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),

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Introduction: Recently, the Vascular Liver Disease Interest Group (VALDIG) proposed the new term porto-sinusoidal vascular disorder to include different conditions sharing three specific histological lesions: obliterative portal venopathy, incomplete septal cirrhosis and nodular regenerative hyperplasia (NRH). However, little is known about the clinical significance of these lesions as well as the outcome of these patients.
Aim: The aim of this study was to describe the epidemiology and the clinical characteristics of patients with a histopathologic diagnosis of NRH.
Methods: From January 2015 to March 2021, adult patients with a histopathologic diagnosis of NRH were included. They were extracted from the database of the pathology department of Cliniques universitaires Saint-Luc, Brussels. Baseline characteristics, conditions associated with NRH, clinical and laboratory findings at diagnosis were recorded. Complications of portal hypertension and mortality were analyzed during a 1 to 6-year follow-up.
Results: Our study included 100 patients with a histopathologic diagnosis of NRH from January 2015 to March 2021. Liver tissue was obtained by transjugular liver biopsy (52%), percutaneous liver biopsy (21%), liver resection specimen (25%) or by intraoperative liver biopsy (2%). There were 55 men and 45 women. The indications for liver tissue sample were diagnostic evaluation of portal hypertension (32%), abnormal liver tests (25%), liver resections for metastasis (22%) , protocol biopsies after liver transplantation (12%) and others (9%). 44% had clinical features of portal hypertension at histological diagnosis (splenomegaly, oesophageal or gastric varices). Conditions related to NRH were immunosuppressive status (62%), cardiovascular diseases (6%), genetic diseases (4%), and autoimmune/inflammatory conditions (4%). No associated conditions could be identified in 24% of the cases. We selected patients in whom liver tissue was obtained during the evaluation of clinical and biological signs of portal hypertension or abnormal liver tests (group 1, n = 57). Among them, 5 patients were excluded due to missing data. The remaining patients were compared with patients who had an incidental histologic diagnosis of NRH (group 2, n = 34) : patients undergoing liver resection for metastasis (n=22) and patients with protocol biopsies after liver transplantation (n=12). We excluded 8 patients who had a protocol liver biopsy after transplantation because of abnormal liver tests. 9 patients in whom the diagnosis of NRH was made in another setting were not included in the analysis. Compared to patients with clinical manifestations (group 1), patients with incidental diagnosis of NRH (group 2) had more often an immunosuppressive status (drug or disease related) (100% versus 40%, $p<0.01$), were more often treated by oxaliplatin (69% versus 2%, $p<0.01$) and developed less manifestations of portal hypertension during follow-up (0% versus 31% ($p<0.02$). Liver-related mortality was 2% in group 1 compared to 0% in group 2.
Conclusions: Our study shows that NRH is a unique histologic lesion associated to different clinical manifestations: about 50% of patients presented signs of portal hypertension or abnormal liver tests. Oxaliplatin treatment was frequently associated to NRH (20% of patients). Notably, the majority of patients with NRH related to oxaliplatin didn't experience liver-related complications.

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UNRAVELING THE INDIVIDUAL CONTRIBUTIONS OF THE PPAR ISOTYPES TO THE PAN-PPAR AGONIST LANIFIBRANOR-INDUCED IMPROVEMENTS OF THE VASCULAR ALTERATIONS AND LIVER HISTOLOGY IN A RAT MODEL OF EARLY NAFLD

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Introduction: Nonalcoholic fatty liver disease (NAFLD) is currently the most prevalent chronic liver disease worldwide, with no pharmacological therapy licensed yet. The pan-peroxisome proliferator-activated receptor (pan-PPAR) agonist Lanifibranor has demonstrated beneficial effects in NAFLD and preclinical models of cirrhosis and portal hypertension. In a previous study we showed that in a preventive set-up Lanifibranor improves the increased intrahepatic vascular resistance (IHVR), the related endothelial dysfunction and the associated liver histology in a rat model of early NAFLD. The PPAR receptors consists of 3 isotypes (alpha, delta and gamma), differentially expressed across tissues and operating through a complex physiological interplay.
Aim: This study aimed to provide more insights in the underlying mechanism through which Lanifibranor acts on the functional intrahepatic vascular alterations and on the histological features in early NAFLD, by exploring the mono-PPAR agonists GW501516 (PPAR-delta agonist) and Rosiglitazone (PPAR-gamma agonist) in the same experimental set-up as before with 100 mg/kg Lanifibranor using a rat model of early NAFLD.
Methods: Male Wistar rats (n=8 per group) were fed a methionine-choline-deficient diet (MCD) or control diet (CD) for 4 weeks simultaneously with either vehicle, 10 mg/kg GW501516, or 5 mg/kg Rosiglitazone treatment via oral gavage daily QD. In vivo haemodynamics and blood pressures of the carotid artery, portal vein and inferior caval vein