Performance evaluation of the Elecsys PIVKA-II and Elecsys AFP assays for hepatocellular carcinoma diagnosis

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Abstract

Background and Aims: Prothrombin induced by vitamin K absence-II (PIVKA-II) is a serum biomarker linked to hepatocellular carcinoma (HCC), showing superiority to alpha-fetoprotein (AFP) for early disease detection. We aimed to assess the clinical and analytical performance of the Elecsys® PIVKA-II immunoassay in diagnosing HCC and evaluate PIVKA-II’s technical performance.

Methods: Serum samples from adult cases (i.e., patients with a first-time HCC diagnosis; n = 168) and disease controls (i.e., patients without HCC with an at-risk condition; n = 208) were assessed. An AFP cut-off of 20 ng/mL was used to differentiate between HCC cases and disease controls. Clinical performance of the Elecsys PIVKA-II assay was compared with that of comparator assays (Lumipulse G PIVKA-II, μTASWako DCP, ARCHITECT PIVKA-II) using receiver operating characteristic curve analysis to determine the area under the curve (AUC) values.

Results: The Elecsys PIVKA-II assay compared favorably with comparator assays. Using a 28.4 ng/mL cut-off, the Elecsys PIVKA-II assay detected HCC with 86.9% sensitivity and 83.7% specificity. Clinical performance of the Elecsys PIVKA-II assay (AUC: 90.8%) was equivalent to that of comparator assays (AUC: 88.3–89.6%). Relatively high PIVKA-II concentrations were observed for cholangiocarcinoma and pancreatic cancer with the Elecsys assay in specificity panel analyses, indicating that high PIVKA-II concentrations should not be used alone in the absence of other clinical data.

Conclusions: The Elecsys PIVKA-II assay showed good analytical performance under routine laboratory conditions, comparing favorably with comparator assays. These findings support the suitability of the Elecsys PIVKA-II assay as an aid in HCC diagnosis.
More recently, non-alcoholic steatohepatitis (NASH) has been shown to be an independent predictor of microvascular invasion in HCC. \textsuperscript{13,14} and superior to AFP for the early detection of HCC, being highly sensitive and specific. \textsuperscript{11,15} Serologic statistical models including serum AFP and PIVKA-II measurements have previously been developed to aid in the prognosis and diagnosis of HCC. A statistical model involving sex, age, AFP isoform L3 (AFP-L3), AFP, and DCP (GALAD) was developed to determine the risk of HCC in patients with chronic liver disease. \textsuperscript{16} An international case–control study showed that the GALAD score can detect early-stage HCC and may aid in monitoring patients with NASH. \textsuperscript{17} The GALAD score has the potential to be used as a screening tool for the detection of HCC in patients with NASH; however, further validation in a large, prospective study is warranted. A second model combining bilirubin, albumin, AFP-L3, AFP, and DCP (BALAD) was developed to aid prognostication in HCC, and was further refined through the use of continuous variables (BALAD-2).\textsuperscript{18,19} BALAD-2 has since been validated in an international setting and across different disease stages, supporting its use in staging and prognostication for patients with HCC.\textsuperscript{20,21}

A new immunoassay for the quantitative measurement of PIVKA-II in human serum and plasma, to be used as a diagnostic aid in HCC, has been developed to complement the tumor marker portfolio on the Elecsys\textsuperscript{10} automated immunoassay platform. The aim of this study was to assess the clinical performance of the Elecsys PIVKA-II and AFP immunoassays in aiding the diagnosis of HCC and to evaluate the technical performance of PIVKA-II, including the determination of reference range(s) for healthy adults.

**Methods**

**Participants.** This was a multicenter, prospective study designed to assess the clinical and analytical performance of the new Elecsys PIVKA-II immunoassay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) as an aid in the diagnosis of HCC. Patients with HCC and disease controls were prospectively enrolled at seven clinics in China, Germany, Japan, and Thailand. All participants were aged 18 years and older, and provided written, informed consent prior to enrollment. One additional site provided banked HCC samples. Additional study details are provided in Methods section, Supporting information.

