Research Article

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FibroGENE: A gene-based model for staging liver fibrosis☆

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Background & Aims: The extent of liver fibrosis predicts long-term outcomes, and hence impacts management and therapy. We developed a non-invasive algorithm to stage fibrosis using non-parametric, machine learning methods designed for predictive modeling, and incorporated an invariant genetic marker of liver fibrosis risk.

Methods: Of 4277 patients with chronic liver disease, 1992 with chronic hepatitis C (derivation cohort) were analyzed to develop the model, and subsequently validated in an independent cohort of 1242 patients. The model was assessed in cohorts with chronic hepatitis B (CHB) (n = 555) and non-alcoholic fatty liver disease (NAFLD) (n = 488). Model performance was compared to FIB-4 and APRI, and also to the NAFLD fibrosis score (NFS) and Forns' index, in those with NAFLD.

Results: Significant fibrosis (⩾F2) was similar in the derivation (48.4%) and validation (47.4%) cohorts. The FibroGENE-DT yielded the area under the receiver operating characteristic curve (AUROCs) of 0.87, 0.85 and 0.804 for the prediction of fast fibrosis progression, cirrhosis and significant fibrosis risk, respectively, with comparable results in the validation cohort. The model

Keywords: Chronic hepatitis C; Chronic hepatitis B; Non-alcoholic steatohepatitis; NASH; IFNL; Fibrosis; Data mining analysis.
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performed well in NAFLD and CHB with AUROCs of 0.791, and 0.726, respectively. The negative predictive value to exclude cirrhosis was >0.96 in all three liver diseases. The AUROC of the FibroGENE-DT performed better than FIB-4, APRI, and NFS and Forns' index in most comparisons.

Conclusion: A non-invasive decision tree model can predict liver fibrosis risk and aid decision making.

Introduction

Irrespective of the underlying disease, hepatic fibrosis culminating in cirrhosis is the principal cause of chronic liver disease related morbidity and mortality [1]. Among such diseases, chronic hepatitis B (CHB), chronic hepatitis C (CHC) and non-alcoholic fatty liver disease (NAFLD) are the most common worldwide [2,3].

Studies over several decades have established that the severity of hepatic fibrosis affects long-term outcomes, and hence, clinical management and treatment, as reviewed elsewhere [4]. For example, the complications of advanced fibrosis such as portal hypertension, ascites and hepatocellular carcinoma are all associated with reduced survival [4]. Thus, clinical decision making is to a large extent based on accurate staging of liver fibrosis. Once this has been established, clinicians typically also consider baseline predictors of fibrosis progression to come to a personalized management algorithm.

Although invasive, limited by sampling error and inter-observer variability, liver biopsy is still considered the 'gold standard' for the assessment of liver disease stage [5–7]. At present, several non-invasive methods for the assessment of liver fibrosis based on panels of serum markers, or the measurement of liver stiffness by elastography, are widely used as surrogate measures. However, these methods are also not free of limitations. For example, biomarker panels fluctuate during concurrent illnesses, reproducibility is a concern, and many components of such panels do not directly reflect the underlying disease process [8,9]. Likewise, transient elastography (TE) is limited by reduced performance in obese patients, and interference by concomitant inflammation and steatosis [10,11]. These concerns about reproducibility and over- or under-estimation of fibrosis stage are even more problematic for non-CHC diseases such as CHB [12,13] and NAFLD [14,15].

In this context, incorporation of an invariant genetic marker of liver fibrosis risk to algorithms for fibrosis prediction could be useful, but has not previously been described. We recently reported that single-nucleotide polymorphisms (rs12979860) in the intronic region of the interferon-λ4 (*IFNL4*) gene modulate liver inflammation and fibrosis, in an etiology independent manner [16]. Thus, it could be hypothesized that this polymorphism, a test for which is widely used and commonly available, might have a role in algorithms that predict fibrosis. We sought to apply an unbiased data mining approach to build a novel *IFNL*-dependent fibrosis prediction algorithm, in combination with routinely available clinical and laboratory data. We envisaged that the algorithm could be utilized in clinical practice to assess the risk for significant fibrosis and to aid in decision making. We compared our model to two existing non-invasive indices, FIB-4 and AST (aspartate aminotransferase) to platelet ratio index (APRI), and in the cohort with NAFLD, also to the NAFLD fibrosis score (NFS) and the Forns' index that does not require additional laboratory testing.

Methods

Patient cohort

The cohort comprised 4277 patients with CHC, CHB and NAFLD accrued from contributing centers to the International Liver Disease Genetics Consortium (ILDGC). The ILDGC included patients from 20 tertiary and academic centers from seven countries (Australia, the United Kingdom, Italy, Germany, Spain, Egypt and Hong Kong). The details of the cohort and inclusion criteria have been reported previously [16]. Briefly, for patients with CHC, all consecutive patients who had a liver biopsy before antiviral therapy, with scoring for fibrosis stage and disease activity between 1999 and 2011, were included. Patients were excluded if they had evidence of other liver diseases by standard tests.

