**Supplementary Table 1: Characteristics of patients with NAFLD with normal aminotransferase levels (ALT and AST <40 U/L) separated by the definite steatohepatitis diagnosis**

|  |  |  |
| --- | --- | --- |
|  | **Definite steatohepatitis** | **P-value** |
| **Yes****(N=188)** | **No****(N=346)** |
| **Demographics** |  |  |  |
| Age at enrollment (yrs) | 53 (11) | 52 (12) | 0.07 |
| GenderMaleFemale | 40 (21%)149 (79%) | 104 (30%)242 (70%) | 0.03 |
| RaceWhiteBlackAsianOther/Refused | 157 (84%)5 (3%)6 (3%)20 (11%) | 273 (79%)17 (5%)20 (6%)36 (10%) | 0.34 |
| Hispanic/Latino | 15 (8%) | 49 (14%) | 0.04 |
| **Anthropometric** |  |  |  |
| BMI (kg/m2) | 36.0 (6.3) | 34.2 (7.1) | 0.003 |
| Weight (kg) | 100 (21) | 94 (22) | 0.004 |
| Waist (cm) | 113 (14) | 108 (15) | <0.001 |
| **Comorbidities** |  |  |  |
| Type 2 diabetes | 107 (57%) | 113 (33%) | <0.001 |
| Hypertension | 129 (69%) | 196 (57%) | 0.006 |
| Dyslipidemia | 119 (64%) | 199 (58%) | 0.18 |
| Hypothyroidism | 48 (26%) | 63 (18%) | 0.04 |
| Metabolic syndrome | 134 (72%) | 181 (53%) | <0.001 |
| **Laboratory measures** |  |  |  |
| Platelet count (109/L) | 233 (75) | 249 (70) | 0.01 |
| AST/ALT ratio | 0.96 (0.30) | 0.91 (0.27) | 0.03 |
| FIB-4 | 1.4 (0.9) | 1.1 (0.6) | <0.0001 |
| HgA1c (%) | 6.6 (1.3) | 6.0 (1.1) | <0.0001 |
| Insulin (μU/mL) | 29.9 (29.3) | 19.2 (15.1) | <0.0001 |
| HOMA-IR Mean (SD)Median (IQR) | 9.4 (10.1)5.7 (3.3, 10.5) | 5.3 (5.2)3.8 (2.5, 6.1) | <0.0001 |
| Total cholesterol (mg/dL) | 180 (39) | 185 (42) | 0.16 |
| Triglycerides (mg/dL) | 183 (141) | 160 (89) | 0.04 |
| HDL (mg/dL) | 43 (11) | 46 (13) | 0.003 |
| LDL (mg/dL) | 104 (34) | 111 (36) | 0.05 |

¶Data are presented as number (percent) or means with standard deviation, unless specified otherwise.

**Abbreviations: BMI:** Body mass index; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **HOMA-IR:** Homeostatic model assessment for insulin resistance; **HDL:** high density lipoprotein; **LDL:** Low density lipoprotein

**Supplementary Table 2: Multiple logistic regression analysis for definite steatohepatitis in NAFLD patients with normal aminotransferase levels (ALT and AST <40 U/L)**

|  |  |
| --- | --- |
|  | **Definite steatohepatitis vs. NAFLD/borderline steatohepatitis\*** |
|  | **Odds ratio** | **95% CI** | **P** |
| **Characteristics (N=526)** |  |  |  |
| Type 2 diabetes | 2.1 | 1.4 – 3.1 | <0.001 |
| Female vs. male | 2.2 | 1.3 – 3.6 | 0.002 |
| Platelets – per 20 unit (109/L) decrement | 1.1 | 1.0 – 1.2 | 0.003 |
| Waist circumference – per 5 cm increment | 1.1 | 1.0 – 1.2 | 0.003 |
| HDL – per 5 mg/dL decrement | 1.1 | 1.0 – 1.2 | 0.01 |
| History of hypothyroidism | 1.4 | 0.9 – 2.2 | 0.18 |
|  |  |  |
| **Clinical model for P, probability of definite steatohepatitis.** Coefficients and SEs shown as b(SE): log(P/1-P) = -1.746(0.913) + 0.754(0.198) if type 2 diabetes + 0.781(0.257) if female - 0.004(0.001) X platelet count (109/L)+ 0.020(0.007) X waist (cm) – 0.023(0.009) X HDL (mg/dL) + 0.316(0.237) if hypothyroid |
|  |  |  |  |

