

**Supplementary Table S1. The full participant sites list, and number of subjects enrolled for the prospective study (DB2).**

<b>Site</b>	<b>City, State</b>	<b>Number of subjects</b>
Arizona Liver Health (Tucson)	Tucson, AZ	34
Henry Ford Hospital System	Detroit, MI	23
Arkansas Gastroenterology	North Little Rock, AR	16
Premier Medical Group	Clarksville, TN	16
Gastrohealth (Ohio GI)	Cincinnati, OH	15
Baylor College of Medicine	Houston, TX	15
South Texas Research Institute (STRI)	Edinburg, TX	14
Arizona Liver Health (Chandler)	Chandler, AZ	14
Methodist Health System	Dallas, TX	12
Pinnacle Clinical Research (San Antonio)	San Antonio, TX	12
Pinnacle Clinical Research (Austin)	Austin, TX	10
Northwell Health, Inc.	Manhasset, NY	9
Arizona Liver Health (Peoria)	Peoria, AZ	8
Delta Research Partners, LLC	Bastrop, LA	7
GI Alliance (Baton Rouge)	Baton Rouge, LA	7
Liver Associates of Texas, P.A.	Houston, TX	5
GI Alliance (Webster)	Webster, TX	5
DHR Health Institute for Research and Development	McAllen, TX	5
Houston Methodist Research Institute	Houston, TX	4
Liver Center of Texas	Dallas, TX	3
Saint Louis University	St. Louis, MO	2
GI Alliance (Flowood)	Flowood, MS	2
Quality Research	San Antonio, TX	2
GI Alliance (Garland)	Garland, TX	1

**Supplementary Table S2. The full inclusion/exclusion criteria list for each of the individual datasets.**

<b>Subject Inclusion/Exclusion Criteria</b>	
<b>Inclusion criteria</b> (Subjects may be included in the study if they meet the following inclusion criteria)	<b>Exclusion criteria</b> (Subjects may not be included in the study if they meet any of the following exclusion criteria)
<b>Prospective collection (DB2)</b>	
<ul style="list-style-type: none"> <li>• Consenting patients with established NAFLD and in any stage and degree of fibrosis, steatosis and inflammatory activity, having undergone liver biopsy as part of the routine management simultaneously or within six (6) months from the day of blood sampling for LIVERFAS<sub>t</sub> test</li> <li>• Aged 18 to 80 years old, inclusive</li> <li>• Male or female from any ethnicity</li> <li>• Consenting patients that have undergone liver biopsy with NAFLD suspected diagnosis (by any means) within six (6) months of enrollment and blood sampling for LIVERFAS<sub>t</sub> test</li> <li>• Willing to undergo blood sampling for LIVERFAS<sub>t</sub> testing after 6 to 12 hours fasting</li> <li>• Willing and able to allow access to requested data and who were informed and signed the consent form</li> <li>• Willing to allow histological lecture by a pathologist for NASH-CRN and SAF scoring systems analysis of the liver biopsy</li> </ul>	<ul style="list-style-type: none"> <li>• Inability to provide informed consent</li> <li>• Patients who may be uncooperative with the sample collection procedures</li> <li>• History of known severe coagulopathy</li> <li>• History of known hepatic abscess</li> <li>• Renal failure undergoing dialysis (GFR&lt;45)</li> <li>• History of malignancy in the past 2 years</li> <li>• Previous liver transplantation</li> <li>• Suffering with a terminal illness or any other conditions or diseases that the investigator considers inappropriate for study participation</li> <li>• Secondary causes of hepatic fat accumulation such as significant alcohol consumption, long-term use of a steatogenic medication</li> <li>• Ongoing or recent alcohol consumption defined as &gt;21 alcoholic drinks per week in a man or &gt;14 alcoholic drinks per week on average per week in women. Approximately 10g of alcohol equals one ‘drink’ unit. One unit equals 1 ounce of distilled spirits, one 12-oz beer, or one 4-oz glass of wine</li> <li>• Total parenteral nutrition within 3 months of interview</li> <li>• Short bowel syndrome</li> <li>• History of gastric of jejunoileal bypass preceding the diagnosis of NAFLD. Bariatric surgery performed concomitant with or following the diagnosis of NAFLD does not exclude enrollment of patients</li> <li>• History of biliopancreatic diversion</li> <li>• Evidence of advanced liver disease defined as Child-Pugh-Turcotte score of equal to or greater than 10</li> <li>• Evidence of chronic hepatitis B as marked by the presence of HBsAg in serum (patients with isolated antibody to hepatitis B core antigen, anti-HBc, are not excluded). Evidence of chronic hepatitis C as marked by the presence of anti-HCV and HCV RNA in serum. Patients with anti-HCV with PCR negative should not be excluded</li> </ul>