For the current analysis, predictors of significant fibrosis were determined from an initial derivation cohort with hepatitis C (n = 1992). These data were subject to several machine learning techniques to develop the best model for the prediction of significant fibrosis (F2/3/4 by METAVIR) [17]. For independent validation, a further 1242 patients with CHC were enrolled from two additional centers.

Based on our recent hypothesis that IFNL genotype is a core variant associated with liver fibrosis independent of disease etiology [16], we tested the validity of the model in 555 patients with CHB and 488 with NAFLD.

Ethics approval was obtained from the Human Research Ethics Committees of the Sydney West Local Health District and the University of Sydney. All other sites had ethics approval from their respective ethics committees. Written informed consent for genetic testing was obtained from all participants. The study was conducted in accordance with ethical guidelines of the International Conference on Harmonization Guidelines for Good Clinical Practice [18].

Clinical and laboratory assessment

The following data were collected at time of liver biopsy for all patients: gender, age, ethnicity, recruitment center, alcohol intake, body mass index (BMI), and routine laboratory tests. Alcohol consumption was assessed by two separate interviews with the patient and close family members. BMI was calculated as weight divided by the square of the height (kg/m^2). Data on cardio-metabolic status was collected for the non-alcoholic steatohepatitis (NASH) cohort and considered as variables in the construction of models for prediction of fibrosis. The diagnosis of arterial hypertension was according to standard international criteria [19].

The FibroGENE-DT model we developed was compared to four popular non-invasive indexes that do not require additional laboratory testing. The APRI was calculated according to the formula: (AST (IU/L)/upper normal limit) × 100/platelets (10^9/L)) [20]. The FIB-4 index was calculated as follows: age (years) × AST (IU/L)/(platelets (10^9/L) × (alanine aminotransferase: ALT(IU/L))^{½} [21]. In the NAFLD cohort, we additionally compared the decision tree model to the widely used NAFLD fibrosis score (NAFLD-FS) [22] calculated as: −1.675 + 0.037 × age (years) + 0.094 × BMI + 1.13 × hyperglycemia or diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet (×10^9/L) − 0.66 × albumin (g/dL) and the Forns' index calculated according to the formula: 7.811 − 3.131.ln (platelet count) + 0.781.ln (gamma-glutamyl transferase: GGT) + 3.467.ln (age) − 0.014.(cholesterol) [23]. The values for the upper limit of normal for AST were set according to the International Federation of Clinical Chemistry, that is, 35 U/L for men and 30 U/L for women [24]. The values for the upper limit of normal for ALT were 19 U/L in women and 30 U/L in men [24]. The fibrosis risk stratifications for each score were created based on the two cut-offs, as described in the original publications. They are 0.5 and 1.5 for the APRI [20]; 1.30 and 2.67 for the FIB-4 score [21] and − 1.455 and 0.676 for the NAFLD-FS [22] and Forns' index <4.2.

Genotyping

Genotyping for *IFNL* single nucleotide polymorphisms was undertaken using the TaqMan SNP genotyping allelic discrimination method (Applied Biosystems, Foster City, CA, USA). Detailed procedures have been described previously [25]. All genotyping was blinded to clinical variables.

Liver histopathology

Liver histopathology for patients with CHC and CHB was scored according to METAVIR [17]. Fibrosis was staged from F0 (no fibrosis) to F4 (cirrhosis). Necroinflammation (A) was graded as A0 (absent), A1 (mild), A2 (moderate), or A3 (severe). For NAFLD, the Kleiner classification was used [26]. Biopsies were interpreted by a single expert liver pathologist in each center who was blinded to patient clinical characteristics, serum measurements and genotyping. All biopsies had a minimum of 11 portal tracts, and inadequate biopsies were excluded. The inter-observer agreement between pathologists was studied previously and was good (κ = 77.5) for METAVIR staging using κ statistics [25].

Statistical analysis

Statistical analyses were performed using the statistical software package SPSS for Windows, version 21 (SPSS, Chicago, IL), SAS version 9.1 and SAS Enterprise 9.4 (SAS Institute Inc., Cary, NC, USA).

Results are expressed as mean ± SD (standard deviation) or number (percentage) of patients. The Student's *t* test or non-parametric, i.e. Wilcoxon-Mann-Whitney *U* test or Kruskal-Wallis tests were used to compare quantitative data, as appropriate. X^2 and Fisher exact tests were used for comparison of frequency data and to evaluate the relationships between groups. All tests were two-tailed and *p* values <0.05 were considered significant. Fibrosis progression rate (FPR) was calculated by taking the ratio between the fibrosis stage and the estimated disease duration (in years). Patients were stratified into two groups of stage-constant FPR, according to the median rate (0.076 fibrosis units/year) which was used as a cut-off [16].

Development of predictive models using data mining analysis

For the formulation of predictive models, a search for the optimal models was conducted by applying several machine learning techniques, including Multivariate Logistic Regression Analysis (MLRA), Artificial Neural Network (ANN), Decision Tree (DT) and Nearest Neighborhood (KNN) [27–29]. These statistical methods have been described previously in the field of hepatic diseases [30–32]. The performance of these techniques was compared.

