Elecsys PIVKA-II and Elecsys AFP assays demonstrate good clinical performance for hepatocellular carcinoma (HCC) diagnosis across different disease stages and aetiologies

Category: Diagnosis, imaging and biomarkers

Henry LY Chan,[1] Arndt Vogel,[2] Thomas Berg,[3] Enrico N De Toni,[4] Masatoshi Kudo,[5] Jörg Trojan,[6] Arndt Weinmann,[7] Anja Eiblmaier,[8] Hans-Georg Klein,[9] Johannes Kolja Hegel,[10] Dave Morgenstern,[11] Kairat Madin,[12] Vinzent Rolny,[12] Marcus-Rene Lisy,[12] Teerha Piratvisuth[13]

[1]The Chinese University of Hong Kong, Hong Kong, China; [2]Medizinische Hochschule Hannover, Hannover, Germany; [3]Universitätsklinikum Leipzig, Leipzig, Germany; [4]Department of Medicine II, University Hospital, Ludwig Maximilian University of Munich, Munich, Germany; [5]Kindai University, Osaka, Japan; [6]Goethe Universitat Frankfurt, Frankfurt, Germany; [7]Universitätsmedizin der Johannes Gutenberg, Mainz, Germany; [8]Microcoat Biotechnologie GmbH, Bernried, Germany; [9]Zentrum fur Humangenetik und Laboratoriumsdiagnostik, Munich, Germany; [10]Labor Berlin Charité Vivantes Services GmbH, Berlin, Germany; [11]Roche Diagnostics Operations, Indianapolis, IN, USA; [12]Roche Diagnostics GmbH, Penzberg, Germany; [13]Prince of Songkla University, Hat Yai, Thailand

Introduction

- Protein induced by Vitamin K absence or antagonist-II (PIVKA-II) and alpha-fetoprotein (AFP) are considered to be effective serum biomarkers for early detection of HCC.[1]
- Controversies exist regarding the value of AFP in the diagnosis of HCC, with some data suggesting that PIVKA-II is superior to AFP for early detection of HCC, being highly sensitive and specific.[2,3]
- An immunoassay for quantitative measurement of PIVKA-II in serum and plasma was developed to complement the tumour marker portfolio on the Elecsys® automated immunoassay platform including to aid in HCC diagnosis.
- Results from an evaluation of the analytical performance and method comparison of the Elecsys PIVKA-II immunoassay are presented in poster P-19 at this congress. Here, we report results on the clinical performance of the assay.

Objective

- **To evaluate the clinical performance of the new Elecsys PIVKA-II immunoassay and the previously launched Elecsys AFP assay (Roche Diagnostics) to aid in the diagnosis of HCC, according to disease stage and aetiology.**

Methods

- This was a multicentre, prospective study designed to assess the clinical performance of the new Elecsys PIVKA-II immunoassay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) as an aid in the diagnosis of HCC.
- Patients and disease controls aged ≥18 years were prospectively enrolled at seven clinics in the People's Republic of China, Germany, Japan and Thailand, with all patients providing written, informed consent prior to enrolment. One additional site in Germany provided banked HCC samples.
  - Eligible HCC cases had first-time HCC diagnosis confirmed radiologically according to national guidelines, or by liver biopsy. Key exclusion criteria were: presence of any other cancer, except non-melanoma skin cancer (NMSC); recurrent HCC; current or previous treatment for HCC.
  - Eligible disease controls had absence of HCC confirmed by imaging within 12 months prior, and presence of: cirrhosis; non-cirrhotic chronic hepatitis B virus (HBV) infection; non-cirrhotic chronic hepatitis C virus (HCV) infection; non-cirrhotic non-alcoholic steatohepatitis (NASH). The key exclusion criterion in disease controls was presence of any cancer except NMSC.
  - Aetiology groups for HCC cases and controls were: cirrhotic; non-cirrhotic chronic HBV, HCV, NASH or alcoholic liver disease (ALD); other. HCC cases were also grouped according to Barcelona Clinic Liver Cancer (BCLC) staging (early, stages 0/A; late, stages B/C/D).
- Serum samples were collected ≥1 day prior to general anaesthesia and surgery, and frozen prior to analysis.
- PIVKA-II and AFP concentrations were measured using the Elecsys PIVKA-II and AFP assays, respectively, on the cobas e 601 analyser, in three experimental runs at Microcoat GmbH (Bernried, Germany). HCC diagnosis was determined by a cut-off of 28.4 ng/mL for PIVKA-II and 20 ng/mL for AFP.
  - AFP concentrations were measured in IU/mL and converted to ng/mL by applying a conversion factor of 1.21.
- All clinical information was collected in an electronic data capture system. All patients provided written informed consent, and the study was conducted according to the principles of the Declaration of Helsinki.