Eligible HCC cases had a first-time HCC diagnosis, confirmed radiologically according to national guidelines, or by liver biopsy. Key exclusion criteria were the presence of any other cancer (except non-melanoma skin cancer), recurrent HCC, or current or previous treatment for HCC.

Eligible disease controls had absence of HCC confirmed by imaging within 12 months before the study and presence of one of the following: cirrhosis; non-cirrhotic chronic hepatitis B virus (HBV); non-cirrhotic chronic hepatitis C virus (HCV); or NASH. The key exclusion criterion was the presence of any cancer except non-melanoma skin cancer.

Etiology groups for HCC cases and controls were classified as cirrhotic, non-cirrhotic HBV, non-cirrhotic HCV, non-cirrhotic NASH, non-cirrhotic alcoholic liver disease (ALD), or other. HCC cases were also grouped according to the Barcelona Clinic Liver Cancer (BCLC) staging (early, stages 0/A; late, stages B/C/D).
Sample handling and analysis. Serum samples were collected by venous blood draw ≥1 day before general anesthesia/surgery and frozen before shipping (see Methods section, Supporting information).

Repeatability (within-run), intermediate precision (within-laboratory), and reproducibility were calculated and compared against prespecified acceptance criteria. For samples with concentrations from the limit of detection to 30 ng/mL, the acceptance criteria were standard deviation (SD) ≤1.5 ng/mL (repeatability), ≤2.25 ng/mL (intermediate precision), and ≤4.5 ng/mL (reproducibility). For samples with concentrations >30 ng/mL, the acceptance criteria were coefficient of variation (CV) ≤5% (repeatability), ≤7.5% (intermediate precision), and ≤15% (reproducibility).

Samples were analyzed at three laboratories in Germany with three cobas e 411 analyzers (the master instruments) and two cobas e 601 analyzers, with one run per day for 5 days using one reagent lot (five replicates for each of the seven samples [five human serum pools plus two PreciControl samples covering the measuring range of 3.5–12 000 ng/mL]; Clinical & Laboratory Standards Institute [CLSI] EP05-A3 criteria). Reference ranges were determined in serum samples collected from healthy individuals 20–79 years of age in Munich (n = 399) and Nuremberg (n = 412) on one cobas e 411 analyzer and two cobas e 601 analyzers.

Method comparison experiments were conducted with samples from HCC cases and disease controls on the Elecsys PIVKA-II assay using the cobas e 601 analyzer at Microcoat GmbH (Bernried, Germany); assays using comparator platforms (Lumipulse G PIVKA-II, μTASWako DCP, and ARCHITECT PIVKA-II) were performed at the Life Science Research Institute (Yokohama, Japan).

Data handling. Details on sample size determination are included in the Methods section, Supporting information.

The clinical performance of the Elecsys PIVKA-II and AFP assays was determined by co-testing the same aliquot of HCC cases and disease control samples on the cobas e 601 analyzer. Specificity panel samples from patients with other benign/malignant diseases were also measured at two sites with the Elecsys PIVKA-II and AFP assays simultaneously and the μTASWako DCP platform.

Analytical comparison of methods was performed using weighted Deming regression (CLSI EP09-A3 criteria). Clinical performance of the different methods was assessed using receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC) values were calculated. An AFP cut-off of 20 ng/mL was prespecified to assess clinical performance of AFP to accurately differentiate between HCC cases and disease controls of 376 participants in total. For Elecsys PIVKA-II, the clinical performance at specified specificity and sensitivity values (between 70 and 95%) was calculated, with the 95th percentile used to define the cut-off.

Results

Study participants. In total, 473 patients were screened. Of these, 168 HCC cases and 208 disease controls were enrolled in the study; 97 screened patients were excluded (56 HCC cases and 41 disease controls) because of either inclusion/exclusion criteria not being met, incomplete sample processing, or physician or sponsor decision.

