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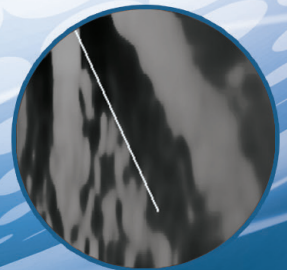
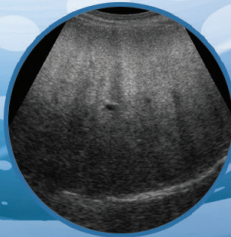
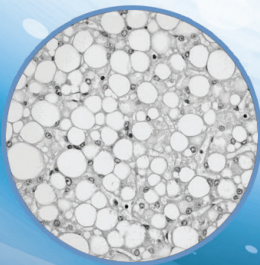
Croatian Society for Diabetes and Metabolic Diseases of the Croatian Medical Association,
Croatian Society for Ultrasound in Medicine and Biology of the Croatian Medical Association,
European Federation of Societies for Ultrasound in Medicine and Biology
and European Association for the Study of the Liver

organize

**3rd Seminaria hepatologica –
Adriatic Liver Forum:
NON-ALCOHOLIC
FATTY LIVER
DISEASE**

Friday, March 3th, 2017

University Hospital Dubrava
Avenija Gojka Suska 6, Zagreb, Croatia



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Dear colleagues,

It is my great pleasure to announce the third *Seminaria hepatologica* symposium that will be devoted to Non-alcoholic fatty liver disease (NAFLD), and will be held on March 3rd 2017 at University Hospital Dubrava, Zagreb, Croatia.

NAFLD is becoming frequent problem in clinical practice, that is according to trends expected to become the leading cause of cirrhosis and liver failure, as well as the most common indication for liver transplantation. Fatty liver is closely related to epidemiology of obesity, as well as metabolic syndrome and is considered one of its components. Dilemmas in diagnostic approach and lack of simple and effective treatment present challenge for clinicians and scientific community, as well as for health systems due to epidemiological trends.

The speakers at the symposium will be eminent experts in the field from Croatia and other European countries. Since this kind of platform has been well accepted from the speakers and participants as a forum for exchange of knowledge and experience in hepatology in this part of Europe, we decided to change the name of the event from *Seminaria hepatologica* to Adriatic Liver Forum. By emphasising the need for interdisciplinary approach to NAFLD, we are delighted to announce endocrinologists, radiologists and pathologists as speakers at the symposium together with hepatologists.

The scientific value of this event is recognized by the most eminent national and international professional organizations and we are proud to state that the event will be endorsed by European Association for the Study of the Liver (EASL), European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB), Croatian Society for Gastroenterology, Croatian Society for Diabetes and Metabolic Diseases of the Croatian Medical Association and Croatian Society for Ultrasound in Medicine and Biology of the Croatian Medical Association. Participation will be accredited according to Regulations of Croatian Medical Chamber. During the symposium *hands-on* ultrasound on models will be available and all participants will be granted certificate of attendance by EFSUMB.

Looking forward to meeting you in Zagreb!

Ivica Grgurevic

President of the Organizing Committee
Seminaria hepatologica - Adriatic Liver Forum



08:00-08:30 REGISTRATION

08:30-08:45 Opening

**08:45-10:00 SESSION 1 (Altabas V.,
Kujundzic M., Ostojic R.,
Simunic M.)**

- 08:45-09:00 Epidemiology of NAFL /
Duvnjak M.
- 09:00-09:15 Pathogenesis of NAFLD /
Milic S.
- 09:15-09:30 Diabetes and NAFLD: vitious
circle / Cigrovski-Berkovic M.
- 09:30-09:45 Natural history of NAFLD /
Grgurevic I.
- 09:45-10:00 Discussion

**10:30-12:00 SESSION 2 (Bevanda M.,
Manolev A., Stimac D.)**

- 10:30-10:45 NAFLD and viral hepatitis /
Vince A.
- 10:45-11:00 NAFLD and HCC / Luetic K.
- 11:00-11:15 Alcohol, coffee, fat burners
and NAFLD /
Stabuc B./Drnovsek J.
- 11:15-11:30 Medications and NAFLD /
Bevanda M.
- 11:30-11:45 NAFLD in lean individuals
(and healthy liver in obese) /
Hrstic I.
- 11:45-12:00 Discussion

12:00-13:30 LUNCH

**13:30-15:00 SESSION 3 (Grgurevic I.,
Mrzljak A.)**

- 13:30-13:45 From fat to fibrosis: is it a
straight line? / Pinzani M.
- 13:45-14:00 Hystological features of
NAFLD / Skrtic A.
- 14:00-14:15 Imaging & elastography in
NAFLD / Berzigotti A.

- 14:15-14:30 Noninvasive biochemical
methods / Premuzic M.

- 14:30-14:45 NAFLD: to biopsy or not? /
Puljiz Z.

- 14:45-15:00 Discussion

**15:15-16:30 SESSION 4 (Husic-
Selimovic A., Kardum D.)**

- 15:15-15:30 Insulin-senzitising agents for
NAFLD / Marusic S.
- 15:30-15:45 Statins in NAFLD and portal
hypertension / Bosch J.
- 15:45-16:00 Herbal products as potential
drugs for NAFLD /
Males Z./Turcic P.
- 16:00-16:15 NAFLD and liver
transplantation /
Filipec-Kanizaj T.

- 16:15-16:30 Discussion

**16:45-18:00 SESSION 5 (Banic M.,
Krzrnaric Z.)**

- 16:45-17:00 Novel therapies for NAFLD /
Alempijevic T.
- 17:00-17:15 Gut microbiota: the key
player of gut-liver liaison in
NAFLD / Papp M.
- 17:15-17:30 Endoscopic treatment of
obesity: impact on liver /
Salkic N./Nikolic M.
- 17:30-17:45 Impact of diet & exercise on
NAFLD / Bokun T.
- 17:45-18:00 Discussion & Concluding
remarks and closure of
symposium

08:30-16:30 Hands-on ultrasound

20:00 DINNER

EPIDEMIOLOGY OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) is negative definition of a very common disease that refers to the presence of hepatic steatosis when no other causes for secondary hepatic fat accumulation are present. By its definition, it is subdivided into 2 groups: non-alcoholic fatty liver (NAFL), where hepatic steatosis is present without evidence of inflammation) and non-alcoholic steatohepatitis (NASH), where hepatic steatosis is associated with hepatic inflammation that is histologically undistinguishable from alcoholic steatohepatitis).¹ NAFLD is the most common liver disorder in Western industrialized countries with prevalence in general population of 6-35% (median 20%) worldwide. In Europe (including Croatia), the median prevalence is 25-26%. Prevalence in obese patients is 70-80% and 75% in patients with diabetes mellitus type 2.² According to the studies, there are uncertainties regarding the influence of gender on NAFLD. It is estimated highest prevalence among Hispanics and lowest prevalence among non-Hispanic blacks, while African Americans have less steatosis than whites.³ Some of the mechanisms that may contribute to gender and racial difference are: insulin resistance, distribution of adiposity, lifestyle – dietary habits and alcohol use, sex hormones and genetic variations. NAFLD is hepatic component of the metabolic syndrome. Major risk factors include central obesity, type 2 diabetes mellitus, dyslipidaemia, insulin resistance and histological evidence of hepatic inflammation, which is one of the most important risk factors. Cardiovascular and not hepatic disease is the most common cause of death among patients with NAFLD/NASH. Progression to cirrhosis or hepatocellular carcinoma (HCC) is usually slower than with other chronic liver diseases and occurs in 2.5% of patients with NASH. Duration of progression to cirrhosis is 57 years from NAFLD to cirrhosis and 28 years from NASH to cirrhosis. It is not clear yet if patients with NAFLD have increased overall mortality rates compared to the general population. The largest study in the US suggests that the overall mortality is not increased, while smaller studies and studies in other populations suggest a slight increase in mortality.⁴ Hepatocellular carcinoma (HCC) is associated with cirrhosis because of NAFLD or NASH (HCC-NASH). In a recently published systematic review of 61 studies and case series of patients with NAFLD or NASH, the risk of HCC in those with cirrhosis ranged from 2.4 percent over 7 years to 12.8% over 3 years, the risk of mortality from HCC in patients without cirrhosis was 0-3% after follow-up periods of up to 20 years. NAFLD is disease that accompanies the epidemic of metabolic syndrome.⁵ Therefore, in future, it is very important to develop new protocols for the diagnosis and treatment of NASH and new policies for the surveillance of patients with NAFLD.

REFERENCES

1. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. *Liver Int.* 2017;37:81-84.
2. Younossi ZM, Stepanova M, Afendy M, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. *Clin Gastroenterol Hepatol.* 2011;9:524-530.
3. Pan JJ and Fallon MB. Gender and racial differences in nonalcoholic fatty liver disease. *World J Hepatol.* 2014; 6(5): 274-283.
4. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver versus nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol.* 2015;13:643-654.
4. White DL, Kanwal F, El-Serag HB. Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin Gastroenterol Hepatol.* 2012;10:1342-1348.

