

# Evaluation of AGP Fucosylation as a Marker for Hepatocellular Carcinoma of Three Different Etiologies

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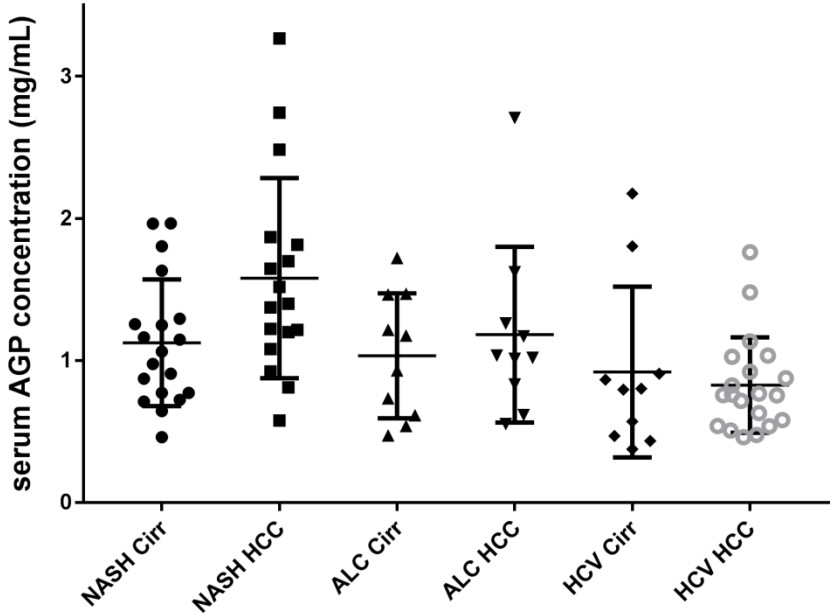
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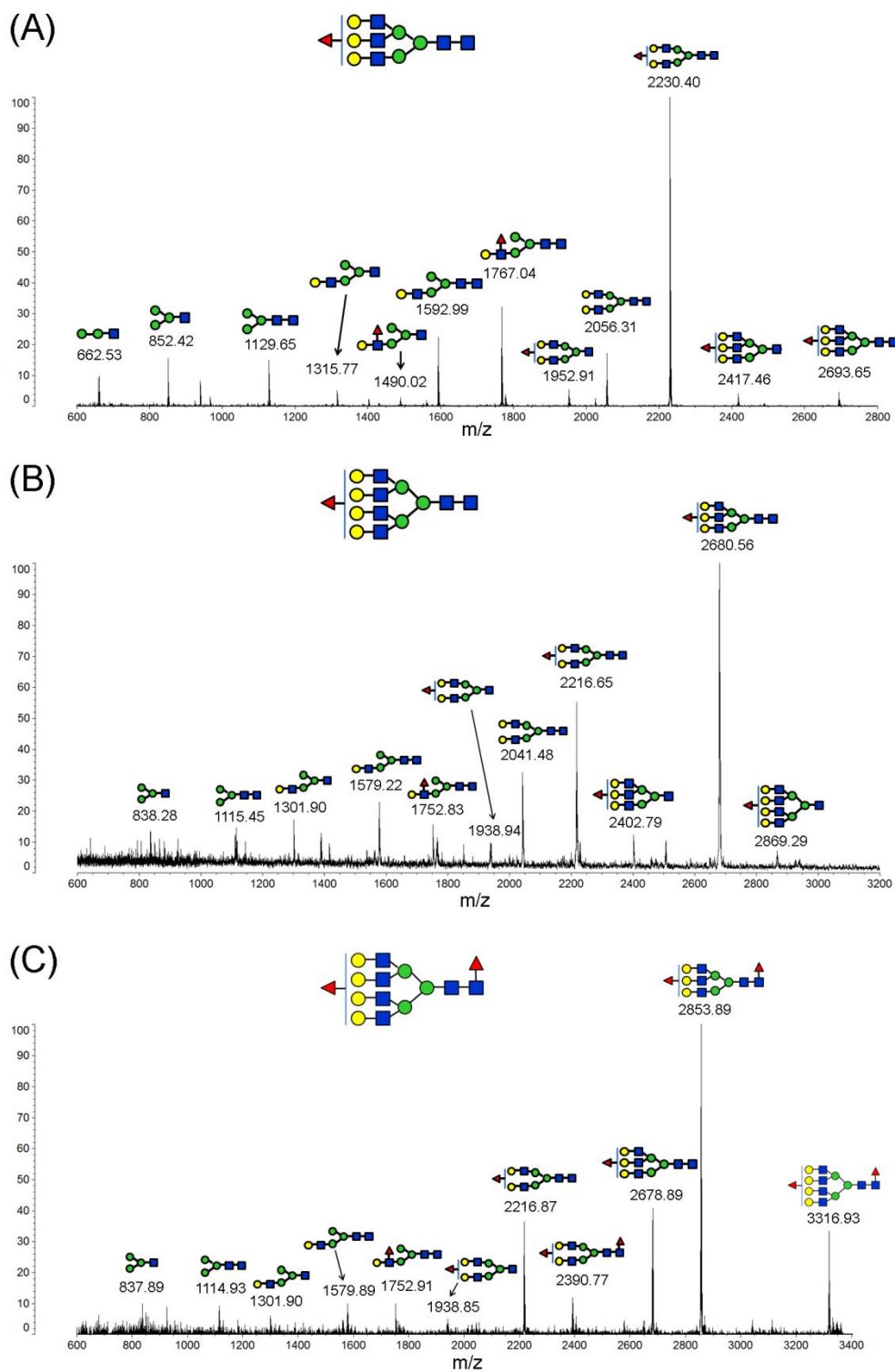
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Supplemental Information