In the HCC cohort, the mean patient age was 62.86 years; 141 (83.9%) patients were men and 139 (82.7%) had cirrhotic etiology (Table S1, Supporting information). Seventy-seven (45.8%) patients had early-stage HCC (BCLC stages 0/A) and 91 (54.2%) had late-stage HCC (BCLC stages B/C/D). In total, 122 (72.6%) patients in the HCC cohort had a Child–Pugh score of A, and 98 (58.3%) had an albumin-bilirubin (ALBI) grade of 2.

In the control cohort, the mean age was 52.18 years; 126 (60.6%) participants were men and 79 (38.0%) had cirrhotic etiology. Child–Pugh scores were not available for the control cohort; however, 153 (73.6%) patients had an ALBI grade of 1.

Analytical performance. The Elecsys PIVKA-II assay demonstrated high repeatability, intermediate precision, and reproducibility when compared against prespecified acceptance criteria. For low-concentration samples (mean: 7.52–26.55 ng/mL), SDs ranged from 0.278 to 1.32 ng/mL for repeatability (within the ≤1.5 ng/mL criterion), from 0.334 to 1.44 ng/mL for intermediate precision (within the ≤2.25 ng/mL criterion), and from 0.619 to 1.79 ng/mL for reproducibility (within the ≤4.5 ng/mL criterion). For high-concentration samples (mean: 359.1–10 294 ng/mL), the CV ranged from 2.05 to 2.99% for repeatability (within the ≤5% criterion), from 3.28 to 3.74% for intermediate precision (within the ≤7.5% criterion), and from 5.28 to 5.82% for reproducibility (within the ≤15% criterion).

The reference range population comprised 811 individuals: 431 (53.1%) were men and the mean age was 47.1 years. Mean PIVKA-II concentration was 19.7 ng/mL, with values ranging from 19.1 to 20.7 ng/mL across age groups. The 95th percentile was 28.4 ng/mL. Therefore, 28.4 ng/mL was used as a cut-off for PIVKA-II in the clinical performance analyses.

Method comparison was performed using 391 samples, although 10 nonclinical samples prepared from leftovers were excluded because of outlying behaviors. Weighted Deming regression analyses showed moderate agreement between the Elecsys PIVKA-II assay and Lumipulse G PIVKA-II (y = 5.81x + 0.57; Pearson’s r = 0.883; P < 0.001); μTASWako DCP (y = 8.11x + 0.60; Pearson’s r = 0.866; P < 0.001); and ARCHITECT PIVKA-II (y = 4.68x + 0.59; Pearson’s r = 0.875; P < 0.001) assays (Fig. 1).

Clinical performance. Clinical performance of the Elecsys PIVKA-II assay (AUC: 90.8%) was equivalent to that of comparator assays (AUC: 88.3–89.6%; Fig. 2a). PIVKA-II and AFP concentrations were clearly elevated in HCC cases compared with disease controls (Fig. 3a,b). The median PIVKA-II concentration was 301.19 ng/mL in HCC cases compared with 19.39 ng/mL in disease controls. The median AFP concentration was 24.55 ng/mL in HCC cases compared with 2.92 ng/mL in disease controls.