	<ul style="list-style-type: none"> <li>• Low alpha-1-antitrypsin level and ZZ phenotype (both determined at the discretion of the investigator)</li> <li>• History of Wilson’s disease</li> <li>• Known glycogen storage disease</li> <li>• Known dysbetalipoproteinemia</li> <li>• Known phenotypic hemochromatosis (determined at the discretion of the investigator). History of primary biliary cholangitis (PBC)/primary biliary sclerosis (PSC), hepatic vascular lesions (determined at the discretion of the investigator)</li> <li>• History of liver granulomas (sarcoidosis, and infectious diseases such as tuberculosis)</li> <li>• Congenital hepatic fibrosis, polycystic liver disease.</li> <li>• Other metabolic / congenital liver disease</li> <li>• Known HIV positive</li> <li>• Disseminated or advanced extrahepatic malignancy</li> <li>• Conditions that could interfere with LIVERFASt parameters and could lead to risk of false positive / false negative results: Drug-induced liver injury (DILI), acute alcoholic hepatitis, acute inflammatory syndrome or acute sepsis (e.g. urinary tract infection, etc.)</li> <li>• Active drug use or dependence that, in the opinion of the study investigator, would interfere with adherence to study requirements</li> </ul>
<b>Retrospective collection DB1</b>	
<ul style="list-style-type: none"> <li>• Data of consented SLD subjects from the liver center registry with available liver biopsy reading that confirmed NAFLD and with available inputs for LIVERFASt test calculation</li> <li>• Aged 18 years or older</li> <li>• Male or female from any ethnicity</li> </ul>	<ul style="list-style-type: none"> <li>• Subjects with alcohol consumption defined as &gt;21 alcoholic drinks per week in a man or &gt;14 alcoholic drinks per week on average per week in women</li> <li>• Subjects with HCV, HBV or HIV chronic infection</li> <li>• Subjects with known auto-immune, genetic-storage diseases</li> <li>• Biopsy histopathology reading missing or incomplete (unable to establish the MASLD diagnosis)</li> <li>• Biopsy reading indicating the presence of other chronic or acute liver diseases</li> <li>• Biopsy quality assessment by the agreement with vibration-controlled transient elastography missing or disagreement</li> <li>• Biopsy quality assessment by the sample size missing or less than twenty millimeters in non-cirrhotic patients</li> </ul>

	<ul style="list-style-type: none"> <li>• LIVERFAS<sub>t</sub> test (blood biomarkers and anthropometrics) missing data</li> <li>• Conditions that could interfere with LIVERFAS<sub>t</sub> test biomarkers (e.g. hemolysis)</li> </ul>
<b>Retrospective collection DB3</b>	
<ul style="list-style-type: none"> <li>• All are adult selected subjects that have a historical report of histopathological results (biopsy) and bio-sample available (stored at -80°C) for the dates of interest from the (NAFLD Adult) Non-alcoholic Fatty Liver Disease (NAFLD) Adult Database and (NASH) Non-alcoholic Steatohepatitis Clinical Research Network</li> <li>• Having serum or plasma samples [0.5ml (minimum amount 0.25 ml)] at the baseline visit with: <ul style="list-style-type: none"> <li>◊ available historical report of biopsy with sample size <math>\geq 20</math> mm</li> <li>◊ at least histopathology fibrosis scoring available (steatosis and necro-inflammation histopathology reports are requested equally when available)</li> <li>◊ Bio-samples should be frozen at -80°C and, ideally, not have been thawed more than once</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Bio-samples that have been thawed more than once.</li> <li>• Participants identified as having risk factors for false positive/negative results for LIVERFAS<sub>t</sub> (severe intravascular hemolysis - if condition is known - acute hepatitis or severe cytolysis with <math>\geq 600</math> ALT values)</li> </ul>

**Supplementary Table S3. Diagnostic performance to identify advanced fibrosis according to sensitivity analysis in the overall MASLD cohort (n=497) according to age (>65 years/≤65 years), sex (M/F), BMI >30/≤30 kg/m<sup>2</sup>), and ALT (>50, ≤50 IU/l). Significance between groups is highlighted (p <0.05).**

