ISSN No:-2456-2165

Nash Clinical Study

Palmieri B.[1,2], Vadalà M.[1,2]
[1]Department of General Surgery and Surgical Specialties, University of Modena and Reggio Emilia Medical School, Surgical Clinic, Modena, Italy;
[2]Second Opinion Medical Network, Modena, Italy

Abstract:- The Non-alcoholic fatty liver disease (NAFLD) affects the 30% of worldwide population with greater incidence (60-80%) in type 2 diabetic patients and progresses to not alcoholic steatohepatitis (NASH) with subsequent severe outcome (fibrosis cirrhosis, liver cancer). Several clinical studies showed that bile acids administration at sustained dosages can improve the NAFLD syndrome counteracting it's worsening and death risk; we thus planned to compare the benefits and side effects of oral versus intravenous treatment of UDCA on 100 overweight not-diabetic volunteers (41 males and 59 females), with strictly similar biochemical–clinical expressions of nonalcoholic fatty liver disease (NAFLD). The patients were divided in two groups: one with 50 mg / kg /daily/os and the other with 3500 mg in 500ml saline perfusion each other day (except the weekend) for a total of 24 intravenous sessions. The results in terms of tolerability, symptoms relieve, and liver enzymes improvement defined the parenteral treatment as the most effective, being the oral burden somehow troublesome with some untoward effects not appearing in the intravenous route.

I. INTRODUCTION

Nash is a widespread western world liver disease connected with obesity, type II diabetes, and insulin resistance whose steps start with Non-alcoholic fatty liver disease (NAFLD), showing long standing abnormal liver enzymes leading to chronic liver disease and cirrhosis **[1,2]**. It affects 30% of the general population and 60–80% of the type 2 diabetic population **[3]**. NAFLD histological landmarks are hepatic steatosis with scanty inflammation defined as nonalcoholic fatty liver (NAFL), with a benign clinical course **[4]**, that can remain steady for decades or progress to NAFLD, and along the life to not alcoholic steatohepatitis (NASH).

NASH in 15–20% of cases is transformed to fibrosis and cirrhosis being also a major risk factor for developing hepatocellular carcinoma (HCC) **[5]**.Thus requiring early treatment and adequate follow up; patients with NAFLD have an higher mortality mainly due to cardiovascular disease if matched with control population, while patients with NASH have higher liver-related mortality. Nash is an area orphan of specific drugs; strong recommendations to weight loss 10% or more, improve insulin sensitivity with proper nutritional instructions (no carbohydrates, no alcohol, add to the diet non-saturated fats, fruits vegetables and omega 3).

The concept that bile acids-based treatment can improve the NAFLD and NASH has very few literature reports notwithstanding the evidence that homeostatic imbalance of bile turnover is supposed to be the common pathway of liver dysfunction.

As a matter of fact the bile acids act as ligand of Farnesoid X Receptor, that is stated in kidneys, liver, ileum and in adrenal glands, and with great affinity with CDCA, and at lesser extent with DCA, CA, and LCA **[6]**,which is the pivotal regulator of bile acid, glucose, and lipid homeostasis and hence their imbalance can trigger the degenerative/inflammatory cascade involving steatosis, fibrosis and cirrhosis. They bind also to other nuclear hormone receptors (NHR), such as vitamin D receptor (VDR), and Pregnane X receptor (PXR) **[6-7]**. Farnesoid X receptor or FXR controls the expression of various BA transporters, such as the Na^+ taurocholate cotransporting polypeptide (NTCP), the bile salt export pump (BSEP), and ileal BA transporters, including organic solute transporter, e.g. OSTβ **[8-9]**, and improves the transcription of ileal bile acid binding protein or I-BABP **[10]**.

On the other hand, BA synthesis self-suppression is achieved through different steps, in the transcription of FGF15/19 **[11]**, penetrating into the liver parenchyma through the portal vein to phosphorylate FGFR4. Successively, the extracellular signal-regulated kinases (ERK1/2) controls Cyp7a1/CYP7A1 gene expression through the intestine-initiated pathway **[11-13]**.

FGFR4 is supposed to promote NAFLD progression, to bang the FGFR4 KO mice refractory to hepatic steatosis **[14]**. On the contrary, Fibroblast growth factor 19 (FGF19) rises FA oxidation by means of ACC2 repression, a strong antagonist of mitochondrial fatty acid oxidation **[15]**. Another G-protein coupled membrane receptor, TGR5, is present in gallbladder, ileum, colon, liver, BAT, muscle and nervous system and it is activated by BA **[16].** A TGR5, like a FXR, rises energy expenditure, by means of WAT pathway and induces BAT gene expression in thermogenesis. Indeed, it plays a significant role in blood glucose monitoring, reduction of hepatic steatosis and increase of energy expenditure in surgical patients **[17-19]**. It has a significant role in improvement of blood glucose monitoring, lower amount of hepatic steatosis, and increase of energy expenditure in post-surgical patients **[18]**. BAs pathways are implicated in drug therapies for several liver diseases, including NAFLD and NASH, acting as ligands of FXR and TGR5. BAs help regulate glucose metabolism via FXR and TGR5, rise secretion of GLP-1 and reduce insulin resistance, as showed in obese mice **[20]**. Moreover, FXR reduces hepatic gluconeogenesis,