Serum PIVKA-II and AFP concentrations correlated with HCC disease stage (Fig. 3c,d). Median PIVKA-II and AFP concentrations were 63 and 11.7 ng/mL for early-stage HCC, increasing to 1486 and 144 ng/mL for late-stage HCC, respectively.
Both assays demonstrated good clinical performance for the detection of HCC (Table 1). The Elecsys PIVKA-II assay showed high sensitivity and good specificity; sensitivity was higher for late stage versus early stage HCC (94.5 vs 77.9%). The Elecsys AFP assay showed excellent specificity and moderate sensitivity; sensitivity was higher for late stage versus early stage HCC (64.8 vs 36.4%). Cumulative data analysis resulted in an AUC value of 0.908 for Elecsys PIVKA-II and 0.88 for Elecsys AFP, confirming their clinical performance. Clinical performance of both Elecsys PIVKA-II and Elecsys AFP for specificity, ranging between 70 and 95%, and sensitivity, ranging between 70 and 95%, was determined (summarized in Table 2). At 95% specificity, cut-offs for Elecsys PIVKA-II and Elecsys AFP were 86.7 and 11.52 ng/mL, respectively, and sensitivity was 67.9 and 61.9%. Cut-offs for Elecsys PIVKA-II and Elecsys AFP at 90% specificity were 35.9 and 8.22 ng/mL, and sensitivity was 81 and 64.9%. Specificity at 95 and 90% sensitivity was similar between Elecsys PIVKA-II and Elecsys AFP.

Specificity and sensitivity for discrimination of HCC in early-, late-, and all-stage patients at rounded cut-offs from 20 to 1000 mAU/mL are summarized in Tables S2–S4. Supporting information for the Elecsys PIVKA-II, Lumipulse G PIVKA-II, µTASWako DCP, and ARCHITECT PIVKA-II assays. At a cut-off of 40 mAU/mL, specificity and sensitivity were comparable between the Elecsys PIVKA-II assay (specificity: 90.9%, sensitivity: 78%) and the Lumipulse G PIVKA-II, µTASWako DCP, and ARCHITECT PIVKA-II assays (specificity: 84.6–89.9%, sensitivity: 81.0–82.7%) for discriminating all-stage HCC cases.

The Elecsys PIVKA-II assay also demonstrated high specificity in other analyses. For instance, high specificity was seen across non-cirrhotic etiologies, ranging from 90.3% in HBV+ samples to 93.3% in samples with NASH and to 100% in HCV+ samples; specificity was lower (68.4%) for cirrhotic cases. Specificity was not determined for ALD and other factors due to small sample sizes.

The AUC for all samples was 90.8%. The AUC was highest for HBV/HCV samples (97.3%) and lowest for cirrhotic cases (85.6%; Fig. 2b). Additionally, specificity panel analyses showed that PIVKA-II was found in lower concentrations for most other conditions tested compared with HCC, with the exception of cholangiocarcinoma and pancreatic cancer, on both Elecsys PIVKA-II and µTASWako DCP assays (Fig. 4). In samples from patients with cholangiocarcinoma, a median PIVKA-II concentration of 143 ng/mL (range: 14.5–22 463 ng/mL) was observed using the Elecsys PIVKA-II assay. In samples from patients with pancreatic cancer, the median PIVKA-II concentration was 211 ng/mL (range: 17.6–3034 ng/mL) using the Elecsys PIVKA-II assay.

Analysis of concordance between a PIVKA-II cut-off of 28.4 ng/mL and AFP cut-offs of 8.22 and 11.5 ng/mL (corresponding to 90 and 95% specificity, respectively) showed that 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; Table S5, Supporting information).

Using a combination PIVKA-II (at a cut-off of 28.4 ng/mL) and AFP (at a cut-off of 20 ng/mL), the 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.

Discussion

This study demonstrated that the Elecsys PIVKA-II assay compares favorably with commercially available comparator assays. Using a cut-off of 28.4 ng/mL, the Elecsys PIVKA-II assay detected HCC with a sensitivity of 86.9% and specificity of 83.7%. Clinical performance of the Elecsys PIVKA-II assay (AUC: 90.8%) was equivalent to that of comparator assays (AUC: 88.3–89.6%).

