Performance of protein induced by vitamin K absence or antagonist-II assessed by chemiluminescence enzyme immunoassay for hepatocellular carcinoma detection: a meta-analysis

Performance of PIVKA-II assessed by chemiluminescence enzyme immunoassay for hepatocellular carcinoma detection: a meta-analysis

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ABSTRACT

Objectives: In the setting of surveillance for hepatocellular carcinoma (HCC) detection, the use of serum biomarkers in addition to ultrasonography (US) is still a matter of debate. Hence, we performed a meta-analysis to evaluate the diagnostic accuracy of protein induced by vitamin k absence or antagonist II (PIVKA-II) and alpha-fetoprotein (AFP) alone or in combination for HCC detection in patients at risk of tumor development.

Materials and methods: We performed a systematic search in PubMed and Scopus database for original articles published in English from 2011 to 2017, investigating the accuracy of PIVKA-II versus AFP (reported as area under the curve [AUC]) for HCC detection among patients at risk of tumor development. Furthermore, we focused on studies in which serum PIVKA-II was assessed by highly sensitive chemiluminescence immunoassay (CLEIA).

Results: A total of 10 studies (818 patients with HCC and 1136 patients with advanced liver disease/cirrhosis) were included in the meta-analysis. The weighted summary (s)AUC of PIVKA-II and AFP for the discrimination between patients with HCC and those without was 0.776 (0.732-0.820) and 0.763 (0.723-0.803), respectively. The combination of PIVKA-II + AFP results in a sAUC of 0.860 (0.836-0.883). The performance for HCC detection of PIVKA-II + AFP was significantly superior to each biomarker used alone (ΔsAUC = 0.084, $p = 0.001$ and ΔsAUC = 0.097, $p < 0.001$, respectively).

Conclusions: In clinical practice, the use of PIVKA-II + AFP in addition to US examination may improve the effectiveness of surveillance among patients at risk for HCC development.

KEYWORDS: alpha-fetoprotein; hepatocellular carcinoma; protein induced by vitamin k absence or antagonist II; surveillance; meta-analysis

Introduction

Despite the availability of direct-acting antiviral agents (DAAs) and potent nucleos(t)ide analogues (NAs) with high genetic barrier to resistance for treatment of patients with chronic hepatitis C and B, respectively, the prognosis of patients with advanced liver disease/cirrhosis responder to antiviral treatment is still characterized by a significant risk of hepatocellular carcinoma (HCC) development.[1,2]

Currently, surveillance programs for HCC detection in high risk population are mainly based on abdominal ultrasonography (US).[3,4] However, US has no predictive power on HCC development. Indeed, hepatocarcinogenesis is a gradual process characterized by genetic and molecular alterations progressively accumulating within hepatocytes before the appearance of a neoplastic lesion detectable by imaging.[5] Thus, additional tools allowing early HCC detection or prediction are needed to complement US screening. Among traditional serum markers, alpha-fetoprotein (AFP) is the most used worldwide despite showing suboptimal performance for HCC detection.[6] Conversely, novel classes of biomarkers involved in epigenetic machinery such as microRNAs (miRNAs) showed promising results.[7,8] However, their use in clinical practice may be limited by the absence of a standardized analytical method and by a complex miRNA-messenger RNA interference network reflecting different genetic and epigenetic features, that in turn may significantly alter miRNAs expression.[9]

Protein induced by vitamin K absence or antagonist II (PIVKA-II), also known as des-gamma-carboxy prothrombin, is a biomarker specific for HCC firstly described in 1984.[10] PIVKA-II is an hypo-carboxylated prothrombin released by the liver in absence of vitamin K or in presence of malignant cells; higher PIVKA-II serum levels are associated to tumor size, microvascular invasion and predict HCC recurrence.[11-13] The combination of PIVKA-II with AFP is currently used in Japan for surveillance of patients at risk of HCC development as recommended by the local HCC guidelines.[14] However, results regarding PIVKA-II performance in comparison or in combination with AFP are

conflicting and available data mainly derive from studies involving Asiatic patients;[15,16] results from Western studies are limited by the relatively small sample size. Furthermore, methods for PIVKA-II measurement in serum evolved over time from competitive radioimmunoassay and enzyme immunoassay to fully automated chemiluminescence enzyme immunoassay (CLEIA).[17] Thus, technical improvement may have affected analytical performance.