Results

Participants

- A total of 473 patients were screened; of these, 168 HCC cases and 208 disease controls were enrolled in the study (**Table 1**).
- In the HCC cohort, mean age was 62.86 years, 141 (83.9%) were male and 139 (82.7%) had cirrhosis; 77 (45.8%) had early-stage HCC and 91 (54.2%) had late-stage HCC.
- In the disease control cohort, mean age was 52.18 years, 126 (60.6%) were male and 79 (38.0%) had cirrhosis.

Table 1. Participant demographics and clinical characteristics

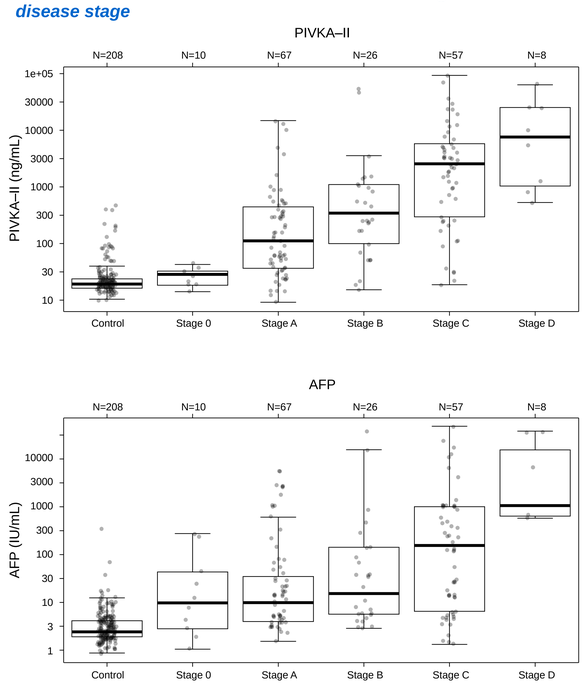
Characteristic	All (N=376)	Control (n=208)	HCC (n=168)
Mean ± SD age, years	56.95 ± 12.42	52.18 ± 12.27	62.86 ± 9.82
Male, n (%)	267 (71.0)	126 (60.6)	141 (83.9)
Race, n (%)			
Asian	170 (45.2)	99 (47.6)	71 (42.3)
White	196 (52.1)	101 (48.6)	95 (56.6)
Black or African American	3 (0.8)	3 (1.4)	0
Other	1 (0.3)	0	1 (0.6)
Missing	6 (1.6)	5 (2.4)	1 (0.6)
Disease aetiology, n (%)			
Cirrhosis	218 (58.0)	79 (38.0)	139 (82.7)
HBV	86 (22.9)	72 (34.6)	14 (8.3)
HCV	30 (8.0)	27 (13.0)	3 (1.8)
NASH	31 (8.2)	30 (14.4)	1 (0.6)
ALD	2 (0.5)	0	2 (1.2)
Other	9 (2.4)	0	9 (5.4)
BCLC stage, n (%)			
0	–	–	10 (6.0)
A	–	–	67 (39.9)
B	–	–	26 (15.5)
C	–	–	57 (33.9)
D	–	–	8 (4.8)

SD, standard deviation.

Clinical performance

- Serum PIVKA-II and AFP concentrations were elevated in HCC cases compared with disease controls (median PIVKA-II concentration: 301.19 vs 19.39 ng/mL; median AFP concentration: 24.55 ng/mL vs 2.92 ng/mL).
- Serum PIVKA-II and AFP concentrations correlated with HCC disease stage (**Figure 1**). Median PIVKA-II and AFP concentrations were 63 ng/mL and 11.7 ng/mL for early-stage HCC (n=77), increasing to 1486 ng/mL and 144 ng/mL for late-stage HCC (n=91), respectively.
- Distribution of serum PIVKA-II and AFP concentrations in the HCC and disease control cohorts by aetiology group is shown in **Table 2**.
  - Serum PIVKA-II and AFP concentrations were elevated in HCC cases with cirrhosis versus disease controls with cirrhosis (median PIVKA-II concentration: 277 vs 20.7 ng/mL; median AFP concentration: 20.1 vs 3.19 ng/mL).
  - There were too few patients with non-cirrhotic aetiology within the HCC cohort to draw effective comparisons with the disease control cohort.

Figure 1. Distribution of PIVKA-II and AFP according to HCC BCLC disease stage



For each box, the black line represents the median value and whiskers represent the minimum and maximum values.