<b>Endpoints</b> (liver biopsy)	<b>Age &gt;65 years vs. ≤65 years</b> n=384 vs. n=113	<b>BMI &gt;30 kg/m<sup>2</sup> vs. ≤30 kg/m<sup>2</sup></b> n=149 vs. n=348	<b>Sex Male vs. Female</b> n=215 vs. n=282	<b>ALT &gt;50 IU/L vs. ≤50 IU/L</b> n=263 vs. n=234
	<b>AUOCs (SE)</b>			
Diagnostic of cirrhosis, F4 stage	0.822 (0.041) vs. 0.859 (0.041), p=ns	0.912 (0.027) vs. 0.846 (0.029), p=ns	0.852 (0.036) vs. 0.883 (0.025), p=ns	0.879 (0.027) vs. 0.859 (0.032), p=ns
Diagnostic of fibrosis stage ≥3	0.828 (0.040) vs. 0.833 (0.021), p=ns	0.877 (0.028) vs. 0.839 (0.021), p=ns	0.847 (0.026) vs. 0.845 (0.023), p=ns	0.849 (0.024) vs. 0.844 (0.025), p=ns
Diagnostic of fibrosis stage ≥2	0.744 (0.050) vs. 0.725 (0.026), p=ns	0.838 (0.032) vs. 0.730 (0.028), p<0.05	0.802 (0.028) vs. 0.735 (0.029), p=ns	0.758 (0.030) vs. 0.740 (0.032), p=ns

**Supplementary Table S4. Standard empirical area under the receiver-operating curves (AUROC) according to the histological feature's endpoint (fibrosis) across DB1 and pooled DB2 and DB3 population.** MASLD, metabolic dysfunction-associated liver disease; T2DM, type 2 diabetes mellitus; AUROC, area under the receiver-operating characteristics curve; DB, database; CI, confidence interval.

	Cohorts						
	DB1 cohort			Pooled DB2 and DB3 cohorts			
Group	Fibrosis Prevalence	AUROC P-value	95% CI	Fibrosis Prevalence	AUROC P-value	95% CI	P Value versus DB1
<b>Cirrhosis (Fibrosis stage 4)</b>							
<i>All MASLD</i>	59 / 191	<b>0.868</b> <0.001	0.799 0.914	41 / 306	<b>0.843</b> <0.001	0.756 0.901	NS
<i>Without T2DM</i>	20 / 94	<b>0.914</b> <0.001	0.819 0.960	12 / 153	<b>0.826</b> <0.001	0.615 0.927	NS
<i>With T2DM</i>	39 / 97	<b>0.817</b> <0.001	0.704 0.889	29 / 153	<b>0.848</b> <0.001	0.739 0.914	NS
<b>Advanced fibrosis (fibrosis stages ≥ 3)</b>							
<i>All MASLD</i>	102 / 191	<b>0.888</b> <0.001	0.834 0.926	117 / 306	<b>0.803</b> <0.001	0.747 0.849	<0.05
<i>Without T2DM</i>	36 / 94	<b>0.903</b> <0.001	0.824 0.947	47 / 153	<b>0.867</b> <0.001	0.786 0.918	NS
<i>With T2DM</i>	66 / 97	<b>0.864</b> <0.001	0.772 0.921	70 / 153	<b>0.730</b> <0.001	0.640 0.800	<0.05
<b>Fibrosis (Fibrosis stages ≥ 2)</b>							
<i>All MASLD</i>	144 / 191	<b>0.779</b> <0.001	0.701 0.839	199 / 306	<b>0.721</b> <0.001	0.659 0.667	NS
<i>Without T2DM</i>	58 / 94	<b>0.711</b> <0.001	0.592 0.800	82 / 153	<b>0.722</b> <0.001	0.631 0.793	NS
<i>With T2DM</i>	86 / 97	<b>0.765</b> <0.001	0.598 0.869	117 / 153	<b>0.683</b> <0.001	0.581 0.763	NS

**Supplementary Table S5. LIVERFASt Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) according to sensitivity analysis in the pooled DB2 and DB3 versus DB1 for different fibrosis endpoints; cirrhosis, advanced fibrosis ( $\geq$  F3) and clinically significant fibrosis ( $\geq$  F2), stratified according to the presence of type 2 diabetes. T2DM, type 2 diabetes mellitus; DB, database.**

