The natural history and risk factors for progression of non-alcoholic fatty liver disease and steatohepatitis

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Abstract. – Nonalcoholic fatty liver disease (NAFLD) is a condition of increasing incidence in western Countries seldom associated to other diseases of high prevalence in general population (i.e. diabetes and obesity). NAFLD ranges from simple fatty liver to steatohepatitis (NASH), which may lead to cryptogenic cirrhosis and in some cases hepatocellular carcinoma (HCC). Natural history of NAFLD in humans is poorly understood and progression of liver disease seems to be due to interaction between hosting (i.e. genetic, gut flora, insulin resistance) and environmental factors (social and eating behaviours) that should be responsible of increased oxidative stress within hepatocytes. Even if we need non-invasive markers able to describe the progression of liver disease, only meaning of liver biopsy is useful to characterize the stigmata of worsening such as inflammation and fibrosis.

Key Words:

Metabolic syndrome; NAFLD, Non-Alcoholic Fatty Liver Disease; NASH, Non-Alcoholic Steatohepatitis; Natural history.

Introduction

The term non-alcoholic fatty liver disease (NAFLD), like a "matrioska", includes a wide range of liver diseases ranging from the simple accumulation of fat into the liver (fatty liver) to different degree of inflammation and fibrosis (NASH), the most severe form, to cryptogenic cirrhosis with its clinical sequaele (HCC, liver decompensation). Several factors involving genetic predisposition, insulin resistance, oxidative stress and gut flora may be responsible of progression.

NAFLD is arising as the most common cause of chronic liver disease in the Western countries[1] with a prevalence ranging from 10 to 24% in general population and reaching 75% in obese group, while its most severe form, non-alcoholic steatohepatitis (NASH), affects 3% of lean subjects, 19% of obese and 50% of severe obese people[2,3].

It is well accepted that simple fatty liver has a benign course[4] while the presence of inflammation, ballooning degeneration and fibrosis, typical features of NASH, may lead to cirrhosis and its complications, in particular hepatocellular carcinoma (HCC)[5].

Natural History

Natural history of NAFLD is poorly understood and is not clear why even if it is a condition common in diseases with high prevalence in general population (i.e. diabetes, obesity, metabolic syndrome, chronic assumption of drugs) only few patients develop progressive fibrosis. Onset of inflammation in a fatty liver is the first step of progression and the key point in the natural history of NAFLD while developing fibrosis is a consequence of a prolonged injury mainly resulting from oxidative stress.

In human, difficulties in describing natural history of fatty liver is a consequence of the prerequisite of liver biopsy for staging disease, as this procedure is the only mean to assess the presence and extent of necroinflammatory changes and fibrosis. Age and duration of exposure to oxidative stress seem to be the most important factor for fibrosis on-

set and progression. The point prevalence of cirrhosis in patients with NASH is 7-16%[6], and increasing prevalence of chronic liver diseases in diabetics[7] may be responsible of a future raising incidence of cryptogenic cirrhosis in clinical settings.

The small number of cases reported in papers and the lack of consensus on the role of liver biopsy joined to the problems of repeating histology in patients with NAFLD are the main causes of this delay of clinical hepatology. In fact, there is no general consensus on performing liver biopsy in the routine clinical setting and a surrogate marker of disease progression is still lacking[8].

Serum liver enzyme tests and ultrasound have limited sensitivity and specificity for depicting progression of inflammation and fibrosis. Serum transaminases are pointed out as predictor of fibrosis and in particular AST/ALT ratio of greater than 1 is related to increase fibrosis stage in patients with NASH[2]. Among a large series of subjects enrolled on the National Health And Nutrition Examination Survey III study, Ruhl and Everhart, by means of an increased ALT activity as the only "surrogate marker" for suspected NAFLD, found 2.8% of subjects with ALT above normal range. There was a significant relationship between ALT and BMI, waist/hip ratio (WHR), serum insulin, triglycerides, and leptin levels. In particular: high WHR and leptin levels were more strongly associated with elevated ALT than BMI suggesting the role of visceral fat storage in the development of NAFLD[9].

Searching for a non invasive surrogate marker for NAFLD, it is far to believe that serum transaminases are the best one able to describe disease progression. In a large retrospective cohort of > 7,000 blood donors, serum ALT activity was independently related to body mass index and to laboratory indicators of abnormal lipid or carbohydrate metabolism even if as Authors stated, the revision of transaminases levels in NAFLD patients is advisable[10]. A retrospective study by Mofrad et al. underlined the lack of correspondence between normal ALT level and histological features, according to fibrosis staging, no differences between NAFLD with normal or elevated ALT and evidence of advanced fibrosis in 5/15 patients with normal ALT level[11].

From an epidemiological point of view the worldwide growing of overweight is a prerequisite for increase of fatty liver detection. Analysing data concerning the prevalence of NAFLD and NASH in general population and in obese people, we can extrapolate the pivotal role of metabolic and nutritional status in the natural history of both conditions and also in the eventual progression from NAFLD to NASH.

Obesity, defined by body mass index (BMI = Weight [kg]/height [m²]) above 30, seems to be the most important risk factor in the development of NAFLD. In northern Italy, Bellentani et al. in the Dyonisos study, showed that the prevalence of liver steatosis increase from 16.2% in normal weight population to 80% in obese, and the relative risk is higher in obese than in heavy drinkers[12]. Loguercio et al. in a group of 84 patients coming from southern part of Italy with clinical and laboratory features of NAFLD, reported an high prevalence of males with a BMI above the range of normality in about 90%, while a clear obesity was found only in four patients[13]. On the other hand, NASH seems to be strictly related to central obesity and metabolic syndrome, a cluster of insulin resistance and hyperinsulinemia[14,15].