The Dempster-Shafer method [33] which combines the performance of more than one classifier (MLRA, ANN, DT, KNN) was also investigated for its ability to improve the recognition accuracy of the prediction model. To do so, those variables showing a *p* <0.05 at univariate analysis (Student *t* test for parametric variables, and X^2 or Fisher exact test for frequencies) were included in building the model. The interaction between these variables was tested. Variables explaining a statistically significant proportion of the variance (*p* <0.05) were maintained in the model using the likelihood ratio (LR) test. The model variables were selected using the leave-one-out method to facilitate the calculation of over-fit bias reduced estimates [35]. To avoid over-fitting, 10-fold cross validations were used in the tree building process.

The discriminative ability of the different classifier techniques for the identification of significant fibrosis ($\geqslant$F2), cirrhosis (F4), and fast FPR ($\geqslant$0.076 fibrosis units/year) was assessed by means of receiver operating characteristic curve analysis and expressed as area under the receiver operating characteristic curve (AUROC). Estimates of AUROCs and comparisons between AUROCs used the empirical (non-parametric) method of DeLong *et al.* [34]. In a further analysis, the discriminative ability of the generated DT for the identification of patients classed as slow progressors, intermediate progressors, or rapid progressors according to the tertiles of rate of fibrosis progression was also assessed.

External validation was conducted in an independent validation cohort with CHC (n = 1242). To ascertain that the validation cohort is well powered to detect a statistically significant difference in model performance considering the number of events (significant fibrosis), sample size estimation was calculated with standard formulas based on the normal distribution [36]. A sample size of n = 1066 was required to achieve 80% power, with 95% confidence interval (CI). Some of the characteristics of the validation population were different from those of the derivation population. Hence, to ensure robustness of the model, randomly selected sub-cohorts were generated and this process was repeated 10 times; the average of AUROCs in each sub-cohort is reported.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratio (LHR^+, LHR^-) and 95% CIs were calculated.

To overcome both spectrum effect and ordinal scale issues, we undertook two approaches [37,38]. Firstly, we used the Obuchowski measure proposed by Lambert *et al.* [37], which is essentially a multinomial version of the AUROC. Each pairwise comparison is weighted to take into account the distance between fibrosis stages (i.e., the number of units on the ordinal scale). A penalty function proportional to the difference in METAVIR units between stages was defined: the penalty function was 0.25 when the difference between stages was 1, 0.50 when the difference was 2 and 1 when the difference was 3. The Obuchowski measure can be interpreted as the probability that the non-invasive index will correctly rank two randomly chosen patient samples from different fibrosis stages according to the weighting scheme, with a penalty for misclassifying patients. In the second method, we standardized the AUROC for the distribution of fibrosis stages as proposed by Poynard *et al.* [38]. Briefly, the DANA (Difference between advanced and non-advanced fibrosis = [(prevalence F2 $\times$ 2 + prevalence F3 $\times$ 3 + prevalence F4 $\times$ 4)/(prevalence F2 + prevalence F3 + prevalence F4)] − [prevalence F1/(prevalence F0 + prevalence F1)]) was calculated [39] and the adjusted AUROCs determined as follows: AdjAUROC = obAUROC + (0.1056) (2.5 − DANA).

The prognostic significance of the new model was evaluated by univariate analysis followed by multivariate prognostic analysis using the stepwise Cox proportional hazard regression model in the sub-cohort of patients with an estimated duration of the infection.

The missing data on predictive factors were filled in to exclude potential bias derived from missing data by multiple imputation using a stochastic switching regression approach with five repeated imputations [40]. Imputation was performed separately on the derivation cohort and the validation cohort, with the predictor variables chosen using the same strategy [40]. Each of the imputed sets was then analyzed as if it were complete, and the results pooled by the method presented by Rubin [41] to create inferences that validly reflects sampling variability as a result of imputation. The imputation method we used is well documented and accepted [40,41].

Results

Patients' characteristics

Baseline characteristics of the 1992 CHC patients used to develop the model (derivation cohort) and the 1242 used to test the model (validation cohort) are shown in Table 1. The derivation and validation cohorts were similar with respect to age at time of biopsy, sex, BMI and HCV RNA levels, while platelet counts were lower in the validation cohort. The distribution of the *IFNL rs12979860* responder genotype (CC), daily alcohol consumption over 50 g and moderate/severe steatosis were significantly lower in the validation cohort and they had a lower ALT and AST (Table 1). The prevalence of significant fibrosis was similar between the two cohorts (Table 1). The distribution of HCV genotype 1 was the same in the derivation and validation cohorts, while the derivation cohort had more HCV genotype 2 and the validation cohort more HCV genotype 4.

An IFNL genotype based fibrosis prediction model using data mining analysis

Based on our previous finding that IFNL genotype is associated with fibrosis [16], we sought to build a model for the prediction of significant fibrosis based on IFNL genotype, age, gender and routinely assessed clinical and laboratory variables. Using data mining analysis, four classifiers MLRA, ANN, DT and KNN, and the combination of more than one classifier were investigated in a systematic unbiased approach. The diagnostic accuracy of the four classifiers (MLRA, ANN, KNN and DT) to distinguish between patients in the derivation cohort with and without significant fibrosis ($\geqslant$F2), as indicated by AUROCs, was 0.767, 0.774, 0.768 and 0.804 respectively. The sensitivity, specificity, PPV, NPV, LHR + and LHR- of all models for prediction of significant fibrosis ($\geqslant$F2) are summarized in Supplementary Table 1.