PATHOGENESIS OF NAFLD

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Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world, but only a small minority of affected patients develops inflammation and fibrosis, while most of them present with simple steatosis. NAFLD is characterized by fat accumulation, mainly as triglycerides, in the hepatocytes. It is associated with clinical factors such as obesity, dyslipidemia, and diabetes. NAFLD represents the hepatic expression of the metabolic syndrome, and its physiopathology involves several mechanisms, such as, glucose intolerance, insulin resistance (IR), enhanced lipogenesis and lipotoxicity, hepatic and systemic inflammation, and oxidative stress. The “multiple-hit” hypothesis is the most accepted for understanding the pathogenesis of NAFLD and their progression to non-alcoholic steatohepatitis and cirrhosis. This hypothesis proposes that many simultaneous hits derived from the gut and adipose tissue may promote inflammation and liver injury. Free fatty acid (FFA) and hepatic triglyceride (TG) accumulation is a cardinal feature of NAFLD, and commonly occurs in the setting of insulin resistance and obesity. Liver injury usually occurs in the presence of these features, mediated by inflammatory cytokines, mitochondrial dysfunction secondary to nutrient excess and oxidative stress. Also, with IR, there is decreased ability of insulin to suppress adipose tissue lipolysis. De novo lipogenesis, during fasting, is increased by 3 fold in patients with NAFLD as compared to those with lean liver. This represents hyperinsulinism, which induces sterol response element-binding protein (SREBP)-1c and peroxisome proliferator-activated receptor (PPAR)- γ that in turn promote the expression of several lipogenic genes. Excess energy intake through a diet rich in fat and carbohydrates leads to failure of adipocytes to adapt in terms of proliferation and differentiation. In the liver, FFAs are the main source for the synthesis of triglycerides.

Similarly, excess dietary fat and de novo lipogenesis are responsible for lipotoxicity. FFAs and cholesterol can also accumulate in the mitochondria leading to inflammation and liver injury mediated by tumor necrosis factor alpha and reactive oxygen species. The extent of hepatic inflammatory damage is also influenced by extrahepatic factors such as adipose tissue signalling, the effect of gut microbiota and genes polymorphisms such as PNPLA3 and TLF613. Despite advances in the knowledge of pathogenesis of NAFLD, some pathways are still unknown.

REFERENCES:

1. Buzzati E, Pinzani M, Tshotzacis A. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism*. 2016;65:1038-48.
2. Ratziu V, Goodman Z, Sanyal A. Current efforts and trends in the treatment of NASH. *J Hepatol* 2015; 68:65-75.
3. Goh GB, McCullough AJ. Natural history of nonalcoholic fatty liver disease. *Dig Dis Sci* 2016;61:1226-33.

VICIOUS CIRCLE

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Type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD) are common conditions affecting millions of people worldwide. Although the prevalence of NAFLD varies widely in different populations, it has been shown to affect up to 70% of patients with diabetes, most likely quite significant proportion of them being unrecognized and asymptomatic. NAFLD and diabetes share two common denominators; physical inactivity and obesity. There is a strong association between NAFLD and diabetes risk. According to some observations, individuals' risk was up to 5-fold higher to contract type 2 diabetes in the setting of NAFLD. Unfortunately there are currently no predictive findings on which individual with NAFLD will develop diabetes so annual surveillance with 2-h oGTT and HbA1c are prudent. Pathogenesis of T2DM is complex, but mainly involves chronic hyperinsulinemia due to hepatic and peripheral tissue insulin resistance (IR), which ultimately leads to beta-cell failure. Whether T2DM increases ones risk of developing NAFLD is less clear cut and difficult to study due to insidious disease onset, although common sense would suggest positive correlation, or to be more precise bi-directional link. NAFLD increases the likelihood of macrovascular complications in an individual with T2DM by almost 2-fold. Similar is seen with the microvascular risk (including retinopathy and chronic kidney disease). Moreover, hepatic fat content, a hallmark of NAFLD is associated with higher insulin requirements, leading to weight gain. On the other hand, people with coexisting T2DM and NAFLD have detrimental liver outcomes; such as more severe inflammation and cirrhosis. Finally, in a meta-analysis, coexisting diabetes was associated with poorer prognosis in individuals with NAFLD-

induced hepatocellular carcinoma. One of the pathophysiologic mechanisms leading to NAFLD is IR at the liver level and peripheral tissues (fat and muscle) leading to liver fat accumulation and availability of glucose and lipid substrates, respectively. Although treating IR seems as a promising concept also for targeting NAFLD, current data on agents such as metformin, DPP-4 inhibitors, GLP-1RA, SGLT-2 inhibitors did not provide enough evidence for these agents to be used in the setting of NAFLD without diabetes. Mainstay of treatment currently relies on dietary and life-style changes, while drugs in the pipeline and those under clinical investigation are offering promise for future NAFLD treatment. Studies examining more severe NAFLD, including NASH with fibrosis and cirrhosis are needed, as this represents the disease spectrum with significant increase in both liver and cardiovascular mortality.

REFERENCES

1. Hazelhurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. *Metabolism*; 2016;65:1096-1108.
2. Adams LA, Waters OR, Knuiman MW, Elliot RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. *Am J Gastroenterol* 2009;104:861-867.
3. Jäger S, Jacobs S, Kröger J, Stefan N, Fritsche A, Weikert C et al. Association between the fatty liver index and risk of type 2 diabetes in the EPIC-potsdam study. *PLoS One* 2015;10:e0124749.
4. Ballestri S, Nascimbeni F, Romagnoli D, Baldelli E, Targher G, Lonardo A. Type 2 diabetes in non-alcoholic fatty liver disease and hepatitis C virus infection-liver: The “musketeer” in the spotlight. *Int J Mol Sci* 2016;17:355.
5. Lonardo A, Ballestri S, Marchesini G, Angulo P, Loria P. Nonalcoholic fatty liver disease: A precursor of the metabolic syndrome. *Dig Liver Dis* 2015;47:181-190.

NATURAL HISTORY OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Natural history of Non-alcoholic fatty liver disease (NAFLD) has traditionally been considered evolving process starting with fat accumulation in hepatocytes, followed by second hit that triggers inflammatory response resulting in scar formation, leading finally to cirrhosis, liver failure, HCC development and death. Overall mortality is increased in patients with NAFLD, including cardiovascular and liver-related mortality. However, until recently many uncertainties existed with regard to the subgroups and proportion of patients that follow this pathway, as well as to the risk factors for deterioration of

liver function. Non-alcoholic fatty liver disease comprises distinct histological forms based on the presence of inflammatory component: Non-alcoholic fatty liver (NAFL) and Non-alcoholic steatohepatitis (NASH). In both cases fibrosis may or may be not present. Presence and the stage of liver fibrosis is the most important histological feature independently related to the overall and liver-related mortality in NAFLD patients. Patients with NAFL without fibrosis (bland steatosis) have very good prognosis, comparable to the general population. However, patients with fibrosis have much worse prognosis irrespective of the presence of inflammatory component within the liver. Even patients with bland steatosis when accompanied by a certain stage of fibrosis have worse prognosis compared to patients with NASH but without fibrosis. Fibrosis stage, age, presence of type 2 diabetes mellitus (T2DM) and current smoking have been identified as factors independently related to the overall mortality in multivariate analysis, with exponential increase of liver-related mortality with increasing fibrosis stage. Interestingly, the use of statins was inversely related to overall mortality. From these data it becomes clear that precise determination of the fibrosis presence and stage has utmost prognostic importance. Certain features point to the NAFLD patients under risk of having fibrosis at presentation: (a) clinical features: age >50, obesity, presence of T2DM; (b) histological features: presence of necroinflammation and probably steatosis; (c) biochemical or combined biochemical and clinical features: increased values of FIB-4 score, NAFLD fibrosis score. In the meta-analysis of paired-biopsy studies fibrosis progressed in 36.1% of NAFLD patients with almost equal proportion of both NAFL and NASH patients showing progression. However, the progression was faster in NASH cohort (1 stage over 7.1 years vs. 1 stage over 14.3 years in NAFL). Among NAFLD patients who progressed around 80% progressed slowly and 20% progressed rapidly (from stage 0 to stage 3-4 over mean period of 5.9 years). Baseline risk factors associated with progressive fibrosis were presence of arterial hypertension (OR 1.94, $p=0.05$), AST/ALT ratio (OR 0.08, $p=0.06$) and 2 of 4 studies observed that patients with a higher steatosis grade were more likely to develop progressive fibrosis. In this meta-analysis no association was found between baseline severity of necroinflammation and risk of progressive fibrosis. Other baseline factors have been identified by other authors as related to the risk of fibrosis progression such as older, higher weight and more diabetes, presence of fibrosis either by histological analysis or FIB-4 score, whereas conflicting data exist on the impact of necroinflammation on this issue. Factors associated with progressive liver disease at follow-up are increased AST and ALT levels, lower Plt, weight gain exceeding 5 kg, more insulin resistance, hepatic fatty infiltration and presence of T2DM.

REFERENCES

1. Mattias Ekstedt, Hannes Hagstrom, Patrik Nasr, Mats Fredrikson, Per Stal, Stergios Kechagias, and Rolf Hultcrantz. Fibrosis Stage Is the Strongest Predictor for Disease-Specific Mortality in NAFLD After Up to 33 Years of Follow-Up. *Hepatology* 2015;61:1547-1554
2. Paul Angulo, David E. Kleiner, Sanne Dam-Larsen, Leon A. Adams, Einar S. Bjornsson, Phunchai Charatcharoenwittaya, Peter R. Mills, Jill C. Keach, Heather D. Lafferty, Alisha Stahler, Svanhildur Hafliadottir, and Flemming Bendtsen. Liver Fibrosis, but No Other Histologic Features,

Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015;149:389-397.

3. Parambir S. Dulai, Siddharth Singh, Janki Patel, Meera Soni, Larry J. Prokop, Zobair Younossi, Giada Sebastiani, Mattias Ekstedt, Hannes Hagstrom, Patrik Nasr, Per Stal, Vincent Wai-Sun Wong, Stergios Kechagias, Rolf Hultcrantz, Rohit Loomba. Increased risk of mortality by fibrosis stage in non-alcoholic fatty liver disease: Systematic Review and Meta-analysis. *Hepatology* 2017; doi: 10.1002/hep.29085
4. S Dam-Larsen, M Franzmann, I B Andersen, P Christoffersen, L B Jensen, T I A Sørensen, U Becker, F Bendtsen. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut* 2004; 53: 750-755.
5. Stuart McPherson, Tim Hardy, Elsbeth Henderson, Alastair D. Burt, Christopher P. Day, Quentin M. Anstee. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. *Journal of Hepatology* 2015 ; 62: 1148-1155.