\* Factors were selected using AIC criteria from a multiple logistic regression model regressing definite steatohepatitis on a candidate set of 15 variables: age, sex, race, Hispanic/Latino ethnicity, BMI, waist circumference, type 2 diabetes, hypertension, hypothyroidism, metabolic syndrome, AST/ALT, platelets, triglycerides, HDL, and LDL.

**Supplementary Table 3: Characteristics of NAFLD patients with normal aminotransferase levels (ALT and AST <40 U/L) categorized by the presence of advanced fibrosis**

|  |  |  |
| --- | --- | --- |
| **Patient characteristics** | **Advanced fibrosis** | **P-value** |
| **Yes****(N=110)** | **No****(N=424)** |
| **Demographic** |  |  |  |
| Age at enrollment (yrs) | 55 (10) | 52 (12) | 0.005 |
| GenderMaleFemale | 27 (25%)83 (75%) | 117 (28%)307 (72%) | 0.52 |
| RaceWhiteBlackAsianOther/Refused | 100 (91%)0 (0%)1 (1%)9 (8%) | 330 (78%)22 (5%)25 (6%)47 (11%) | 0.002 |
| Hispanic/Latino | 9 (8%) | 55 (13%) | 0.19 |
| **Anthropometric** |  |  |  |
| BMI (kg/m2) | 35.8 (6.5) | 34.6 (7.0) | 0.08 |
| Weight (kg) | 100 (21) | 96 (22) | 0.09 |
| Waist (cm) | 112 (14) | 109 (15) | 0.05 |
| **Comorbidities** |  |  |  |
| Type 2 diabetes | 62 (57%) | 158 (37%) | <0.001 |
| Hypertension | 83 (76%) | 242 (57%) | <0.001 |
| Dyslipidemia | 60 (55%) | 258 (61%) | 0.26 |
| Hypothyroidism | 21 (19%) | 90 (21%) | 0.65 |
| Metabolic syndrome | 70 (65%) | 245 (58%) | 0.23 |
| **Laboratory measures** |  |  |  |
| Platelet count (109/L) | 200 (70) | 255 (69) | <0.0001 |
| AST/ALT ratio | 1.06 (0.38) | 0.90 (0.23) | <0.0001 |
| FIB-4 | 1.8 (1.2) | 1.0 (0.5) | <0.0001 |
| HgA1c (%) | 6.6 (1.3) | 6.2 (1.2) | <0.001 |
| Insulin (μU/mL) | 32.9 (31.6) | 20.4 (17.6) | <0.001 |
| HOMA-IR Mean (SD)Median (IQR) | 10.7 (11.1)6.5 (3.8 – 11.7) | 5.7 (6.0)3.9 (2.5 – 6.4) | <0.0001 |
| Total cholesterol (mg/dL) | 177 (39) | 185 (41) | 0.06 |
| Triglycerides (mg/dL) | 174 (120) | 166 (108) | 0.51 |
| HDL (mg/dL) | 44 (12) | 45 (12) | 0.30 |
| LDL (mg/dL) | 100 (33) | 110 (35) | 0.009 |

¶Data are presented as number (percent) or means with standard deviation, unless specified otherwise

**Abbreviations: BMI:** Body mass index; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **HOMA-IR:** Homeostatic model assessment for insulin resistance; **HDL:** high density lipoprotein; **LDL:** Low density lipoprotein