ISSN No:-2456-2165

glycolysis, and rises glycogen synthesis **[21]**. These mechanisms antagonize effectively the diabetes 2 induction. FXR mediates glucose metabolism via modulation of Phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) and by means of upregulation of the expression of genes associated with glycogen synthesis **[22]**. Same pathway of action mechanism on glucose liver metabolism is induced by the BA through activation of FXR **[23].**In addition, FXR activates the transcription and release of several cytokines/hormones, such as adiponectin (APN) that is an adipokine produced in adipocytes with anti-inflammatory and anti-fibrotic characteristics. This adipokine stimulates the absorption of glucose in multiple tissues, involving a reduction of gluconeogenesis in the liver and inhibition of production pro-inflammatory cytokine like IL-6 **[24]**.

The adiponectin is adversely regulated by bile acids, since the NASH patients had low adiponectin levels and high BAs levels **[25]**. However, Balmer et al discovered high APN values in cirrhosis patients, probably due to activation of ceramidase by APN, that could be involved in NAFLD-NASH progression **[26-29].** Further studies on interaction between BAs and adipokines are needed.

Under conditions of very low or no dietary cholesterol, bile contains cholesterol that comes from de novo hepatic synthesis, regulated by low-density lipoprotein (LDL) and high-density lipoprotein (HDL) **[30]**. Its catabolism passes through BA conversion by inducing Cyp7a1 transcription **[31]**.

ABCA1 cholesterol transport activates the first step from the peripheral tissues to apolipoproteins **[32]**. The class B type I-scavenger receptor (SR-B1) that binds HDL into the liver, mediating the selective HDL-associated cholesteryl esters uptake **[33]**. FXR decreases also cholesterol levels by means of SR-B1 expression and helps to remove HDL from the blood into the liver, and it is transcriptionally activated by bile acids **[34]**. Repa et al showed that RXR agonists dispensing in mice, fed with high-cholesterol alimentation, reduced cholesterol absorption in a dose-dependent manner, probably due to rise of reverse cholesterol transport and reduction of BAs synthesis by means of FXR-RXR **[35]**. With the aim to maintain cholesterol homeostasis, the expression of genes involved in cholesterol synthesis, such as ABCG5/G8, that transports cholesterol in the intestinal lumen, is upregulated **[36].**

BAs support lipid absorption from the intestine and the activation of nuclear hormone receptors, such as VDR or PXR **[37-39]**. FXR induces the expression of the small heterodimer partner (SHP) in the liver, that reduces a transcription of SREBP1c **[37]**.

Treatment of human hepatocytes with chenodeoxycholic acid (CDCA) showed variation in expression of genes that regulate lipid homeostasis, such as LDLR and APOL3 **[40-42]**. In the same way, the fed primary BAs, such as chenodeoxycholic acid (CDCA), lithocholic acid (LCA) and deoxycholic acid (DCA), increase LDL receptor gene expression by means of MAP kinase pathway **[43].**

FXR has been connected to changes in HDL level, but the relationship is controversial. Several in *vivo* studies confirmed the protective action of FXR in atherosclerosis formation **[44-45].** Li et al observed, in FXR mice, that SR-B1 induction displayed high level of total and HDL cholesterol proposing that opposite cholesterol transport is disrupted without FXR **[34]**. On the other hand, Sinal et al showed in FXR knock-out mice, high HDL and phospholipids levels but reduced ApoA-1 (a mayor protein component of HDL) values **[46-47]**. This ApoA-1 employees opposite cholesterol transport, by that means it reduces cholesterol values. Claudel and coworkers displayed that BAs downregulate ApoA-1 expression via FXR to reduce HDL level **[48]**. The same authors propose that FXR antagonists can play a cardioprotective role, increasing serum HDL levels, even though the precise procedure by which this would arise still involves other pathways not yet discovered.

In our opinion, based on the rationale of the introductory remarks and the huge impending and steadily expanding literature burden, exploring the genomics and proteomic involvement of biliary acids in the healthy liver physiology and physiopathology, we planned a comparative study between oral and parenteral administration of these compounds to observe the possible benefits in this orphan area of NAFL versus NASH.

II. MATERIALS AND METHODS:

100 patients volunteers (41 males and 59 females), coming from outpatients hospital office, aged between 30 and 80 years, appealed to our "Second Opinion Medical Consulting Network, Medical Centre (Modena, Italy), because of overweight and normoglycemic with strictly similar biochemical–clinical expressions of nonalcoholic fatty liver disease (NAFLD).

The Second Opinion Medical Network is a consultation referral web and Medical Office System recruiting suddenly a wide panel of real-time available specialists, to whom any patient affected by any disease or syndrome and not adequately satisfied by the diagnosis or therapy can apply for an individual clinical audit **[49]**. Due to the doctor-patient communication gap, most of the patients usually wander around the medical websites looking for proper answers to their health problems. However, their search often becomes compulsive and obsessive and often ambiguous and frustrating **[50]**. Palmieri et al. **[51]** describe this borderline or even pathological behavior as the "Web Babel Syndrome" – a psychological imbalance affecting young and elderly patients, especially those with multiple synchronous diseases who receive from their caregivers heterogeneous and misleading information or advices, including confused, contradictory statements and prescriptions **[52]**. To deal with this problem, the Second Opinion Network aims to be

ISSN No:-2456-2165

a useful "problem-solving" support revisiting each diagnostic and therapeutic step and properly re-addressing tailored treatments and prognoses, as well as preventing unnecessary investigational procedures and unhelpful and expensive medical and surgical interventions **[53]**.