NON-ALCOHOLIC FATTY LIVER DISEASE AND VIRAL HEPATITIS

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Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of liver changes from steatosis to hepatocellular injury and inflammation (NASH). The interaction of NAFLD and chronic hepatitis C (HCV) has been extensively studied in past 15 years. HCV replicates in the cytoplasm of the hepatocyte and its life cycle has been mostly discovered. A variety of metabolic disorders have been described during the course of HCV infection including insulin resistance, liver steatosis or disturbed fatty acid metabolism. The unique feature of HCV biology is association between HCV and lipids, HCV relies on host lipid metabolism and transport pathways at all stages of its lifecycle, for example hepatocyte entrance via endocytosis is dependent on membrane cholesterol levels. Different studies have shown a specific interaction of the HCV core protein with the signaling pathway of insulin in the hepatocyte, which may lead to insulin resistance and accumulation of fat in the liver. Chronic HCV itself causes progressive liver inflammation and fibrosis, which can progress to cirrhosis over several decades. Additional role of inflammatory cytokines in the pathogenesis of the HCV-associated IR state has been suggested, mostly TNF- α and the suppressor of cytokine signaling 3 (SOCS 3) as well as IL-18. Patients who regularly drink alcohol, patients with HIV/HBV coinfection and patients with NAFLD progress to cirrhosis faster. On the average NAFLD is found in 20-30% of patients with chronic HCV. NASH occurs in 33% of patients with the metabolic syndrome. Both chronic HCV and metabolic syndrome share common features. Patients with a normal body mass index, no hypertension

nor hyperlipidemia are more likely to have steatosis from HCV, which can be also attributed to some genetic polymorphisms. The presence of the PNPLA3/Adiponutrin rs738409 C/G single nucleotide polymorphism resulted in a risk of steatohepatitis similar to that generated by metabolic factors in obese individuals. On the contrary patients with obesity are more likely to have both NASH and chronic HCV. Hepatic steatosis in patients with chronic HCV can be a direct result of genotype 3 or from IR and T2DM in other genotypes. Identifying patients with HCV and coexisting NAFLD is important because they remain at risk of progressive fibrosis, cirrhosis and HCC even after HCV has been eradicated with antiviral therapy. However the high efficacy of new antivirals does not appear to be affected by steatosis. On the other hand the eradication of HCV cannot reverse the established metabolic syndrome, and those patients have to be monitored for HCC continuously even after the clearance of the virus. Evaluation of histology has become rare in patients with chronic HCV, and the stage of liver fibrosis is usually defined by non-invasive techniques, most commonly transient elastography. The Controlled Attenuation Parameter (CAP) technology using a process based on transient elastography specifically targets liver steatosis and allows the parallel evaluation of liver steatosis in patients with viral hepatitis.

REFERENCES

1. Schiffman ML, Gunn NT. Impact of hepatitis C virus therapy on metabolism and public health. *Liver International* 2017;37: 13-18.
2. Stepanova M, Lam B, Younossi Y, Srishod MK, Younossi ZM. Association of hepatitis C with insulin resistance and type 2 diabetes in US general population; the impact of the epidemic of obesity. *J Viral Hepat* 2012;19:341-345.
3. Sheikh MY, Choi J, Quadri I, Friedman JE, Sanyal AJ. Hepatitis C virus infection: molecular pathways to metabolic syndrome. *Hepatology* 2008;47:2127-2133.
4. Vanni E, Abate ML, Gentilecore E, Hickman L, Gambino R, Cassader M, Smedile A et al. Sites and mechanisms of insulin resistance in nonobese, nondiabetic patients with chronic hepatitis C. *Hepatology* 2009;50: 697-706.
5. Petta S, Vanni E, Bugianesi E, Rosso C, Cabibi D, Cammà C, Di Marco V, Eslam M, Grimaudo S, Macaluso FS, McLeod D, Pipitone RM, Abate ML, Smedile A, George J, Craxi A. PNPLA3 rs738409 I748M is associated with steatohepatitis in 434 non-obese subjects with hepatitis C. *Aliment Pharmacol Ther* 2015; 41: 939-941.
6. Bochud PY, Cai T, Overback K et al. Genotype 3 is associated with accelerated fibrosis progression in chronic hepatitis C. *J Hepatol* 2009;51:655-666.
7. Kurosaki M, Hosokawa T, Matsunaga K, et al. Hepatic steatosis in chronic hepatitis C is a significant risk factor in developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy. *Hepatol Res* 2010;40:870-877.

NON-ALCOHOLIC FATTY LIVER DISEASE AND HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma is the 5th most frequently diagnosed cancer in adult men and 7th in adult women worldwide. Liver cancer is the 3rd cause of cancer-related death (692,000 cases) and accounts for 7% of all cancers. HCC represents more than 90% of primary liver cancers and is one of the deadliest malignancies with five-year survival of 10% and an extremely rare longer survival.¹ Incidence in the USA has increased during the past two decades, possibly due to large pool of people with longstanding chronic hepatitis C. The rate began to accelerate in mid 1980s, most likely because of the increased incidence of cirrhosis due to chronic HCV infection and NAFLD. HCC incidence rate per 100,000 in Croatia in 2014 was 10.5.

The main risk factors for development of HCC are HCV and HBV infection (the percentage depends on the continent), alcohol use, inherited metabolic diseases such as hemochromatosis or alpha-1-antitrypsin deficiency and NAFLD. All etiologic forms of cirrhosis may be complicated by tumor formation, but the risk is higher in patients with hepatitis infection.² At a major US referral center, in a research conducted from 2007 to 2009, the predominant HCC etiologies were HCV, alcohol use and NAFLD.³ In at least one study it was found that HCC can occur in patients with NAFLD who do not have cirrhosis. They identified a national cohort of 1500 patients with verified HCC during 2005-2010 in the US Veterans Administration. Approximately 13% of the patients with HCC did not have cirrhosis and NAFLD and metabolic syndrome were the main risk factors.⁴

One other study confirmed that older age and advanced fibrosis were important risk factors for HCC and that HCC was the major cause of mortality in NASH patients with advanced fibrosis. At present, NAFLD is the second leading indication for HCC-related transplantation in the USA. Although NAFLD is a risk factor for HCC, which may also develop in the pre-cirrhotic stage, and the risk is further increased by the presence of the PNPLA3rs738409 C>G polymorphism, no recommendation can be currently made on the timing of surveillance and its cost-effectiveness.⁵

REFERENCES

1. Blechacz B, Mishra L. Hepatocellular carcinoma biology. Recent Results Cancer Res 2013; 190: 1-20
2. EASL-EORTC Clinical Practice Guidelines: Management of hepatocellular carcinoma Europe- an Association for the Study of the Liver, European Organisation for Research and Treatment of Cancer Journal of Hepatology Volume 56, Issue 4, Pages 908-943 (April 2012)
3. Yang JD, Harmsen WS, Slettedahl SW, et al. Factors that affect risk for hepatocellular carcinoma and effects of surveillance. Clin Gastroenterol Hepatol 2011; 9:617.

4. Mittal S, El-Serag HB, Sada YH, et al. Hepatocellular Carcinoma in the Absence of Cirrhosis in United States Veterans is Associated With Nonalcoholic Fatty Liver Disease. Clin Gastroenterol Hepatol 2016; 14:124.
5. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease, Journal of Hepatology, Volume 64, Issue 6, Pages 1388-1402 (June 2016)

DIABETES AND NON-ALCOHOLIC FATTY LIVER DISEASE: ALCOHOL, COFFEE, FAT BURNERS AND NAFLD

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Non-alcoholic fatty liver disease (NAFLD) has become the most common cause for chronic liver diseases in the Western world and is estimated to impact at least 25% of the world population, with the highest prevalence in the Middle East and South America and lowest in Africa (1). Since the global obesity epidemic continues, the clinical and economic burden of NAFLD and its more severe form, non-alcoholic steatohepatitis (NASH), will increase as well.

The absence of approved therapies in high prevalence and rising incidence of NAFLD is striking. Lifestyle modifications such as weight loss and low-fat healthy diet are the only effective treatments of NAFLD; however, the long-term compliance is very low. Therefore, several pharmacological treatments have been proposed, among them also natural substances with antioxidant properties.

Natural polyphenols are a wide class of phytochemicals sharing a common phenolic structure and classified as flavonoids and non-flavonoids. Flavonoids are formed by two phenolic rings connected by a three carbon bridge. The diversity in functional groups leads to the differences in biological properties of different subgroups of flavonoids (2).

Coffee is a complex mixture of biologically active compounds, including caffeine, chlorogenic acid, cafestol and kahweol. These compounds interfere with lipid and glucose metabolism, improve insulin sensitivity and possess anti-oxidative and anti-fibrotic properties, which may protect against a range of liver diseases (3,4).

Green tea is rich in flavanols, in particular catechins, among them epigallocatechin-3-gallate (EGCG). Several studies showing the benefit of EGCG on metabolic status. It reduces oxidative stress, inflammation and hepatic fibrosis.

The market is bursting with dietary supplements, among them *fat burners*. The main substance is gamma-hydroxybutyric acid (GHB), which is a neurotransmitter, regulator of energy metabolism and growth promoter. However, there are insufficient clinical data,

supporting it's benefit as a target treatment for NAFLD. Nevertheless, not only being useles, fat burnes are also potentially harmful, leading to acute liver injury.

The intake of alcohol is a well known risk factor for liver damage. However, *quercetin* is found in red wine. It is a flavonol with potent antioxidant activity. It improves insulin sensitivity, increases plasma adiponectin and reduces liver fat accumulation by stimulating omega-oxidation of fatty acids. Since all studies were using animal models, we lack clinical trials which would support the benefit of quercetin and therefore wine intake is not recommended as a part of a healthy diet.

