

The role of PIVKA-II in hepatocellular carcinoma surveillance in an Asian population

Dear Editor,

Alpha-fetoprotein (AFP) is the most established biomarker for surveillance of hepatocellular carcinoma (HCC) in at-risk individuals. However, its sensitivity and specificity are not very satisfactory.¹ Protein induced by vitamin K absence or antagonist-II (PIVKA-II) is a newer biomarker for HCC but without a widely established cut-off.^{2,3} Recent data suggested that the cut-off may be different for HCC due to chronic hepatitis C (CHC) and chronic hepatitis B (CHB) infection.⁴ Two different reference intervals (RIs), Japan and European Union (EU), are provided in the assay kit (Architect PIVKA-II, Abbott Laboratories, Chicago, US).⁵ In Singapore, CHB is the most prevalent association with HCC.⁶

Hence, we studied the distribution of PIVKA-II to determine which RI was more applicable to our Singapore patients. We also examined for significant associations between HCC and PIVKA-II alone, AFP alone, and the combination of both PIVKA-II and AFP.

Study subjects had blood drawn on the day of their 6-monthly HCC surveillance ultrasound for up to a maximum of 3 visits. PIVKA-II, AFP (Abbott Laboratories, Chicago, US) and AFP (Roche Elecsys; Roche Diagnostics, Basel, Switzerland) were assayed at the same time. Ultrasound and blood results were reviewed by a clinician. Suspicious lesions were investigated and HCC was diagnosed in accordance with international guidelines. Patient consent was obtained. SingHealth Centralised Institutional Review Board approved the study (reference CIRB 2017/2577).

Six hundred and ten patients in our HCC surveillance programme, who were without HCC for the prior 12 months, were enrolled. Majority were males (60.7%). Median age was 62 years (interquartile range [IQR] 55–69). Most had CHB (89.5%), of which 59.6% were on antiviral treatment with good control of the disease in 98.8%. Baseline median levels of alanine transaminase and aspartate transaminase of the cohort were 22U/L (IQR 17.0–32.0) and 25U/L (IQR 22.0–32.0), respectively. Median follow-up was 12.2 months, with 632 patient-years. About half (54.4%) had the second and 19.2% the third visit blood specimens taken.

Five patients developed HCC during the study period at a median age of 60 years (IQR 51.5–70.0). All had PIVKA-II levels measured at the time of diagnosis of HCC. Median PIVKA-II level was 28.5mAU/mL (IQR 19.7–42.3). Median AFP levels were 4.7µg/L (IQR 2.4–43.3) and 3.9µg/L (IQR 2.3–36.8) for Roche and Abbott assays, respectively.

Biomarker distribution. Distributions of PIVKA-II, AFP (Abbott), and AFP (Roche) in 605 study subjects without HCC are shown in Table 1A. The current cut-off, according to the manufacturer, is 40mAU/mL. The 97.5th percentile value of 48.0mAU/mL in our study cohort was closer to EU PIVKA-II of 50.9mAU/mL than Japan's 32.0mAU/mL provided by the manufacturer. The distribution of our AFP aligned more with the recommended cut-off value of AFP (Abbott) than AFP (Roche).

Diagnostic performance of PIVKA-II with/without AFP. Specificity and sensitivity were studied for PIVKA-II alone with the various cut-offs: 40mAU/mL (current), 32.0mAU/mL (Japan), 50.9mAU/mL (EU) and our study's 48.0mAU/mL. The combination of AFP (Roche) and AFP (Abbott) was also tested. (Table 1B).

All the tests had good specificity ($\geq 82\%$). However, sensitivity was less ideal. Both Roche and Abbott AFP alone were statistically significant for the detection of HCC (Fisher's test, $P=0.01$ and $P=0.03$, respectively). When combining PIVKA-II and AFP, only the combination of PIVKA-II using our study's or EU's cut-offs with AFP (Abbott) was significant (Table 1B). However, the sensitivity of using combined biomarkers was the same as AFP alone.

Discussion and conclusion. We have established for the first time the distribution of PIVKA-II in the Singapore population. The distribution of PIVKA-II values in our non-HCC patients was more similar to the healthy individuals in the EU than in Japan. This could be due to the large proportion of CHB patients in our cohort, which is more akin to the EU cohort, as the Japanese cohort was largely CHC patients. Unfortunately, we could not compare between CHB and CHC due to our small number of CHC patients.

Table 1A. Distribution of tumour biomarkers in non-HCC patients

Biomarker	Percentile	Overall cohort	Chronic hepatitis B	Reference interval (manufacturer)	
PIVKA-II, mAU/mL	2.5th	14.2	14.4	11.1 (JP)	17.4 (EU)
	97.5th	48.0	45.1	32.0 (JP)	50.9 (EU)
AFP (Abbott), µg/L	0	1.15	1.13	-	-
	97.5th	9.44	8.54	8.78	8.78
AFP (Roche), µg/L	0	1.30	1.30	-	-
	95th	9.80	9.07	7.00	7.00

AFP: alpha-fetoprotein; EU: European Union; HCC: hepatocellular carcinoma; JP: Japan; PIVKA-II: protein induced by vitamin K absence or antagonist-II
 Bold values denote the cut-off values for determining positive levels