Considering the availability of novel CLEIA-based methods for PIVKA-II measurement and the lack of consensus regarding the usefulness of PIVKA-II in the setting of HCC surveillance, we performed a meta-analysis on the diagnostic accuracy of PIVKA-II alone or in combination with AFP for HCC detection among patients at risk of tumor development.

Methods

Search strategy

The meta-analysis of observational studies in epidemiology (MOOSE) reporting guidelines for the performance of meta-analyses have been followed.[18] Original research articles published in English on accuracy of PIVKA-II for the discrimination of patients with HCC from those with advanced liver disease/cirrhosis were identified through PubMed (https://www.ncbi.nlm.nih.gov/pubmed) and Scopus (https://www.scopus.com) database. The search strategy was based on the use of the following terms: "PIVKA-II" or "des-gamma-carboxy prothrombin" or "DCP" and "HCC" or "hepatocellular carcinoma". Since we focused on articles in which PIVKA-II was tested by CLEIA method, only papers published from 1 Jan 2011, were screened. For both databases the search was performed on 30 August 2017. Patients with HCC were considered the "case group" whereas patients with advanced chronic liver disease or cirrhosis or with non-neoplastic liver nodules were considered as the "control group". No restriction was set for age and sex of the patients.

Study selection

Two authors (GPC and DGR) independently reviewed the titles and the abstracts of the studies retrieved from electronic search and selected those potentially relevant for the meta-analysis. The full-text of selected studies was assessed by three authors (GPC, DGR and MLA) to determine whether the inclusion criteria were satisfied.

Inclusion criteria were: (1) original research articles published in English; (2) studies reporting PIVKA-II diagnostic accuracy for the discrimination between patients with HCC and patients with advanced liver disease/cirrhosis in comparison to AFP; and (3) studies investigating PIVKA-II performance assessed by CLEIA method. Duplicates studies and studies lacking of data of interest were excluded. The quality of included studies was assessed by the quality assessment of diagnostic accuracy studies (QUADAS) tool.[19] Accordingly, a maximum of 14 points could be awarded answering questions related to biases, variability and reporting.

Data extraction

From selected papers, the same two authors (GPC and DGR) extracted data regarding authors, Country, year of publication, number of patients, underlying liver disease etiology, biomarkers performance (area under the curve [AUC] and 95% confidence interval [CI]), sensitivity (Se) and specificity (Sp) at the corresponding cut-off value.

Statistical analysis

The meta-analysis was performed using MedCalc® software version 15.8.1 (Ostend, Belgium). Chi-square test was performed to evaluate difference of categorical variables between groups. Test for inter-rater agreement (Cohen Kappa statistics) was used to evaluate the agreement between investigators. The weighted summary (s)AUC was calculated including each AUC value and the

corresponding standard error (SE) from all included studies. SE was calculated with the following formula: SE = (ln UB – ln LB)/2*1.96, where UB and LB were the upper and lower bound of the 95%CI of AUC, respectively.

Forrest plots showing the overall effect and funnel plots for publication bias assessment were constructed. According to the presence of heterogeneity, a fixed or random effects model was preferred. Cohcran's Q and I^2 statistics were used to detect heterogeneity; a *p*-value < 0.1 and I^2 value > 25% were considered as indicative of heterogeneity, respectively.

To measure funnel plot asymmetry, Egger regression analysis was performed.[20] Accordingly, the standard normal deviate, defined as the natural logarithm of estimate divided by its SE, was regressed against the estimate's precision, defined as the inverse of the SE. The intercept of the regression line and the corresponding *p*-value provided the measure of asymmetry.

Results

A total of 10 studies were included in the meta-analysis (Table I).[21-30] The strategy search is depicted in Figure 1. There was no disagreement among authors regarding eligibility of original articles finally included in the meta-analysis (K statistics = 1.0). Overall, 1954 patients were included: 818 patients with HCC (Case group), 655 patients with cirrhosis and 481 with chronic liver disease (Control group). The underlying liver disease etiology was mainly viral, with a significant higher proportion of patients with chronic hepatitis B virus (HBV) infection ($p < 0.001$).