**Supplementary Table 4: Multiple logistic regression analysis for advanced fibrosis in NAFLD patients with normal aminotransferase levels (ALT and AST<40 U/L)**

|  |  |
| --- | --- |
|  | **Clinical Model for Advanced Fibrosis\*** |
|  | **Odds ratio** | **95% CI** | **P** |
| **Characteristics (N=514)** |  |  |  |
| Platelets – per 10 unit (109/L) decrement | 1.2 | 1.1 – 1.2 | <0.001 |
| AST/ALT ratio | 4.0 | 1.6 – 10.1 | 0.003 |
| White race vs. non-white | 2.4 | 1.1 – 5.0 | 0.02 |
| LDL – per 10 mg/dL decrement | 1.1 | 1.0 – 1.2 | 0.02 |
| History of hypertension | 1.8 | 1.0 – 3.1 | 0.047 |
| Female vs. male | 1.8 | 1.0 – 3.5 | 0.06 |
| Waist circumference – per 5 cm increment | 1.1 | 1.0 – 1.2 | 0.14 |
| Type 2 diabetes | 1.4 | 0.8 – 2.4 | 0.19 |
|  |  |  |  |
| **Clinical model for P, probability of advanced fibrosis.** Coefficients and SEs shown as b(SE): log(P/1-P) = -1.770(1.316) – 0.014(0.002) X platelet count (109/L) + 1.384(0.474) X AST/ALT + 0.865(0.383) if white – 0.008(0.004) X LDL (mg/dL) + 0.565(0.284) if hypertension + 0.615(0.321) if female + 0.013(0.009) X waist (cm) + 0.347(0.262) if type 2 diabetes  |
|  |  |  |  |

\* Factors were selected using AIC criteria from a multiple logistic regression model regressing advanced fibrosis on a candidate set of 15 variables: age, sex, race, Hispanic/Latino ethnicity, BMI, waist circumference, type 2 diabetes, hypertension, hypothyroidism, AST/ALT, platelets, triglycerides, HDL, and LDL.

**Supplementary Table 5: Description of NAFLD patients with normal ALT level according to Prati criteria (<30 U/L for males, <19 U/L for females) within 3 months of liver biopsy (N=143)**

|  |  |
| --- | --- |
| **Study**Database 1Database 2PIVENSFLINT | 23 (16%)115 (80%)1 (1%)4 (3%) |
| ALT (U/L)Males (N=69)Mean (SD)Median (IQR)Range Females (N=74)Mean (SD)Median (IQR)Range | 24.5 (4.5)26 (21, 28)10-3015.6 (2.1)16 (15-17)9-18 |
| Time between liver panel and biopsy (days)Mean (SD)Median (IQR)Range | 33 (26)28 (9-53)0-91 |
| **Histology – N (%)** |  |
| Phenotypes: |  |
| Definite steatohepatitis | 34 (24%) |
| Definite steatohepatitis and fibrosis stage 2-3 | 17 (13%) |
| Advanced fibrosis | 29 (20%) |
| Cirrhosis | 14 (10%) |
| Steatohepatitis diagnosisNAFLD onlyBorderline 1aBorderline 1bDefinite | 70 (49%)37 (26%)2 (1%)34 (24%) |
| Fibrosis stage01a1b1c234 | 65 (45%)15 (10%)8 (6%)11 (8%)15 (10%)15 (10%)14 (10%) |

**Abbreviations:**  **NAFLD:** Nonalcoholic fatty liver disease; **AST:** Aspartate aminotransferase; **ALT:** Alanine aminotransferase; **SD:** Standard deviation; **IQR:** Interquartile range

