INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the hepatic pandemic of the XXI century, being the number one cause of chronic hepatic disease in the occidental world ***(Bellentani et al., 2010).***

Although usually benign, fatty liver may associate with serious injury, inflammation, hepatocyte necro-apoptosis and non-alcoholic steatohepatitis (NASH) in 20–30% of subjects. Those patients are at risk of developing fibrosis, one fifth progressing to liver cirrhosis ***(Angulo et al., 1999).***

NAFLD could be both the result and the cause of metabolic syndrome, with a vicious cycle operating between these conditions. Remaining challenges are 1- the lack of clear threshold alcohol intake for defining(nonalcoholic) 2-a lacking consensus for the classification of fatty liver disease, and 3-absence of a histological definition of NASH, which currently remains the gold standard for the diagnosis ***(Hashimoto et al., 2015).***

The gold standard for the diagnosis and staging of NAFLD is liver biopsy (LB), although as it will be discussed later, it may have been dethroned by more accurate methods in what concerns steatosis. However, it is the only way to directly diagnose NASH and fibrosis, even if several assays and models try to predict it with reasonably good accuracy. LB has several drawbacks. It is an invasive procedure, frequently associated with distress and

discomfort. Although generally safe, it comes with a risk for major complications in 1–3% and even death in 0.01%.

Due to the remarkable increase in the prevalence of NAFLD and the concomitant efforts in developing novel therapies, a non-invasive, simple and reproducible technique is needed in the clinical practice. Transient elastography is a non-invasive technique for liver stiffness measurement (LSM) as a function of the extent of hepatic fibrosis ***(Abenavoli and Beauqrand, 2012).***

Several non-invasive methods aim at diagnosing and quantifying hepatic steatosis, while others were designed to predict NASH or significant advanced fibrosis.

AIM OF THE WORK

The aim of this work is to evaluate the ability of transient elestography to stage hepatic fibrosis and to identify histological parametres, including steatosis, in patients with NAFLD and NASH.

Chapter I

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND NON-ALCOHOLIC STEATOHEPATITIS

Introduction:

Non-Alcoholic Steatohepatitis and non-alcoholic fatty liver disease are common causes of chronic liver disease world wide(fiuire1), and hepatic steatosis is the most common pathological feature ***(Ludwig et al., 1980).***

NAFLD comprises a disease spectrum ranging from benign hepatic steatosis to non-alcoholic steatohepatitis with inflammation (NASH) and liver cirrhosis. Although simple steatosis appears to be benign, NASH can progress to cirrhosis with its resultant complications, including hepatocellular carcinoma (HCC). It is increasingly recognised that NASH accounts for a significant proportion of "cryptogenic" or "idiopathic" cirrhosis ***(Hui-Hui and Jason., 2010).***

Primary NAFLD often co-exists with at least one feature of the metabolic syndrome (impaired glucose tolerance, central obesity, hypertension, hypertriglyceridaemia, low high-density lipoprotein [HDL] cholesterol) ***(Hui-Hui et al., 2010).***

History of NAFLD discovery:

In 1958, Zelman studied liver function and liver biopsy findings in 29 overweight patients, and concluded that Fatty liver, fatty hepatitis, fatty fibrosis and fatty cirrhosis were seen with equal frequency in those patients and diabetes was also present with an equal incidence in each of these four pathologic groups. ***(AGA, 2002)***

Finally in 1980, Ludwig et al. introduced the term "nonalcoholic steatohepatitis" (NASH) to describe these histologic findings in those who did not consume alcohol ***(AGA, 2002).***

Definitions:

1. **Alcoholic liver disease (ALD)** is caused by long-term heavy drinking of alcohol (generally more than 5 years) in which the amount of alcohol drunk is ≥40 g/day in men and ≥20 g/ day in women) and usually manifests initially as a fatty liver, then develops progressively to alcoholic hepatitis, alcoholic liver fibrosis and alcoholic liver cirrhosis. Excessive drinking of alcohol may induce widespread hepatocellular necrosis and even liver failure ***(Lok et al., 2004).***
2. **Non alcoholic fatty liver disease (NAFLD)** is a clinicopathological entity with histological features resembling alcohol-induced liver injury, though occurring in patients with little or no history of alcohol consumption ***(Flavio et al., 2009)***.