The sAUC of PIVKA-II for the discrimination between patients with HCC and those without was 0.776 (0.732-0.820) (Figure 2). Since the studies showed heterogeneity $p < 0.001$ of Cohcran's Q and $I^2 = 71.3\%$), a random effects model was applied. The sAUC of AFP for the discrimination between patients with HCC and those without tumor was 0.763 (0.723-0.803) (Figure 3). Considering that the studies showed heterogeneity ($p = 0.061$ of Cohcran's Q and $I^2 = 44.7\%$), a random effects

model was applied. The sAUC of PIVKA-II in combination with AFP for the discrimination between patients with HCC and those without was 0.860 (0.836-0.883) (Figure 4). A fixed effects model was applied because the results of the studies showed no heterogeneity ($p = 0.578$ of Cohcran's Q and $I^2 = 0\%$). No differences were observed between PIVKA-II and AFP diagnostic accuracy (ΔsAUC = 0.013, $p = 0.668$), whereas the combination of PIVKA-II + AFP was significantly superior to each biomarker used alone (ΔsAUC = 0.084, $p = 0.001$ and ΔsAUC = 0.097, $p < 0.001$, respectively).

Publication bias

Egger test for publication bias showed that the risk of having missed or overlooked studies was minimal for the assessment of sAUC of PIVKA-II and AFP ($p = 0.035$ and $p = 0.049$, respectively), whereas no publication bias was observed for the analysis of biomarkers combination ($p = 0.583$) (Figure 5).

Discussion

The use of serum biomarkers for HCC surveillance is a matter of debate since no unequivocal evidence in improving early HCC detection has been produced.[31] Nevertheless, conventional biomarkers such as AFP and PIVKA-II are used in clinical practice, although not universally recommended by scientific guidelines.

AFP is the widest used biomarker in the setting of surveillance of high risk population albeit showing a large range of Se (40%-65%) and Sp (76%-96%) values for HCC detection.[32] Furthermore, AFP values are often increased in patients with chronic liver disease or cirrhosis and without HCC, as a consequence of inflammation, necrosis and regeneration.[33] Nonetheless, as suggested by Asian Pacific Association for the Study of the Liver, AFP used in combination with US, may be useful to increase Se without decreasing Sp for tumor recognition.[34]

To date, several biomarkers have been proposed as potential alternative or diagnostic complement to AFP.[35-39] Amongst these, PIVKA-II has been extensively investigated either alone or in combination with AFP, but results concerning performance for HCC detection are conflicting.[40,41] In the present meta-analysis including 10 studies, we found no difference between AFP and PIVKA-II diagnostic accuracy for the discrimination between patients with HCC and those without, but the combination of both biomarkers led to a significant improvement in the performance of HCC detection. Consistently, several studies focused either on the combination of biomarkers or on the development of scores that include different classes of biomarkers and even demographical or clinical characteristics,[28,42-46] in order to improve reliability and performance for HCC detection. As a matter of fact, these strategies showed promising results. Furthermore, some of these scores seem able to accurately predict HCC development among high risk patients.[28,47]

Another major issue is represented by the method used for biomarker assessment. Most of novel biomarker proposed for HCC detection have been assessed by non-standardized methods, not allowing to reliably reproduce or compare results from different studies and thus, still far from any potential use in clinical practice.[48] For this reason, only biomarkers evaluated by highly sensitive standardized methods may be recommended.

The results of this meta-analysis may be limited by lack of pathological characterization of HCC cases. Since data regarding HCC classification (*i.e.* staging according to Barcelona Clinic Liver Cancer system) or nodules features (such as number, size and vascular invasion) were absent or not limited to very early/early tumor stages, we could not assess the performance of biomarkers for early HCC detection. Another potential limitation is represented by the presence of non-cirrhotic patients in control group, as cirrhosis is considered the principal risk factor for HCC development. However, we included studies that enrolled as controls only patients with advanced liver diseases/cirrhosis in order to

obtain a control group representative of the real population under surveillance for the risk of HCC development.

