Study for the Evaluation of Risk of Hepatocellular carcinoma in NonAlcoholic fatty liver

A multicenter prospective cohort study for the evaluation of the interaction between congenital and acquired risk factors in the pathogenesis of hepatocellular carcinoma in patients with nonalcoholic fatty liver and disease risk stratification.

Principal investigator: Luca Valenti  
Co-Investigator: Serena Pelusi  
Other investigators: Silvia Fargion, coordinators of participating centers, Guido Baselli, Anna Fracanzani.

Background: Nonalcoholic fatty liver disease (NAFLD) is projected to become the leading cause of hepatocellular carcinoma (HCC) in Western countries within 2025. NAFLD-HCC frequently develops without overt cirrhosis suggesting that steatosis directly promotes hepatic carcinogenesis. However, progression of liver disease to cirrhosis and HCC is more frequent in the subgroup of patients who develop non-alcoholic steatohepatitis (NASH)9. Established risk factors include older age, obesity, severe insulin resistance - type 2 diabetes (T2D), hypertension, non-alcoholic steatohepatitis (NASH)5. Due to the very high prevalence of NAFLD, the occurrence of HCC in patients without advanced fibrosis, and lack of disease awareness, classic screening strategies for the detection early HCC are ineffective. As a consequence, most of patients who developed NAFLD-HCC are diagnosed at an advanced stage, when curative treatments are not anymore possible. This renders the development of new noninvasive biomarkers able to stratify the risk of HCC development in NAFLD patients a public health priority.

Genetic factors have been shown to influence disease progression in NAFLD, and family history remains the main risk factor for HCC development. The common genetic polymorphism rs738409 C>G encoding for the I148M variant in PNPLA3 is the main common genetic determinant of hepatic fat content and of progressive NAFLD, and NAFLD-HCC, but it has a low sensitivity to be used as single prognostic biomarker. The rs58542926 E167K variant in TM6SF2, and the rs641738 C>T variant in MBOAT7 may also be involved. Moreover, rare mutations inducing Mendelian diseases due to severe derangements in the function of encoded proteins may predispose to NAFLD-HCC.

Aims: The overall aim is to quantify the impact of genetic risk factors on the risk of NAFLD-HCC, as compared to other major outcomes, and their interaction with acquired triggers on disease in a prospective cohort of at risk patients. Specific aims will be:
1. Validation of the inclusion criteria as able to identify the individuals at risk of NAFLD-HCC among NAFLD patients in follow-up.
2. Identification of the impact of single genetic variants on HCC risk, and development of a score to predict NAFLD-HCC and select patients for whom the screening may be cost-effective.
3. The study cohort will allow the detailed characterization of the natural history of advanced liver disease related to NAFLD (including patients with moderate alcohol intake) in a multicenter Italian cohort, with complete clinical, metabolic and most importantly genetic characterization, and the identification of inherited risk factors for progression to HCC and other major outcomes (in particular hepatic decompensation, cardiovascular events, cancer, and overall mortality).

Study design: To maximize the study power, we will select different categories of NAFLD patients > 45 years old at higher risk of HCC because of already advanced liver disease (Fibrosis stage F3-F4), family history and/or confirmation of rare genetic mutations with a strong impact on HCC, or with strong acquired risk factors (type 2 diabetes or obesity) carrying at least three common genetic risk variants for the disease. We plan to enroll 500 patients with advanced...
NAFLD, who will be followed for 5 years, to have a >80% power to detect a significant association of the genetic score with HCC risk in the first study phase, accounting also for the occurrence of other competitive major clinical outcomes. Candidate common risk variants will be determined and their impact on clinical outcomes analyzed in a first study phase. Next, we will also consider a panel of rare variants, which are being identified as associated with NAFLD-HCC in ongoing studies (second stage by targeted resequencing of candidate genes). Finally, we will request funding to proceed to a discovery phase of new risk variants (third stage: whole exome sequencing).

**Participating centers:**

1. Internal Medicine and Metabolic Diseases, Policlinico, Milano; coordinating center (L. Valenti): enrolling (n=35)
2. Gastroenterology and Hepatology, Policlinico, Milano (P. Lampertico): enrolling (n=2)
3. Hepatology, Rozzano (A. Aghemo): obtained ethical committee approval
4. Internal Medicine, Udine (G. Soardo): requesting ethical committee approval
5. Internal Medicine, Roma (L. Miele): requesting ethical committee approval
6. Gastroenterology, Ancona (G. Svegliati-Baroni): requesting ethical committee approval
7. Internal Medicine, Bologna (F. Piscaglia): requesting ethical committee approval
8. Gastroenterology, Torino (E. Bugianesi): agreed
9. Gastroenterology, Palermo (S. Petta): agreed

Others centers are being contacted to join the study group, and endorsement is being requested from the Italian Association for the Study of Liver Disease (AISF).
REFERENCES