Table 1B. Specificity and sensitivity of PIVKA-II diagnostic performance

Biomarker	Sensitivity	Specificity	P value
AFP (Roche) 7.00µg/L	0.40	0.94	0.01
AFP (Abbott) 8.78µg/L	0.40	0.96	0.03
Singular: PIVKA-II			
PIVKA-II (current) 40mAU/mL	0.20	0.93	0.29
PIVKA-II (JP) 32.0mAU/mL	0.40	0.82	0.22
PIVKA-II (EU) 50.9mAU/mL	0.20	0.97	0.14
PIVKA-II (study) 48.0mAU/mL	0.20	0.97	0.16
Combination: PIVKA-II + AFP			
PIVKA-II (current) + AFP (Roche)	0.40	0.89	0.10
PIVKA-II (current) + AFP (Abbott)	0.40	0.90	0.08
PIVKA-II (JP) + AFP (Roche)	0.60	0.78	0.07
PIVKA-II (JP) + AFP (Abbott)	0.60	0.80	0.07
PIVKA-II (EU) + AFP (Roche)	0.40	0.92	0.06
PIVKA-II (EU) + AFP (Abbott)	0.40	0.93	0.04
PIVKA-II (study) + AFP (Roche)	0.40	0.91	0.06
PIVKA-II (study) + AFP (Abbott)	0.40	0.96	0.04

AFP: alpha-fetoprotein; EU: European Union; HCC: hepatocellular carcinoma; JP: Japan; PIVKA-II: protein induced by vitamin K absence or antagonist-II

Bold P values are statistically significant

Diagnostic cut-offs to relate HCC associations used in 2x2 contingency analysis

HCC: 5, non-HCC: 605

There were 5 cases of incident HCC during the study period of 632 patient-years of follow-up. This is in concordance with our institution's previously reported HCC rate of 0.8% per year.⁷

Our study also showed that PIVKA-II alone was specific but not significant for HCC diagnosis. On the other hand, AFP alone was significant for HCC diagnosis. Combining PIVKA-II and AFP was significant in diagnosing HCC in our population but the sensitivity was the same as using AFP alone.

A systematic review of des-γ-carboxyprothrombin (DCP), another name for PIVKA-II, has shown a moderate diagnostic utility for HCC.⁸ DCP/PIVKA-II level has also been reported to be related to HCC size.⁹ This may explain the low diagnostic utility of PIVKA-II in our HCC surveillance study cohort, where incident HCCs are early HCCs.

Previous studies have shown that adding PIVKA-II to AFP is useful for HCC screening.¹⁰ In our cohort, this was significant only when our study's PIVKA-II cut-off was used with AFP. However, the sensitivity was the same as AFP alone. During surveillance, ruling in possible HCC cases is more important than ruling out, and hence, the sensitivity of screening biomarkers is more pertinent than specificity. Thus, combining PIVKA-II and AFP was not superior to AFP alone for HCC surveillance in our population.

Our study had limitations. There were too few incident HCC cases to allow a more robust analysis of PIVKA-II alone, compared to a combination with AFP for early diagnosis of HCC. As the HCC incidence is very low even in at-risk populations, a very large study population is necessary to yield sufficient HCC cases. There were also too few CHC patients in our cohort to allow for comparison between CHB and CHC cases. The manufacturer's different RIs of PIVKA-II in the EU and Japan may be due to different

preponderances of CHB and CHC in both these places. Our study supported the notion that diagnostic cut-off is likely population specific. We are cognisant that, unlike the manufacturer's RIs that were based on normal individuals, ours was based on an at-risk population. PIVKA-II is not a screening test for the general population. So in the real world, it would only be used in at-risk populations, which makes our basis of derivation of RIs in at-risk populations most relevant.

In conclusion, we have derived our own PIVKA-II RIs. The combination of PIVKA-II and AFP did not appear to be better than using AFP alone for HCC surveillance in our Singapore population.

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Wai-Yoong Ng ^{*1}PhD, Daniel Yan-Zheng Lim ^{*2,3}MRCP (UK),
Si-Yu Tan ¹PhD, Jason Pik-Eu Chang ⁴MRCP (UK),
Thinesh Lee Krishnamoorthy ⁴MRCP (UK),
Chee-Hooi Lim ⁴MD (Leeds),
Damien Meng-Yew Tan ⁴MRCP (UK),
Victoria Sze-Min Ekstrom ⁴MRCP (UK),
George Boon-Bee Goh ⁴MRCP (UK),
Mark Chang-Chuen Cheah ⁴MRCP (UK),
Rajneesh Kumar ⁴MRC P(UK), Chin-Pin Yeo ¹FRCPA,
Chee-Kiat Tan ⁴FRCP (Edinburgh)

¹ Department of Clinical Pathology, Singapore General Hospital, Singapore

² Health Services Research Unit, Medical Board, Singapore General Hospital, Singapore

³ Medicine Academic Clinical Programme, Duke-NUS Medical School, Singapore

⁴ Department of Gastroenterology and Hepatology, Singapore General Hospital, Singapore

Correspondence: Dr Chee-Kiat Tan, Department of Gastroenterology and Hepatology, Academia, Level 3, 20 College Road, Singapore 169856.

Email: tan.chee.kiat@singhealth.com.sg

* Joint first authors