Non alcoholic fatty liver disease (NAFLD) includes two histological entietes:

a) **Simple steatosis (fatty liver):** defined as an excess of fat in the liver in which at least 5% of hepatocytes display lipid droplets that exceed 5%-10% of liver weight without lobular inflammation or pericellular fibrosis ***(Yongzhong et al., 2008).***

b) **Non-alcoholic steatohepatitis (NASH):** defined as part of the spectrum of NAFLD characterized by steatosis, lobular inflammation and progressive pericellular fibrosis ***(Jia et al., 2009).***

A study based on routine health care of Japanese government employees revealed an overall incidence of non-alcoholic hypertransaminasemia of 31 cases per 1000 person-years ***(Suzuki et al., 2005).***

Another Japanese study reported follow-up data on 3147 individuals without NAFLD at baseline; of these, 308 (10%) developed new cases of NAFLD over 414 days ***(Hamaguchi et al., 2005).***

The clear discrepancy among these rates suggests that accurate global incidence rates for NAFLD require further study.

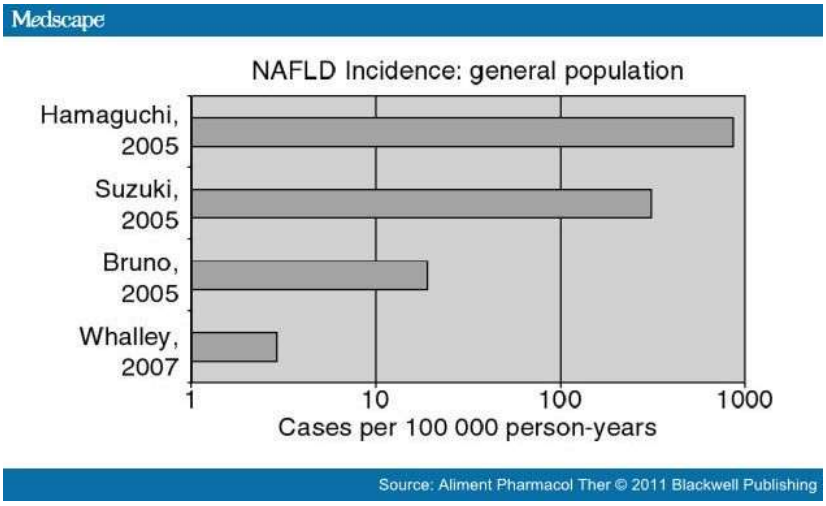

Figure (1): Non-alcoholic fatty liver disease incidence in the general population.

Prevalence of NAFLD and NASH:

The prevalence of NAFLD in the general population has been assessed with a variety of diagnostic tools. Liver biopsy, the current gold standard for NASH diagnosis and staging.

In addition to liver biopsy-proven fatty liver, several non-invasive diagnostic methods for NAFLD and NASH have been introduced recently. Nevertheless, these methods are somewhat less definitive than histology-based evaluations of biopsies and autopsies. On the other hand, some important conclusions can be drawn from non-invasive population-wide samples studied non-invasively ***(Vernon et al., 2011).***

Non-invasive radiological modalities used to assess the prevalence of fatty liver include magnetic resonance imaging (MRI) and ultrasonography.

Other studies have used elevations in the liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as non-invasive indicators of NAFLD. Data from the US National Health and Nutrition Examination Survey (1988–1994) reported elevated ALT levels in 3% of the population. These liver enzyme elevations are about twice as prevalent as those reported for the Nutrition Examination Survey (NHANES) 1988–1994 study ***(Ruhl et al., 2003).***

The issue of normal range for aminotransferases and their relationship with gender is described in more detail in following sections. Nevertheless, it is important to emphasise that although elevated ALT is generally associated with histological NASH, a number of patients with normal ALT levels may also have NAFLD and even advanced fibrosis. Therefore, ALT activity alone cannot be used to rule out significant liver disease in patients suspected of having NAFLD, especially those with type II diabetes or hepatomegaly ***(Kojima et al., 2003).***

Most of the US studies report a 10–35% prevalence rate of NAFLD; however, these rates vary with the study population and the modality used to establish the diagnosis (Figures 2 and 3). Because approximately one-third of the US population is considered obese, the prevalence of NAFLD in US population is likely to be about 30% (Figures 2 and 3).

