

Performance Comparison of GP73, Fucosylated Hemopexin, PIVKA-II and AFP in a Cohort of Liver Disease Specimens

P. M. Hemken¹, Kaori Morota², M. Nakagawa², Rika Sekiya², Lori J. Sokoll², Debra J. Elliott² and B. L. Dowell¹

Diagnosics Research and Development, Abbott Diagnostics, Abbott Park, IL, USA¹ and Matsudo, Japan²,
Division of Clinical Chemistry, Department of Pathology, and Johns Hopkins Medical Institutions, Baltimore, MD, USA³

Abstract

Aim: The goal of this study was to evaluate the clinical performance of AFP (alpha-fetoprotein), PIVKA-II (protein induced by vitamin-K absence), GP73 and fucosylated hemopexin (fuc-HPX) in a cohort of liver disease specimens.

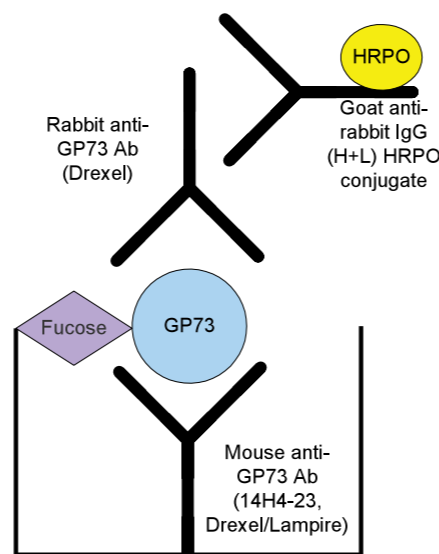
Methods: GP73 is a 73 kDa Golgi protein that is upregulated by viral infection and reported to be elevated in patients with HCV or HBV infection and hepatocellular carcinoma (HCC). Since an increase in core fucosylation of serum proteins has been reported with the development of HCC, fuc-HPX was measured. Specimens (n=229) were tested using the Tosoh AFP and ARCHITECT PIVKA-II (in development at Abbott Diagnostics) assays at Johns Hopkins and the GP73 and fuc-HPX assays at Abbott Diagnostics, Japan. The specimens included HCC (n=70, pre treatment), cirrhosis (n=35), hepatitis (n=52) and normals (n=12). Additional normals (n=60) were obtained from ProMedDx (Norton, MA). GP73 was measured using a sandwich ELISA using a mouse monoclonal (Mo) anti-GP73 for capture and a with a rabbit (Rb) polyclonal Rb anti-GP73 second antibody (Drexel University). A fuc-HPX microtiter chemiluminescence qualitative assay was used with Mo anti-HPX (Antibody Shop) for capture and biotinylated Aleuria Aurantia Lectin (AAL) (Vector Labs) for binding to fucose. Acridinium-streptavidin was used for detection.

Materials and Methods

Assay Format for in-house GP73 ELISA

GP73 microtiter plate ELISA (quantitative assay):

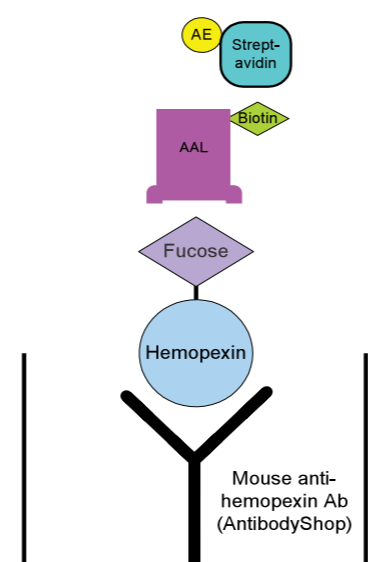
- **Mouse anti-GP73 antibody** (14H4-23, Drexel University/ Lampire)
- **Rabbit anti-GP73 antibody** (Drexel University)
- **Detection:** Goat anti-rabbit IgG (H+L) HRPO conjugate (Seikagaku Kogyo)
- **Calibrators:** rGP73-Fc from HEK293 (Abbott)



Assay Format for Fuc-HPX

Fuc-HPX microtiter chemiluminescence assay (qualitative assay):

- Mouse monoclonal anti-hemopexin antibody (AntibodyShop ABS013-04)
- Fucosylated-Hemopexin in human serum or plasma
- Lectin: biotinylated Aleuria Aurantia Lectin (AAL) (Vector Lab)
- CPSP Acridinium ester to Streptavidin