In view of the apparent superiority of the decision tree FibroGENE-DT, compared to other classifiers and its simplicity, this was used in subsequent analysis (Fig. 1A).

Table 1. Demographic and clinical characteristics of the derivation (n = 1992) and validation (n = 1242) cohort of patients with chronic hepatitis C.

Variables	Initial cohort (n = 1992)	Replication cohort (n = 1242)	Overall (n = 3234)	*p* value initial *vs.* replication
Age (years)	44.08 ± 10.7 44.1 (18-69)	44.7 ± 10.9 44 (18-62)	44.38 ± 10.7 44 (18-69)	0.1
Male (%)	1251 (62.8)	815 (65.6)	2066 (63.9)	0.1
Caucasian (%)	1827 (91.7)	1057 (85.1)	2884 (89.1)	<0.0001
BMI (kg/m^2)	26.4 ± 4.9 25.7 (16-45)	26.1 ± 4.31 25.6 (17-46)	26.3 ± 4.65 25.7 (16-46)	0.1
ALT (IU/L)	112.6 ± 89.9 83.2 (12-709)	86.83 ± 79.02 65 (14-864)	101 ± 82.13 72 (12-864)	<0.0001
AST (IU/L)	77.5 ± 60.1 59 (11-490.5)	69.8 ± 57 53 (14-678)	72.7 ± 59.55 54 (11-678)	0.002
GGT (IU/L)	77. 9 ± 65.6 50 (7-683)	75 ± 62.1 50 (6-688)	78.21 ± 61.82 50 (6-688)	0.2
Platelet (x10^9/L)	226.7 ± 68.6 217 (56-577)	206.05 ± 69.8 203 (54-503)	215.77 ± 69.97 211 (54-577)	<0.0001
HCV-RNA $\log_{10}$	5.9 ± 0.7 5.87 (2.38-7.95)	5.9 ± 0.7 5.91 (2.40-7.95)	5.92 ± 0.76 5.92 (2.38-7.95)	0.1
HCV-genotype (%) 1, 2, 3, 4, 6	1370 (68.8), 210 (10.5), 374 (18.8), 33 (1.7), 5 (0.3)	789 (63.5), 32 (2.6), 162(13), 258 (20.8), 1 (0.1)	2159 (66.8), 242 (7.5), 536 (16.5), 318 (9.8), 6 (0.1)	0.05
Liver fibrosis (%)				
F0	264 (13.3)	152 (12.23)	416 (12.9)	0.08
F1	762 (38.3)	531 (42.77)	1293 (39.9)	
F2	518 (25.9)	312 (25.12)	830 (25.7)	
F3	248 (12.45)	126 (10.14)	374 (11.6)	
F4	200 (10.05)	121 (9.74)	321 (9.9)	
Inflammation score (%)				
A0	75 (3.77)	49 (3.9)	124 (3.83)	0.057
A1	1048 (52.61)	698 (56.3)	1746 (54)	
A2	707 (35.49)	383 (30.8)	1090 (33.7)	
A3	162 (8.13)	112 (9)	274 (8.47)	
IFNL3 *rs12979860* (%)				
CC	807 (40.51)	409 (33)	1216 (37.6)	<0.0001
CT	947 (47.54)	630 (50.7)	1577 (48.8)	
TT	238 (11.95)	203 (16.3)	441 (13.6)	
Alcohol history (%)				
None or less than 50 g/daily	1471 (73.8)	1242 (100)	2713 (83.88)	<0.0001
≥50 g/daily	521 (26.2)	0 (0)	521 (16.12)	
Steatosis degree (%)				
None/mild	1756 (88.2)	933 (75.1)	2689 (83.1)	<0.0001
Moderate severe	236 (11.8)	309 (24.9)	545 (16.9)	

*Data are mean ± standard deviation, median and range or as %. Liver biopsy data are according to METAVIR score.

Fig. 2 depicts the final tree generated by classification and regression tree (CART) analysis along with the significant fibrosis data for each node of this tree. These branch points permit patient stratification into 9 risk groups, and in a simple 3- to 4-step process. There was high specificity (86%) and PPV (80% certainty of presence of significant fibrosis). The AUROC of the model for prediction of severe fibrosis (⩾F3) was 0.821 (95% CI, 0.78–0.86, $p = 0.0001$), with 70% sensitivity and 90% NPV and for cirrhosis (F4) was 0.85 (95% CI, 0.79–0.87, $p = 0.0001$), with 86% sensitivity and 98% NPV.

Independent validation of the decision tree

To ascertain the validity of our FibroGENE-DT model generated by CART analysis of the derivation cohort, we tested its ability to predict significant fibrosis in an independent validation cohort (n = 1242). The baseline characteristics of the validation cohort are shown in Table 1.