Risk Factors for NAFLD and NASH: Predisposing Demographical and Clinical Factors:

1. Age

NAFLD can be found in all age groups; however the prevalence appears to increase with age ***(Hui-Hui et al., 2010)***.

In addition to the association between age and the prevalence of NAFLD, older patients with NAFLD have a higher likelihood of disease progression or mortality. Older age also increases the risk of developing related problems such as severe hepatic fibrosis, hepatocellular carcinoma and type II diabetes mellitus ***(Vernon et al., 2011).***

Gender:

Although early reports which suggested a predominance of NAFLD in females, recent larger population studies have established that NAFLD occurs in both genders ***(Hui-Hui et al., 2010).***

2. Metabolic Conditions:

Non-alcoholic fatty liver disease is more prevalent in cohorts of patients with pre-existing metabolic conditions than the general population

Primary causes of NAFLD:

1) Obesity.
2) Diabetes.
3) Hyperlipidemia

Secondary causes NAFLD due to:

1. Drug induced: (e.g., Tamoxifen, amiodarone, oestrogens, glucocorticoids).
2. Nutritional causes.
3. Toxin exposure.
4. Other causes

1) *Primary causes of NAFLD:*

Metabolic syndrome:

The term metabolic syndrome refers to a cluster of cardiovascular risk factors associated with insulin resistance

Metabolic syndrome is a strong predictor of NAFLD and NAFLD is less likely to regress in those participants with the metabolic syndrome at the baseline ***(Hamaguchi et al., 2005).*** Abdominal obesity, hypertension, dyslipidemia and type II diabetes mellitus are pathological conditions frequently associated with NAFLD, and their coexistence in the same individual increases the likelihood of having more advanced forms of liver disease ***(Angulo, 2007)***.

A. Obesity:

Obesity is associated with NAFLD; the prevalence of simple steatosis in obese individuals ranges from 30% to 37%, and NAFLD ranges from 57% of overweight individuals attending out-patient clinics to 98% of nondiabetic obese patients ***(Vernon et al., 2011).***

The prevalence of NAFLD and cirrhosis in a cohort of obese patients undergoing gastric bypass was 63% and 2%, respectively. However, Ong and colleagues showed that over 95% of bariatric surgery patients had fatty liver, 20–30% had NASH, and 10% had advanced fibrosis ***(Ong et al., 2008).***

The median prevalence of NASH in the obese population is 33%, ranging from 10% to 56%. The prevalence of fibrosis in obese patients depends on the type of fibrosis; 67% of gastric bypass surgery patients had portal fibrosis, but only 4% of bariatric surgery patients had perisinusoidal fibrosis. ***(vernon et al., 2011)***

B. Type 2 diabetes mellitus:

Generally, NAFLD is very common in the type 2 diabetes population with between 50 and 75% of subjects demonstrating fat in the liver by ultrasound. NAFLD, as manifest by elevated alanine aminotransferase levels, predicts the future development of diabetes. Additionally, the presence of diabetes has been identified as a risk factor for precence of aggressive form of

NAFLD and NASH, with one autopsy series showing a 2.6-fold increased risk of steatohepatitis in individuals who were hyperglycemic ***(Utzschneider et al., 2009)***.

This study showed that the prevalence of NASH increases in parallel with components of the metabolic syndrome. Nevertheless, it is important to note that NASH and advanced fibrosis are often observed in diabetic patients without symptoms, signs, or liver enzyme abnormalities. ***(Prashanth et al., 2009)***

C. Hyperlipidemia:

As with type 2 D.M., hyperlipidemia also consider as a risk factor for nonalcoholic fatty liver disease (NAFLD), which concurrently with the metabolic syndrome is increasing in prevalence in the same parts of the world ***(Angelika et al., 2005).***

Table (1): Genetic causes of NAFLD.

