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Diagnostic accuracy of a new ultrasound software compared to liver biopsy for Non Alcoholic Fatty Liver Disease (NAFLD) and Non Alcoholic Steato Hepatitis (NASH) in morbidly obese candidate to bariatric surgery and/or cholecystectomy.

Diagnostic accuracy of a new ultrasound software compared to liver biopsy for Non Alcoholic Fatty Liver Disease (NAFLD) and Non Alcoholic Steato Hepatitis (NASH) in morbidly obese candidate to bariatric surgery and/or cholecystectomy. Pilot study.

PILOT PROSPECTIVE STUDY

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Introduction

The non-alcoholic fatty liver disease (NAFLD) is a systematic disease characterized by the presence of at least 5% of liver tissue, in the absence of other known causes of hepatopathy. To the present day, the NAFLD represents the first chronic hepatopathy cause. (1) It is a benign clinical condition that might evolve in non-alcoholic steato hepatitis (NASH) if inflammation and fibrosis occur along with the hepatic steatosis, with possible evolution in hepatic cirrhosis (in 25-30% of cases) and, subsequently, in hepatocellular carcinoma (7%) (2, 3).

The pathophysiological mechanisms that foster the progression from simple steatosis to NASH are complex and still not entirely clarified. It is certain that multiple genetic factors and acquired factors – such as insulin resistance, high ferritin and adiponectin reduction) are involved. NAFLD prevalence is directly proportional to obesity: it is estimated that 30% of the US population and 24% of the European population is affected by NAFLD and that within the obese population candidate to bariatric surgery the prevalence is at 90%. (4) In the western world, the prevalence of type 2 diabetes patients is at 60-70%. NAFLD shows a strong association with all the components of the metabolic syndrome, enough to be considered the hepatic manifestation of the metabolic syndrome (5).

Key elements of the NAFLD pathogenesis are hyperinsulinemia and insulin resistance. Insulin has three main actions in the liver: it increases glycogen synthesis, inhibits gluconeogenesis, and promotes lipogenesis. In patients with NAFLD, insulin resistance determines:

1) An increase in lipolysis in adipose tissue, resulting in increased circulating levels of free fatty acids, which convey to the liver level;
2) A reduction in glycogen synthesis and an increase in gluconeogenesis in the liver.

In response to insulin resistance, the body produces higher levels of insulin, which further increase liver lipogenesis, resulting in an intrahepatic accumulation of lipids and increased liver secretion of triglycerides in the form of VLDL lipoproteins.

In hepatocytes, the infestation of lipids causes a lipotoxicity that further worsens insulin resistance, increases oxidative stress and promotes inflammation and fibrosis (6,7).

The diagnosis of NAFLD is based on the combination of a number of clinical and radiological features. Hepatic enzymes are not a diagnostic criterion, as more than 60% of NAFLD patients and normal GPT values may have advanced degrees of steatohepatitis and 53% of NAFLD and increased GPT patients do not have NASH.Hepatic enzyme levels, therefore, are not reliably correlated with liver histology (8).

Currently, validated methods for quantization of fibrosis, steatosis and hepatic necroinflammatory phenomena are: Elastography for the evaluation of hepatic fibrosis(9), contrastless MRI (10,11)or ecoguidated percutaneous biopsy for the evaluation of hepatic steatosis (12), ecoguidated percutaneous biopsy for the evaluation of necroinflammatory phenomena (13).

The NAFLD Activity Score (NAS) is the most widely used validated classifications for staging the NAFLS from a histological point of view(14), which assigns a score based on the following parameters:

- The steatosis level (with a score from 0 to 3)
- The degree of lobular inflammation (with a score from 0 to 3)
- The degree of balloning, i.e. hot air balloon hepatocytes (with a score from 0 to 2)

According to the score, we can distinguish among:

- NAS 0 patients: with NAS Score < 3, in which the diagnosis of steatohepatitis is excluded (NO NASH)
- NAS 1 patients: with NAS Score between 3 and 4, indicative of probable pathology or borderline
- NAS 2 patients: with NAS Score ≥ 5 where steatohepatitis (NASH) diagnosis is made

Purpose of the pilot study:

Evaluate the diagnostic accuracy of the new ultrasound software vs. liver histology, current reference standard for the diagnosis of NAFLD and NASH in obese patients (BMI > 30) candidates for bariatric laparoscopic surgery and/or cholecystectomy.

Type of study:

Monocentric, open, sequential enrollment pilot study of 20 patients.

Datas will be analyzed with descriptive statistics, absolute values and percentages of concurrent and conflicting cases according to the two methods.

The concordance between the new ultrasound method and the liver biopsy will be evaluated and tested by the Mcnemar test.

As the pilot study is used to assess whether there is good agreement between the two methods and in case of clinically significant response a study will be set up diagnostic with formal calculation of the sample size, with adequate power statistics and level of significance.

