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A non-invasive prediction model for non-alcoholic steatohepatitis in paediatric patients with non-alcoholic fatty liver disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease encompasses a spectrum of diseases that range from simple steatosis to the aggressive form of non-alcoholic steatohepatitis. Non-alcoholic steatohepatitis is currently diagnosed through liver biopsy.
Aim: To develop a non-invasive predictive model of non-alcoholic steatohepatitis in children with non-alcoholic fatty liver disease.
Methods: Anthropometric, laboratory, and histologic data were obtained in a cohort of children with biopsy-proven non-alcoholic fatty liver disease. Multivariable logistic regression analysis was employed to create a nomogram predicting the risk of non-alcoholic steatohepatitis. Internal validation was performed by bootstrapping.
Results: Three hundred and two children were included in this analysis with a mean age of 12.3 ± 3.1 years, a mean body mass index percentile of 94.3 ± 6.9, and non-alcoholic steatohepatitis was present in 67%. Following stepwise variable selection, total cholesterol, waist circumference percentile, and total bilirubin were included as variables in the model, with good discrimination with an area under the receiver operating characteristic curve of 0.737.
Conclusions: A nomogram was constructed with reasonable accuracy that can predict the risk of non-alcoholic steatohepatitis in children with non-alcoholic fatty liver disease. If validated externally, this tool could be utilized as a non-invasive method to diagnose non-alcoholic steatohepatitis in children with non-alcoholic fatty liver disease.

1. Introduction

The incidence of non-alcoholic fatty liver disease (NAFLD) has been increasing, and it has now become the most common cause of chronic liver disease in the paediatric population [1,2]. NAFLD encompasses a spectrum of diseases that can range from simple steatosis to non-alcoholic steatohepatitis (NASH), of which NASH can ultimately progress to cirrhosis and even end-stage liver disease [3]. Furthermore, children with NASH may benefit from NASH-specific therapy that may include vitamin E, metformin, and newer agents that are being tested [4,5]. It is therefore important to be able to identify those patients with NASH.

At the present time, there are no clinically available biomarkers to reliably differentiate between steatosis and NASH, and instead this differentiation is made through histologic evaluation by liver biopsy. There are drawbacks to liver biopsy, however, which include sampling error, variability in histopathology interpretation by a pathologist, inadequate biopsy size, cost, and associated morbidity. Liver biopsy is an invasive diagnostic procedure with its own inherent risks that include pain, bleeding, and even other organ perforation. Serious complications with percutaneous liver biopsy have been found in 0.3% of cases, with a reported mortality rate of 0.01% [6]. Given these inherent drawbacks to liver biopsies, an ideal test would be a non-invasive test that is reproducible, simple, readily available, and less costly. In our current study, we have developed a nomogram that utilizes clinical variables and routine

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laboratory tests to predict the risk of NASH in paediatric patients with NAFLD.

2. Materials and methods

2.1. Patient population

Between January 2003 and December 2009 at the Bambino Gesù Children's Hospital (Rome, Italy), paediatric patients with biopsy proven NAFLD were seen and enrolled in this study. The study was carefully explained to the patients' guardians and written consent was obtained for all patients. This study was approved by the ethics committee at the Bambino Gesù Children's Hospital and Research Institute.

To be included in the study, subjects had characteristics suspicious for NAFLD based on persistently elevated serum aminotransferase levels and imaging studies that were suspicious for fatty liver with a diffusely hyperechogenic liver, with a final diagnosis made on liver biopsy of NAFLD. For patients with a diagnosis of NAFLD, they were excluded if they had any of the following: (1) hepatic viral infections (such as hepatitis A, B, C, D, and E; cytomegalovirus; and Epstein–Barr virus); (2) alcohol consumption; (3) use of drugs that are known to induce steatosis (e.g.: valproate, prednisone, or amiodarone), affect carbohydrate metabolism or body weight; (4) history of parental nutrition; and (5) known liver disease, such as autoimmune hepatitis, metabolic liver disease, Wilson's disease, and alpha-1-antitrypsin associated liver disease. These were ruled out using standard laboratory, clinical, and/or histologic criteria.

2.2. Patient characteristics

Patient clinical variables were recorded, which included standard height and weight, as well as the body mass index (BMI) and *Z*-score, which were calculated [7]. Patients with a BMI greater than or equal to 95th percentile adjusted for age and sex were defined as obese. The waist circumference (WC) of patients was measured at the highest point of the iliac crest with a standing subject [8]. A WC to height ratio was also calculated.