The FibroGENE-DT predicted significant fibrosis with AUROC 0.78 (95% CI: 0.75–0.81) (Supplementary Fig. 1) with 83% specificity and PPV 79%. The AUROCs for the prediction of cirrhosis (F4) was 0.83 (95% CI, 0.7–0.87, $p = 0.0001$), with 82% sensitivity and 96% NPV, reflecting the reliability of the model.

In the joint cohort (n = 3234), the FibroGENE-DT predicted significant fibrosis with AUROC 0.79 (95% CI, 0.76–82, $p = 0.0001$) and the AUROC for the prediction of cirrhosis (F4) was 0.838 (95% CI, 0.782–0.86, $p = 0.0001$) with 84% sensitivity and 96% NPV.

Adjusted AUROCs (Obuchowski) showed similar results (Supplementary Table 2). The sensitivity, specificity, PPV, NPV, LHR+ and LHR- of FibroGENE-DT for the prediction of significant fibrosis (⩾F2), severe fibrosis (⩾F3) and cirrhosis (F4) in the

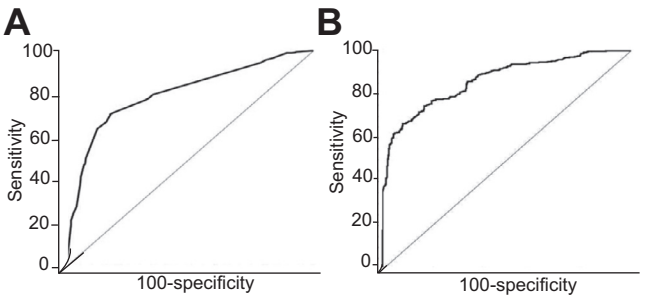

Fig. 1. Receiver operating characteristics (ROC) analysis showing the predictive value of FibroGENE-DT for significant fibrosis (F ⩾2) and for fast fibrosis progression (FPR) in patients with chronic hepatitis C virus infection. (A) ROC curve for FibroGENE-DT in the derivation cohort. Area under the ROC curve (AUROC) = 0.804 (95% confidence interval (CI), 0.77–0.83, $p = 0.0001$). (B) ROC curve showing the predictive value of FibroGENE-DT for fast fibrosis progression in patients with chronic HCV infection. AUROC = 0.869 (95% CI, 0.85–0.89, $p = 0.0001$).

derivation, validation and joint cohort are summarized in Supplementary Table 2.

Some of the characteristics of the validation population were different from those of the derivation population. Hence, to ensure comparability of the two populations, a random distribution was applied. The average of the diagnostic accuracy of the FibroGENE-DT in 10 random sub-cohorts had an AUROC 0.792 (95% CI, 0.763–821, $p = 0.0001$) and the AUROC for the prediction of cirrhosis (F4) was 0.837 (95% CI, 0.788–0.858, $p = 0.0001$). These were virtually identical to the full sample estimate (Table 2).

An IFNL-based fibrosis prediction model predicts fibrosis progression

We next investigated the validity of the FibroGENE-DT model for the prediction of fast FPR in the sub-cohort of 1242 patients with an estimated date of infection, and hence a known duration of infection. Again, the chosen model demonstrated excellent overall performance with AUROC of 0.87 (95% CI: 0.85–0.89, $p = 0.0001$) for the prediction of risk of fast FPR (Fig. 1B). The sensitivity, specificity, PPV, NPV, LHR^+ and LHR^- were 81.89%, 91.35%, 92.89%, 78.90%, 9.47 and 0.20, respectively. The prognostic value of the model was evaluated in our FPR cohort comparing the cumulative significant fibrosis rates in the high and intermediate/low risk groups over time using a Cox regression model. The high risk group compared to the intermediate/low risk group (created by pooling data from the low and intermediate risk groups) had a significantly higher hazard ratio (HR) for significant fibrosis (HR: 3.2, 95% CI: 2.79–4.54, $p = 0.0001$) (Fig. 3). Finally, the FibroGENE-DT model shows an AUROC of 0.847 (95% CI: 0.817–0.875, $p = 0.0001$) for the identification of patients classed as slow progressors, intermediate progressors, or rapid progressors.

Subdividing the overall cohort according to HCV genotype (genotype 3 *vs.* non-3) based on the reported association of genotype 3 with fibrosis [16], the FibroGENE-DT model performance was not significantly different according to HCV genotype (data not shown).

Comparison of the performance of the IFNL fibrosis prediction model with other serum biomarker panels

In both the derivation and validation cohorts, the FibroGENE-DT model was superior ($p < 0.05$, for all comparisons) to two popular scores (FIB-4 and APRI index) for the prediction of significant fibrosis (⩾F2) and severe fibrosis (⩾F3) (Table 2). FibroGENE-DT was superior to APRI for the prediction of cirrhosis in the derivation cohort. It was also significantly superior to FIB-4 in prediction of FPR.