Cohorts	Subgroup stratification	Sensitivity	Specificity	PPV	NPV
<b>Cirrhosis (fibrosis stage 4)</b>					
<b>DB1</b>	Overall	79.66	79.55	63.51	89.74
	<i>Non-T2DM</i>	80.00	85.14	59.26	94.03
	<i>T2DM</i>	79.49	72.41	65.96	84.00
<b>Pooled DB2 and DB3</b>	Overall	51.22	95.47	63.64	92.67
	<i>Non-T2DM</i>	66.67	95.04	53.33	97.10
	<i>T2DM</i>	44.83	95.97	72.22	88.15
<b>Advanced fibrosis (fibrosis stages <math>\geq</math> 3)</b>					
<b>DB1</b>	Overall	80.39	73.03	77.36	76.47
	<i>Non-T2DM</i>	72.22	79.31	68.42	82.14
	<i>T2DM</i>	84.85	61.29	82.35	65.52
<b>Pooled DB2 and DB3</b>	Overall	46.15	92.06	78.26	73.42
	<i>Non-T2DM</i>	53.19	96.23	86.21	82.26
	<i>T2DM</i>	41.43	85.54	70.73	63.39
<b>Clinically significant fibrosis (fibrosis stages <math>\geq</math> 2)</b>					
<b>DB1</b>	Overall	84.03	53.19	84.62	52.08
	<i>Non-T2DM</i>	70.69	61.11	74.55	56.41
	<i>T2DM</i>	93.02	27.27	90.91	33.33
<b>Pooled DB2 and DB3</b>	Overall	61.31	70.09	79.22	49.34
	<i>Non-T2DM</i>	57.32	77.46	76.40	61.11
	<i>T2DM</i>	64.10	55.56	82.42	32.26

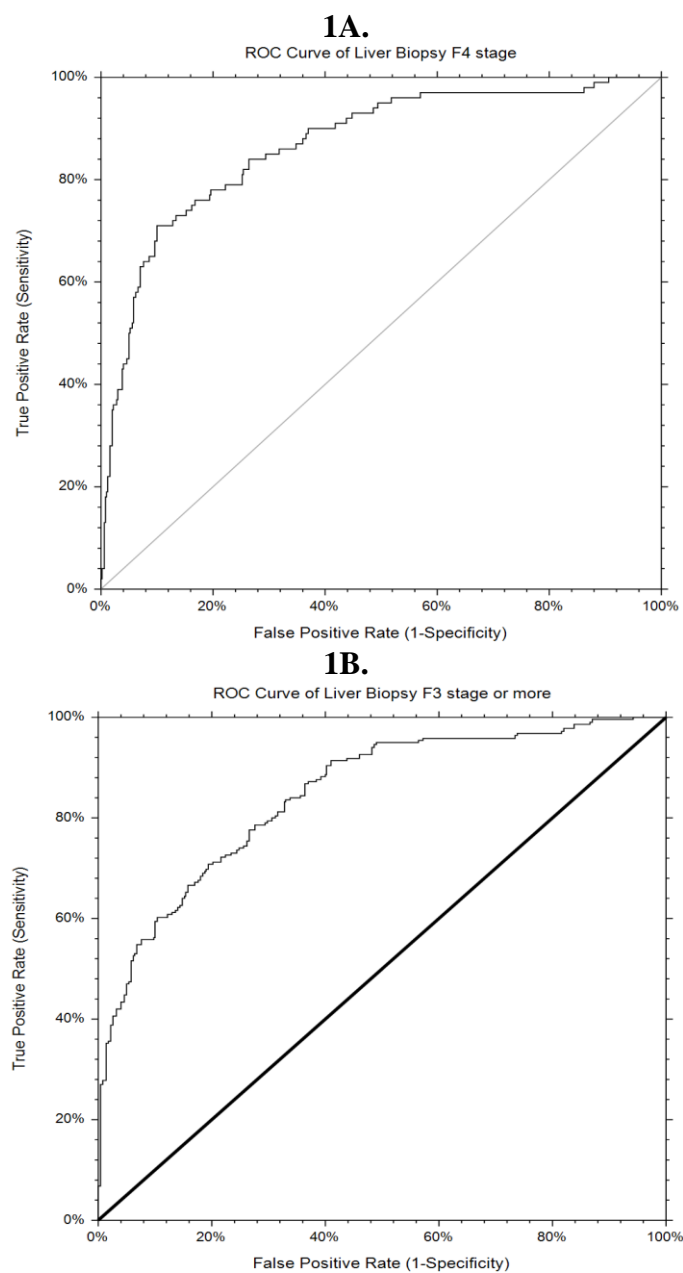
**Supplementary Table S6. Standard empirical area under the receiver-operating curves (AUROC) according to the histological feature's endpoint (fibrosis) and time interval between liver biopsy and blood draw for LIVERFASt.** T2DM, type 2 diabetes mellitus; AUROC, area under the receiver-operating characteristics curve; DB, database; CI, confidence interval.