In conclusion, the results of this meta-analysis highlight the added value of PIVKA-II and AFP combination for HCC detection rather than a single biomarker used alone. In clinical practice, the use of this combination in addition to US examination may be considered to improve the effectiveness of surveillance of patients at risk for HCC development. Nonetheless, prospective multicenter studies including a large cohort of patients with advanced liver disease/cirrhosis are needed to evaluate PIVKA-II + AFP performance for tumor prediction, thus allowing the identification of patients at higher risk of HCC development.

Declaration of interest statement

All authors have nothing to disclose regarding the material discussed in the present manuscript.

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Table 1. Studies included in meta-analysis.

Study	Country	Year of publication	No. of patients	Etiology	Quality (QUADAS)
Potè et al. [21]	France	2015	HCC = 85	32 HCV/ 17 HBV / 36 n.a.	13
			LC = 43	30 HCV/ 13 HBV	
Yu et al. [22]	China	2015	HCC = 134	121 HBV / 3 HCV / 10 non-viral	11
			LC = 100	100 HBV	
			CLD = 247	247 HBV	
Viggiani et al.[23]	Italy	2016	HCC = 60	60 n.a.	7
			CLD = 60	HCV / HBV / SOL (number n.a.)	
Sultanik et al.[24]	France	2016	HCC = 46	26 HCV / 13 HBV / 7 HCV+HBV	13
			LC = 116	113 HCV / 2 HBV / 1 HCV+HBV	
Ji et al.[25]*	China	2016	HCC = 200	200 HBV	11
			LC = 41	41 HBV	
			CLD = 56	56 HBV	
Yu et al.[26]	China	2016	HCC = 51	51 HBV	12
			LC = 101	101 HBV	

			CLD = 27	27 HBV	
Saitta et al.[27]	Italy	2017	HCC = 40	31 viral / 9 non-viral	12
			LC = 50	27 viral / 23 non-viral	
Caviglia et al.[28]	Italy	2017	HCC = 33	33 HBV	12
			LC = 30	30 HBV	
Gentile et al.[29]	Italy	2017	HCC = 56	56 HCV	12
			LC = 72	72 HCV	
			CLD = 32	32 HCV	
Wang et al.[30]	China	2017	HCC = 113	113 HBV	11
			LC = 102	102 HBV	
			CLD = 59	59 HBV	

*Only cohort B (high risk population surveillance) was included in meta-analysis.

CLD: chronic liver disease; n.a.: not available; QUADAS: quality assessment of diagnostic accuracy studies; SOL: solid occupying lesion.

Table 2. Raw data of the studies included.

