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Clinical and genetic markers of NAFLD and prediction of liver disease mortality. Ready for population screening?

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Selected Summary: Unalp-Arida A and Ruhl CE. PNPLA3 I148M and liver fat and fibrosis scores predict liver disease mortality in the United States population. Hepatology 2020 in press

In the United States, chronic liver disease and cirrhosis was the 11th leading cause of mortality in 2017, when it caused more than 40 thousand deaths and ranked fifth at age 45-54 (Kochanek *et al*, 2019). Mortality from end stage liver disease has been increasing in selected states in the South and West over the last decade (Tapper & Parikh, 2018). In the same time period, the aetiologic profile of the offending liver diseases is being reshuffled, due to the climbing incidence of non-alcoholic fatty liver disease (NAFLD) (Allen *et al*, 2018; Younossi *et al*, 2016). NAFLD has been, in fact, on the raise among all age categories mainly in relation to the epidemic of obesity among the general U.S. population, a condition that has been predicted to cause more than 78 thousand deaths by 2030 (Estes *et al*, 2018).

Understanding the natural history of NAFLD, therefore, has important implications in terms of public health. Yet, it stands as a major undertaking, given the complexity of this disease that ranges from benign non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH) with progressive fibrosis, where disease severity appears to be driven by a dynamic interplay of environmental, lifestyle and genetic factors (Rinella *et al*, 2019). NAFLD contributes to both liver-related and all-cause mortality, as it is associated with progression to severe and decompensated liver diseases, such as cirrhosis and hepatocellular carcinoma, and to cardiometabolic diseases such as cardiovascular disease and type 2 diabetes (Rinella *et al*, 2019). In the U.S., mortality from NAFLD-related cirrhosis and liver cancer is on the rise, with NAFLD standing now as the second-leading aetiology among adults listed for liver transplantation (Kim *et al*, 2019; Wong *et al*, 2015). With all the caveats due to the suboptimal accuracy of the available staging procedures, fibrosis, but no other histologic liver characteristics, has been found to independently predict both all-cause and liver-related mortality in patients with NAFLD (Angulo *et al*, 2015).

There are, however, significant racial and ethnic disparities in NAFLD prevalence, severity, and outcomes that add complexity to the prognostication of this disease, and further support the view of genetic variation being an important risk modifier of NAFLD severity (Caussy *et al*, 2017; Rich *et al*, 2018). The best characterized genetic determinants of disease severity are genes encoding patatin-like phospholipase domain-containing 3 (PNPLA3) rs738409 c.444C>G (p.I148M) and transmembrane 6 superfamily member 2

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(TM6SF2) rs58542926 c.449C>T (p.E167K). These have been strongly associated with the severity of NAFLD, including development of hepatocellular carcinoma (Kozlitina *et al*, 2014; Romeo *et al*, 2008).

In an elegant, two-phase study based on record-linkage of the third U.S. National Nutrition and Health Examination Survey (NHANES III) database with national mortality data, Unalp-Arida and Ruhl (Unalp-Arida & Ruhl, 2019) provided further insights on the potentially serious consequences of NAFLD and on the disease markers that could be utilized for population screening by primary health care providers. They were, in fact, able to show that such predictors of NAFLD severity as higher steatosis, advanced liver fibrosis and PNPLA3 I148M, predicted both liver-related and all-cause mortality in the general U.S. population, independently from other relevant covariates like body mass index, hypertension and alcohol intake, whereas the 148M allele was not associated with cardiovascular disease mortality (Unalp-Arida & Ruhl, 2019).

The first phase of the study included 13,298 viral hepatitis negative adults who were a representative sample of non institutionalized population in the NHANES III. Between 1988 and 1994, they underwent a structured interview, laboratory testing and examination with liver fat and fibrosis scores thought to predict diagnosis and staging of NAFLD. In the second phase of the study, PNPLA3 I148M was genotyped in the stored DNA of a subset of 5640 participants recruited from 1991 to 1994.

Mortality data were collected during a follow-up to the end of 2015, showing a cumulative total mortality at 27 years of 33.2% (5570 deaths). Based on underlying or contributing cause, mortality was 14.6% (2200 deaths) from cardiovascular disease and 1.1% (196 deaths) from liver disease, including 16 deaths from primary liver cancer. With full adjustment, there was a direct relation between liver disease mortality and severity of NAFLD fat score, with hazard ratios (HR) of 2.84 for intermediate scores and 7.50 for high scores, whereas a high NAFLD fibrosis score was associated with over 6 times the risk (HR, 6.68; 95% confidence interval, CI, 2.62-17.03). These scores were also directly associated to cardiovascular disease mortality. All-cause mortality increased by 16% and 63% respectively with intermediate and high NAFLD liver fat scores and by 53% with high NAFLD fibrosis scores. In addition, among the subset of 5640 subjects providing genetic data, liver disease mortality was over 8 times as high with two 148M alleles (HR, 8.61; 95% CI, 3.28-22.60) in an analysis adjusted for age, sex, and race-ethnicity and based, overall, on 84 deaths from liver disease. In the population under evaluation, the high liver fat score alone explained about 25%, and the combination of intermediate and high scores more than 50% of liver disease mortality. In addition, these explained over 12% of cardiovascular

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disease mortality, and have consequently a relevant impact on total mortality. As for the fibrosis score, intermediate and high scores account for 27% of deaths from liver disease and about 10% of deaths from cardiovascular disease.