Data collection in patients included laboratory evaluation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum gamma-glutamyltransferase (GGT), total bilirubin, albumin, INR, total cholesterol, HDL cholesterol, and triglycerides. This was obtained utilizing standard laboratory methods.

Metabolic syndrome in this patient population was present if there were three of more of the following: (1) impaired fasting glucose ($\geq$110 mg/dL), impaired glucose tolerance, or known type 2 diabetes mellitus [9]; (2) hypertriglyceridemia, defined as triglyceride level >95th percentile for age and sex [10]; (3) low HDL-cholesterol, defined as concentrations <5th percentile for age and sex [10]; (4) hypertension, defined as systolic or diastolic blood pressures >95th percentile for age and sex [11]; and (5) abdominal obesity, defined as WC $\geq$90th percentile for age [12].

2.3. Liver histology

Liver biopsy was performed in all patients who met criteria because of an indication to assess for the presence of NASH, degree of fibrosis, and/or to identify if other liver diseases were present. After fasting overnight and under general anaesthesia, a liver biopsy was performed utilizing ultrasound guidance and the automatic core biopsy 18 gauge needle (Biopince, Amedic, Sweden). The Sonoline Omnia ultrasound machine (Siemens, Munich, Germany) was used, which is equipped with a 5-MHz probe (5.0C 50, Siemens) and biopsy adaptor. For each patient, two biopsy passes in different liver segments were made, and the length of the biopsy specimen was recorded in millimetres. Only samples that included at least 5–6 complete portal tracts and had a minimal length of $\geq$15 mm met the requirements for this study [13]. Biopsies were processed and routine staining of liver tissue included haematoxylin-eosin, Periodic acid-Schiff diastase, Van Gieson, and Prussian blue stain. A single hepatopathologist was blinded to the clinical and laboratory data and reviewed the biopsies.

A single expert paediatric hepatopathologist reviewed the biopsies and established the histopathological diagnosis of NASH. Based on this diagnosis, patients were divided into two groups: (1) "NASH" or (2) diagnosis not compatible with NASH or "not NASH". Liver histology was scored using the NAFLD activity scoring (NAS) system developed by the NASH Clinical Research Network [14]. The grade of steatosis (0–3), hepatocyte ballooning (0–2), and lobular inflammation (0–3) were added together to determine the NAS score (0–8). Portal inflammation (PI) was graded from 0 to 2 (0 = no PI, 1 = mild PI, and 2 = more than mild PI). Fibrosis was staged as the following: 0 = no fibrosis, 1 = periportal or perisinusoidal, 2 = perisinusoidal and portal/periportal fibrosis, 3 = bridging fibrosis, and 4 = cirrhosis.

2.4. Statistical analysis

Descriptive statistics were computed for all variables. These include means, standard deviations and percentiles for continuous variables and frequencies and percentages for categorical factors. A univariate analysis was done to assess differences between subjects with and without NASH; Student's *t*-tests or the non-parametric Wilcoxon rank sum tests were used to compare continuous and ordinal factors and Pearson's chi-square tests were used to compare categorical variables. Multivariable logistic regression analysis was performed to build a model for prediction of NASH. An automated stepwise variable selection method performed on 1000 bootstrap samples was used to choose the final model. All non-invasive factors were assessed and variables with inclusion rates of 30% or more were included in the final model. Bilirubin was modelled with restricted cubic spline to relax linearity assumptions and an inverse transformation for waist circumference percentile ((1/WC percentile) * 1000) was used.

Discrimination and calibration for model performance were used for internal model validation. Discrimination is the ability to rank patients by risk of NASH such that patients with a higher predicted risk are more likely to have NASH. Discrimination was measured by the area under the receiver operating characteristics curve (AUROC). Calibration refers to the accuracy of prediction of the model compared to observed outcome in our dataset; this was assessed by constructing a calibration curve (a 45° curve would represent perfect prediction). The method described by Harrell et al. was used to compute the validation metrics with over-fitting bias corrected through bootstrap resampling [15]. A thousand bootstrap samples (B = 1000) were drawn from the original data set and a new model with the same model settings was built on each bootstrap resample. Prediction on patients that were not chosen in the resample was calculated. An optimism factor was calculated over the 1000 new models and the bias-corrected validation metric was obtained by subtracting this optimism value from the validation metric directly measured from the original model. A p value <0.05 was considered statistically significant. All analyses were performed using SAS (version 9.2, The SAS Institute, Cary, NC) and R (version 2.13.1, The R Foundation for Statistical Computing, Vienna, Austria; packages used: Design and ROCR).