All the patients were visited and informed during a personal interview, gave their permission, and signed an informed consent.

We thus subdivided the subjects in two groups (n=50 patients/each group), after having carefully standardized the diet to a 1300 calories /day (30% proteins, 30% carbohydrates and 40% fat) **(TABLE 2)**.

The challenge was to administer high dosages of UDCA either orally or parenterally in order to define the optimal tolerated concentration of the drug and the clinical endpoint outcome after an intensive treatment of 2 months.

The fixed dose was 50 mg/kg/die for 2 months in multiple 500 mg capsules swallowed after the meals (on average 2-3 after breakfast, 3-4 after lunch and 3-4 after dinner) and the second treated with parenteral schedule, infusing 3500 mg each other day (except the weekend) for a total of 24 intravenous sessions.

Instrumental and lab exams were performed at:

- **TIME T0:** at the beginning of the study,
- **TIME T1**: at the end of the second month.

Each component of the two groups was submitted to standard echographic classification as follows: **-GRADE-0**: No fatty liver

- **GRADE-1 (Mild):** Mild diffuse increase in the echogenicity of liver parenchyma or increased hepatorenal contrast with normal diaphragm and intrahepatic vessel borders.
- **GRADE-2 (Moderate):** Moderate diffuse increase in the echogenicity of liver parenchyma and increased hepatorenal contrast with slight impairment of diaphragm and intrahepatic vessel borders
- **GRADE-3 (Severe):** In addition to moderate steatosis there was no visualization of posterior portion of the right lobe of liver, intrahepatic vessel borders and diaphragm.

Values were presented as mean ± standard deviation for quantitative variable and percentages for categorical variables. The two groups were compared by student's t-test for quantitative variable and chi-square for the categorical variables. Changes from baseline to 12 weeks were compared by paired t-test within each group. Wilcoxon test was used for rating variable comparison before and after treatment in each group.

To evaluate variation in symptoms pre- and post-treatment, we asked to answer a self-administered questionnaire consisting of type, frequency, intensity, and time of symptom. Scale ranging from 0 (minimal symptom) to 5 (severe symptom).

Baseline Characteristics	Parenteral bile salts therapy Group (n=50)	Oral bile salts therapy Group (n=50)
Age (years)	40±8.75	42±9.42
Female gender	30	35
Male gender	20	15
BMI (Body Mass index kg/m2)	29.06±4.6	28.18 ±3.8
Serum Total Cholesterol-Total CHL (mg/dl)	145±35.8	156±22.6
Serum Triglycerides -TRGs (mg/dl)	95±35.2	110±45.5
Systolic Blood pressure-SBP (mm hg)	120±8.2	115±6.9
Diastolic Blood pressure-DBP (mm hg)	84±8.2	78±9.0

Table 1:- Baseline Characteristics of study groups

A. Histology

Only 4 cases of each group accepted to undergo liver biopsy before and after the treatment and we reported the histological results; in the parenterally treated group, regression of fat embedded liver a leucocytes infiltration into the portal spaces cells was striking and more evident than in the oral group **(TABLE 2).**

ISSN No:-2456-2165

	Parenteral bile salts therapy Group (n=4)-Grade								Oral bile salts therapy Group (n=4)-Grade							
Patietn	Periportal +/- bridging		Intralobular		Portal inflammation		Fibrosis		Periportal +/- bridging		Intralobular		Portal inflammation		Fibrosis	
	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.
#1	3	1	0	0	3	1	3	2	1	1	0	0	3	2	4	3
#2	2	0	0	0	2	0	3	1	3	1	0	0	2	1	4	2
#3	1	0	0	0	2	0	4	1	2	1	0	0	3	2	4	3
#4	1	0	0	0	3	2	4	1	1	1	0	0	3	2	3	2

Table 2:- Hystological results-Knodell hystology activity index (HAI)-Score

B. Elastography In Liver

Ultrasound imaging is fundamental in the diagnosis and monitoring drug therapy of liver pathologies, because it 1) provides morphological examination of the liver parenchyma, 2) examines the risk of chronic liver disease, 3) detects liver lesions; 4) evaluates local treatments and connected response. The main histological score, used to evaluate the fibrosis severity, is the METAVIR, which identifies five degrees of fibrosis: 1) no fibrosis (F0), 2) minimal fibrosis (F1), 3) moderate fibrosis or clinically significant fibrosis (F2), 4) severe fibrosis (F4) and 5) cirrhosis (F5). This score is important because:

- Allows to establish the specific treatment based on severity of liver damage;
- Controls the progression or regression of liver fibrosis during pharmacological therapy;

To date, the conventional ultrasound, such as liver biopsy (LB) that is invasive method, cannot differentiate, with precision, the several liver fibrosis stages.