Although many studies and clinical trials have shown the benefit of natural polyphenols in NAFLD, there are some restricting clinical issues. The main remaining question is the right dosage needed to prevent hepatotoxicity. Further issue is the varying bioavailability, which depends on a variety of factors, such as the production techniques, chemical structure, intestinal transit time and probably colonic microbiota as well. We need more prospective controlled trials assessing histological outcomes, but repeated liver biopsies may not be possible because of ethical and practical considerations. Therefore, upcoming new non-invasive markers of liver damage may be a promising alternative for the evaluation of clinical outcomes (5).

REFERENCE:

1. Younossi, Z. M. et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*, 64: 73–84. doi:10.1002/hep.28431
2. Del Rio D, Rodriguez-Mateos A, Spencer JP, et al. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid Redox Signal* 2013; 18: 1818–92.
3. Grosso G et al. Factors associated with metabolic syndrome in a mediterranean population: role of caffeinated beverages. *J Epidemiol* 2014; 24: 327–33.
4. Xiao Q et al. Inverse associations of total and decaffeinated coffee with liver enzyme levels in National Health and Nutrition Examination Survey 1999–2010. *Hepatology* 2014; 60: 2091–8.
5. Vitaglione P et al. Coffee reduces liver damage in a rat model of steatohepatitis: the underlying mechanisms and the role of polyphenols and melanoidins. *Hepatology* 2010; 52: 1652–61.

MEDICATIONS AND NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) is defined as fat infiltration of the liver in people who don't consume alcohol in hepatotoxic quantities (less than 30 g/day for men and 20

g/day for women) and it is the major cause of liver diseases. NAFLD includes a wide spectrum of liver diseases from simple hepatic steatosis, nonalcoholic steatohepatitis (NASH) to liver cirrhosis and hepatocellular carcinoma (HCC). Two principal phenotypes are nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). It is frequently joined with metabolic syndrome, diabetes mellitus type 2, obesity and hyperlipidemia. In general population, prevalence ranges from 20 to 30%. Drug-induced liver injuries (DILIs) are liver injuries caused by drugs or other foreign compounds. Steatosis and steatohepatitis are rare form of DILI and drugs account for fewer than 2% of cases of NASH. The diagnosis of DILI is challenging. It is necessary to establish a strong connection between medication and liver disease and exclude other possible etiologies. Objectifying of DILI is facilitated with development of scoring systems like Roussel-Uclaf Causality Assessment Method (CIOMS/RUCAM), which is used most often, Maria and Victorino causality assessment scale and Naranjo algorithm. There is few pathophysiological mechanisms of hepatic steatosis and injury in steatohepatitis induced with medications. Inhibition of entry of long-chain fatty acids into mitochondria may lead to β -oxidation inhibition and to increased free fatty acids, which are esterified into triglycerides. Some medications can block exit of triglycerides from hepatocytes. Blocking the flow of electrons through the electron transport chain causes accumulation of electrons which can interact with oxygen to produce reactive oxygen species. Certain medications induce DILI by direct damage of mitochondrial DNA and can induce mitochondrial permeability transition pore formation. By histopathological point of view drugs effect can be divided into three groups- into those that cause microvesicular steatosis and those that predominantly lead to macrovesicular steatosis, and third that cause steatohepatitis. Drugs with true cause-effect relationship with steatosis and steatohepatitis are amiodarone, irinotecan, fluorouracil, oxaliplatin, cituximab and bevacizumab. Tamoxifen, metotrexate and corticosteroids can independently cause steatohepatitis and lead to worsening of underlying NAFLD. Valproic acid, tetracycline (intravenous administration of high doses), aspirin (Reye's syndrome), nucleoside reverse transcriptase inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs) and total parenteral nutrition also can induce DILI. There is not enough controlled clinical trials which can be implemented in everyday clinical work. Also there are no clear guidelines how to manage patients with DILI. It is necessary to discontinue medication whenever is possible and consider alternative therapy if it is available. If there is no alternative medication, risks and benefits must be carefully weighed in consultation with the patient. Recent studies suggest that steatosis and steatohepatitis are improved when medications are discontinued. Liver function tests and imaging should be used to confirm improvement.

REFERENCES

1. Farrell G. Drugs and Steatohepatitis. *Seminars in Liver Disease*. 2002;22(2):185-194.
2. Fromenty B, Pessayre D. Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacology & Therapeutics*. 1995;67(1):101-154.
3. Patel V, Sanyal A. Drug-Induced Steatohepatitis. *Clinics in Liver Disease*. 2013;17(4):533-546.

4. Poordad F. Nonalcoholic fatty liver disease: a review. *Expert Opinion on Emerging Drugs*. 2005;10(3):661-670.
5. Rabinowich L. Shibolet O. Drug Induced Steatohepatitis: An Uncommon Culprit of a Common Disease. *BioMed Research International*. 2015;2015:1-14.

NAFLD IN LEAN INDIVIDUALS AND HEALTHY LIVER IN OBESE

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Nonalcoholic fatty liver disease (NAFLD) is strongly associated with metabolic syndrome (MetS) and is more common in obese patients. Term “nonobese fatty liver disease” (NOFLD) and synonym “lean NAFLD” have been recently proposed for individuals who are not overweight by the standard body mass index (BMI) and have fatty liver. Traditionally this condition was considered unique in Asia but NOFLD has been found in 10% of lean Americans. Clinical relevance, factors associated with disease activity, disease progression and prognosis of NOFLD according to so far known data is inconclusive. Study conducted by Dela Cruz and coauthors on 125 lean (BMI 23.1±1.7) and 965 non-lean (BMI 33.3±6.6) patients suggest that NOFLD patients have a higher overall mortality than obese NAFLD patients despite presenting with a healthier metabolic profile, less insulin resistance and less advanced fibrosis. This observation was not confirmed in some other studies published in recent years probably due to low number of lean patients. In the very recently published study, which represent one of the largest histological cohorts, Leung et al. prospectively followed 72 lean and 235 obese patients. They found that lean patients had slightly less-severe steatosis and fibrosis but equal overall proportion of patients with NASH and advanced fibrosis. Authors suggested that factors other than adiposity play a role in the progression of the disease like hypertriglyceridemia and higher creatinine level. On the other hand, some obese individuals (measured via BMI) do not have clinical components of MetS despite having excessive body fat and are described as “metabolically healthy obese” (MHO). The definitions of MHO are quite heterogeneous and absence of NAFLD has been proposed as a potential identifier of MHO. Exploring the available literature it is questionable whether these obese individuals without components of MetS are truly healthy. For example, Lee et al. in the retrospective study of 523 patients who underwent bariatric surgery identified 150 (28.7%) individuals with mean BMI of 49 kg/m² and without diabetes and hypertension. Among them 88.7% had liver steatosis while only 7.3% nonalcoholic steatohepatitis and „only“ 19.3% had liver fibrosis on histology. In further analysis of 44 complete MHO individuals (without diabetes, hypertension and hypertriglyceridemia) similar results of liver injury have been found (84.1% had liver steatosis, 4.6% nonalcoholic steatohepatitis and 15.9% had liver fibrosis). Since NAFLD is progressive disease leading to cirrhosis and hepatocellular carcinoma it is important to recognize

patients with lean fatty liver disease. Further, metabolically healthy obese is questionable healthy entity since liver steatosis can be found in majority of such individuals

REFERENCES:

1. Balistreri WF. Lean patients, yet still at risk for fatty liver disease. *Medscape* Sep 20, 2016.
2. Dela Cruz AC, Bugianesi E, George J, et al. Characteristics and long-term prognosis of lean patients with nonalcoholic fatty liver disease. *Gastroenterology* 2014;146:S-909.
3. Leung JC, Loong TC, Wei JL, et al. Histological severity and clinical outcomes of nonalcoholic fatty liver disease in nonobese patients. *Hepatology*. 2017;65(1):54-64.
4. Roberson LL, Aneni EC, Maziak W, et al. Beyond BMI: The “Metabolically healthy obese” phenotype & its association with clinical/subclinical cardiovascular disease and all-cause mortality – a systematic review. *BMC Public Health*. 2014;14:14.
5. Lee CJ, Clark JM, Asamoah V, Schweitzer M, Magnuson T, Lazo M. Prevalence and characteristics of individuals without diabetes and hypertension who underwent bariatric surgery: lessons learned about metabolically healthy obese. *Surg Obes Relat Dis* 2015;11(1):142-6.

FROM FAT TO FIBROSIS: IS IT A STRAIGHT LINE?

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Non-alcoholic fatty liver disease (NAFLD) covers a spectrum of lesions ranging from simple steatosis to a complex pattern with hepatocellular injury and inflammation (non-alcoholic steatohepatitis; NASH) in the absence of alcohol intake. In general, the sequence steatosis-cell damage-inflammation-fibrosis is assumed as the paradigm of disease evolution. It is however increasingly clear and common clinical observation that, although liver fibrosis is the almost obligate end-point of NASH, different patterns of evolution may occur. In absence of evident hepatocellular necrosis, which typically induce a chronic wound healing reaction leading to tissue fibrosis, oxidative stress represents a predominant pro-fibrogenic mechanism and perisinusoidal fibrosis may develop independently of evident tissue necrosis and inflammation due to the direct pro-fibrogenic action of products of lipid peroxidation such as reactive oxygen species and reactive aldehydes. In this context, hepatocyte mitochondrial dysfunction may be an additional factor favouring further lipid peroxidation and reduction of the natural anti-oxidant response. From the clinical point of view, NASH can be suspected by the presence of constant liver enzyme alterations associated with an ultrasonographic evidence of fatty liver. However, the diagnosis of NASH requires liver biopsy and the evaluation of the stage of fibrosis is even more fundamental than necro-inflammation since it is the main prognostic factor of this disease. The histopathological evaluation of liver biopsy samples

is indeed central in the diagnosis of NAFLD and NASH in the absence of sufficiently accurate non-invasive tests because a precise definition of each group is a key issue. When at least 5% of hepatocytes display steatosis, patients can be defined as having NAFLD in an appropriate clinical context. When, in addition, lobular inflammation and liver cell clarification/ballooning are present, then the lesion is usually defined as NASH. Semi-quantitative histological scoring systems have been proposed for NAFLD, but they are not useful in clinical practice and each has certain limitations. For comprehensive purposes, we suggest describing histopathological lesions in NAFLD using the SAF (Steatosis, Activity, Fibrosis) score which assesses separately the grade of steatosis (S, from S0 to S3), the grade of activity (A from A0 to A4 by adding grades of ballooning and lobular inflammation, both from 0 to 2) and the stage of fibrosis (F from F0 to F4).

