



# AISF

## ASSOCIAZIONE ITALIANA PER LO STUDIO DEL FEGATO

Riconosciuta con D.M. del 7.5.1998, G.U. del 20.6.1998

Iscritta nell'Elenco di cui all'art. 1, comma 353, della Legge 23.12.2005 n. 266, D.P.C.M. 15.4.2011

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### Sintesi del progetto

#### BACKGROUND AND AIMS

Oxidative stress plays a pivotal role in the development and progression of liver injury in patients with NAFLD [1]. Lipid and protein peroxidation is directly associated with the degree of liver fibrosis and, similarly, DNA oxidative damage is involved in the onset of hepatocellular carcinoma [1]. Polyphenols are potent antioxidants contained in fruits and vegetables whose intake has been demonstrated to influence health outcomes [2]. A low dietary polyphenolic intake is emerging as a crucial factor in promoting obesity and cardiovascular disease [3]. By contrast, a high polyphenol intake, as in the Mediterranean Diet, may prevent obesity-related morbidity including liver steatosis [4]. Furthermore, there is evidence that polyphenols exert anti-fibrogenic effects in experimental models of NAFLD [5].

To date, no study has assessed if dietary polyphenol intake may be involved in the pathogenesis of NAFLD. The aim of this study is to establish if it may influence oxidative stress and the degree of liver damage in patients with NAFLD.

#### MATERIALS AND METHODS

The design of the study is cross-sectional, multicenter (with a potential extension to a prospective observational cohort). The study population will include outpatients with biopsy-proven NAFLD, consecutively seen in secondary and tertiary centers in Italy. During routine clinical evaluation for NAFLD, patients will be invited to participate in the study and will sign a written informed consent. Dietary polyphenol intake will be assessed by determining urinary polyphenolic excretion (UPE) by the Folin-Ciocalteu (F-C) method that is the reference assay to measure polyphenols in foods as well as their excretion in urine. For this aim, a spot morning urine sample will be collected and stored for each patient. Urine will be collected in the common steril jars and should be aliquoted and



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stored at  $-80\text{ C}^{\circ}$  to avoid the oxidations of polyphenols. Urine samples will be also used to measure the levels of 8-isoprostanes that are considered the gold standard marker to assess lipid peroxidation in the clinical setting (8-isoprostanes ELISA kit Cayman #516351). In addition to 8-isoprostanes, oxidative stress will be also evaluated by measuring a panel of circulating markers. DNA oxidative damage will be evaluated by 8-OH-deoxyguanosine (8-OH-deoxyguanosine ELISA kit Cayman #589320); protein preoxidation will be assessed by 3-Nitrotyrosine (3-NT ELISA Kit Abcam #ab212166); total antioxidant capacity will be assessed by a colorimetric assay (Cayman #709001). In order to assess the different contribution of the several classes of polyphenols, dietary habits will be investigated by a semi-quantitative food frequency questionnaire and polyphenol content of each food will be measured by the Phenol Explorer Database [6]. Association of UPE levels with histological, biochemical and anthropometric variables related to NAFLD at baseline will be firstly explored by Spearman's correlation and then after adjustment for main confounders by regression analysis. If baseline analysis will show an independent association of UPE with oxidative stress levels and liver injury degree, the association between variations in UPE and clinical outcomes will be further determined at 12-month follow-up.

### REFERENCES

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