RESEARCH ARTICLE

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Establishing a Decision-Making Threshold for PIVKA II in the Diagnosis of Pancreatic Ductal Adenocarcinoma (PDAC): A Single Centre Experience

Antonella Farina, Sara Tartaglione, Isabella Oliviero, Valentina Viggiani, Antonio Angeloni, Emanuela Anastasi*

Abstract

Objective: Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related deaths worldwide. Due to the crucial importance of an earlier identification of PDAC, there is substantial ongoing effort to identify serological biomarkers that would facilitate the timely detection of this malignancy. Protein Induced by Vitamin K Absence II (PIVKA II), a modified prothrombin, has been recently suggested as promising diagnostic marker for PDAC detection: however, in literature no diagnostic cut-off have been proposed for PDAC diagnosis yet. Aim of this research was to identify PIVKA II decisional threshold in PDAC patients, comparing their serum values to those found in HCC patients since the clinical-diagnostic validity of PIVKA II in HCC has already been widely demonstrated. **Methods:** Serum PIVKA II levels were measured in 91 PDAC patients, 92 HCC patients, and 59 healthy donors using an automated chemiluminescent enzyme immunoassay. Statistical analysis, including Receiver Operating Characteristic (ROC) curves, was used to establish sensitivity, specificity, and the optimal diagnostic threshold. **Results:** The optimal PIVKA II cut-off for PDAC diagnosis was 69 ng/mL, yielding a sensitivity of 80% and specificity of 94%. Comparable performance was observed in HCC patients (sensitivity 87%, specificity 94%). PIVKA II levels distinguished PDAC patients from healthy controls and demonstrated diagnostic potential similar to HCC, suggesting a shared biomarker profile. **Conclusion:** This study establishes a novel cut-off for PIVKA II in PDAC, supporting its utility as a biomarker. Further research is needed to validate these findings across diverse populations.

Keywords: PIVKA II- cancer biomarkers- PDAC- HCC- cut-off

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Introduction

Pancreatic adenocarcinoma (PADC) is commonly referred to as the “silent killer” because its asymptomatic nature, or alternatively the manifestation of non-specific symptoms during the early stages, results in a delayed diagnosis at an advanced or metastatic phase, subsequently compromising patients’ prognosis [1]. Currently, diagnostic imaging techniques are the primary resource to determine tumor’s localization, mass extension, and clinical staging but this diagnostic tools fall short in facilitating the timely detection of PDAC, necessitating support from cytology and the assessment of circulating tumor biomarkers [2]. To date, suggested neoplastic markers, i.e. CA 19.9 (considered the gold-standard test), CEA, and CA 242, often exhibit suboptimal sensitivity and specificity, prompting the scientific community to find novel circulating molecules with superior analytical performance [3]. In this context, the investigation of PIVKA II, a modified prothrombin produced by the liver, in the absence of vitamin K, is gaining particular attention and opening new avenues of research. PIVKA II is a nonfunctional prothrombin resulting from incomplete carboxylation of 10 glutamic acids located at the N-terminal portion of the molecule [4].

Currently, PIVKA II is widely employed as a biomarker in hepatocellular carcinoma (HCC), significantly augmenting the sensitivity of the gold standard alpha-fetoprotein in HCC diagnosis and providing a valuable tool for both the diagnosis and prognosis of this neoplasm [5]. However, to date, the mechanisms underlying the overexpression of PIVKA II in HCC have not yet been well characterized, thus, it is plausible that high circulating levels of this protein could be triggered by a multifactorial network.Moreover, it has been observed that vitamin K can limit the oncogenesis of pancreatic cancer, both directly by suppressing tumor growth and inducing apoptosis in tumor cells, and indirectly through the post-translational activation of specific proteins such as PIVKA II [6]. Recent evidences suggest that high levels of PIVKA II in HCC,