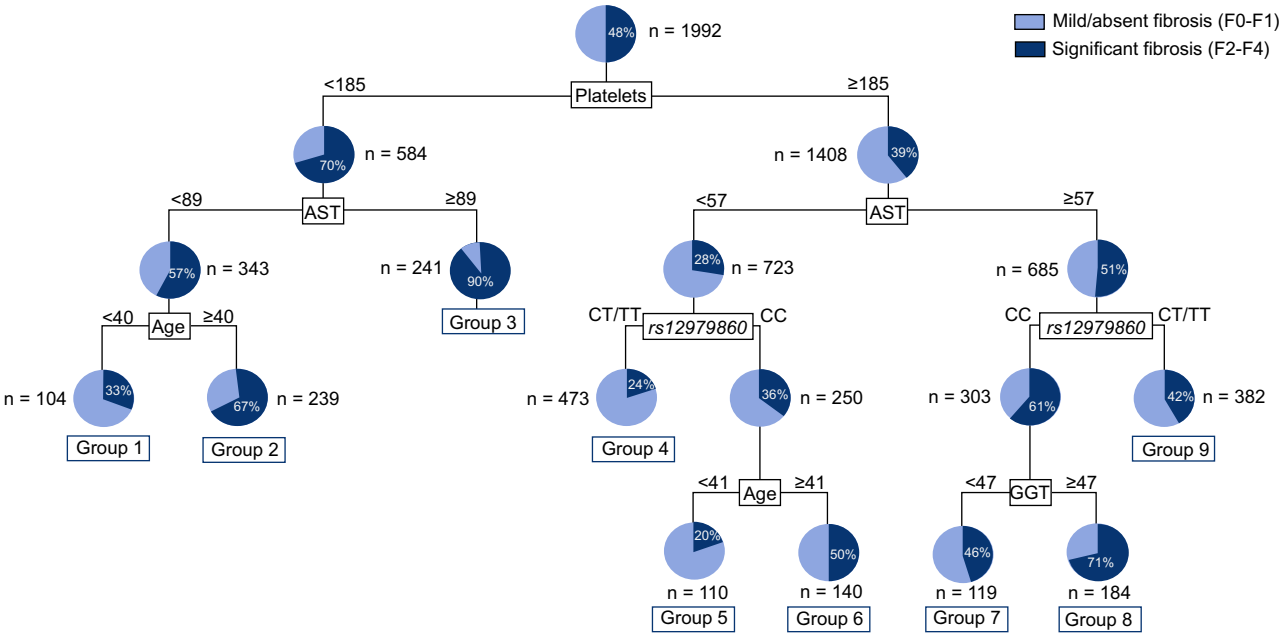

Fig. 2. FibroGENE-DT for the prediction of significant fibrosis in patients with chronic hepatitis C. The boxes indicate the factors used for decision making. Pie charts indicate the rate of significant fibrosis for each group of patients (based on the derivation cohort of n = 1992) after they have been segregated.

Table 2. Areas under the receiver operating characteristics curve (AUROCs) for significant fibrosis (⩾F2), severe fibrosis (⩾F3), cirrhosis (F4) and fast fibrosis progression rate (FPR) for the AST to platelet ratio index (APRI), FIB-4 and the FibroGENE-DT in patients with chronic viral hepatitis C.

	APRI	FIB-4	FibroGENE-DT
Derivation cohort			
≥F2	0.732 (95% CI, 0.692-0.772)	0.747 (95% CI, 0.699−0.790)	0.804 (95% CI, 0.772-0.833)*†
≥F3	0.76 (95% CI, 0.741-0.779)	0.778 (95% CI, 0.75-0.796)	0.821 (95% CI, 0.781-0.862)*†
F4	0.79 (95% CI, 0.757- 0.820)	0.806 (95% CI, 0.774-0.835)	0.845 (95% CI, 0.794-0.871)*
Fast FPR	0.805 (95% CI, 0.784-0.827)	0.779 (95% CI, 0.754-0.81)	0.872 (95% CI, 0.851-0.889)†
Validation cohort			
≥F2	0.75 (95% CI, 0.734-0.805)	0.743 (95% CI, 0.717-0.798)	0.780 (95% CI, 0.754-0.805)*†
≥F3	0.748 (95% CI, 0.709-0.799)	0.75 (95% CI, 0.715-0.783)	0.803 (95% CI, 0.774-0.851)*†
F4	0.812 (95% CI, 0.766-0.860)	0.809 (95% CI, 0.787- 0.857)	0.829 (95% CI, 0.784- 0.867)
Fast FPR	0.801 (95% CI, 0.778-0.822)	0.775 (95% CI, 0.751-0.80)	0.866 (95% CI, 0.845-0.886)†
Joint cohort			
≥F2	0.74 (95% CI, 0.73-0.78)	0.75 (95% CI, 0.69-0.80)	0.79 (95% CI, 0.76-82)*†
≥F3	0.761 (95% CI, 0.75-0.78)	0.775 (95% CI, 0.76-0.789)	0.816 (95% CI, 0.793-0.858)*†
F4	0.80 (95% CI, 0.78-0.85)	0.81 (95% CI, 0.79-0.83)	0.838 (95% CI, 0.782-0.86)*
Fast FPR	0.803 (95% CI, 0.780-0.825)	0.777 (95% CI, 0.753-0.80)	0.869 (95% CI, 0.849-0.887)†

Data are presented as AUROCs (95% confidence interval), *p <0.05 compared to APRI; †p <0.05 compared to FIB-4, #p <0.05 compared to NAFLD-FS, ¶p <0.05 compared to Forns' index.