Relatively high PIVKA-II concentrations were also observed for cholangiocarcinoma and pancreatic cancer using the Elecsys PIVKA-II assay in the specificity panel of this study, although this may be attributable to underlying conditions in these patients (e.g. cholestatic disease, cholangitis, biliary stenosis, or bile duct stone). The small number of measurable samples from patients with cholangiocarcinoma (n = 27) and pancreatic cancer (n = 10) should also be considered as a limitation. However, the elevated concentrations were confirmed using the μTASWako DCP platform; previous studies have also reported high levels of expression of PIVKA-II in patients with...
intrathelial cholangiocarcinoma and pancreatic cancer. Berhane et al. found that neither PIVKA-II nor AFP-L3 was elevated in patients with cholangiocarcinoma or pancreatic cancer compared with healthy controls when measured using microchip capillary electrophoresis and a liquid-phase binding assay on a \( \mu \) TASWako i30 analyzer. Therefore, the relatively high concentrations of PIVKA-II observed here are more likely due to differences in the patient population than differences between assays. These findings indicate that high concentrations of PIVKA-II should not be used as a stand-alone diagnostic tool for HCC in the absence of other clinical data, such as patient symptoms, imaging, and other laboratory tests. Currently available diagnostic biomarkers for cholangiocarcinoma are considered to be inaccurate, and the only approved biomarker for pancreatic cancer, CA 19-9, is limited by poor sensitivity and specificity. Therefore, it may be beneficial to investigate the specificity and sensitivity of PIVKA-II for these two malignancies in a future study in the setting of healthcare systems that have not incorporated PIVKA-II in routine clinical practice.

The use of AFP and other biomarkers to detect HCC has been previously shown to be complementary to surveillance techniques and can benefit diagnostic models. Our findings support the use of AFP as a biomarker for HCC. This study demonstrated the discrimination of patients with HCC from those with non-HCC conditions using the Elecsys AFP assay. Furthermore, the results reported here for the increased overall sensitivity for HCC detection when combining PIVKA-II and AFP assays support previously published evidence. A study of the usefulness of PIVKA-II, AFP, and AFP-L3 for diagnosing HCC found that the AUC was significantly \( P = 0.001 \) higher for the combination of PIVKA-II >40 mAU/mL and AFP >10 ng/mL versus the combination of PIVKA-II, AFP at the same concentrations, plus AFP-L3 >10%. A real-world study investigating the effectiveness of PIVKA-II in detecting HCC in clinical practice demonstrated that PIVKA-II effectively increases the detection rate of HCC and is a valid complement to AFP; these results and others suggest that PIVKA-II could therefore provide a more sensitive means of differentiating HCC from other diseases.

We used AFP 20 ng/mL as the cut-off for HCC diagnosis, with a sensitivity of 51.8% and specificity of 98.1%. AFP 200 ng/mL, an alternative cut-off recommended as an aid to HCC diagnosis by APASL, was also analyzed but was found to be notably less sensitive (data not shown). AFP cut-offs of 11.52 and 8.22 ng/mL, corresponding to 95 and 90% specificity, had a sensitivity of 67.9 and 81% for PIVKA-II at the same concentrations, plus AFP-L3 for diagnosing HCC found that the AUC was significantly \( P = 0.001 \) higher for the combination of PIVKA-II >40 mAU/mL and AFP >10 ng/mL versus the combination of PIVKA-II, AFP at the same concentrations, plus AFP-L3 >10%. A real-world study investigating the effectiveness of PIVKA-II in detecting HCC in clinical practice demonstrated that PIVKA-II effectively increases the detection rate of HCC and is a valid complement to AFP; these results and others suggest that PIVKA-II could therefore provide a more sensitive means of differentiating HCC from other diseases.

### Table 1: Clinical performance of Elecsys PIVKA-II and Elecsys AFP assays by HCC stage

| Assay (cut-off)     | Metric, % (95% CI) | 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) |