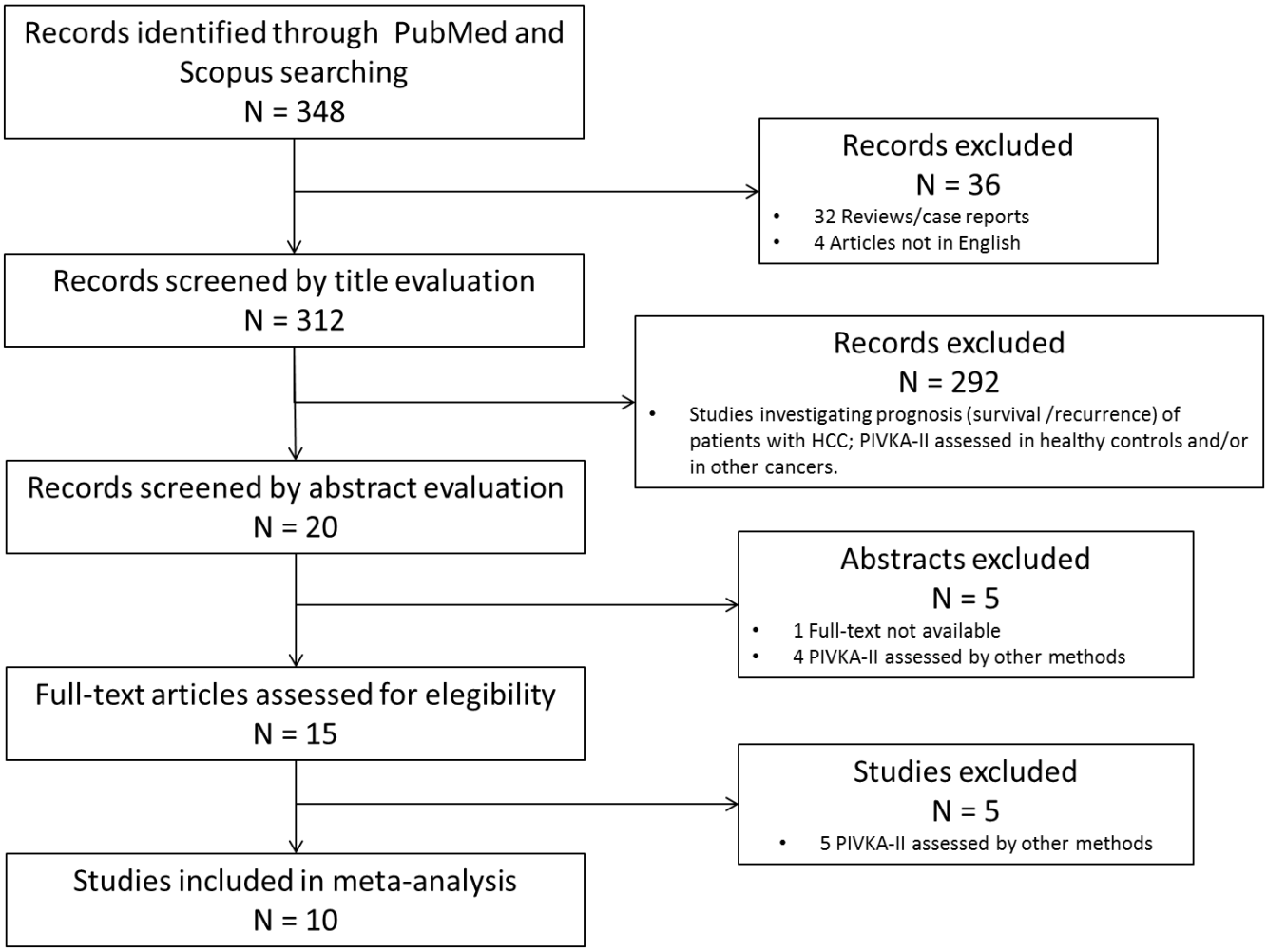
Study	Biomarker	AUC (95%CI)	SE	Cut-off	Se	Sp
Potè et al. [21]	PIVKA-II	0.810 (0.697 - 0.924)	0.072	42 mAU/mL	77%	82%
	AFP	0.582 (0.443 - 0.722)	0.125	5.5 ng/mL	61%	50%
	PIVKA-II + AFP	0.826 (0.722 - 0.929)	0.064	/	/	/
Yu et al. [22]	PIVKA-II	0.760 (0.699 - 0.820)	0.041	200 mAU/mL[a]	64.2%	90.8%
	AFP	0.826 (0.784 - 0.869)	0.026	192.2 ng/mL[b]	60.4%	89.6%
	PIVKA-II + AFP	0.846 (0.804 - 0.888)	0.025	[a] or [b]	73.1%	83.3%
Viggiani et al.[23]	PIVKA-II	0.814 (0.735 - 0.890)	0.049	47 mAU/mL	60%	90%
	AFP	0.618 (0.516 - 0.720)	0.085	20 ng/mL	55%	55%
	PIVKA-II + AFP	n.a.	/	/	75%	61%
Sultanik et al.[24]	PIVKA-II	0.890 (0.820 - 0.960)	0.040	128 mAU/mL[a]	74%	92%
	AFP	0.770 (0.680 - 0.860)	0.060	20 ng/mL[b]	63%	82%
	PIVKA-II + AFP	0.900 (0.840 - 0.960)	0.034	[a] or [b]	87%	76%
Ji et al.[25]*	PIVKA-II	0.913 (0.884 - 0.941)	0.016	40 mAU/mL	82.6%	90.7%
	AFP	0.691 (0.638 - 0.743)	0.039	20 ng/mL	62.0%	69.1%
	PIVKA-II + AFP	0.840 (0.796 - 0.885)	0.027	/	78.5%	93.8%