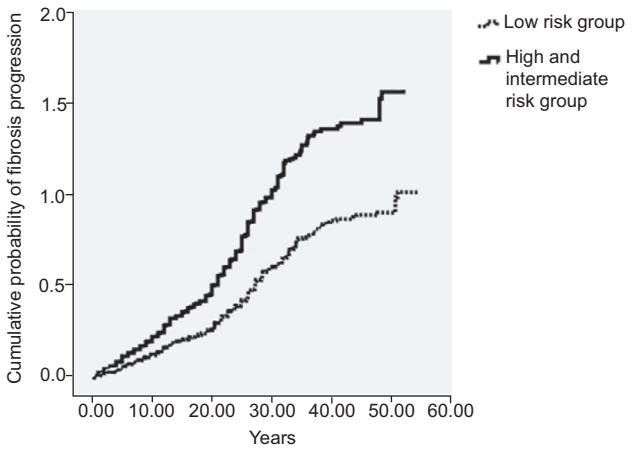

Fig. 3. Cumulative probability of progression to moderate/severe (⩾F2 fibrosis) in 1242 patients with a known duration of HCV infection in specific subgroups of patients defined by the decision tree model. The high and intermediate risk group created by pooling the data from the high and intermediate risk groups have a significantly higher cumulative probability of progression to moderate/severe (⩾F2) than the low risk group (HR: 3.2, 95% CI: 2.79–4.54, p = 0.0001).

Performance of the model in other liver diseases

Hepatitis B

The FibroGENE-DT-model performed reasonably well in the cohort with CHB with AUROCs of 0.726 (95% CI: 0.681–0.767) for prediction of severe fibrosis (F3–F4) (Supplementary Fig. 2), which was higher than the AUC for FIB-4 and APRI indexes (0.655 and 0.705, p <0.05 with FIB-4). The AUROC of 0.819 (95% CI: 0.785–0.851) for cirrhosis (F4) was also higher than the AUROC for FIB-4 and APRI indexes (0.724, 0.786, p <0.01, respectively). Adjusted AUROCs (Obuchowski) showed similar results (Supplementary Table 3). The FibroGENE-DT model had 79% and 92% sensitivity and 86% and 99% NPV for severe fibrosis and cirrhosis, respectively.

NAFLD

We used the same approach for the development of a predictive score in NAFLD as described above for viral hepatitis. The FibroGENE-DT-metabolic model incorporated HOMA-IR and thus included IFNL genotype, HOMA-IR, GGT, AST, ALT and platelets (Supplementary Fig. 3A). The AUROCs of the FibroGENE-DT-metabolic model for prediction of significant fibrosis (F2-4) was 0.791, 95% CI: 0.749–0.828, p = 0.0001 (Supplementary Fig. 3B) which was higher than that of FIB-4, APRI, NAFLD-FS and the Forns' index (0.733, 0.696, 0.743 and 0.721, respectively p <0.05 for all four) (Supplementary Table 4). For the prediction of severe fibrosis (⩾F3), AUROCs of the FibroGENE-DT-metabolic model, FIB-4, APRI, NAFLD-FS and Forns' index were 0.807, 0.783, 0.727, 0.79 and 0.786, respectively (p <0.05 with APRI). For the prediction of cirrhosis (F4), AUROCs of the FibroGENE-DT-metabolic model, FIB-4, APRI, NAFLD-FS and Forns' index were 0.839, 0.832, 0.819, 0.821 and 0.816, respectively (p = n.s). Adjusted AUROCs (Obuchowski) showed similar results (Supplementary Table 4). The IFNL metabolic model had 80% and 98% NPV for significant fibrosis and cirrhosis, respectively.

In all cohorts (HCV, HBV and NAFLD), the overall measure using the standardization of the AUROC for the distribution of fibrosis stages as described in the methods, of FibroGENE-DT, FIB-4, APRI, NAFLD-FS and Forns' index was similar to observed AUROC curve (data not shown).

Discussion

This is the first study to incorporate host genetic polymorphisms into an easy-to-use data mining based index for the prediction of fibrosis stage in liver disease. Our analysis of 4277 patients with disease of different etiologies demonstrates that the risk of fast fibrosis progression, significant fibrosis and cirrhosis can be reliably estimated using the IFNL genotype, together with routinely

available clinical and laboratory data. Overall, the algorithm showed good performance, and although the study was not aimed at comparing the accuracy of this model with other models, it showed higher AUROCs than APRI, FIB-4, NFS and the Forns' index in all comparisons and in all cohorts, being applicable not only to CHC, but also to CHB and NAFLD.