**Supplementary Table 6: Diagnostic statistics for clinical predication models based on the Prati ALT criteria for normal ALT level in the prediction sample**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Model fixed at 90% specificity** |  | **Model fixed at 90% sensitivity** |  | **Model fixed at maximum** **Youden’s Index\*** |
|  | **AUROC (95% CI)** | **Sens** | **PPV†** | **NPV‡** |  | **Spec** | **PPV†** | **NPV‡** |  | **Sens** | **Spec** | **PPV†** | **NPV‡** |
| **Definite steatohepatitis** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| LACSNA | 0.64 (0.53-0.74) | 0.21 | 0.41 | 0.78 |  | 0.30 | 0.29 | 0.91 |  | 0.79 | 0.44 | 0.31 | 0.87 |
| FIB-4 | 0.67(0.55 – 0.79) | 0.35 | 0.55 | 0.82 |  | 0.07 | 0.24 | 0.73 |  | 0.71 | 0.61 | 0.36 | 0.87 |
| NFS | 0.69(0.57 – 0.80) | 0.30 | 0.44 | 0.83 |  | 0.11 | 0.21 | 0.85 |  | 0.85 | 0.48 | 0.30 | 0.93 |
| **Definite steatohepatitis with stage 2-3 fibrosis** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| LACSNA | 0.78 (0.65-0.91) | 0.56 | 0.45 | 0.94 |  | 0.42 | 0.19 | 0.98 |  | 0.63 | 0.87 | 0.40 | 0.94 |
| FIB-4 | 0.67(0.51 – 0.84) | 0.27 | 0.29 | 0.90 |  | 0.02 | 0.12 | 0.67 |  | 0.67 | 0.66 | 0.22 | 0.93 |
| NFS | 0.69(0.57 – 0.82) | 0.20 | 0.23 | 0.89 |  | 0.36 | 0.17 | 0.97 |  | 0.93 | 0.44 | 0.19 | 0.98 |
| **Advanced fibrosis** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| LACSNA | 0.87 (0.78-0.95) | 0.66 | 0.63 | 0.91 |  | 0.32 | 0.26 | 0.95 |  | 0.83 | 0.88 | 0.63 | 0.95 |
| FIB-4 | 0.84(0.74 – 0.94) | 0.59 | 0.61 | 0.90 |  | 0.17 | 0.22 | 0.91 |  | 0.79 | 0.86 | 0.59 | 0.94 |
| NFS | 0.80(0.70 – 0.90) | 0.43 | 0.45 | 0.89 |  | 0.43 | 0.23 | 0.96 |  | 0.67 | 0.80 | 0.39 | 0.93 |
| **Cirrhosis** |  |  |  |  |  |  |  |  |  |  |  |  |  |
| LACSNA | 0.99 (0.97-1.00) | 1.00 | 0.54 | 1.00 |  | 0.93 | 0.77 | 0.99 |  | 1.00 | 0.93 | 0.61 | 1.00 |
| FIB-4 | 0.94(0.89 – 0.98) | 0.79 | 0.48 | 0.98 |  | 0.81 | 0.34 | 0.99 |  | 1.00 | 0.81 | 0.36 | 1.00 |
| NFS | 0.94(0.88 – 1.00) | 0.78 | 0.37 | 0.98 |  | 0.00 | 0.07 | -- |  | 1.00 | 0.78 | 0.25 | 1.00 |

LACSNA**: L**ow **A**LT **C**linically **S**ignificant **NA**FLD , FIB-4: Fibrosis-4, NFS: NAFLD Fibrosis Score.

N’s ranged from 127 – 143 due to missing values in the outcome (definite steatohepatitis with fibrosis stage 2-3 and excluding patients with cirrhosis) and covariates.

\* Youden’s Index=sensitivity+specificity-1

† PPV = Positive Predictive Value: the probability that the disease is present when the test is positive

‡ NPV = Negative Predictive Value: the probability that the disease is not present when the test is negative.

**Supplementary Table 7: Comparison of clinical predication models based on the Prati criteria for normal ALT in the prediction and validation samples**

|  |  |
| --- | --- |
|  | **AUROC (95% CI)** |
|  |  |  | **Validation Models** |
|  | **Prediction Model** |  | **Cross-validated** | **Split-sample Validation\*** | **Random** **Split-sample Validation**† |
| Definite steatohepatitis | 0.64 (0.53-0.74) |  | 0.55 (0.44-0.67) | 0.52 (0.34–0.69) | 0.64 (0.46-0.82) |
| Definite steatohepatitis with stage 2-3 fibrosis | 0.78 (0.65-0.91) |  | 0.67 (0.50-0.83) | 0.54 (0.26-0.82) | 0.39 (0.20-0.58) |
| Advanced fibrosis | 0.87 (0.78-0.95) |  | 0.83 (0.73-0.94) | 0.60 (0.33-0.87) | 0.77 (0.53-1.00) |
| Cirrhosis | 0.99 (0.97-1.00) |  | 0.95 (0.90-1.00) |   |  |

\* Split-sample validation, where the prediction model was applied to 60% of the sample selected on calendar time, and the performance characteristics were then calculated in the 40% validation sample using the predicted probabilities from the 60% training sample.

† Split-sample validation was repeated with a randomly selected 40% of the sample.