The main non-invasive method to assess liver fibrosis is based on a physical parameter that measures the tissue elasticity and is called elastography. It can replace subjective palpation and is intended to image the mechanical properties of tissues and more particularly their stiffness. The common strategy of all the elastography methods is the measurement of deformation induced in a tissue by a force. This technique is based on external mechanical device or an internal acoustic radiation force, such as shear wave elastography (SWE) and acoustic radiation force impulse (ARFI) to induce shear waves in the tissue to be explored. The diagnostic action mechanism is based on shear wave, which is generated by an external mechanical impulse and whose speed is measured by an ultrasound one-dimensional probe (3.5MHz), that is assembled in the axis of an electro-dynamic transducer (vibrator). In this way it is possible to define the liver stiffness by measuring the velocity of elastic shear waves in the liver parenchyma generated by the mechanical impulse. The propagation velocity is directly related to the inflexibility of the medium, defined by the Young modulus. Stiff tissues manifest higher shear wave velocities than soft tissues. The elasticity is expressed in kilopascals (kPa) and is measured at depth ranging from 25 to 65mm in a 1×4cm area: the assessed liver volume is therefore two hundred times greater than the volume examined in a LB. The obtained values range from 2.5kPa to 75kPa. Mean liver elasticity in "normal" subject is 5.81±1.54 (for men) and 5.23±1.59kPa (for women). The time of measurement, that is painless, is 5-10 minutes **[48]**.

We used in our study one of the first prototypes of "Fibroscan" kindly supplied by Fibro Scan® (Echosens, Milano, Italy), an instrument that became in the following years the worldwide gold standard of liver stiffness evaluation.

We reported the results of our measurements in the 4 cases of each group that accepted to undergo liver biopsy (**TABLE 3**).

	Parenteral bile salts		Oral bile salts therapy	
	Pre-treat.	Post-treat.	Pre-treat.	Post-treat.
F≥3 (n. patients =3)	20.2±5.7	9.9±5.6	19.9±7.1	12.5±6.9
F≥4 (n. patients =5)	25.3±6.5	18.5±5.9	23.6±6.8	19.7±5.8

Table 3:- Elastography, kPa (mean ± standard deviation)

ISSN No:-2456-2165

III. RESULTS

The oral burden of high dosage biliary salts gave origin to diarrhea in 38% of the patients, nausea 15%, bloating 20%, moderate colicky pain, reduced appetite (10%).

The switch to intravenous administration (3500mg every third day, 20 sessions/month), was optimally tolerated and largely preferred in terms of patients compliance compared with the cumbersome burden of capsules to be swallowed; no local or systemic side effects were noted during and after the infusion; the vein access remained patent except in a couple of Cases, were the small size of the cubital veins, gradually obstructed probably due to the chemical irritation of endothelial layer after repeated catheterization.

Specific quality-of-life questionnaire administered at the end of treatment to all the patients evidenced improvement of the symptoms, such as narcolepsy, weakness, sweating, tachycardia, dizziness, dyspepsia insomnia, reflux, where more quickly and efficiently controlled in the parenterally treated group since the first month of therapy; in the orally treated group the benefits started later and were less marked **(FIG.1-2)**.

Fig.1-2: Graphic Representation of clinical symptoms in parenteral and oral bile salts therapy groups.