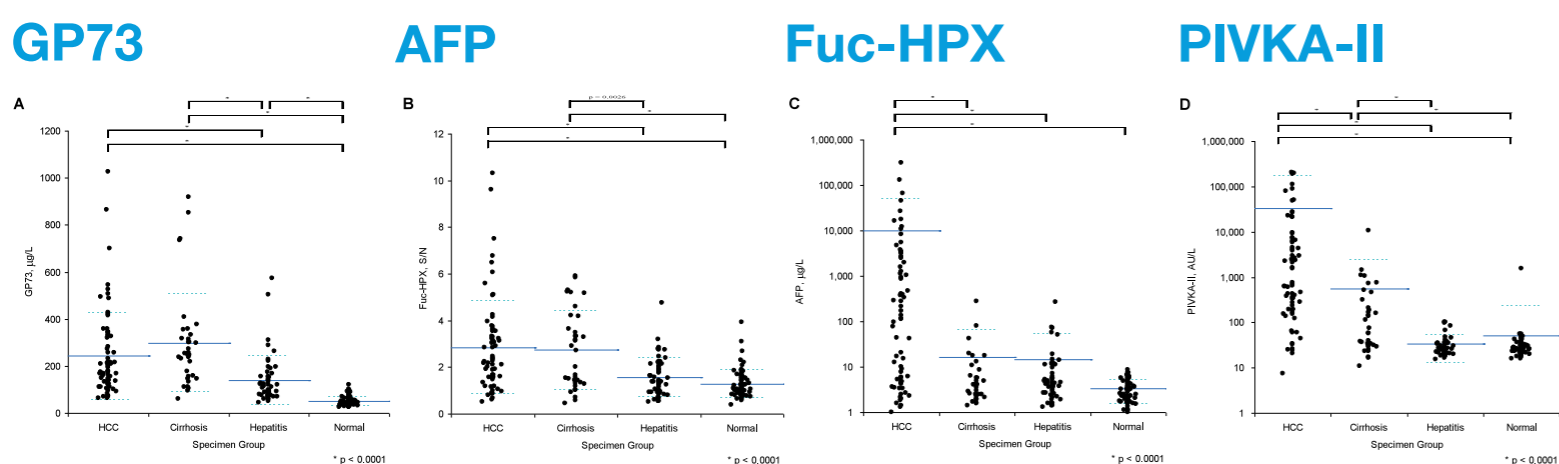


Results

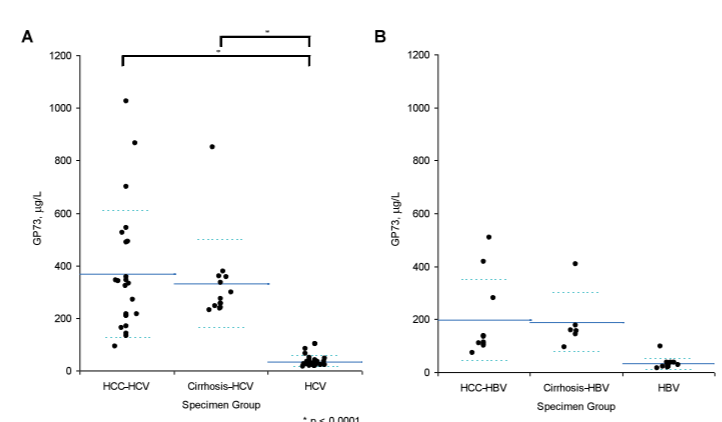
Levels of the four serum markers for HCC, cirrhosis, hepatitis, and normal patients.

	HCC (n=70)		Cirrhosis (n=35)		Hepatitis (n=52)		Normal (n=72)	
	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	Median
GP73, µg/L	244.8 ± 183.1	177.0	298.4 ± 207.9	247.4	138.1 ± 101.3	110.0	51.0 ± 18.4	47.3
Fuc-HPX, S/N	2.85 ± 1.96	2.35	2.73 ± 1.68	2.70	1.56 ± 0.83	1.37	1.29 ± 0.60	1.12
AFP, µg/L	9853.3 ± 41313.3	127.3	16.3 ± 47.9	4.0	14.8 ± 38.8	4.3	3.3 ± 1.8	3.0
PIVKA-II, AU/L	32942.0 ± 138308.2	720.1	552.4 ± 1827.2	67.3	33.5 ± 20.4	27.0	51.1 ± 181.7	28.0

Dot plots of serum levels of (A) GP73, (B) Fuc-HPX, (C) AFP, and (D) PIVKA-II in patients with HCC, cirrhosis, hepatitis, and in normal patients. The values of GP73 and Fuc-HPX are shown on a linear scale, while the values of AFP and PIVKA-II are shown on a log scale due to a wide range of reported concentrations. The solid and dotted lines represent the mean and +/-1 SD, respectively.