Yu et al.[26]	PIVKA-II	0.718 (0.619 - 0.818)	0.071	32 mAU/mL[a]	58.3%	92.6%
	AFP	0.829 (0.749 - 0.909)	0.049	5.0 ng/mL[b]	75.0%	91.7%
	PIVKA-II + AFP	0.886 (0.826 - 0.945)	0.034	[a] or [b]	88.9%	85.2%
Saitta et al.[27]	PIVKA-II	0.710 (0.596 - 0.823)	0.082	60 mAU/mL	60%	88%
	AFP	0.718 (0.613 - 0.823)	0.075	6.5 ng/mL	67%	68%
	PIVKA-II + AFP	0.764 (0.665 - 0.862)	0.066	/	70%	94%
Caviglia et al.[28]	PIVKA-II	0.846 (0.734 - 0.924)	0.059	58 mAU/mL	91%	71%
	AFP	0.791 (0.671 - 0.882)	0.070	9.5 ng/mL	61%	87%
	PIVKA-II + AFP	0.890 (0.786 - 0.954)	0.050	/	91%	77%
Gentile et al.[29]	PIVKA-II	0.788 (0.707 - 0.868)	0.052	36 mAU/mL[a]	78.6%	66.3%
	AFP	0.756 (0.676 - 0.836)	0.054	12 ng/mL[b]	60.0%	77.2%
	PIVKA-II + AFP	/	/	[a] and [b]	92.5%	51.4%
Wang et al.[30]	PIVKA-II	0.756 (0.698-0.814)	0.039	32.6 mAU/mL	52.2%	81.5%
	AFP	0.781 (0.726-0.836)	0.036	17.6 ng/mL	64.6%	73.3%
	PIVKA-II + AFP	0.868 (0.822-0.913)	0.027	50.23	74.3%	89.4%

*Only cohort B (high risk population surveillance) was included in meta-analysis.

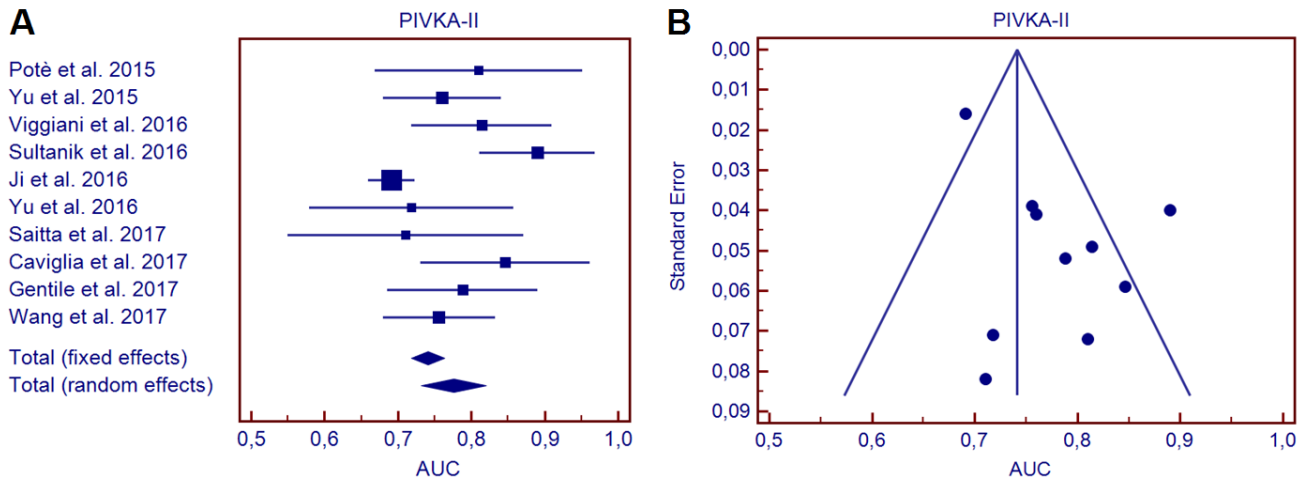
AFP, alpha-fetoprotein; AUC, area under the curve; CI, confidence interval; n.a.; not available; PIVKA-II, protein induced by vitamin absence or antagonist II; Se, sensitivity; SE, standard error; Sp, specificity.