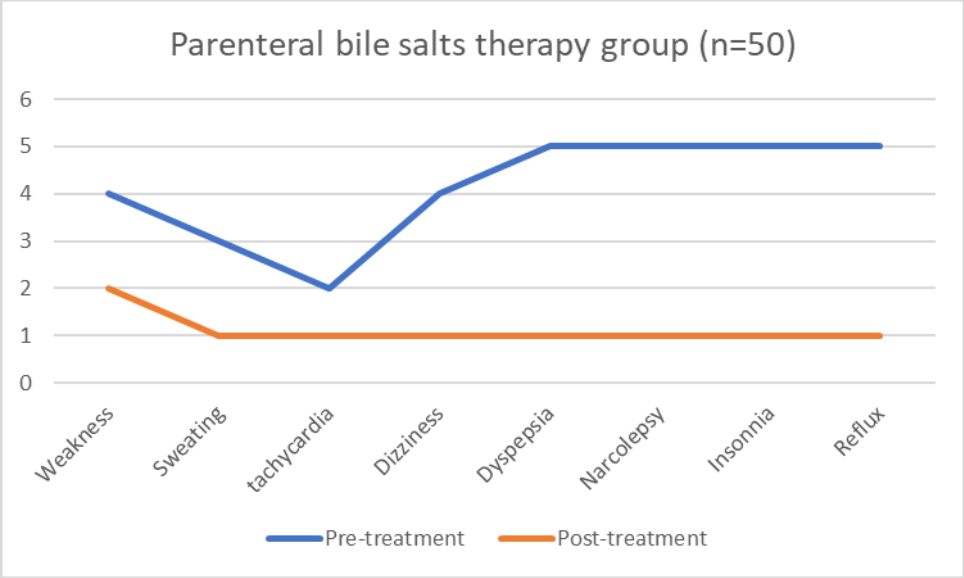

Fig 1

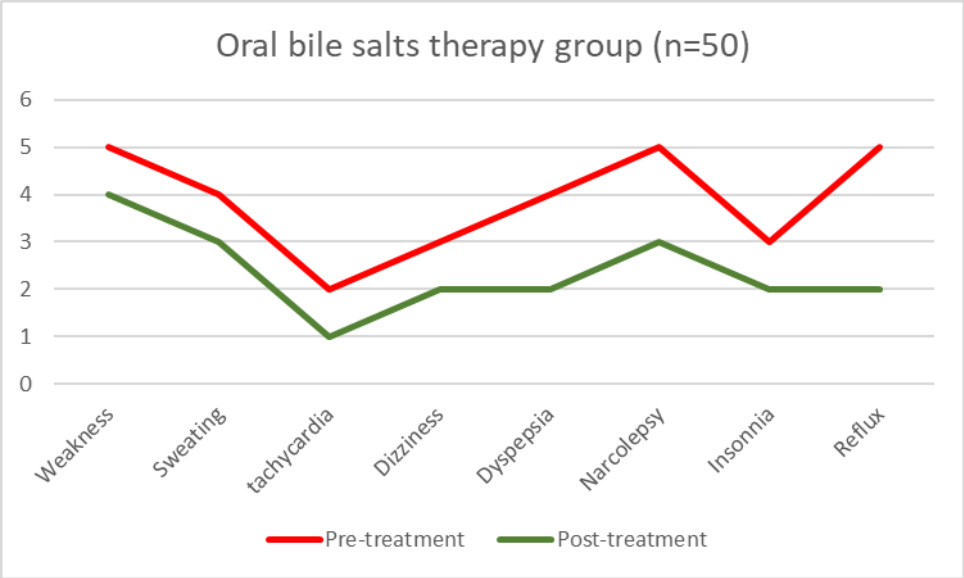

Fig 2

ISSN No:-2456-2165

We observed also a significant reduction of liver parameters (GGT, AST, ALT) in the parenteral group that oral group **(TABLE 5, FIG.3-4).**

Parameters	Parenteral bile salts therapy Group (n=50)		Oral bile salts therapy Group (n=50)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
BMI(kg/m2)	29.06±4.6	26.25±6.2	28.18 ±3.8	27.67±4.2
GGT (IU/L)	19.52±5.82	16.77±7.76	22.71±6.43	20.83±7.56
AST(IU/L)	65.54±4.56	44.56± 5.52	63.25±5.43	59.43±3.39
ALT(IU/L	78.05±5.52	52.6±5.65	76.48±4.95	74.32±5.58
Liver Ultrasound-G	3±2	1±0	3±2	2±1

Table 4:- Results of parenteral and oral bile salts therapy groups (pre and post treatment)