Disease	Genetic mutation	Age of presentation	Other clinical Symptoms	Management
Abetalipoproteinemia	Microsomal triglyceride transfer protein	Infancy	Growth problems, mental retardation	Low-fat diet; fat soluble vitamin supplementation
Familial hypobetalipoproteinemia	apoB100	Infancy	Failure to thrive steatorrhea, spinocerebellar degenerative ataxia	Low-fat diet; fat soluble vitamin supplementation
Familial combined hyperlipidemia	USF1	Infancy	Hypertriglyceridemia, hypercholesterolemia,	Low-fat diets, exercise, smoking cessation, weight loss
Glycogen storage disease	PHKA2, PHKB	Infancy	Growth retardation, lactic acidosis, and development delay	Avoidance of fasting, ingestion of corn starch, liver transplantation
Weber–Christian disease	unknown	Childhood	Fever, arthralgias, myalgias, skin lesions, and painful subcutaneous nodules	Immunosuppressive reagents, NSAIDs, glucocorticoids
Lipodystrophy (congenital)	AGPAT2, BSCL2	Infancy	Severe fat loss, voracious appetite, accelerated linear growth, and advanced bone age	Low-fat diet

2) *Secondary causes of NAFLD and NASH due to:*

1- **Drug induced:** (e.g., HAART, amiodarone, Tamoxifen, Methotrexate, Corticosteroids).

Establishing the diagnosis of drug-induced liver disease may be difficult. It requires: (a) absence of other causes of liver disease, and (b) that the ingestion of drug precedes the onset of liver disease ***(Das et al., 2006)***.

E.g.

HAART

After the development of antiretroviral therapies to treat HIV disease, the mortality rate due to AIDS decreased while the rate due to liver disease increased. Ultrasonographic evidence of steatosis is found in 31% of patients with HIV infection. Treatment of patients with HIV using nucleoside reverse transcriptase inhibitors such as didanosine, fialuridine, and zidovudine may induce microvesicular steatosis. Nucleoside analogs lead to hepatotoxicity and steatosis by inhibiting the mitochondrial polymerase-gamma gene and preventing mitochondrial replication, decreasing the net rate of beta-oxidation of fatty acids and facilitating the accumulation of fat ***(Price and Thio, 2010).***

Available alternative therapies that reduce the risk of progressive liver damage while maintaining viral suppression.

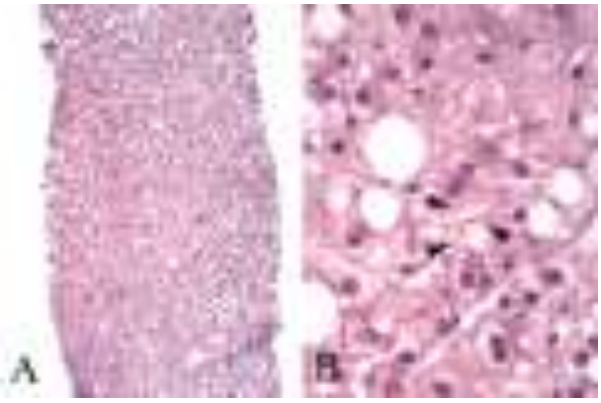

Figure (2): Liver biopsy in a patient on nucleoside analogs. (A) The low-power view shows moderate steatosis in a nonzonal pattern, hepatocyte pallor, and mild hepatic plate thickening suggestive of reactive changes. (B) The high-power view shows the hepatocytes.

Amiodarone

Nearly a quarter of patients on long-term amiodarone develop mild LFT abnormalities but only 1–3% have significant inflammation on liver biopsy ***(Lewis and Zimmerman, 1989).***

Amiodarone is associated with a pattern of NASH morphologically similar to alcohol hepatitis. Liver biopsies are characterized by macrovesicular and microvesicular steatosis, ballooning degeneration, neutrophilic infiltration, Mallory–Denk bodies, and sinusoidal fibrosis (Figure 3). A characteristic ultrastructural lesion of amiodarone use is phospholipidosis, in which lysosomes are densely packed with concentric membranous arrays with a fingerprint pattern Alternative medications should be considered to treat cardiac arrhythmias in patients who suffer from amiodarone-related liver injury ***(Guigui et al., 1988).***