Figure 1. Study screening and selection.



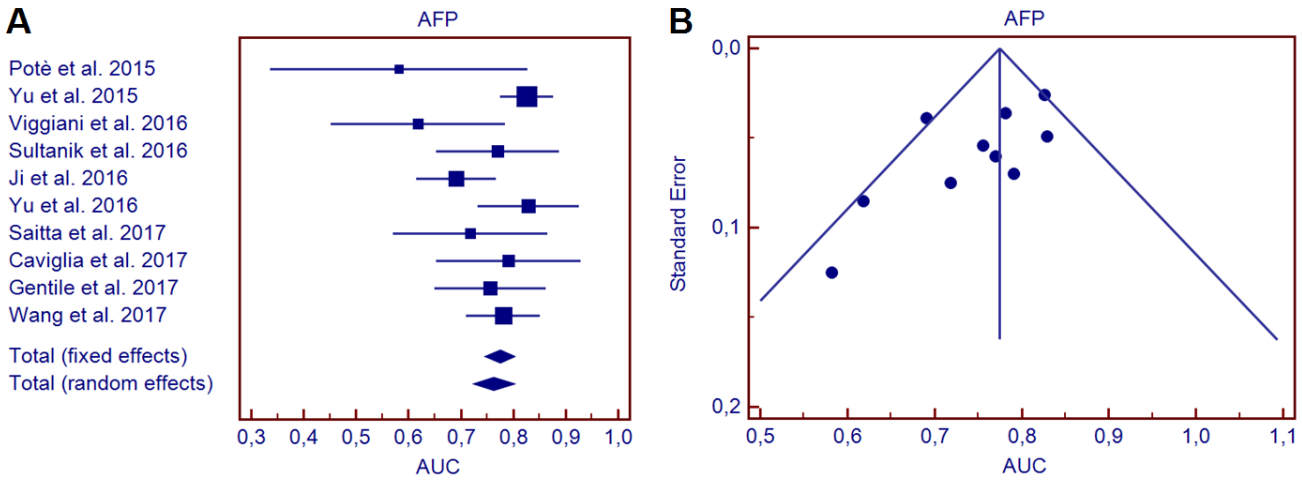
PIVKA-II: protein induced by vitamin k absence or antagonist II.

Figure 2. Forrest plot (A) and funnel plot (B) of PIVKA-II accuracy for HCC detection.



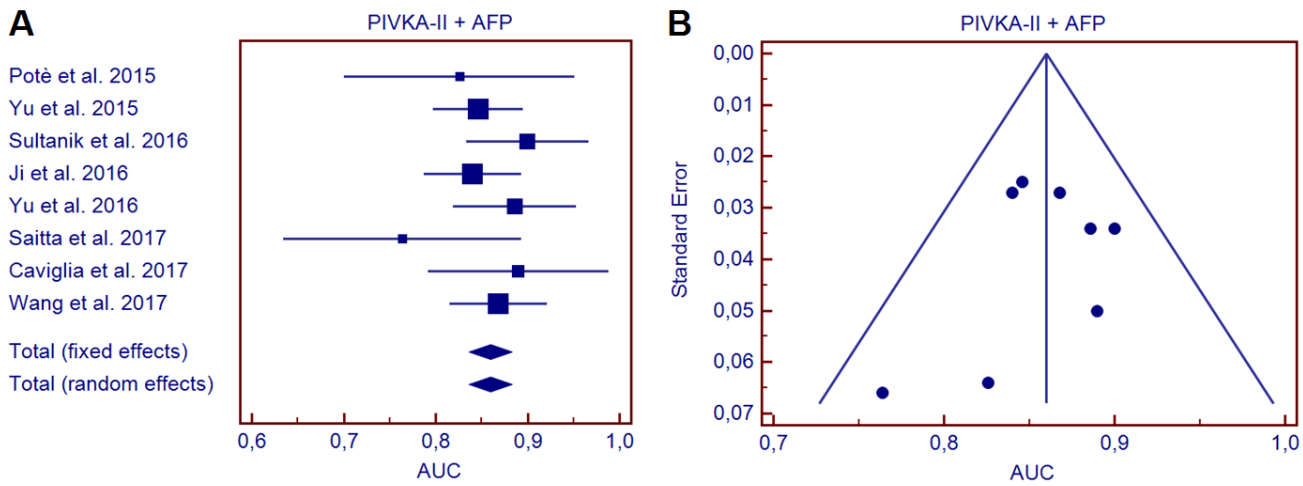
AUC: area under the curve; PIVKA-II: protein induced by vitamin k absence or antagonist II.