Fig.3-4: Liver parameters in parenteral and oral bile salts therapy group

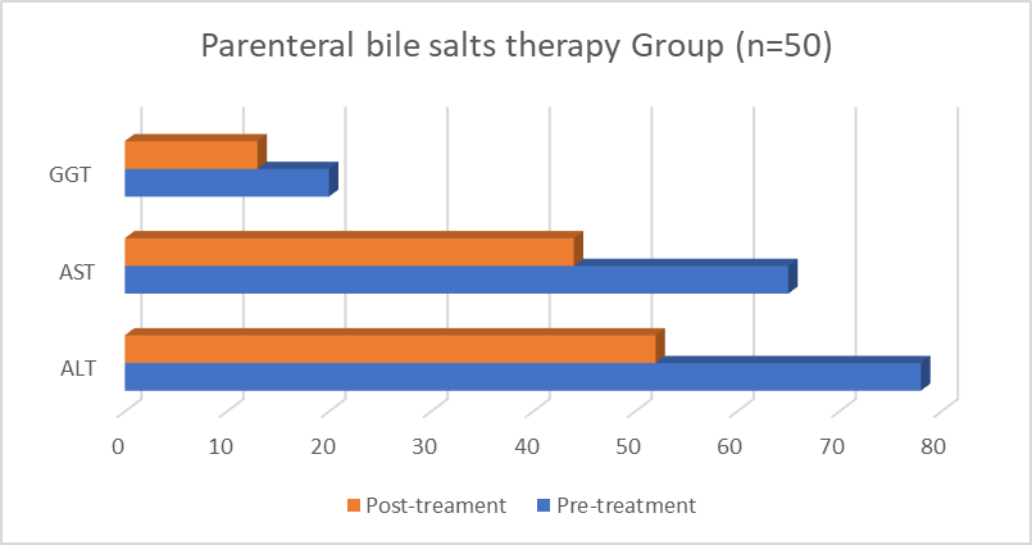

Fig 3

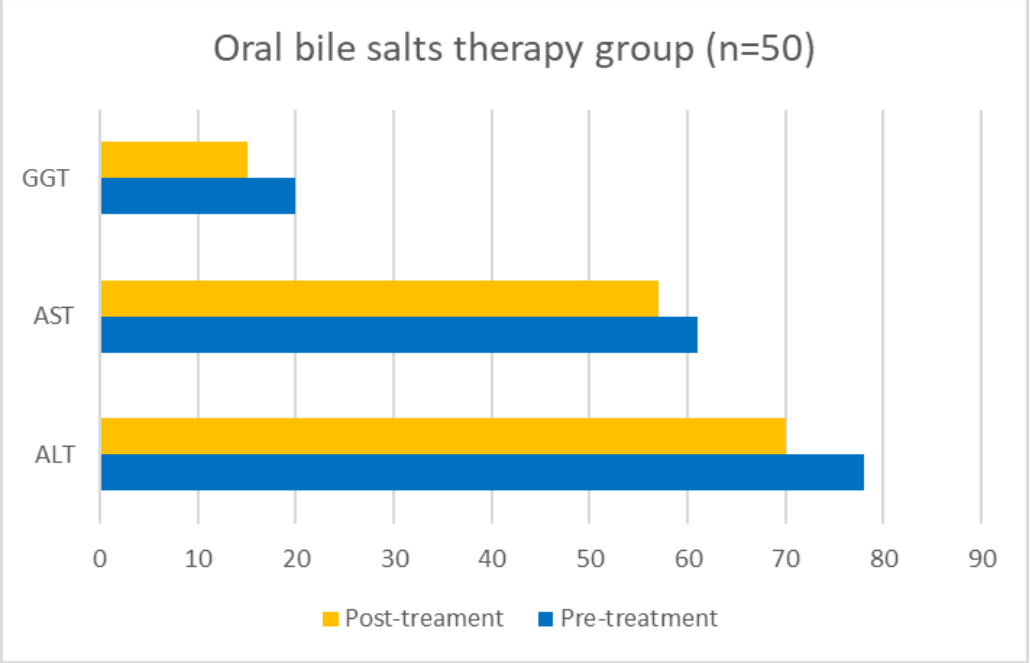

Fig 4

IV. DISCUSSION AND CONCLUSIONS

In our study the oral administration of high UDCA dosages caused, minor, but troublesome untoward effects in 35% of the patients; the benefits of the treatment in this cohort were moderate in terms of symptoms improvement and contemporarily the pre-post biopsy samples didn't show effective regression of the liver cells fat storage.

The parenterally treated group did not declare any side effects during or after BA perfusion.

On the contrary symptom's improvement was early observed since the third session: notably tiredness, fatigue, dyspepsia, flatulence insomnia, postprandial sleepiness almost disappeared; the 4-pre-post treatment histological samples showed a restoration of normal liver cells morphology with fat droplets reabsorption.

Conclusively, in our study, the hepatomegaly NAFL appearance takes great advantage from high dosage parenteral biliary salts administration, based on the hypothesis that intravenous delivery carries more diffusely and homogeneously the drug into the sinusoidal spaces via the arterial hepatic artery, compared to the oral route; this implies a substantial ubiquitous uptake of UDCA by every damaged cell and subsequent early activation of the liver restoration action mechanisms previously reported; the adequate prompt timing of symptoms remission and liver segments recovery is in our experience very relevant, effective and utmost safe; in this perspective, expanding the number of the treated patients if our pilot trial will be furtherly confirmed the intravenous biliary salts administration might become a new helpful therapeutic approach especially when the sudden or progressive worsening patient's clinical conditions due to environmental or endogenous causes require to be effectively and promptly counteracted and neutralized.

REFERENCES

[1]. Sayiner M, Koenig A, Henry L, Younossi ZM. *Epidemiology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis in the United States and the Rest of the World*. Clin Liver Dis 2016; 20: 205-214,

[2]. Kanwar P, Kowdley KV. *The Metabolic Syndrome and Its Influence on Nonalcoholic Steatohepatitis.* Clin Liver Dis 2016; 20: 225-243,

[3]. Poonawala A, Nair SP, Thuluvath PJ. *Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study*. Hepatology 2000; 32: 689-692

[4]. Teli MR, James OF, Burt AD, Bennett MK, Day CP. *The natural history of nonalcoholic fatty liver: a follow-up study*. Hepatology 1995; 22: 1714-1719,

[5]. Patel V, Sanyal AJ, Sterling R. *Clinical Presentation and Patient Evaluation in Nonalcoholic Fatty Liver Disease.* Clin Liver Dis 2016; 20: 277-292

[6]. Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM et al. (2002). *Vitamin D receptor as an intestinal bile acid sensor*. Science 296 (5571): 1313–1316.

[7]. Guo GL, Lambert G, Negishi M, Ward JM, Brewer HB, Jr, Kliewer SA et al. (2003). *Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity*. J Biol Chem 278 (46): 45062–45071.

[8]. Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM et al. (2002). *Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor, and constitutive androstane receptor*. J Biol Chem 277 (4): 2908–2915.

[9]. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ (2000). *Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis*. Cell 102 (6): 731–744.

[10]. Huang L, Zhao A, Lew JL, Zhang T, Hrywna Y, Thompson JR et al. (2003a). *Farnesoid X receptor activates transcription of the phospholipid pump MDR3*. J Biol Chem 278 (51): 51085–51090.

[11]. Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG et al. (2005). *Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis*. Cell Metab 2 (4): 217–225.