	<b>Lesser than 6 months (&lt;6) between liver biopsy and LIVERFASt</b>			<b>6 months or more (≥ 6) between liver biopsy and LIVERFASt</b>			
<b>Group</b>	<b>Prevalence</b>	<b>AUROC P-value</b>	<b>95% CI</b>	<b>Prevalence</b>	<b>AUROC P-value</b>	<b>95% CI</b>	<b>P Value &lt; 6 vs. ≥ 6 months</b>
<b>Cirrhosis (Fibrosis stage 4)</b>							
<i>Overall</i>	74 / 401	<b>0.857</b> <0.001	0.797 0.901	26 / 96	<b>0.889</b> <0.001	0.797 0.940	NS
<i>Without T2DM</i>	24 / 198	<b>0.957</b> <0.001	0.795 0.992	8 / 49	<b>0.866</b> <0.001	0.752 0.929	NS
<i>With T2DM</i>	50 / 203	<b>0.847</b> <0.001	0.763 0.903	18 / 47	<b>0.801</b> <0.001	0.629 0.898	NS
<b>Advanced fibrosis (fibrosis stages ≥ 3)</b>							
<i>Overall</i>	172 / 401	<b>0.811</b> <0.001	0.764 0.849	47 / 96	<b>0.959</b> <0.001	0.907 0.982	P<0.001
<i>Without T2DM</i>	69 / 198	<b>0.967</b> <0.01	0.889 0.991	14 / 49	<b>0.864</b> <0.01	0.800 0.908	P<0.01
<i>With T2DM</i>	103 / 203	<b>0.751</b> <0.001	0.677 0.810	33 / 47	<b>0.952</b> <0.001	0.851 0.985	P<0.001
<b>Fibrosis (Fibrosis stages ≥ 2)</b>							
<i>Overall</i>	276 / 401	<b>0.721</b> <0.001	0.667 0.768	67 / 96	<b>0.842</b> <0.001	0.740 0.906	P < 0.05
<i>Without T2DM</i>	115 / 198	<b>0.733</b> <0.001	0.550 0.849	25 / 49	<b>0.712</b> <0.001	0.633 0.776	NS
<i>With T2DM</i>	161 / 203	<b>0.687</b> <0.001	0.820 0.983	42 / 47	<b>0.943</b> <0.001	0.581 0.763	P<0.001

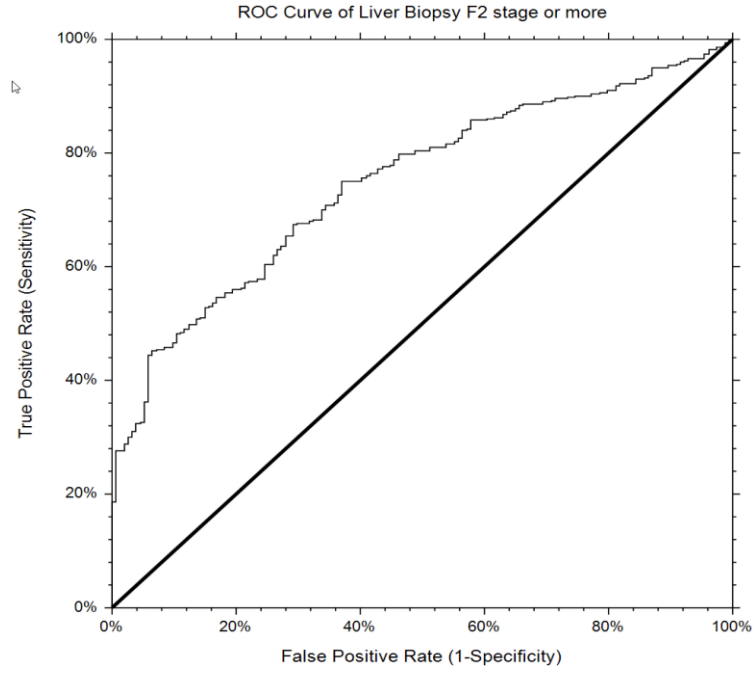
**Supplementary Figure S1. Empirical ROC curves in the derivation of the LIVERFAST Fibrosis score for the diagnostic cirrhosis (stage F4), fibrosis stages  $\geq 3$  and  $\geq 2$ , respectively.**