Table 2. Distribution of PIVKA-II and AFP by aetiology group

	Median concentration (range), ng/mL	
	PIVKA-II	AFP
HCC cohort (n=168)		
Cirrhotic (n=139)	277 (9.39–89,918)	20.1 (1.26–54,071)
Non-cirrhotic HBV (n=14)	625 (37.3–24,432)	46.2 (2.73–2070)
Non-cirrhotic HCV (n=3)	32.3 (19–571)	52.1 (2.25–1209)
Non-cirrhotic NASH (n=1)	–	–
Non-cirrhotic ALD (n=2)	633 (460–807)	20,141 (4.69–40,278)
Other (n=9)	1620 (22.3–23,205)	254 (2.4–19,499)
Disease control cohort (n=208)		
Cirrhotic (n=79)	20.7 (11.7–465)	3.19 (1.03–80.9)
Non-cirrhotic HBV (n=72)	18.2 (10.3–39)	2.79 (1.25–3.97)
Non-cirrhotic HCV (n=27)	18.2 (9.92–24.9)	3.01 (1.57–7.66)
Non-cirrhotic NASH (n=30)	21.3 (16.7–86.7)	3 (1.22–13.2)
Non-cirrhotic ALD (n=0)	–	–
Other (n=0)	–	–

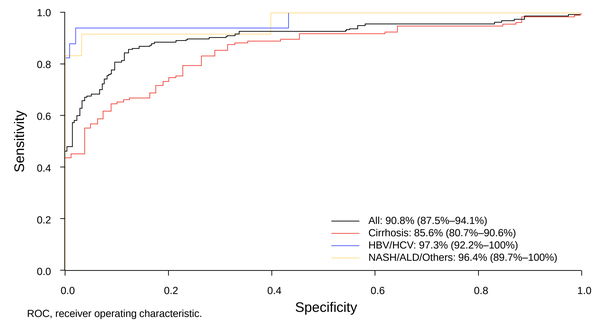
- Both assays demonstrated good clinical performance for detection of HCC (**Table 3**). The Elecsys PIVKA-II assay showed high sensitivity and good specificity in detecting HCC; sensitivity was higher for late-stage versus early-stage HCC. The Elecsys AFP assay showed excellent specificity and moderate sensitivity in detecting HCC; sensitivity was higher for late-stage versus early-stage HCC.

Table 3. Clinical performance of Elecsys PIVKA-II and Elecsys AFP assays by HCC BCLC stage

Assay (cut-off)	Metric, % (95% confidence interval)	HCC BCLC stage		
		Early (n=77)	Late (n=91)	Overall (n=168)
PIVKA-II (28.4 ng/mL)	Sensitivity	77.9 (67.0–86.6)	94.5 (87.6–98.2)	86.9 (80.8–91.6)
	Specificity	83.7 (77.9–88.4)	83.7 (77.9–88.4)	83.7 (77.9–88.4)
AFP (20 ng/mL)	Sensitivity	36.4 (25.7–48.1)	64.8 (54.1–74.6)	51.8 (44.0–59.5)
	Specificity	98.1 (95.1–99.5)	98.1 (95.1–99.5)	98.1 (95.1–99.5)

- The Elecsys PIVKA-II assay also demonstrated high specificity in other analyses. High specificity was observed across non-cirrhotic aetiologies ranging from 90.3% in HBV+ samples, to 93.3% in samples with NASH and 100% in HCV+ samples; specificity was lower (68.4%) for cirrhotic cases.
- In ROC analysis, area under the curve for all samples was 90.8%, being highest for HBV/HCV (97.3%), and lowest for cirrhotic cases (85.6%; **Figure 2**).

Figure 2. ROC plot of Elecsys PIVKA-II for discriminating between HCC patients and disease controls, overall and by aetiology group



ROC, receiver operating characteristic.

- Using a combination of PIVKA-II and AFP, overall sensitivity for HCC detection was 92%, versus 87% using the Elecsys PIVKA-II assay alone or 52% using the Elecsys AFP assay alone. The corresponding specificities were 82%, 84% and 98%, respectively.
- In analysis of concordance between a PIVKA-II cut-off of 28.4 ng/mL, and AFP cut-offs of 8.22 ng/mL and 11.5 ng/mL (corresponding to 90% and 95% specificity, respectively), concordance was highest for a PIVKA-II cut-off of >28.4 ng/mL and an AFP cut-off of >8.22 ng/mL in patients with early- and late-stage HCC (43% and 70%, respectively).

Conclusions

- **The Elecsys PIVKA-II and AFP assays demonstrated good clinical performance as aids in the diagnosis of HCC, across all disease stages and aetiologies.**
- **Combining the two biomarkers may further increase diagnostic performance.**

References

1. Omata M, et al. Hepatol Int. 2010;4(2):439–74.
2. Llovet JM. Clin Gastroenterol Hepatol. 2006;4(1):29–35.
3. Zakhary NI, et al. J Adv Res. 2013;4(6):539–46.

Acknowledgements

This study was funded by Roche Diagnostics International Ltd (Rotkreuz, Switzerland). Third-party medical writing support, under the direction of the authors, was provided by Tina Morley (contract medical writer at Gardiner-Caldwell Communications, Macclesfield, UK) and Chloe Fletcher (Gardiner-Caldwell Communications, Macclesfield, UK), and was funded by Roche Diagnostics International Ltd (Rotkreuz, Switzerland).