*Department of Experimental Medicine, Sapienza University of Rome, Policlinico Umberto I, Rome, Italy. *For Correspondence: emanuela.anastasi@uniroma1.it. Antonella Farina and Sara Tartaglione have equal contribution in this study.*

correlate with a series of phenomena such as hypoxia of the surrounding microenvironment, reduced activity of gamma-glutamyl carboxylase, altered metabolism of vitamin K (references 20 and 21), associated with the downregulation of the VKORC1 gene [7, 8]. Further studies, emphasized the novel role for PIVKA II as a biomarker in pancreatic ductal adenocarcinoma (PDAC): it have been suggested that PIVKA II could serve as a promising biomarker for detecting pancreatic head cancer and may offer superior diagnostic performance compared to other biomarkers [8, 9]. The evidence supporting the role of PIVKA II as a biomarker for pancreatic adenocarcinoma is further reinforced by recent in vitro studies demonstrating its overexpression in pancreatic adenocarcinoma tissue. These studies provide valuable insights into the molecular mechanisms underlying PIVKA-II's involvement in pancreatic cancer pathology. Moreover, the observation that PIVKA II is produced and released by Panc-1 cell lines, which are commonly used as a model for pancreatic adenocarcinoma in laboratory research, adds another layer of support to its potential utility as a biomarker [10, 11]. Unfortunately, the lack of a standardized cut-off value represents a limit to the implementation of PIVKA II in the diagnosis of PDAC. Establishing a threshold for an effective PDAC biomarker such as PIVKA II may contribute to tempestive diagnosis and appropriate treatment of patients. According to these premises, the aim of the present study was to identify the best PIVKA II decision threshold or cut-off value for diagnosing PDAC.

Materials and Methods

Patients

This retrospective observational study included patients with PDAC and HCC recruited at the Oncologic Unit, of Policlinico Umberto I, Rome, Italy, from September 2022 to October 2023.

We analyzed 242 serum samples subdivided as follows:

- (Group 1) 91 from PDAC patients
- (Group 2) 92 from HCC patients
- (Group 3) 59 healthy blood donors.

Inclusion criteria comprised first occurrence of neoplastic pathology, absence of diabetes, and no prior neoadjuvant therapy. Exclusion criteria encompassed high alcohol consumption, active hepatopathy, anticoagulant therapy, or coagulopathy.

All participants provided written informed consent. The study was approved by the Policlinico Umberto I Review Board and adhered to the Declaration of Helsinki.

HCC AND PDAC were diagnosed by histology or by imaging methods (multiphasic computed tomography or dynamic contrast-enhanced magnetic resonance): when the presence of the neoplasms was confirmed, each serum sample was analyzed for PIVKA-II. The PDAC and HCC patients and healthy control cohorts had similar demographics in terms of ratio of men to women, with the majority of all subjects being Caucasian. Table 1 shows patients and controls characteristics.

Blood sampling

Blood collection was performed following a standard protocol. After signing the informed consent for participation in the study and acquisition of personal pathological anamnesis, all patients underwent peripheral venous puncture: blood samples were collected in a yellow top Vacutainer (Becton, Dickinson and Company, Plymouth, UK) and after clotting 60-90 minutes were centrifuged for 10 minutes at 1300xg. Following the collection of the serum samples, an amount of 500 µL of each serum fraction was aliquoted in Eppendorf tubes (Eppendorf srl, Milano, Italy) and stored at– 80°C until analysis.

Serum biomarker determination

Serum PIVKA II levels were assessed in ng/ mL with the Elecsys PIVKA-II kit (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) on Roche® COBAS e411, a full automated analyzer based on a one-step sandwich assay with elettrochemiluminescence (ECLIA) technology. Elecsys PIVKA-II immunoassay is characterized by a detection range between 3.5 – 12,000 ng/mL, while the LoD and LoQ were respectively ≤ 3.5 ng/mL and ≤ 4.5 ng/mL [12]. The instructions of the manufacturer were followed to perform the assays. All tests were conducted in duplicate.

Statistical analysis

Descriptive statistics were used to calculate mean or number (percentage) of the study population. ROC curve analysis was performed to determine the diagnostic accuracy of PIVKA II, including sensitivity, specificity, and area under the curve (AUC). A p-value < 0.05 was considered statistically significant.

For AUC we estimated the 95% confidence interval (95% CI). We considered statistically significant a two tailed p value < 0.05. All statistical analyses were performed using StatsDirect 3.0.187 statistical software (StatsDirect software, Cheshire, England).

A 2x2 contingency table was used to evaluate the performance of the test, calculating sensitivity, by comparing the test results with the gold standard.

Results

Demographics and Clinical Characteristics

In this retrospective observational study, we analyzed 242 serum samples subdivided as follows: 91 from PDAC patients, 92 from HCC patients, 59 healthy blood donors. The demographics of the study cohort are summarized in Table 1.

PDAC and HCC patients had similar age ranges, gender distribution, and clinical profiles. Included patients with PDAC and HCC have been recruited at the Oncologic Unit, of Policlinico Umberto I, Rome, Italy, from September 2022 to October 2023.