The overall MASLD pooled cohort (1A, 1B, 1C) and according to the presence of T2DM\* in the overall MASLD pooled cohort (1D, 1E, 1F).

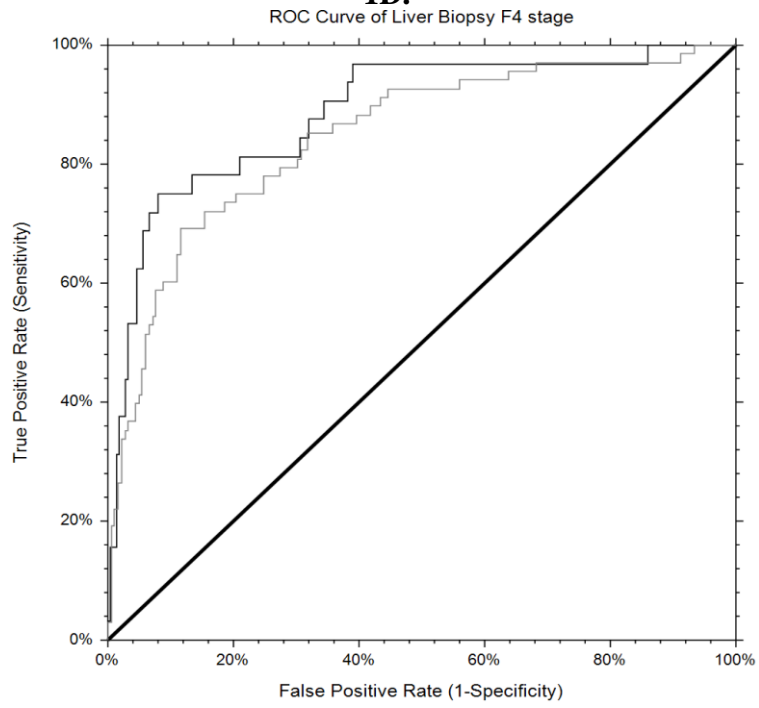
\*Empirical ROC gray line indicates the group with type 2 diabetes and the ROC black line indicates the group without type 2 diabetes.



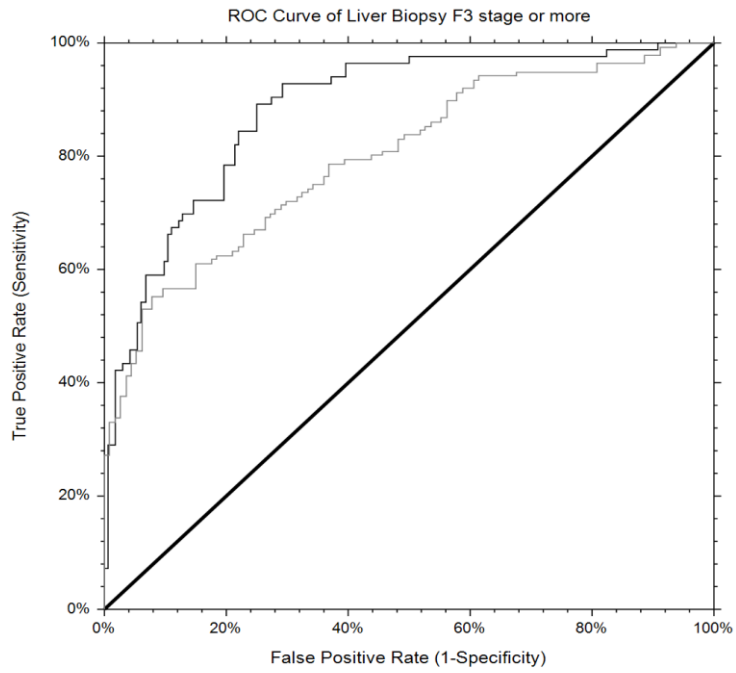
**1C.**



**1D.**



**1E.**



**1F.**