HISTOLOPATHOLOGICAL FEATURES OF NONALCOHOLIC FATTY LIVER DISEASE

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Nonalcoholic fatty liver disease (NAFLD) requires an evidence of hepatic steatosis, either by imaging or by histology and, with no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders. NAFLD is further histologically dichotomized into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFL is characterized with the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes. The diagnosis of NASH is established by the presence of a characteristic pattern of steatosis, inflammation and hepatocellular ballooning with or without fibrosis. Currently, a liver biopsy is the only generally acceptable method for the diagnosis of NASH. The value of establishing a diagnosis of NASH is that it identifies individuals who are at risk for progressive liver disease to the point of cirrhosis and death from chronic liver disease (1). It should be stressed out that the dichotomous assessment of liver biopsies is less helpful in treatment trials of therapeutic agents because it cannot identify patients with significantly decreased NASH, but who continued to fulfil diagnostic criteria for NASH. For these purposes, two contemporary classifications and scoring systems are used. They should be able also to determine changes in the underlying disease process independent of the diagnosis of NASH. A scoring system for NAFLD was developed and validated by the National Institute of Diabetes and Digestive and Kidney Diseases sponsored Nonalcoholic Steatohepatitis Clinical Research. The histologic categorization of disease states in patients at risk NAFLD comprises five categories. The fibrosis score reflects the patterns of fibrosis that may occur in the variety of NAFLD. A separate system of scoring the features of NAFLD called the NAFLD Activity Score (NAS) was developed as a tool to measure changes in NAFLD during therapeutic

trials (2). Based on the definition of NASH which is combination of tree histological features, the FLIP consortium (fatty liver inhibition of progression) has created a simple histological algorithm (FLIP algorithm) of mild and significant disease based on a scoring system, the SAF score (steatosis, activity, fibrosis) intended for pathologists to reliably diagnose NASH and limit interobserver variation (3). Liver biopsies as part of diagnostic algorithm of NAFLD should be used for the overall disease classification according to the pattern of injury as well as for the grading and staging of the disease. The spectrum of lesions in fatty liver disease has been well described in the literature, differs in adults and children and includes many lesions beyond NASH features. Scoring systems have been developed for a variety of uses, such as for communication of severity of disease to clinicians, comparative use in clinical trials. It is important to note that the process of grading and staging is related to, yet separate from the process of assigning a diagnostic pattern. Both are important in the pathologic evaluation of NAFLD liver biopsies.

REFERENCES

1. Angulo P et al. Gastroenterology. 2015;149(2):389-97.
2. Brunt EM et al; NASH Clinical Research Network (CRN). Hepatology. 2011;53(3):810-20.
3. Bedossa P et al. Hepatology. 2012;56(5):1751-9.

IMAGING AND ELASTOGRAPHY IN NON-ALCOHOLIC FATTY LIVER DISEASE

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The aim of non-invasive assessment of patients with suspected NAFLD is on one hand to confirm the diagnosis by identifying steatosis and ideally quantifying fat content, and on the other hand to stratify the risk of progression to advanced chronic liver disease, namely diagnosing non-alcoholic steato-hepatitis (NASH)(1).

Ultrasound is the first line technique to be used to confirm the presence of steatosis since it holds 90% sensitivity to detect steatosis $\geq 20\%$ of hepatocytes (2); however, ultrasound can provide only qualitative or semi-quantitative information (2). Controlled attenuation parameter (CAP) has been recently described as an objective and numerical parameter to quantify steatosis (2); it is implemented on FibroScan® and showed promising results in patients with NAFLD (3). More sophisticated methods, which currently cannot be used as routine screening methods due to their high cost, include proton density fat fraction (PDFF) on magnetic resonance imaging and H1 MR-spectroscopy, that is currently considered the gold standard (3). In a recent paper, PDFF was proven superior to CAP to quantify liver steatosis in patients with NAFLD (4).

NASH is defined by a score taking into account presence of fat, lobular inflammation, hepatocyte ballooning and fibrosis(1). While inflammation cannot be diagnosed by the existing non-invasive methods and liver biopsy remains key for this aim, fibrosis, which is the most important component of risk in patients with NASH (1), can be identified by different ultrasound and magnetic resonance-based elastography techniques. Liver stiffness measurement (LSM) by transient elastography (monodimensional ultrasound elastography) using the regular M probe allows ruling-out severe fibrosis with 90% negative predictive value in patients with NAFLD at a cut-off of 7.9 kPa. Values of LSM above 9.6 kPa allow confirming severe fibrosis/cirrhosis in this population, while patients with intermediate values should receive further testing (liver biopsy)(1). TE applicability in NAFLD patients is limited by obesity, and a probe specifically designed for obese patients is now available (XL probe); this probe measures systematically around 1 kPa less than M probe, and this should be taken into account on interpreting the results. Liver fat content influences LSM results, leading to overestimation of fibrosis. Therefore, caution should be paid to the interpretation of LSM in patients with severe steatosis on ultrasound or on CAP.

In a head-to-head study in NAFLD/NASH, TE and newer ultrasound elastography methods (pSWE-ARFI and 2D-SWE-SSI) showed a similar diagnostic accuracy for the assessment cirrhosis, while pSWE resulted slightly inferior to the other two for identifying significant fibrosis (5). Magnetic resonance elastography proved marginally superior to TE for fibrosis staging in NAFLD/NASH in one study (4), but results in larger populations with head-to-head comparison is needed due to the much higher cost of MRE making unlikely its wide-scale application.

REFERENCES

1. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1388-1402.
2. Berzigotti A. Non-invasive assessment of non-alcoholic fatty liver disease: ultrasound and transient elastography. *Rev Recent Clin Trials* 2014;9:170-177.
3. Castera L. Noninvasive Evaluation of Nonalcoholic Fatty Liver Disease. *Semin Liver Dis* 2015;35:291-303.
4. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, Fujita K, et al. Magnetic Resonance Imaging More Accurately Classifies Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease Than Transient Elastography. *Gastroenterology* 2016;150:626-637 e627.
5. Cassinotto C, Boursier J, de Ledinghen V, Lebigot J, Lapuyade B, Cales P, Hiriart JB, et al. Liver stiffness in nonalcoholic fatty liver disease: A comparison of supersonic shear imaging, FibroScan, and ARFI with liver biopsy. *Hepatology* 2016;63:1817-1827.

NONINVASIVE BIOCHEMICAL METHODS FOR NAFLD

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Nonalcoholic fatty liver disease (NAFLD) has become an increasingly common problem, it affects approximately 30-40% of adults in western world. Diagnosis and treatment of NAFLD is one of most actual problem in hepatology.

The development of noninvasive markers to diagnose and monitor progression of NAFLD is necessary: simple, inexpensive, and reliable noninvasive means to assess disease severity.

Many clinical variables have been proposed as predictors of severe fibrosis in patients with NAFLD: old age, type 2 diabetes mellitus, obesity, serum transaminase levels, peripheral platelet counts etc. A variety of serologic markers have been evaluated to predict the degree of fibrosis in the liver, and panels have been developed that combine assays of multiple markers to improve predictive ability.

The most studied panels are the NAFLD fibrosis score, FIB-4 index, BARD score, Fibro Test, Hepascore, APRI, Fibrometer, PGA index as indirect markers; and as direct markers Fibro Spect II, SHASTA, ELF...

Studies suggest they have good ability to differentiate patients with significant fibrosis from those without. The panels may also be able to monitor changes in fibrosis over the time, predict liver and non-liver related morbidity and mortality, as markers of liver and systemic disease activity.

REFERENCES:

1. Zelber-Sagi et al. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. *World J Gastroenterol.* 2013 Jan 7;19(1):57-64. doi: 10.3748/wjg.v19.i1.57.
2. Shen FF, Lu LG, Advances in noninvasive methods for diagnosing nonalcoholic fatty liver disease. *J Dig Dis.* 2016. Sep; 17(9):565-571
3. Petta et al. Non invasive indexes for the assessment of patients with non-alcoholic fatty liver disease. *Curr Pharm Des.* 2013;19(29):5193-218.
4. Li et al. The development of a clinical score for the prediction of nonalcoholic steatohepatitis in patients with nonalcoholic fatty liver disease using routine parameters. *Turk J Gastroenterol.* 2015;26:408-16.

NON-ALCOHOLIC FATTY LIVER DISEASE: TO PERFORM BIOPSY OR NOT?

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Despite increasingly better noninvasive biomarkers and feature techniques, liver biopsy is still the gold standard in diagnosing Non-alcoholic fatty liver disease (NAFLD). It is an excellent tool in assessment of the stage of fibrosis as well as the stage of Non-alcoholic steatohepatitis (NASH). On the other hand, liver biopsy is flawed with many drawbacks: pain, sample errors, cost, availability, morbidity and eventually mortality. In case if biomarkers predict fibrosis or the suspicion of fibrosis remains, liver biopsy is recommended. NASH is potentially reversible phase of NAFLD and it's diagnosis is very important for timely treatment. Diabetes mellitus and metabolic syndrome are well known risk factors for necroinflammation (NASH) and these patients should undergo biopsy especially in case of elevated ALT or AST. Furthermore, all patients with AST level higher than ALT, in those with low platelet number, low albumine level as well as elderly, have higher chance of severe liver injury. For them liver biopsy could be helpful in choosing optimal therapy. Up today, no one method is able to precisely score NASH activity (NAS) than patohistology. Finally, individual approach to each patient, his informed consent and ordering right diagnostic instrument will gain the best results.