Pathogenesis

The finest approach to realize the natural history of NAFLD is the application of the "two-hit" hypothesis from James and Day. According to this model NASH results from a "first hit" responsible of fatty accumulation in the liver causing liver steatosis and a "second hit" responsible for apoptosis or necrosis of hepatocytes and inflammation. Multiple "hits" may be guilty of each hit or both. Progression of NASH to cirrhosis may be due to the same factors responsible of the passage from normal liver via fatty liver to steatohepatitis according to a "sequential" model or a "parallel" model for coexistence and contemporary multiple hits.

Insulin resistance and metabolic syndrome is widely accepted as the "primum movens" for development of fatty liver. Marchesini et al. using the HOMA method reported that insulin-resistance was the more strictly asso-

ciated to presence of NAFLD than BMI and fat distribution[16]. Moreover, decreased insulin sensitivity, assessed by the euglycemic clamp, deranges lipid metabolism: in NAFLD patients was observed hypertrigliceridemia with increased free fatty acids levels, whereas insulin mediated suppression of lipolysis is less effective if compared to type 2 diabetes and healthy controls. Means of frequently sampled intravenous glucose tolerance test (FSIGT)[17], confirmed impaired insulin activity (hyperinsulinemia and decreased insulin sensitivity) as an early finding in non obese NASH patients.

The lack of insulin suppression enhances periferic lypolisis from adipocytes with increase of plasma free fatty acids flow from peripheral tissues leading to fat accumulation into hepatocytes. In the liver we observe enhanced gluconeogenesis, due to large amount of available triglycerides and of free fatty acids. The increase of fatty acids synthesis in conjunction with the reduction of delivery of fatty acids from hepatocytes by VLDL for an increased degradation of apolipoprotein B100 cause the unbalance of hepatic fat turnover resulting in steatosis.

The excess of fat is the precondition for the "second hit" resulting in a "chronic stimulus" responsible of inflammation, depletion of the antioxidant pool and consequently progression of fatty liver to steatohepatitis. In fact, fatty acids undergo oxidation by multiple metabolic pathway (microsomes, mitochondria, peroxisome) resulting in reactive oxygen species (ROS) formation. Increased levels of fatty acids provides a source of oxidative stress and damage of mitochondria with increased beta-oxidation and raising levels of ROS[18,19]. In presence of insulin resistance the coexisting of increased beta oxidation and inefficiency in coupling of oxidation with phosphorylation cause decrease of ATP formation and increase of ROS with progressive cytochrome c depletion resulting in apoptosis and cell death[20].

Another source of ROS is microsomial cytochrome P450 (CYP) system. In particular CYP 2E1 is present at high concentration in liver of patients with insulin resistance due the loss of insulin-mediated down regulation[21-23]. Rising microsomial activity promote progression from steatosis to steatohepatitis and fibrosis by lipid peroxidation and cy-

tokine induction via hydrogen peroxide and its reactive species[24]. Serum levels of malonyldialdehyde (MDA) and 4-hydroxynonenal (HNE) are significantly higher in patients with NASH than in patients with fatty liver alone supporting the role of lipid peroxidation products in the activation of cytokines cascade and hepatic stellate cells and in the induction of inflammation and finally to fibrosis[25].

Therefore, initiation of fibrogenesis may be due to noncytokine stimuli, such as products of lipid peroxidation, while perpetuation is a consequence of Tumor Necrosis Factor (TNF)-α that is able to stimulates other cytokines such IL-6, |Tissue Grow Factor (TGF)-β, platelet grow factor (PDGF), all involved in fibrosis progression. Patients with NASH seem to have a dynamic process of the extra cellular matrix supported by high level of serum levels of laminin, TGF-β, TIMP1 and leptin also in the early stages of this disease[26-30].

On the other hand several data on experimental observation in mice[31] and in humans[32,33] support the hypothesis that intestinal bacterial flora may contribute to the pathogenesis of NASH by increasing production of ethanol or by direct activation of cytokines in the lumenal epithelial cells, liver macrophages, or both via release of lipopolysaccharide (LPS). All these factors may cause an activation of liver macrophages with production of TNF-α causing further activation of cytokines cascade and of IKKβ pathway[34]. Preliminary data may show an increase of intestinal permeability in patients with NAFLD[35].

In conclusin, NAFLD may be imagined as a "matrioska" for at least two reasons: first of all, opening the NAFLD, researchers may find NASH, chronic hepatitis and cryptogenic cirrhosis with its complication (decompensation, HCC) and secondly, there is a close link between NAFDL and metabolic syndrome characterized by increase of waist, a "matrioska" stigmata of hyperinsulinemia itself.

The increase of fat within hepatocytes, in presence of mitochondrial dysfunction, is the prerequisite of the oxidative stress. The decrease of ATP availability within hepatocytes is the reason of the high susceptibility of cells to injury if another "hit" is in close proximity. Fat is also a potential source of ROS by mul-

tiple pathways and generation of ROS induces Kuppfer cells to release TNF-α, promoting the proinflammatory pathway via IKK-beta and augmenting insulin resistance. Inflammatory status and recruitment of inflammatory cells incite the wound healing response by fibrogenesis.

Some conditions, as overweight and obesity, are the main guilty partners for the disease progression but also the length of exposure to oxidative stress and the function of antioxidant system are important partners dancing with NAFLD.

Investigation of genetic factors[36] may be helpful for better understanding of the natural history of NASH.

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