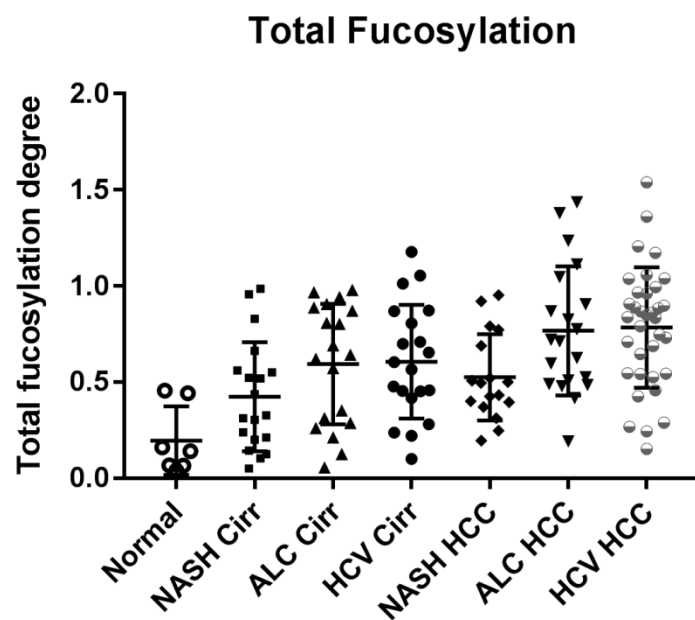


**Figure S1.** Scatter plot of serum AGP concentration measured by ELISA assay in patients with HCC or cirrhosis divided by the etiology, NASH, ALC, and HCV, respectively.



**Figure S2.** Representative MALDI-QIT-TOF MS/MS spectra of glycans at (A)  $m/z$  2693.65, (B)  $m/z$  3142.68, and (C)  $m/z$  3316.93 for glycan composition and core/antennary fucosylation assignment. Fragment ions are labeled with potential structures. (A) The mono-fucosylated tri-antennary glycan ( $m/z$

2693.65) is confirmed as antennary fucosylated, where a diagnostic peak at  $m/z$  1490.02 is the cleavage product of the peak at  $m/z$  1767.04 after loss of core GlcNAc, indicating no core fucose attached originally. (B) Fragment ions at  $m/z$  1938.94, 2402.79, and 2869.29 indicate antennary fucosylation for the mono-fucosylated tetra-antennary glycan ( $m/z$  3142.68). (C) The bifucosylated tetra-antennary glycan ( $m/z$  3316.93) is determined with both core and antennary fucosylation by the presence of a fragment ion at  $m/z$  1938.85 which is the product of the ion at  $m/z$  2390.77 after loss of core Fuc-GlcNAc, with one fucose attached to the antennae.



**Figure S3.** Scatter plot of the total fucosylation degree of AGP *N*-glycans in healthy subjects and patients with cirrhosis or HCC caused by NASH, ALC, and HCV, respectively.

**Table S1.** Branching degree and fucosylation degree of serum AGP in healthy control, HCC and cirrhosis patients of different etiologies.

	<b>Branching Degree (% , mean)</b>			<b>Fucosylation Degree (% , mean)</b>			
	<b>Bi</b>	<b>Tri</b>	<b>Tetra</b>	<b>mono-Fc</b>	<b>bi-Fc</b>	<b>tri-Fc</b>	<b>total Fc</b>
<b>Healthy Control</b>	17.3	47.6	35.2	17.6	0.9	0.0	19.6
NASH	8.6	45.8	45.6	31.1	4.9	0.5	42.4
<b>Cirrhosis</b>							
ALC	10.8	48.1	41.1	42.6	7.2	0.8	59.5
HCV	12.2	41.9	45.9	41.3	7.8	1.3	60.6
NASH	10.6	41.5	47.9	36.5	6.4	1.1	52.6
<b>HCC</b>							
ALC	9.4	44.3	46.2	44.7	12.3	2.4	76.7
HCV	11.7	39.9	48.3	42.1	13.5	3.1	78.4