REFERENCES

1. Ratziu V, Bellentani S, Cortez-Pinto H, A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53:372-384.
2. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012;55:2005-2023.
3. Williams CD, Stengel J, Asike MI, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011;140:124-131.
4. Loomba R, Tech MA, Unalp A, et al.; for NASH Clinical Research Network. Association between diabetes, family history of diabetes and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* 2012; April 13. doi:10.1002/hep.25772.
5. Nouredin M, Vaughn IA, Neuschwander-Tetri BA, et al. Clinical and histological determinants of nonalcoholic steatohepatitis (NASH) and advanced fibrosis in the elderly. *Hepatology* 2011; 54(suppl):1118a.

DRUGS AFFECTING INSULIN RESISTANCE IN THE TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE

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Obesity-associated Insulin resistance is the leading cause of metabolic syndrome. Obesity is a worldwide pandemic and is expected that 10% of the global population will be obese by 2030. Many patients with NAFLD have some elements of metabolic syndrome. This raised the hypothesis that insulin resistance is a basic pathophysiologic mechanism of NAFLD.

Drugs that decrease insulin resistance are effective in the treatment of diabetes mellitus. Some clinical studies indicated that they can be beneficial in the treatment of NAFLD. Evidence suggest that pioglitazone may improve biochemical and histological features of NAFLD. Also, some studies showed that activity of NAFLD can be influenced by metformin and liraglutide. However, level of evidence is too scarce to recommend such therapy.

REFERENCES

1. Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link? *Mol Cell Endocrinol.* 2015 Dec 15;418 Pt 1:55-65.
2. Hossain N, Kanwar P, Mohanty SR. A Comprehensive Updated Review of Pharmaceutical and Nonpharmaceutical Treatment for NAFLD. *Gastroenterol Res Pract* 2016; 2016: 7109270.

STATINS IN NON-ALCOHOLIC FATTY LIVER DISEASE AND PORTAL HYPERTENSION

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Non-alcoholic fatty liver disease (NAFLD) is the cause of cirrhosis that is more rapidly increasing in western countries. As in chronic advanced liver disease of other aetiologies, the cirrhosis due to NAFLD evolves from a good prognosis, asymptomatic compensated stage to a poor prognosis, decompensated stage, characterized by the development of clinical manifestations mostly related to severe portal hypertension, such as ascites, gastrointestinal bleeding from oesophageal and gastric varices and portal hypertensive

gastropathy, hepatic encephalopathy, spontaneous bacterial peritonitis, and other less frequent complications (hepatorenal syndrome, hepatopulmonary syndrome, portopulmonary hypertension). In the absence of a specific treatment for NAFLD that at present is limited to intensive life-style intervention, treatments for advanced chronic liver disease due to NAFLD aim at decreasing or preventing the increase in portal pressure that leads to decompensation.

Portal hypertension in the early stages of cirrhosis is mainly determined by the increase resistance to portal blood flow at the hepatic circulation, due on one hand to the structural abnormalities associated with cirrhosis (distortion of liver vascular anatomy by fibrosis, nodule formation, angiogenesis and vascular occlusion) and on the other hand to a dynamic increase of the hepatic vascular tone due to contraction of activated hepatic stellate cells (HSC) and myofibroblasts as a consequence of hepatic endothelial microvascular dysfunction (with decreased availability of nitric oxide -NO- and increased production of endogenous vasoconstrictors). In more advanced stages, when the portal pressure gradient exceeds 10 mmHg and portal-systemic collaterals develop, portal hypertension is aggravated by an increased portal-collateral blood flow, caused by splanchnic and systemic vasodilatation, expanded plasma volume and increased cardiac index (hyperkinetic syndrome). Therefore, in early cirrhosis treatments should aim at decreasing liver vascular resistance, while in advanced cirrhosis there is potential for drugs decreasing the portal blood flow (non-selective beta-blockers, splanchnic vasoconstrictors).

In the past few years statins have emerged as useful drugs for portal hypertension. This is due to their vascular effects (to a large extent independent from the lipid-lowering effects). Statins enhance the expression of the transcription factor KLF2, which encodes the transcription of endothelial NO synthase (eNOS) and other endothelial protecting genes. This enhances NO-production, mitigating the increased hepatic vascular tone. In addition, simvastatin and atorvastatin have been shown to exert antifibrotic effects in the liver, through a direct effect deactivating HSC, and through the crosstalk of endothelial cells and HSC. Moreover, simvastatin induced KLF2 overexpression has been proved to have liver-protecting effects after liver preservation, warm ischemia reperfusion injury, hypovolemic shock and endotoxemia. Because all of this, we introduced the use of simvastatin in the treatment of portal hypertension. Our studies have shown that simvastatin, given on top of standard of care, ameliorates survival of patients with advanced cirrhosis who already have bled from varices. Because of this simvastatin should be considered in the treatment of any portal hypertensive cirrhotic. This is specially so in patients with NAFLD, because these patients frequently have an indication for statins due to the associated metabolic and cardiovascular disturbances.

REFERENCES

1. Bosch J, Groszmann RJ, Shah VH. Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *J Hepatol.* 2015 Apr;62(1 Suppl):S121-30.

2. Marrone G, Maeso-Díaz R, García-Cardena G, Abalde JG, García-Pagán JC, Bosch J, Gracia-Sancho J. KLF2 exerts antifibrotic and vasoprotective effects in cirrhotic rat livers: behind the molecular mechanisms of statins. *Gut*. 2015 Sep;64(9):1434-43.
3. La Mura V, Pasarín M, Meireles CZ, Miquel R, Rodríguez-Vilarrupla A, Hide D, Gracia-Sancho J, García-Pagán JC, Bosch J, Abalde JG. Effects of simvastatin administration on rodents with lipopolysaccharide-induced liver microvascular dysfunction. *Hepatology*. 2013 Mar;57(3):1172-81.
4. Abalde JG, Villanueva C, Aracil C, Turnes J, Hernandez-Guerra M, Genesca J, Rodriguez M, Castellote J, García-Pagán JC, Torres F, Calleja JL, Albillos A, Bosch J. Addition of simvastatin to standard therapy for the prevention of variceal rebleeding does not reduce rebleeding but increases survival in patients with cirrhosis. *Gastroenterology*. 2016 May;150(5):1160-1170
5. Mohanty A, Tate JP, Garcia-Tsao G. Statins are associated with a decreased risk of decompensation and death in veterans with hepatitis c-related compensated cirrhosis. *Gastroenterology*. 2016 Feb;150(2):430-40.

HERBAL PRODUCTS AS POTENTIAL DRUGS FOR NON-ALCOHOLIC FATTY LIVER DISEASE

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Non-alcoholic fatty liver disease (NAFLD) represents the most common chronic liver disease that can progress to liver cirrhosis and hepatocellular carcinoma, and may lead to the end-stage liver disease. NAFLD is a multifactorial disease, and currently, there is no satisfying therapeutic strategy except lifestyle modification by diet and exercise. Several pharmacological treatments such as insulin sensitizers, antioxidants, lipid-lowering drugs, angiotensin receptor blockers and others have been proposed but none has shown significant efficacy or long-term safety. Over the past decades, herbal medicines have received increasing attention as potential therapeutic agents for NAFLD due to its wide availability, low side effects, variety mechanisms of action and consequential benefits. Evidence from *in vitro* and *in vivo* studies suggests that herbal medicines may prevent cellular damage in hepatocytes associated with NAFLD through different mechanisms of action, including: (1) depressing lipogenesis; (2) increasing β -fatty acid oxidation; (3) increasing insulin sensitivity; (4) depressing oxidative stress and (5) inhibiting activation of inflammatory pathways. Herbal medicines can be used as plant extract (licorice, green tee, milk Thistle, red grapes, coffee...), polyherbal formulations (Kampo and Chinese medical) and phytochemicals (flavonoids, polyphenols, terpenoids, saponins, alkaloids...). Among them, red grape, milk thistle, licorice and green tee are the most promising agents

that can target most of the pathological changes during NAFLD. Silymarin is a lipophilic extract from the milk thistle seeds that contains flavonolignans and flavonoids. It is the most studied herb in the field of hepatology. Two small-randomized clinical trials reported that silibinin, main component of silymarin may improve insulin resistance and liver histology as well as liver enzymes and lipid profile. Resveratrol belongs to stilbene family from red grape that improves insulin sensitivity and glucose tolerance, and reduce plasma lipids, inflammation and oxidative stress in animal studies, but there is conflicting results in clinical trials, and further studies are needed. *Camellia sinensis*, also popularly referred as green tea is rich with catechins that regulate lipid accumulation at multiple levels and prevent inflammation and oxidative stress in non-alcoholic steato-hepatitis patients. Licorice is widely used to treat various diseases including liver diseases. Glycycomarin is active component from licorice that shows protective effect on hepatocyte lipoapoptosis *in vitro* and *in vivo*. Besides aforementioned therapeutic approaches have been observed with *Lycii fructus*, Garlic, Siberian Ginseng, Curcuma longa, Olive etc.

From *in vitro* studies and *in vivo* animal models, we can conclude that herbal medicines may be promising therapeutic agents, but the findings are inconclusive, and rigorously conducted randomised clinical trials are required to establish the efficacy and safety of herbal medicines for NAFLD.

