF administration consisted of 3 peritoneal injections (2 the day before the surgery and one at the time of surgery). Vascular diameter and density were assessed on CD31 staining. Recruitment of endothelial progenitors was assessed using Cdh5-CreERT2 x mTmG mice, expressing GFP in mature endothelial cells when tamoxifen is administered. Liver function was evaluated by qPCR for the xenobiotic metabolism, circulating factor V by ELISA, circulating albumin by colorimetric test and hepatocyte glycogen content by PAS staining. EMT was evaluated by expression of E-Cadherin by immunofluorescence, and gene expression of HNF4A and Snai1 by qPCR. EMT pathway was investigated via immunofluorescence of Hes1 and Notch1.
Results: Administration of granulatory-colony stimulation factor (G-CSF) increased vascular diameter and density and induced the recruitment of endothelial progenitors in regenerating livers compared to non-treated animals. Despite managing vascular defects, administration of G-CSF did not rescue survival. We thus focused on the hepatocyte population. After a SFSS-setting hepatectomy, a large portion of the hepatocytes enters the cell cycle at the same time (around 50% at POD3), especially in peri-portal and peri-central areas, where key metabolizing functions are performed. High proliferation was associated with significantly decreased circulating albumin and factor V, two proteins heavily produced by the liver. Xenobiotic metabolism genes CYP1A2 and CYP2E1 were also decreased in normoxia compared to hypoxia. Finally, PAS staining revealed that only 30% of hepatocytes were PAS+, compared to 60% in mice kept in hypoxia. Loss of epithelial characteristics and in this case hepatocyte signature is a sign of EMT. HNF4A, a master regulator of hepatocyte phenotype, was downregulated in normoxia and its expression was inversely correlated with Snai1, a regulator of EMT. Concordantly, the epithelial marker E-Cadherin was faintly stained in mice kept in normoxia. Finally, we explored through which pathway EMT is induced in hepatocytes. Notch1 intracellular domain was stained inside the nucleus of hepatocytes of mice kept in normoxia while no nuclear staining was found in hypoxia. Similarly, Hes1, a target of the Notch pathway, was more expressed in normoxia compared to hypoxia.
Conclusions: In this study, we found that vascular damage after a SFSS-setting hepatectomy are rescued by administration of G-CSF. However, this was not sufficient to increase survival. Focusing on hepatocytes, we found that SFSS-setting hepatectomy leads to extravagant engagement in the cell cycle in hepatocytes, and this was associated with decreased function of the liver remnant. We found that a large portion of the proliferative hepatocytes engaged in an EMT process through the Notch1/Hes1 pathway. Thus, we think that EMT engagement after a SFSS-setting hepatectomy disrupts the balance between function and proliferation in the remnant, leading to organ failure.

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ALVEOLAR ECHINOCOCCOSIS IS INCREASING IN SOUTHERN BELGIUM: A REPORT OF THE BELGIAN NATIONAL REFERENCE LABORATORY FOR ECHINOCOCCOSIS (BNRLE) AND CLINICAL EXPERIENCE OF ECHINO-LIEGE. O. Detry (1), C. Bihain (2), R. Sacheli (3), S. Egrek (4), N. Blétard (5), P. Meunier (6), P. Lovinfosse (7), J. Delwaide (8), N. Botembe (9), E. Larranaga (10), C. Truyens (11), B. Delaere (12), B. Pirotte (13), J. Giot (14), P. Leonard (14), M. Hayette (3) / [1] Centre Hospitalier Universitaire de Liège, Liège, Belgium, ECHINO-Liege, Dpt of Abdominal Surgery and Transplantation, [2] CHU of Liège, Belgium, Dpt of Abdominal Surgery and Transplantation, [3] CHU of Liège, Belgium, ECHINO-Liege, Dpt of Clinical Microbiology, Belgian National Reference Laboratory for Echinococcosis, (BNRLE), [4] CHU of Liège, Belgium, Dpt of Clinical Microbiology, Belgian National Reference Laboratory for Echinococcosis, (BNRLE), [5] CHU of Liège, Belgium, ECHINO -Liege, Dpt of Pathology, [6] CHU of Liège, Belgium, ECHINO-Liege, Dpt of Radiology, [7] CHU of Liège, Belgium, ECHINO-Liege, Dpt of Nuclear Medicine, [8] CHU of Liège, Belgium, ECHINO-Liege, Dpt of Hepatogastroenterology, [9] Centre Hospitalier des Ardennes, Libramont, Belgium, Dpt of Gastroenterology, [10] Erasme Hospital, Brussels, Belgium, Dpt of Infectiology, [11] Université Libre de Bruxelles Faculté de Médecine, Brussels, Belgium, of Parasitology, [12] CHU Dinant Godinne, Yvoir, Belgium, Dpt of Infectiology, [13] CHR Citadelle, Liège, Belgium, ECHINO-Liege, Dpt of Infectiology, [14] CHU of Liège, , Belgium, ECHINO-Liege, Dpt of Infectiology.

Introduction: Alveolar echinococcosis (AE) is endemic in Southern Belgium where up to 50% of the red foxes might be infected and spread Echinococcus eggs in the environment. In humans, the primary target organ of AE is the liver, in which AE grows as a parasitic tumor and might later develop in other organs as a malignancy and be lethal. In response to the increasing number of AE cases, a multidisciplinary group (ECHINO-Liege) was created in CHU Liege to improve AE management and to discuss the AE cases. In addition, on the top of a retrospective AE registry, ECHINO-Liege is prospectively building a database (ECHINO-Base) and a biobank (ECHINO-Bank) of AE patients managed in CHU Liege, after EC approval and informed consent. Finally, since 2021, the Belgian National Reference Laboratory for Echinococcosis (BNRLE) is based in the department of Clinical Microbiology of CHU Liege.