[12]. Kok T, Bloks VW, Wolters H, Havinga R, Jansen PL, Staels B et al. (2003). *Peroxisome proliferator-activated receptor alpha (PPARalpha)-mediated regulation of multidrug resistance 2 (Mdr2) expression and function in mice*. Biochem J 369 (Pt 3): 539–547.

[13]. Song X, Kaimal R, Yan B, Deng R (2008). *Liver receptor homolog 1 transcriptionally regulates human bile salt export pump expression*. J Lipid Res (5): 973–984.

[14]. Huang W, Ma K, Zhang J, Qatanani M, Cuvillier J, Liu J et al. (2006). *Nuclear receptor-dependent bile acid signaling is required for normal liver regeneration*. Science 312 (5771): 233–236.

[15]. Schreuder TC, Marsman HA, Lenicek M, van Werven JR, Nederveen AJ, Jansen PL, Schaap FG. *The hepatic response to FGF19 is impaired in patients with nonalcoholic fatty liver disease and insulin resistance*. Am J Physiol Gastrointest Liver Physiol. 2010;298(3):G440–445

[16]. Li Y, Jadhav K, Zhang Y. *Bile acid receptors in non-alcoholic fatty liver disease.* Biochem Pharmacol. 2013;86(11):1517–1524

[17]. Scheja L, Heeren J. *Metabolic interplay between white, beige, brown adipocytes and the liver.* J Hepatol. 2016;64(5):1176–1186

[18]. Ding L, Sousa KM, Jin L, Dong B, Kim BW, Ramirez R, Xiao Z, Gu Y, Yang Q, Wang J, Yu D, Pigazzi A, Schones D, Yang L, Moore D, Wang Z, Huang W. *Vertical sleeve gastrectomy activates GPBAR-1/TGR5 to sustain weight loss, improve fatty liver, and remit*

insulin resistance in mice. Hepatology. 2016;64(3):760–773
[19]. Wang YD, Chen WD, Yu D, Forman BM, Huang W. *The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor kappa light-chain enhancer of activated B cells (NF-kappaB) in mice*. Hepatology. 2011;54(4):1421–1432.
[20]. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. *TGR5-mediated bile acid sensing controls glucose homeostasis.* Cell Metab. 2009;10(3):167–177.
[21]. Jiao Y, Lu Y, Li XY. *Farnesoid X receptor: a master regulator of hepatic triglyceride and glucose homeostasis.* Acta Pharmacol Sin. 2015;36(1):44–50.
[22]. Zarrinpar A, Loomba R. *Review article: the emerging interplay among the gastrointestinal tract, bile acids and incretins in the pathogenesis of diabetes and non-alcoholic fatty liver disease*. Aliment Pharmacol Ther. 2012;36(10):909–921.
[23]. Stayrook KR, Bramlett KS, Savkur RS, Ficorilli J, Cook T, Christe ME, Michael LF, Burris TP. *Regulation of carbohydrate metabolism by the farnesoid X receptor.* Endocrinology. 2005;146(3):984–991.
[24]. Silva TE, Colombo G, Schiavon LL. Adiponectin: *A multitasking player in the field of liver diseases.* Diabetes Metab. 2014;40(2):95–107.
[25]. Bechmann LP, Kocabayoglu P, Sowa JP, Sydor S, Best J, Schlattjan M, Beilfuss A, Schmitt J, Hannivoort RA, Kilicarslan A, Rust C, Berr F, Tschopp O, Gerken G, Friedman SL, Geier A, Canbay A. *Free fatty acids repress small heterodimer partner (SHP) activation and adiponectin counteracts bile acid-induced liver injury in superobese patients with nonalcoholic steatohepatitis*. Hepatology. 2013;57(4):1394–1406.
[26]. Balmer ML, Joneli J, Schoepfer A, Stickel F, Thormann W, Dufour JF. *Significance of serum adiponectin levels in patients with chronic liver disease*. Clin Sci (Lond) 2010;119(10):431–436.
[27]. Dasarathy S, Yang Y, McCullough AJ, Marczewski S, Bennett C, Kalhan SC. *Elevated hepatic fatty acid oxidation, high plasma fibroblast growth factor 21, and fasting bile acids in nonalcoholic steatohepatitis.* Eur J Gastroenterol Hepatol. 2011;23(5):382–388.
[28]. Holland WL, Adams AC, Brozinick JT, Bui HH, Miyauchi Y, Kusminski CM, Bauer SM, Wade M, Singhal E, Cheng CC, Volk K, Kuo MS, Gordillo R, Kharitonenkov A, Scherer PE. *An FGF21-adiponectin-ceramide axis controls energy expenditure and insulin action in mice.* Cell Metab. 2013;17(5):790–797.
[29]. Silva TE, Colombo G, Schiavon LL. *Adiponectin: A multitasking player in the field of liver diseases.* Diabetes Metab. 2014;40(2):95–107.
[30]. Russell DW, Setchell KD. *Bile acid biosynthesis.* Biochemistry. 1992;31(20):4737–4749.
[31]. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. *Endogenous bile acids are ligands for the nuclear receptor FXR/BAR*. Mol Cell. 1999;3(5):543–553.
[32]. Oram JF, Lawn RM. ABCA1. *The gatekeeper for eliminating excess tissue cholesterol.* J Lipid Res. 2001;42(8):1173–1179
[33]. von Eckardstein A, Nofer JR, Assmann G. *High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport.* Arteriosclerosis, thrombosis, and vascular biology. 2001;21(1):13–27.
[34]. Li G, Thomas AM, Williams JA, Kong B, Liu J, Inaba Y, Xie W, Guo GL. *Farnesoid X receptor induces murine scavenger receptor Class B type I via intron binding.* PLoS One. 2012;7(4):e35895.
[35]. Repa JJ, Turley SD, Lobaccaro JA, Medina J, Li L, Lustig K, Shan B, Heyman RA, Dietschy JM, Mangelsdorf DJ. *Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers.* Science. 2000;289(5484):1524–1529.
[36]. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. *Endogenous bile acids are ligands for the nuclear receptor FXR/BAR*. Mol Cell. 1999;3(5):543–553.
[37]. Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, Moore DD, Auwerx J. *Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c*. J Clin Invest. 2004;113(10):1408–1418.
[38]. Studer E, Zhou X, Zhao R, Wang Y, Takabe K, Nagahashi M, Pandak WM, Dent P, Spiegel S, Shi R, Xu W, Liu X, Bohdan P, Zhang L, Zhou H, Hylemon PB. *Conjugated bile acids activate the sphingosine-1-phosphate receptor 2 in primary rodent hepatocytes*. Hepatology. 2012;55(1):267–276.
[39]. Schmitt J, Kong B, Stieger B, Tschopp O, Schultze SM, Rau M, Weber A, Mullhaupt B, Guo GL, Geier A. *Protective effects of farnesoid X receptor (FXR) on hepatic lipid accumulation are mediated by hepatic FXR and independent of intestinal FGF15 signal*. Liver Int. 2015;35(4):1133–1144.
[40]. Kwong E, Li Y, Hylemon PB, Zhou H. *Bile acids and sphingosine-1-phosphate receptor 2 in hepatic lipid metabolism.* Acta Pharm Sin B. 2015;5(2):151–157.
[41]. Studer E, Zhou X, Zhao R, Wang Y, Takabe K, Nagahashi M, Pandak WM, Dent P, Spiegel S, Shi R, Xu W, Liu X, Bohdan P, Zhang L, Zhou H, Hylemon PB. *Conjugated bile acids activate the sphingosine-1-phosphate receptor 2 in primary rodent hepatocytes.* Hepatology. 2012;55(1):267–276
[42]. Krattinger R, Bostrom A, Lee SM, Thasler WE, Schioth HB, Kullak-Ublick GA, Mwinyi J. *Chenodeoxycholic acid significantly impacts the expression of miRNAs and genes involved in lipid, bile acid and drug metabolism in human hepatocytes.* Life Sci. 2016;156:47–56
[43]. Nakahara M, Fujii H, Maloney PR, Shimizu M, Sato R. *Bile acids enhance low density lipoprotein receptor gene expression via a MAPK cascade-mediated stabilization of mRNA*. J Biol Chem. 2002;277(40):37229–37234.