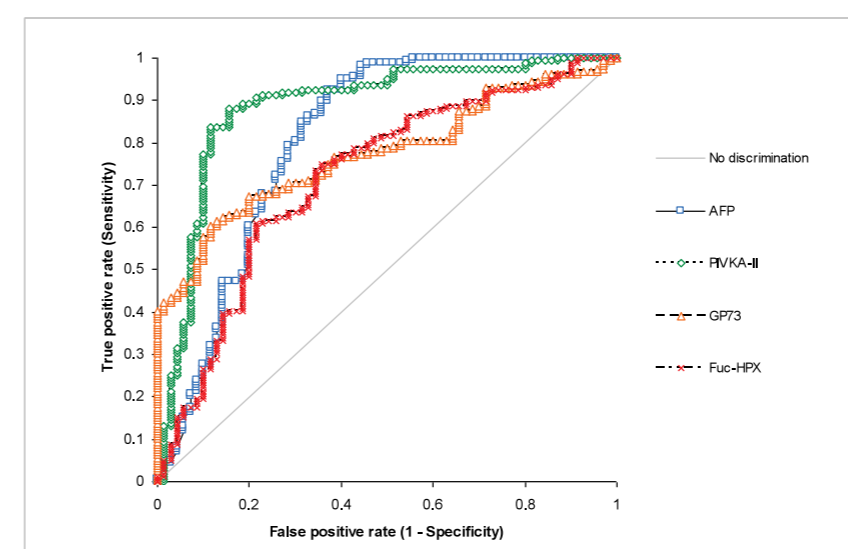
Dot plots of serum levels of GP73 for HCC, cirrhosis, and hepatitis by (A) HCV infection and (B) HBV infection. The solid and dotted lines represent the mean and +/-1 SD, respectively.



Results (cont'd)

AUROC comparison: HCC vs all others

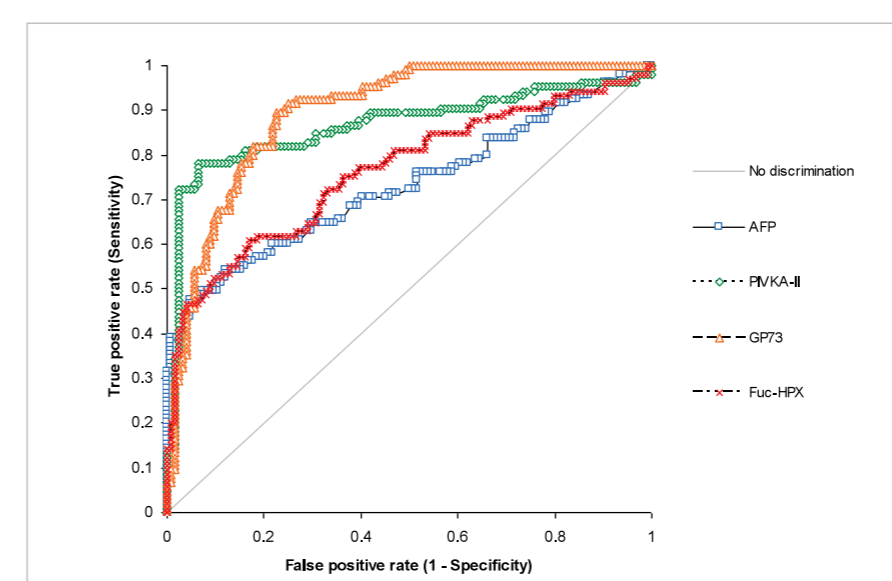
- 229 specimens were evaluated for AFP, PIVKA-II, GP73 and Fucosylated Hemopexin.
- ROC curves for HCC vs. all others were shown below. AUCs of AFP, PIVKA-II, GP73 and Fuc-HPX were 0.81, 0.89, 0.78, and 0.72, respectively. PIVKA-II showed largest AUC among them.



Disease	n	Test		
		Area	95% CI	SE
HCC	70			
Cirr + Hep + Normal	159			
AFP	229	0.81	0.73 to 0.88	0.037
PIVKA-II		0.89	0.83 to 0.94	0.027
GP73		0.78	0.72 to 0.83	0.030
Fuc-HPX		0.72	0.65 to 0.79	0.038

AUROC comparison: HCC + Cirrhosis vs Hepatitis + Normal

- 229 specimens were evaluated for AFP, PIVKA-II, GP73 and Fucosylated Hemopexin.
- ROC curves for HCC + Cirrhosis vs. hepatitis + normals were shown below. AUCs of AFP, PIVKA-II, GP73 and Fuc-HPX were 0.73, 0.87, 0.90, and 0.76, respectively. GP73 showed largest AUC among them.



Disease	n	Test		
		Area	95% CI	SE
Hep + Normal	124			
HCC + Cirrhosis	105			
AFP	229	0.73	0.66 to 0.80	0.035
PIVKA-II		0.87	0.81 to 0.92	0.027
GP73		0.90	0.86 to 0.94	0.020
Fuc-HPX		0.76	0.70 to 0.83	0.032

AUROC Summary		
	HCC	HCC + Cirrhosis
AFP	0.81	0.73
PIVKA-II	0.89	0.87
GP73	0.78	0.90
Fuc-HPX	0.72	0.76

Conclusions

- PIVKA-II followed by AFP showed the highest sensitivity and specificity for HCC
- GP73 followed by PIVKA-II showed the highest sensitivity and specificity for HCC + Cirrhosis

ARCHITECT is a trademark of Abbott Laboratories.