Figure 3. Forrest plot (A) and funnel plot (B) of AFP accuracy for HCC detection.



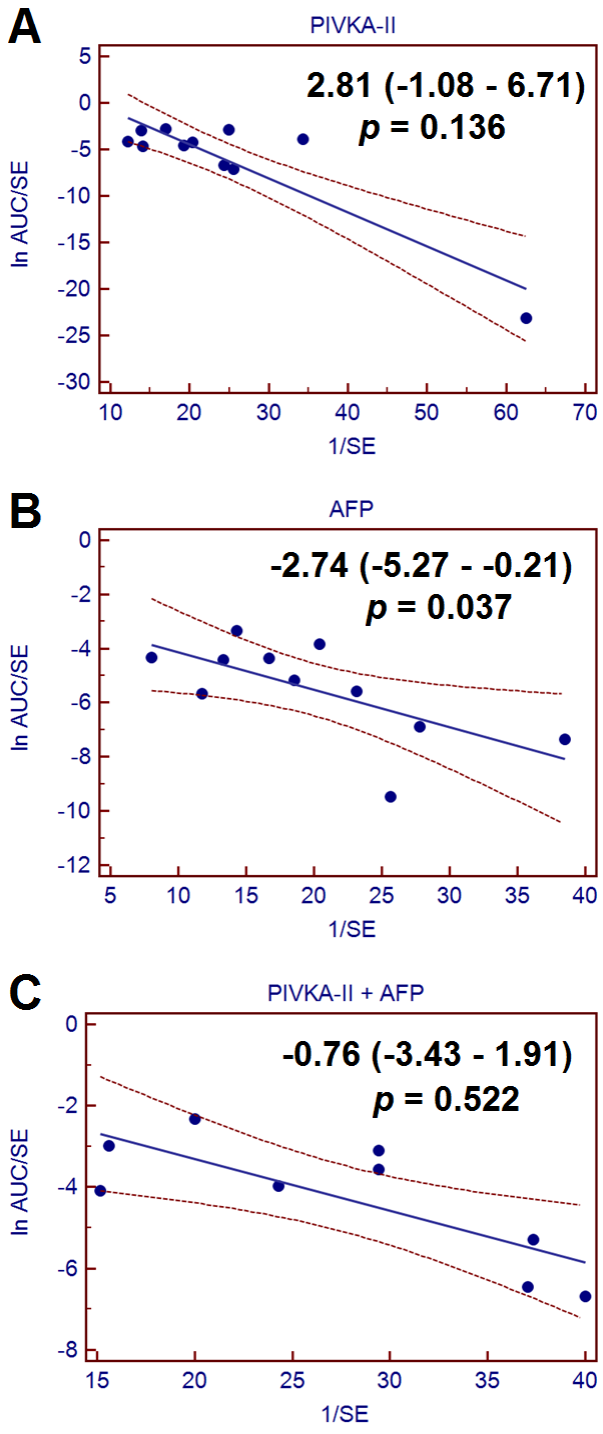
AFP: alpha-fetoprotein; AUC: area under the curve.

Figure 4. Forrest plot (A) and funnel plot (B) of PIVKA-II + AFP accuracy for HCC detection.



AFP: alpha-fetoprotein; AUC: area under the curve; PIVKA-II: protein induced by vitamin k absence or antagonist II.

Figure 5. Egger regression plot of PIVKA-II (A), AFP (B) and PIVKA-II + AFP (C).



AFP: alpha-fetoprotein; AUC: area under the curve; ln: natural logarithm; PIVKA-II: protein induced by vitamin k absence or antagonist II; SE: standard error.