Inclusion criteria:

1. Patients who are candidates for bariatric surgery and are between 18 and 65 years of age,

- BMI > 40 kg/m2, in the absence of any other comorbidity
- BMI > 35 kg/m2, in the presence of comorbidities among those classically considered as associated with obesity (tab,) (15)

Metabolic disease	**Neoplasm**	
Type 2 Diabetes Mellitus	Breast cancer	Esophageal cancer
Dyslipidemia	Pancreas cancer	Gallbladder cancer
Hyperuricemia and Gout	Colorectal cancer	Renal cancer
Female infertility	Prostate cancer	Leukemia
Polycystic ovary syndrome	Endometrial cancer	Ovarian cancer
	Liver cancer	
Cardiovascular Disease	**Others**	
Arterial Hypertension	Bronchial asthma	Psychological disorders
Coronary heart disease	Obstructive sleep apnea syndrome	Gastroesophageal reflux
Congestive heart failure	Non alcoholic steatohepatitis	Urinary incontinence
Pulmonary embolism	Gallbladder disease	Osteoarthritis
Stroke	Intertrigo	Plantar fasciitis

- ICM > 30 kg/m2, in the presence of T2DM not in glycomethabolic compensation

2. Patients who are candidates for laparoscopic cholecystectomy for symptomatic calculosis with BMI > 30

Exclusion criteria:

- Age ≤ 18 or ≥ 65

- Any concomitant surgical procedure except cholecystectomy and repair of iatal hernia
- Conversion into laparotomic surgery
- Post-operative complications requiring new surgical treatment
- Non-compliant patient in follow-up

Methods:

Pre- and post-operative evaluations:

All patients will sign an informed consent ad hoc

Patients will be enrolled according to the PDTA deposited at the Health Directorate of the ICOT

Before surgical treatment all patients will be subjected to:

- standard liver ultrasound, shear wave elastography, attenuation imaging, shear wave dispersion

blood tests: coagulation, GOT, GPT, GGT, alkaline phosphatase, total and fractionated bilirubin, fractionated cholesterol, triglycerides, insulinemia, glycaemia, Cpeptide

Imaging:

The software is designed to be used on the Aplio i800 TUS system.
(already supplied by our structure) and detects the attenuation that the signal
ultrasound meets crossing the hepatic parenchyma and expresses this attenuation
in Decibels on cm of hepatic parenchyma as a function of frequency (dB/cm/MHz) in a
ROI of analysis.
Non-homogeneous zones are automatically excluded from the calculation.
Being a newly introduced software, there is no reference standard with respect to
the ultrasound systems currently used for the analysis of hepatic steatosis
as the transient elastography (Fibroscan, Echosense Paris) or the
CAP (Controlled AttenuationParameter).
There are no studies carried out with this software to date.

The methods that will be used are as follows:

Shear Wave Elastography (SWE)

It quantifies the degree of liver fibrosis by measuring the speed of propagation of SW
A focused ultrasonic pulse generates a tissue movement that creates shear waves (SW).
The speed of movement of the shear waves depends on the rigidity of the crossed tissues.

Its tracking makes it possible to evaluate the rigidity of the tissue (the speed, expressed in m/s, is converted into "stiffness", expressed in kPa, using Young's equation).

Attenuation Imaging (AI)

Quantifies the ultrasound attenuation of the tissue as an indicator of liver steatosis

Ultrasonic waves in the body are attenuated by sound loss, reflection and absorption (heat). Attenuation is frequency-dependent.

Attenuation Imaging is based on the RF information generated by the probe during conventional B-mode examination. The system compares the amplitude of the signal transmitted by the transducer with the return signal from the tissues at different depths, removing from the return ultrasound information all those pre- and post-processing compensations that the system introduces precisely to avoid having an uneven image between surface and depth. This analysis is carried out in a specific region of interest that the operator places on the tissue that is being analyzed. The value obtained represents the attenuation in Decibels per cm as a function of the transmission frequency used (dB/cm/MHz).

Shear Wave Dispersion (SWD)

SWE has some limitations in the presence of steatosis and inflammation because it starts from the assumption that the liver is perfectly elastic.

Increasing the viscosity of the liver (inflammation, steatosis, circulation disorders) increases the phenomena of dispersion.

SWD measures the frequency dispersion of the shear wave propagation in the viscoelastic liver

The dispersion quantification ((m/s)/kHz) allows estimation of liver viscosity

SW signals are converted to SW frequencies with FFT (Fast Fourier Transform) processing

Increasing the viscosity (Pa*s) with constant elasticity (kPa)-> the dispersion increases (steepest slope of the curve).

Surgical technique:

All patients will undergo bariatric surgery according to the standard procedures validated by SICOB (15) and wedge resection of 1.5 cm x 1.5 cm at the level of the left hepatic lobe. In routine surgical practice, a liver biopsy is indicated in cases of hepatomegaly and clinical radiological suspicion of steatosis (16).

Histology:

Histological examination of liver tissue will be performed on material from liver biopsy, fixed in formalin, included in paraffin and finally dissected. The reading of the set preparations will be performed by an expert pathologist who will not have access to the identity, clinical history and biochemical parameters of the patients.

A minimum biopsy sample length of 1.5 x 0.8 cm. or at least 10 complete portal spaces will be required, it is strongly recommended whenever clinically possible to have a separate core for inclusion in cryomold and freezing at -80 C so as to have additional tissue available for possible molecular biology studies.

For liver histology sections of 3-4 μm will be obtained by staining with Hematoxylin/Eosin, PAS, PERLS, Gomoric Reticulum and Masonic Trichrome. NASH will be diagnosed according to Brunt's criteria (17). The NAFLD activity score (NAS) and a staging score capable of quantifying the extent of fibrosis in order to perform statistical correlations will be calculated (17). In addition, patients will be stratified according to the SAF score into no NAFLD, NAFLD or NASH (18).

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