[44]. Guo GL, Santamarina-Fojo S, Akiyama TE, Amar MJ, Paigen BJ, Brewer B, Jr, Gonzalez FJ. *Effects of FXR in foam-cell formation and atherosclerosis development.* Biochim Biophys Acta. 2006;1761(12):1401–1409.
[45]. Zhang Y, Wang X, Vales C, Lee FY, Lee H, Lusis AJ, Edwards PA. *FXR deficiency causes reduced atherosclerosis in Ldlr−/− mice*. Arteriosclerosis, thrombosis, and vascular biology. 2006b;26(10):2316–2321
[46]. Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. *Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis*. Cell. 2000;102(6):731–744.
[47]. D. Roulot, S. Czernichow, H. Le Clesiau, J.L. Costes, A.C. Vergnaud, M. Beaugrand *Liver stiffness values in apparently healthy subjects: influence of gender and metabolic syndrome* J Hepatol, 48 (4) (2008), pp. 606-613
[48]. Claudel T, Sturm E, Duez H, Torra IP, Sirvent A, Kosykh V, Fruchart JC, Dallongeville J, Hum DW, Kuipers F, Staels B. *Bile acid-activated nuclear receptor FXR suppresses apolipoprotein A-I transcription via a negative FXR response element*. J Clin Invest. 2002;109(7):961–971.
[49]. Wunsch, A. and B. Palmieri, *The role of second opinion in oncology: an update*. European Journal of Oncology, 2014. 18(3): p. 117-120.
[50]. Palmieri B, Laurino C., Vadalà M., *The "Second Opinion Medical Network*". Int J Pathol Clin Res 3:056, 2017.
[51]. Palmieri, B. et al. [*Second opinion clinic: is the Web Babel Syndrome treatable?]*. Clin Ter, 2011. 162(6): p. 575-83.
[52]. Palmieri B. and T. Iannitti, *The Web Babel syndrome*. Patient Educ Couns, 2011. 85(2): p. 331-3.
[53]. Di Cerbo, A. and B. Palmieri. *The economic impact of second opinion in pathology*. Saudi Med J, 2012. 33(10): p. 1051-2.