REFERENCES

1. Ben MD, Polimeni L, Baratta F, Pastori D, Angelico F. The role of nutraceuticals for the treatment of non-alcoholic fatty liver disease. *Br J Clin Pharmacol* (2017); 83: 88-95.
2. Yao H, Qiao YJ, Zhao YL, Tao XF, Xu LN, Yin LH, Qi P, Peng JY. Herbal medicines and nonalcoholic fatty liver disease. *World J Gastroenterol* (2016); 22: 6890-905.
3. Salomone F, Godos J, Zelber-Sagi S. Natural antioxidants for non-alcoholic fatty liver disease: molecular targets and clinical perspectives. *Liver Int* (2016); 36: 5-20.
4. Ibrahim MA, Kelleni M, Geddawy A. Nonalcoholic fatty liver disease: Current and potential therapies. *Life Sciences* (2013); 92: 114-118.
5. Zhang E, Yin S, Song X, Fan L, Hu H. Glycycomarin inhibits hepatocyte lipoapoptosis through activation of autophagy and inhibition of ER stress/GSK-3-mediated mitochondrial pathway. *Sci Rep.* (2016); 6: 38138.

LIVER TRANSPLANTATION IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

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The main indications for liver transplantation (LT) in Europe are alcoholic cirrhosis, HCV cirrhosis, and HCC. Introduction of antiviral agents will influence the number of patients requiring LT for HCV and HBV infection. Consequently, we may expect to see a shift in the indications for LT within the next five years among the causes of cirrhosis and the causes of HCC.

LT for NASH is a growing indication in United States. Between 2004 and 2013 in The United States, new LT waitlist registrants with NASH increased by 170% and in 2013, NASH became the second-leading disease among LT waitlist registrants, after HCV.

The absence of a well-documented medical history, disappearance of some histological features of NASH in end stage of liver disease, coexistence of various factors for liver disease and diagnosis of cryptogenic cirrhosis makes diagnosis and estimation of impact on HCC development difficult. In Europe it is representing <5% of the indications.

In countries with long waiting list and longer waiting time, the mortality on waiting list is higher for NASH patients than other indications. It is explained with higher rates of comorbidities (especially cardiovascular and infections). The mean BMI (31.6 kg/m²) and the prevalence of diabetes (46%) is higher in NASH transplant candidates than in patients with other liver diseases. The role of obesity on perioperative mortality and morbidity is controversial. There are many studies with higher rate of perioperative complications in obese, particularly with BMI>40. These patients are more prone to wound dehiscence, ventral hernia, respiratory complications and longer hospital stay. Morbid obesity can be contraindication for LT. In the United States rates of HCC among NASH and HCV recipients is higher than for other indications (21-24% vs. 7%).

Long-term complications are higher in most series on NASH LT recipients. Components of metabolic syndrome are prevalent after transplantation and major source of cardiovascular incidents. The rate of de novo NAFLD or NASH is 10-20%. Presence of liver steatosis in pretransplant liver grafts biopsies is up to 26%. The recurrence of NAFLD on the graft has been reported as >50% within the first two years post-LT and 100% at five years. The impact of potential graft loss is still in evaluation since medium-term survival seems identical to other causes of LT.

Strategies to recognize and correct components of metabolic syndrome and obesity before LT are important and have to be implemented in everyday practice. Prevention and treatment of metabolic syndrome after LT is essential to prevent NAFLD disease recurrence and to decrease cardiovascular related complications.

REFERENCES

1. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015;148:547-555.
2. Barnard A, Konyan P, Saab S. Medical Management of Metabolic Complications of Liver Transplant Recipients. *Gastroenterol Hepatol (NY)*. 2016 Oct;12(10):601-608.

3. Dare AJ, Plank LD, Phillips AR, Gane EJ, Harrison B, Orr D, Jiang Y, Bartlett AS. Additive effect of pretransplant obesity, diabetes, and cardiovascular risk factors on outcomes after liver transplantation. *Liver Transpl.* 2014 Mar;20(3):281-90.
4. VanWagner LB, Lapin B, Levitsky J, Wilkins JT, Abecassis MM, Skaro AI, Lloyd-Jones DM. High Early Cardiovascular Mortality After Liver Transplantation *Liver Transpl.* 2014 Nov;20(11):1306-16.
5. Malik SM, DeVera ME, Fontes P, Shaikh O, Ahmad J. Outcomes After Liver Transplantation for NASH Cirrhosis. *American Journal of Transplantation* 2009; 9: 782-79.

NOVEL THERAPIES FOR NONALCOHOLIC FATTY LIVER DISEASE

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Nonalcoholic fatty liver disease is the most common cause of liver dysfunction in the western world because of its close association with obesity, insulin resistance and dyslipidaemia. Nonalcoholic steatohepatitis (NASH) is a particular health concern due to the increased morbidity and mortality associated with progressive disease. While simple to recommend, diet and lifestyle measures as a first-line therapy for nonalcoholic steatohepatitis (NASH) are hardly a model of successful therapy, as most clinicians can testify. They can be complex to implement, hard to sustain, and of limited efficacy in advanced stages of the disease. The need for specific pharmacotherapy is now acknowledged by practitioners, the pharmaceutical industry, and regulators and is largely expected by patients. The result is a clear move away from products developed second hand for NASH (such as pioglitazone or metformin) or from generic, non-specific hepatoprotectors (such as pentoxifylline, ursodeoxycholic acid, or antioxidants) toward molecules developed and tested specifically for NASH that aim to correct one or several of the pathways of liver injury in this disease. The two most advanced molecules, obeticholic acid and elafibranor, have shown encouraging data on improving hepatic histology. Both compounds appear to clear NASH, with obeticholic acid improving liver fibrosis and elafibranor improving the glycemic and lipid profile. At present, without specific targeted pharmacological therapies, the mainstay of therapy remains weight loss through dietary modification and lifestyle change; thus, the purpose of this review is to summarize the recent evidence for current and emerging therapies in NASH.

GUT MICROBIOTA: THE KEY PLAYER OF GUT-LIVER LIASON IN NAFLD

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Gut and liver have several connections to each others. From early embryology they are intrinsically linked due to the fact that liver budding directly comes from the foregut. In later life, this link is maintained by several connections, such as vascular, neural, biochemical and immunological. Evidence is increasing that disturbances in gut-liver interaction plays an important role in hepatic pathology.

Normal gut microbes perform several beneficial functions for the host. At the same time, dysbiosis, namely qualitative and quantitative alterations in microbiota, has been implicated in a variety of human diseases. Increasing evidence support the key role of gut microbiota in obesity and its related disorders, including NAFLD and the metabolic syndrome. Very first and convincing evidence came from animal experiments showing that phenotype could be altered by transferring of gut microbiota from obese animals to lean littermates. Furthermore descriptive human studies showed an association between NASH and small intestinal bacterial overgrowth. Culture-independent microbiologic technology (16S ribosomal RNA) has facilitated the characterisation of both the composition and diversity of intestinal microbiota. Since than ranges of human studies have also been demonstrated an intriguing association between intestinal dysbiosis and NAFLD. These studies were important early steps towards determining the mechanisms by which dysbiosis affect risk for NAFLD and NASH as well. Changes in the composition of microbiota at phylum level vary between different studies and not being devoid of contradictions. In a number of studies, increase in Firmicutes and reduction in Bacterioidetes were observed. This microbiota constellation results in increased fermentation end products such short-chain fatty acids (SCFAs) that have effects on energy metabolism, immunity and adipose tissue expansion via activation of G-protein coupled receptors. Interestingly, studies in paediatric population with NASH showed a reverse association, with an increase Bacterioidetes content. Certain NASH-associated microbiota however were proved to produce various harmful agents such as ethanol (*E. coli*, Proteobacteria phyla), hepatotoxic volatile organic compounds (VOC) or trimethylamine (TMA). Chronic, low-level exposure to various hepatotoxins enhanced by increased intestinal permeability might put the individuals at risk for disease progression. Dysbiosis contributes to breakdown of intestinal barrier as well that accounts for enhanced translocation of bacteria, bacterial product and injurious components resulting in intestinal, hepatic and systemic inflammation. Mainly it is via activation of Toll-like receptor signaling and inflammasome assembly. More recently severity of the NAFLD was found to associate with gut dysbiosis and a shift in metabolic function of the gut microbiota. Bacteroides abundance was independently associated with NASH, while

Ruminococcus with significant fibrosis. Remarkably differences appeared from the family level but not at the phylum level. Progression to liver cirrhosis had fewer Bacteroidetes and a lower abundance of butyrate producing species contribute to gut health. Invasion of the gut by oral origin species was also observed. Finally, dietary composition is known to influence the balance of microbiota and certain dietary patterns have been linked to specific gut microbial enterotype.

Gut microbiota analysis might add prognostic information to the classical risk factors for NAFLD severity. More complete description of gut microbiota, its metabolic function and interaction with the diet hold the promise that dysbiosis would be a target for therapeutic interventions in NASH

REFERENCES

1. Wieland A, Frank DN, Harnke B, et al. Systematic review: microbial dysbiosis and nonalcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2015;42:1051-63.
2. Leung C, Rivera L, Furness JB, Angus PW. The role of the gut microbiota in NAFLD. *Nat Rev Gastroenterol Hepatol.* 2016;13:412-25.
3. Boursier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology.* 2016;63:764-75.
4. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, Guo J, Le Chatelier E, Yao J, Wu L, Zhou J, Ni S, Liu L, Pons N, Batto JM, Kennedy SP, Leonard P, Yuan C, Ding W, Chen Y, Hu X, Zheng B, Qian G, Xu W, Ehrlich SD, Zheng S, Li L. Alterations of the human gut microbiome in liver cirrhosis. *Nature.* 2014;513:59-64.

ENDOSCOPIC TREATMENT OF OBESITY

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Despite the fact that bariatric surgery is highly efficient in accomplishing significant weight loss in obese patients, a substantial morbidity related to invasive surgical procedures is also a fact that cannot be neglected. Endoscopic bariatric treatment is viable and less invasive alternative to surgery able to accomplish excellent results in well selected patients.

Several experimental techniques are currently under evaluation, however, endoscopic intragastric balloon placement has recently became a well-established and safe procedure which can be performed in ambulatory settings and which can provide clinically significant excess weight loss (EWL). The mechanism of action is based on gastric distension which induces early satiety and loss of appetite by still insufficiently explored interplay of hormones of digestive tract regulating appetite and satiety in humans.