Owing to the lack of specific guidelines of scientific societies, the authors recommend that the above described genetic and clinical markers of disease severity be utilized for lifetime patient surveillance for NAFLD by primary care providers, who will therefore be able to prioritize counselling and treatment of patients at higher risk of complications.

Comment

Our understanding of the natural history of NAFLD has long be hampered by the complex clinical profile of the NAFLD patients resulting from highly variable and fluid evolution of the disease, which is bidirectionally modified by both endogenous and exogenous comorbidities (Rinella *et al*, 2019). Based on cohort studies, it remains unclear whether NAFLD is an independent risk factor for mortality beyond its association with comorbidities like obesity and metabolic syndrome. Those studies, in fact, have shown liver-related mortality to be most pronounced in patients with NASH and advanced fibrosis, whereas the leading causes of death in pre-cirrhotic NAFLD patients are cardiovascular disease (38%) and extrahepatic malignancy (19%), with liver-related death representing 9% of cases, only (Angulo *et al*, 2015). Robust data at population level are lacking, and are influenced by determinants of NAFLD, besides age (Allen *et al*, 2018). In the light of this, Unalp-Arida and Ruhl should be commended for having attempted to fill the knowledge gap with respect to the association between NAFLD and all-cause mortality, by linking the NHANES III database with national mortality data.

This study has several strengths, including the use of a record-linkage design based on routinely available data to derive scores of NAFLD liver fat and fibrosis, which showed strong associations with liver disease, cardiovascular, and consequently total mortality.

Of interest, these scores were independent from fasting time for their fasting components, such as plasma glycemia or serum insulin, supporting the validity of their adoption and use. In addition, inclusion of alcohol, tobacco consumption and total cholesterol in the fully adjusted model did not materially modify any of the results, thus indicating that confounding is unlikely to have had a relevant role in any of the associations. However, owing to the well-established fact that alcohol consumption is underreported on a population level (Parish *et al*, 2017), the definition of NAFLD may include a proportion of alcohol-related liver diseases.

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On top of this, the study has other possible limitations including the reliance on a single collection of data on serum markers and clinical/environmental features, as properly acknowledged by the authors, and the lack of information on drug use, mainly aspirin and statins, which are commonly used for cardiovascular disease prevention and have been favorably related to all-cause mortality and incidence of liver cancer (Tran *et al*, 2020; Wang *et al*, 2019). Treatment with statins improves NAFLD/NASH and reduces cardiovascular morbidity and mortality (Athyros *et al*, 2017), and aspirin use has been associated to a reduced risk of fibrosis progression in patients with NAFLD and hepatocellular carcinoma risk (Simon *et al*, 2019).

The statement that 148M was strongly related to liver disease mortality - but not to cardiovascular one - merits attention given the small number of liver disease deaths. Indeed, there were only 29 liver disease deaths versus about 4 expected, i.e. an excess of 25 deaths in the genotyped subsample out of a total of 1903 deaths, indicating that the impact of 148M is relevant and strong on liver disease mortality while accounting for little over 1% of all deaths. A few dozen additional deaths may have been related to I148M, though its association with liver disease mortality was not significant.

In the end, owing to the small number of liver related deaths (84 in the genotyped subsample) it was not possible to investigate the gene-environment interaction between 148M and NAFLD score, which theoretically is of interest. Another point that deserves attention is the increase – from 8.6 to over 17 - in the HR of liver disease mortality associated to 148M when excluding from the analyses subjects with fasting time <4 hours or positive to viral hepatitis (from 5640 to 5037 subjects). Along this line, the strength of the association between scores of liver fat or fibrosis and mortality from liver disease increases when analyzing the sample of participants with both environmental and genetic markers, with HRs of 2-4 for the intermediate and of 12-13 for the high scores categories, after adjustment for the PNPLA3 I148M genotype and selected demographic and lifestyle characteristics.

Despite these observations and the large confidence intervals, which limit the interpretation of the point estimates and hence quantification of the associations, this study is impactful from a public health perspective, since it shows and quantifies that simple NAFLD scores of liver fat and fibrosis, applicable to large population surveys such as the NHANES III, are strong indicators of liver disease.

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