Aim: The aim of this study was to report the actual epidemiological and clinical situation on AE in Belgium, using the BNRLE data and the clinical experience of ECHINO-Liege.
Methods: All Belgian clinical laboratories were asked to fill epidemiological forms on AE cases detected in 2021 and 2022. All cases confirmed by serology (immunoblot) and/or PCR and/or histology (proved cases) or without microbiological confirmation (probable and possible cases) were included. These cases were added to the retrospective series already published in 2018 and to the cases discussed during the regular meetings of ECHINO-Liege.
Results: AE was newly diagnosed and reported to BNRLE in 16 patients in the time-period of 2 years, added to the 36 patients previously registered (total: 52 patients, 29M/23F) (mean age: 60y, 19-89). Most patients were born and lived in Wallonia or the Brussels area. All cases but 2 are considered contracted in Belgium (1 in France and 1 in Luxembourg). 31 patients underwent liver resection and 1 liver transplantation.
Conclusions: AE appears to be spreading in Southern Belgium. The authorities should be aware of this public health issue. The radiologists and gastroenterologists should be informed of this diagnosis possibility in case of liver tumor. A national multicentric survey will be soon initiated as a collaboration between the different hospitals in the whole country.

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THE SILENCING OF SOX9 INHIBITS THE DUCTULAR REACTION EXPANSION BUT ENHANCES THE DIFFERENTIATION OF DR CELLS INTO HEPATOCYTES IN THE DISEASED LIVER. A. de Schaetzen (1), M. De Rudder (1), A. Pottier (2), I. Leclercq (1) / [1] Université Catholique de Louvain (UCLouvain), Brussels, Belgium, Hepato-Gastro-Enterology, [2] Université catholique de Louvain (UCLouvain), Belgium, Hepato-Gastro-Enterology.

Introduction: After acute damage or liver resection the liver regenerates and each cell compartment proliferates to repopulate the cells that were lost. In chronic liver disease, hepatocytes are found in a state of replicative senescence and are no longer able to proliferate. In such a setting, cholangiocytes proliferate and invade the parenchyma, in a phenomenon called the ductular reaction (DR). Cells of the ductular reaction are in transitional shift between cholangiocytes and hepatocytes, and have the ability to differentiate in hepatocytes, offering an alternative path for regeneration. Observations in animal models show nevertheless that DR cells generate only a modest fraction of hepatocytes. Hence strategies to enhance DR-derived regeneration are needed to significantly support liver function in chronic diseases. Sox9 is a transcription factor that determines the biliary fate of the bipotential precursor to cholangiocytes and hepatocytes during embryogenesis. Sox9 ectopic expression is proposed to direct liver epithelial cells towards the biliary lineage. Here we hypothesize that the silencing of Sox9 in cells of the ductular reaction will ease their shift towards the hepatocyte lineage, thereby enhancing their contribution to liver regeneration.
Aim: We aim to enhance DR cell-to-hepatocyte differentiation by silencing Sox9 in biliary cells.
Methods: We made successive crossings to obtain OpniCreERT2 : Rosa26RYFP : Sox9floxed transgenic mice. In these mice, the injection of Tamoxifen drives the constitutive expression of YFP and the silencing of Sox9 in cholangiocytes alone. Any cell expressing YFP is a cholangiocyte or its progeny. Sox9Chol KO and controls with intact Sox9 expression received carbon tetrachloride (CCl4) 3 times per week for 6 weeks to cause chronic hepatocellular damage. In a separate experiment, we first treated mice with CCl4 to induce DR and then operate Sox9 silencing in cholangiocytes, then mice underwent a 2 weeks recovery period before harvest. We used immunohistochemistry and immunofluorescence (IF) to quantify the number of biliary cells in which Tamoxifen induced YFP expression and Sox9 silencing, and to investigate the effect of the silencing of Sox9 on DR-driven regeneration.
Results: In response to tamoxifen injection, we observed the expression of YFP in 71% (±1,9 %) of cholangiocytes together with expression of Sox9 in none of them in Sox9chol KO mice. In controls mice 82% (±3,5%) of cholangiocytes expressed YFP and all of them expressed Sox9. After 6 weeks of CCl4, the magnitude of the ductular reaction, as measured by the area of Ck19+ cells, was significantly lower in Sox9Chol KO than in controls. Patches of YFP+/HNF4α+ hepatocytes generated from the DR were present in all animals, but similar in density in Sox9Chol KO and in controls, this regardless whether the ductular reaction was weak (Sox9Chol KO) or vigorous (controls). This supports that Sox9 deletion favors differentiation of DR cells. To further support this, we silenced Sox9 after the induction of the DR in chronic hepatocellular injury, and examined livers after a 2 weeks recovery period. We confirmed that tamoxifen injection effectively induced YFP expression and silenced Sox9 in 68% and 100% of DR cells respectively in Sox9Chol KO and in 83% and 0% of DR cells in controls. The number of YFP+ hepatocytes was significiantly increased in Sox9Chol KO compared to controls.
Conclusions: We generated a model for inducible and cholangiocyte-specific YFP expression along with Sox9 silencing: the OpniCreERT2 : Rosa26RYFP : Sox9floxed mice. With this model, we showed that the silencing of Sox9 impairs the expansion of DR cells. Furthermore, the silencing of Sox9 in DR cells enhances their hepatocytic differentiation.

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NODULAR REGENERATIVE HYPERPLASIA OF THE LIVER : AN INSIGHT INTO THE EPIDEMIOLOGY AND THE CLINICAL CHARACTERISTICS OF A RARE AND POORLY UNDERSTOOD ENTITY. E. Kaze (1),