The extent of liver fibrosis is the most important predictor of long-term outcomes in chronic liver diseases, and hence the critical variable for rational decision making [42,43], including for variceal and hepatocellular carcinoma screening. However, the diagnosis of cirrhosis is frequently missed [44], with autopsy studies revealing that up to one-third of patients are not identified during their lifetime [45]. Thus, segregating patients with mild or no fibrosis, and hence little chance of liver-related adverse outcomes, from those with more advanced disease or in fact cirrhosis, is crucial. When using non-invasive algorithms, models that have the highest NPV for cirrhosis are likely to have the most clinical utility [46]. Furthermore, in hepatitis C, accurate fibrosis staging [47,48] will become even more important in the era of direct-acting antiviral treatments, not for assessing eligibility for therapy but for prioritization, particularly in government payer scenarios and in resource poor economies. The high cost of these regimens has already resulted in restrictions to their use in many countries, where only those with advanced disease are eligible for subsidized treatment.

We contend that the FibroGENE-DT has immediate clinical utility for the exclusion of subjects with cirrhosis, and in identifying those with a high likelihood of advanced fibrosis in CHC, CHB and NAFLD, and for patient counseling on the risk of rapid fibrosis progression in CHC. The FibroGENE-DT as a first point of care test excludes the presence of cirrhosis with a NPV >0.96 in CHC, CHB and NAFLD. In other scenarios, it could be used in combination with alternative diagnostic modalities such as TE, however this requires further investigation. Notably, the FibroGENE-DT could predict fibrosis progression with high accuracy (AUROC = 0.87), a character unique from most of the current available non-invasive modalities of liver fibrosis.

The current study has several strengths. Firstly, we used a data mining approach, a group of non-parametric regression methods specifically designed for predictive modeling, unlike conventional statistical analysis. Second, we utilized a systematic unbiased strategy and compared the performance of four different data mining techniques (MLRA, ANN, DT and KNN). We then focused on and validated DT analysis owing to its better performance and simplicity of use, lending the model to bedside clinical practice (Fig. 2). This is in contrast to other commonly used multivariable-generated risk models, which are complex due to the mathematical functions involved to interrelate a number of variables. Usually, such an approach requires generation of a score to determine risk, making them relatively impractical. Even when converted to point scores, the results generated from a multivariate model still needs a nomogram reference to interpret a point score to risk. Of relevance, clinical data are not normally distributed, so an advantage of DT analysis is that it does not require parametric assumptions, and therefore it is well suited to handling numerical data that are highly skewed, or alternatively, categorical and multimodal predictors with either an ordinal or non-ordinal structure. Finally, the present model was externally validated for hepatitis C, using an independent cohort.

Non-invasive assessment of liver fibrosis stage and prediction of fibrosis progression is an increasing focus of clinical research. The most widely utilized approaches are based on serum biomarkers or imaging modalities. With advances in our understanding of the genetic contributions to liver fibrosis risk, the incorporation of genetic variants, alone or in combination with or without other clinical variables, seems a logical extension for the development of novel models for fibrosis prediction. Further, unlike serum biomarkers or imaging, which might fluctuate based on clinical status and operator factors, genetic markers are robust and invariable between clinical settings.

An earlier study [49] has defined a genetic signature-based cirrhosis risk score (CRS) consisting of 7 markers. This was identified by a genome scan that selected 361 markers in a derivation cohort (N = 420), and then validated a 7 gene signature in a validation cohort of 154 patients. The AUROC of the CRS was 0.75 in the training cohort and 0.73 in the validation cohort. Their findings have been shown in other CHC cohorts to be associated with fibrosis progression in patients with mild (METAVIR score of F0–F1) basal fibrosis stage [50,51], but not in other chronic liver diseases [52,53].

Potential limitations of the current analysis must be acknowledged. Patients for all cohorts were recruited at tertiary units, which could represent a selection bias. However, all cohorts were consecutive and the large number of patients makes this representative of the overall population. In addition, each patient's actual risk may be influenced by many factors not measured or considered in the current model. We compared FibroGENE-DT to two existing non-invasive indices, FIB-4 and APRI, and in the cohort with NAFLD, also to the NFS and the Forns' index, that do not require additional laboratory testing. However, we were not able to compare it to other existing panels such as Fibrotest, Hepascore, FibroMeter or ELF, which require non-routine laboratory tests and for which additional stored blood was not available. Finally, the model had a lower AUROC in CHB compared to CHC. However, it is generally accepted that the prediction of fibrosis stage in CHB is more difficult that in CHC, given the fluctuating course of the former [12,13].

In summary, we have developed a simple clinical tool that allows an easy prediction of fibrosis risk in the three most common non-alcoholic causes of chronic liver disease. We suggest that the model has immediate applicability where an assessment of liver disease stage is considered important for clinical decision making.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Authors' contributions

M.E., J.G. conceived the research. Enrolling of patients, clinical phenotype data collation and sample acquisition/DNA preparation was performed by M.E, J.G., C.L., D.B, M.W.D, G.A., M.R-G., T.B, G.J.D., H.L.Y.C, W.L.I, D.S, M.L.A., L.A.A., A.M., M.W., E.B., U.S., O.S., J.F., L.M., W.C., E.P., J.N. and S.R. Genotyping was performed by R.L., Histological analysis of tissues and scoring was conducted by D.M. Statistical analysis and interpretation of results was

performed by M.E., A.H and J.G. The manuscript was principally written and revised by M.E and J.G. All authors critically reviewed the manuscript for important intellectual content and approved the final submitted manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhep.2015.11.008.

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