### Table 2: Cut-offs of Elecsys PIVKA-II and Elecsys AFP at specified specificity and sensitivity values

| Specificity, % | Cut-off, ng/mL | Sensitivity, % (95% CI) | Cut-off, ng/mL | Sensitivity, % (95% CI) |
|---------------|---------------|------------------------|---------------|------------------------|
| 95            | 18.7          | 43.3 (36.4–50.3)        | 2.85          | 45.2 (38.3–52.2)       |
| 90            | 23.1          | 72.1 (65.5–78.1)        | 3.65          | 64.4 (57.5–70.9)       |
| 85            | 31.7          | 87.5 (82.2–91.7)        | 4.45          | 73.1 (66.5–79.0)       |
| 80            | 36.5          | 90.4 (85.5–94.0)        | 5.04          | 77.9 (71.6–83.3)       |
| 75            | 51.5          | 91.8 (87.2–95.2)        | 5.87          | 83.2 (77.4–88.0)       |
| 70            | 63.9          | 93.3 (89.0–96.3)        | 6.45          | 87.0 (81.7–91.4)       |
developing HCC in patients undergoing antiviral treatment for HBV with nucleoside/nucleotide analogs. Similarly, other studies found that AFP 6 ng/mL was an appropriate cut-off, including for patients with HBV receiving entecavir treatment and patients with HCV receiving interferon treatment. Accordingly, future studies investigating whether antiviral nucleoside analog treatments impact the performance of the Elecsys PIVKA-II assay may be beneficial.

Although the Elecsys PIVKA-II assay cannot be directly compared with other platforms because of the use of different technologies, antibodies, and detection of different carboxylated variants, this study achieves the objective of demonstrating clinical performance equivalent to that of the comparator assays. Strengths of the study include the familiarization and quality control measures, which ensure accurate performance of all study...

Figure 4  Distribution of PIVKA-II in the subgroups of the specificity panel cohort on (a) the Elecsys PIVKA-II assay and (b) the µTASWako DCP platform. DCP, des-γ-carboxyprothrombin; PIVKA-II, prothrombin induced by vitamin K absence-II.
procedures. Additionally, the presented reference values may support physicians in their diagnosis of HCC.

As this was a cross-sectional study, it was not possible to assess the timing and amplitude of PIVKA-II rise in relation to early HCC development; such an assessment would require a longitudinal study with serial samples needed for validation. Another limitation of this study is that it aimed to study the performance of HCC biomarkers alone, without considering the role of ultrasound. The performance of PIVKA-II with or without AFP versus ultrasound was not compared, nor was the performance of serum biomarkers with ultrasound on HCC surveillance. Future studies are needed to better inform the application of these biomarkers in clinical practice.

In conclusion, the Elecsys PIVKA-II assay demonstrated good analytical performance under routine laboratory conditions and compared favorably with commercially available comparator assays, demonstrating its suitability as an aid in HCC diagnosis. This translated as good clinical performance, which may aid in the diagnosis of HCC across all disease stages and etiologies. Combining PIVKA-II with AFP may further increase the diagnostic performance.

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Ethics approval

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol received approval from the joint CUHK-NTEC Clinical Research Ethics Committee of the Prince of Wales Hospital, Hong Kong; the ethics committee of the Kinki University School of Medicine, Osaka, Japan; the Human Research Ethics Committee Faculty of Medicine, Prince of Songkla University, Hat Yai, Thailand; the ethics committee of the Medizinische Hochschule Hannover, Germany; the ethics committee of the Medical Faculty of the University of Leipzig, Germany; the ethics committee of the Medical Faculty of Goethe University Theodor-Stern-Kai, Frankfurt, Germany; the ethics committee of the Medical Faculty of LMU Munich, Germany; and the ethics committee of the Rhineland-Palatinate Medical Association, Mainz, Germany. Use of prospectively collected specificity panel samples for the purpose of this study was approved by the following institutional review boards: Kiev City Clinical Oncology Center, Kiev; National Cancer Institute MOH Ukraine, Kiev; Shalimov National Institute of Surgery and Transplantation, Kiev; and Kiev City Hospital Nu, Kiev.

Data availability statement. This study was conducted in accordance with applicable regulations. Qualified researchers may contact the following Leading Ethics Committee to request access: Ethik-Kommission der MHH (Ethikkommission@mhh-hannover.de; Study Protocol RD002542).

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher’s website:

Appendix S1. Supporting information.