Several types of intragastric balloons are being used worldwide with Bioenterics/Orbera intragastric balloon (BIB) as the most prevalent and most researched type. The endoscopic technique of placement and balloon extraction is simple and not demanding. The best candidates for procedure are patients with a BMI between 28 and 40, who have failed to achieve long-term weight loss with conservative weight loss programs and patients in need for weight loss in cases of severe obesity (BMI over 55) before a bariatric procedure in order to reduce risks associated with the excess weight. The balloon is typically left in stomach for 6 months.

Intragastric balloon treatment is a relatively safe procedure with nausea and vomiting being the most frequent side effects, while main complications include gastric ulcers, gastric erosions, esophagitis, spontaneous deflation, persistent vomiting, gastroesophageal reflux and abdominal pain. There have been reports of gastric perforations, small bowel obstructions, and significant gastric dilatation.

Several studies exploring efficacy and safety of BIB have been performed in the last decade. A heterogeneous meta-analysis on 3608 patients in 15 included studies resulted with pooled average weight loss of 14.7 kg or 32.1% of EWL with a good tolerability and safety and a small frequency of significant complications such as intestinal obstruction (0.8%) and perforation (0.1%). Still, there are studies that add to the controversy with reports that weight loss is only but temporary, and that significant number of patients regain weight in the first year after the procedure. It appears that weight loss within the first month from balloon placement is independent predictor of sustained weight loss.

Additional benefits from BIB placement include improvement in metabolic disease; there are studies reporting decrease in prevalence in metabolic syndrome from 34.8% to 11.6% in patients 12 months after balloon extraction with reduction of diabetes mellitus from 32.6% to 21.3%. There is also an increasing pool of evidence that BIB placement treatment can improve histology in NAFLD with significant reduction in non-invasive NAFLD scores after treatment.

Endoscopic bariatric treatments are certainly more than a temporary curiosity, as they allow for minimally invasive and safe treatment with a significant excess weight loss and favourable metabolic effects in properly selected obese patients.

REFERENCES

1. Geliebter A, Westreich S, Gage D. Gastric distention by balloon and test-meal intake in obese and lean subjects. *Am J Clin Nutr.* American Society for Nutrition; 1988 Sep;48(3):592-4.
2. Mathus-Vliegen EMH. Endoscopic treatment: The past, the present and the future. *Best Practice & Research Clinical Gastroenterology.* 2014 Aug;28(4):685-702.
3. Ginsberg GG, Chand B, Cote GA, Dallal RM, Edmundowicz SA, Nguyen NT, et al. A pathway to endoscopic bariatric therapies. *Gastrointest Endosc.* 2011 Nov;74(5):943-53.
4. Abu Dayyeh BK, Kumar N, Edmundowicz SA, Jonnalagadda S, Larsen M, Sullivan S, et al. ASGE Bariatric Endoscopy Task Force systematic review and meta-analysis assessing the ASGE PIVI thresholds for adopting endoscopic bariatric therapies. *Gastrointest Endosc.* 2015 Sep;82(3):425-5.

5. Imaz IA, Mart nez-Cervell C, Garcia-Alvarez EE, Sendra-Guti rrez JM, Gonz lez-Enr quez JS. Safety and Effectiveness of the Intra gastric Balloon for Obesity. A Meta-Analysis. Obesity Surgery. 2008 May 6;18(7):841-6.

TREATMENT OF NAFLD WITH INTAGASTRIC BALLOON: CROATIAN RESULTS

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INTRODUCTION: Intra gastric balloon treatment (ITT) is a non-invasive procedure which proved to be effective in short-term treatment of obesity. Its effect on alterations in metabolic parameters and hepatic steatosis is poorly understood, although several studies indicated a major role in treatment of diabetes mellitus type 2 and non-alcoholic fatty liver disease (NAFLD). Our previous study showed different ghrelin and leptin response between groups of morbidly (M) and non-morbidly (NM) obese patients after ITT. Moreover, we have previously shown that the decrease of insulin resistance may have a key role in improvement of metabolic syndrome in patients undergoing ITT. We aimed to investigate the alteration dynamics in metabolic and liver parameters between M and NM obese patients undergoing ITT. Subjects consisted of 44 patients (age 20-59, BMI 33-61) treated with BioEnterics Intra gastric Balloon (BIB, Inamed and Co. Santa Barbara, USA) for six months. NM obese patients were considered those with BMI <40 kg/m². Glucose, insulin, homeostatic model assessment IR (HOMA-IR), glycated hemoglobin, growth hormone, uric acid, lipidogram, AST, ALT, GGT were determined prior and 1, 3 and 6 months after the procedure. NAFLD was diagnosed based on ultrasonography criteria suggested by Hamaguchi (score > 2) and reassessed at the end of the treatment. Patients with HBV(+), HCV(+), alcohol consumption >20 g/day and with history of hepato-steatogenic drugs were excluded.

RESULTS: BMI significantly decreased in both groups (NM 37.65 vs. 33.05, M 44.60 vs. 39.65 kg/m², p<0.001). Baseline NAFLD rates did not significantly differ (NM 57.1 vs. M 42.9%). HOMA-IR, glycated hemoglobin and uric acid decreased to similar values in both groups, but significantly faster decrease occurred in NM group. Faster increase in growth hormone levels occurred also in NM. NAFLD score and liver enzymes insignificantly decreased in both groups. When we analyzed patients based on the presence of NAFLD, significant decrease of HOMA-IR (3.58 vs. 2.05, p=0.000), ALT (31 vs. 27, p=0.009), GGT (31

vs. 21, $p=0.000$) occurred in patients with NAFLD, while these parameters did not change in patients without NAFLD.

CONCLUSION: These results suggest a pattern of individualization of metabolic parameters between NM and M obese patients which could influence future indications for ITT. Patients with NAFLD might have greater benefit from ITT. Hence, liver ultrasonography might be an additional tool for stratifying patients undergoing ITT. Moreover, ITT might be useful in treatment of NAFLD in obese patients. Further investigations on larger series of patients are needed.

REFERENCES

1. Hamaguchi M et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol.* 2007;102:2708-15.
2. Nikolić M et al. Morbidly obese are ghrelin and leptin hyporesponders with lesser intragastric balloon treatment efficiency : ghrelin and leptin changes in relation to obesity treatment. *Obes Surg.* 2011 Oct;21(10):1597-604.
3. Mirošević G, Nikolić M, Kruljac I, Ljubičić N, Bekavac-Bešlin M, Milošević M, Nikolić BP, Supanc V, Budimir I, Vrkljan M. Decrease in insulin resistance has a key role in improvement of metabolic profile during intragastric balloon treatment. *Endocrine.* 2014 Mar;45(2):331-4.

IMPACT OF DIET AND EXERCISE ON NON-ALCOHOLIC FATTY LIVER DISEASE

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NAFLD is the most frequent liver disease nowadays with increasing prevalence and costs related to treatment especially of the most advantageous stages – cirrhosis with its complications, and HCC. Medical and socio-economic burden is even greater when viewed in the context of NAFLD being tightly connected to obesity, type 2 diabetes mellitus, cardiovascular diseases and cancer. Currently, there are no approved drugs for NAFLD by regulatory agencies. Unhealthy lifestyle including unhealthy diets with excessive calorie intake, sedentary behavior and reduced or no physical activity play a major role in the disease development and progression. The most prominent dietary characteristic of NAFLD patients is higher total daily calorie intake compared to patients without NAFLD, and also NAFLD patients are less physically active.

The evidence base behind recommendations for NAFLD treatment by dietary change and/or exercise is rather weak, with very few well-conducted trials. Reliable outcome

measurements such as histological improvement that would be expected to translate to disease progression stoppage and the better long-term prognosis are lacking in most of the studies, and there is no long-term follow-up. However, there is data supporting dietary change inducing liver fat and ALT reduction, with calorie restriction being most effective when leading to weight loss that can result in NASH resolution and even fibrosis regression, both depending on the level of weight loss. Desirable histological improvements occur when weight loss reaches 7% and more of index weight. However, weight reduction is difficult for the patients in real life. Even in controlled conditions and highly motivated environment such as clinical trials, the percentage of patients that manages to reach (and sustain at) 7% and more of weight reduction is rather small. Weight loss is best achieved by calorie restriction, and healthy diets and exercise should be encouraged to aid weight loss schemes and later sustain the weight at the target level. Mediterranean diet probably has certain benefit as is able to reduce liver fat and increase insulin sensitivity, and its potential in NAFLD has to be better explored, but certainly can be pursued during weight reduction and sustain schemes. Physical activity seems to be very important as NAFLD prevalence decreases with increase in physical activity, independent of obesity or metabolic syndrome. Aerobic exercise is effective in improving various features of NAFLD, with the frequency, intensity and duration not being clearly defined, but the higher the intensity and longer the total time spent exercising, the more beneficial on NAFLD features. EASL recommends a total of 150 to 200 minutes per week arrayed to 3-5 aliquots of at least moderate intensity exercise. Resistance training is also effective and can be pursued as a substitute for or added to aerobic training. Lifestyle modification including weight loss, healthy diets, and the increase in physical activity should be first line therapy at all stages of NAFLD.

REFERENCES

1. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol.* 2016;64(6):1388-402.
2. Ratziu V. Non-pharmacological interventions in non-alcoholic fatty liver disease patients. *Liver Int* 2017;37 Suppl 1:90-96.
3. Hannah WN and Harrison SA. Lifestyle and Dietary Interventions in the Management of Nonalcoholic Fatty Liver Disease. *Dig Dis Sci* 2016;61(5):1365-74.
4. Oni ET et al. Relation of physical activity to prevalence of nonalcoholic Fatty liver disease independent of cardiometabolic risk. *Am J Cardiol* 2015;115(1):34-9.
5. Vilar-Gomez E et al. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015;149(2):367-78.

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