PIVKA II Cut-Off Analysis

The gold standard used for calculating sensitivity and specificity in this study was histopathological confirmation, which definitively classified patients as

Table 1. Demographics of Study Cohort

	Subjects	Age (range)	Gender (M/F)	Smoker N/Y	Caucasian/others (%)	Heart Diseases (%)	Respiratory diseases (%)
PDAC	91	39-92	41/50	80/11	96/4	10	10
HCC	92	37-88	42/50	85/7	95/5	7	8
Healthy	59	25-79	25/34	45/14	100/0	2	1

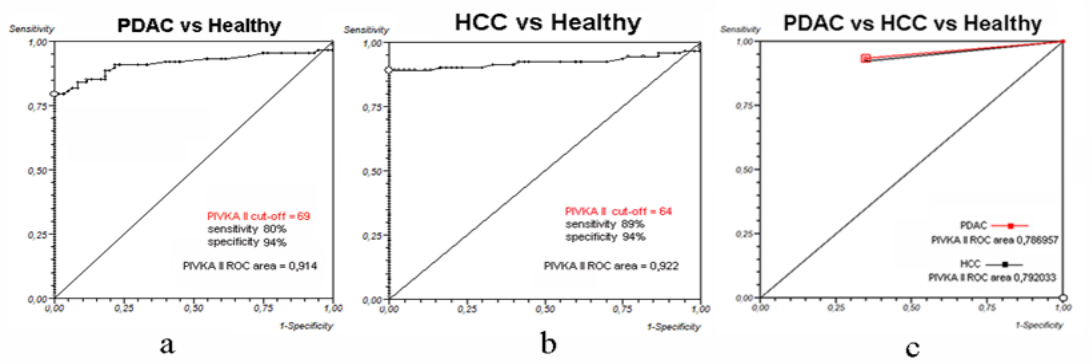


Figure 1. ROC Curves for PIVKA II in PDAC and HCC. (a)(b)(c) Decisional threshold for PIVKA II in PDAC and HCC patients.

having or not having the disease. The ROC curve was constructed by comparing the test results to this gold standard, with sensitivity representing the true positive rate and 1−Specificity representing the false positive rate at various thresholds.

The PIVKA II specificity and sensitivity studied in the PDAC patients showed, by statistical analysis, that the area under the ROC curve was 0.914 (95% CI 0.86–0.96). The statistical analysis for PIVKA II in HCC patients showed that the area under the ROC curve was 0.922 (95% CI 0.871–0.972). In PDAC patients, the PIVKA II ROC curve analysis showed, that the best specificity (0.94) and sensitivity (0.80) are obtained with a cut-off 69 ng/mL(Figure 1a). Whereas the PIVKA II ROC curve in HCC patients showed the best specificity (0.94) and sensitivity (0.87) with a cut-off of 64 ng/mL (Figure 1b). Comparing PIVKA II cut-offs for PDAC vs HCC revealed very similar diagnostic performance and very similar decisional threshold for the studied biomarker in both neoplasms (Figure 1c). Table 2 presents the data on sensitivity and specificity observed in the ROC curves.

Here we also underscore in Table 3 the high sensitivity of PIVKA-II in detecting HCC and PDAC when compared to the gold standard. Out of 92 HCC patients with confirmed histological disease, 82 were correctly identified as positive (true positives), while 10 were misclassified as negative (false negatives). Among the 60 patients without the disease (negative), all were accurately classified as negative (true negatives), resulting in no false positives. The overall sensitivity for HCC detection was calculated to be 89%. In regards to PDAC, among 91 patients with confirmed disease (positive), 73 were correctly identified as positive (true positives), while 18 were misclassified as negative (false negatives). Among the 60 disease-free individuals, all were accurately classified as negative (true negatives), with no false positives recorded. The overall sensitivity for PDAC detection was calculated to be 80%.

Table 2. Curves ROC Data

	Sensitivity	Specificity	AUC
a			
PIVKA II Cut-off ≤ 69	80%	94%	0.914
b			
PIVKA II Cut-off ≤ 64	89%	94%	0.922

Comparison with Literature Cut-Offs in PDAC patients, HCC patients and healthy subjects

Applying a literature-based threshold (28.4 ng/mL) to our PDAC cohort resulted in higher sensitivity but lower specificity. The optimal cut-off value for PIVKA II to distinguish between normal healthy subjects and patients with HCC was previously determined to be 28.4 ng/mL [13], and we used it to preliminarily investigate

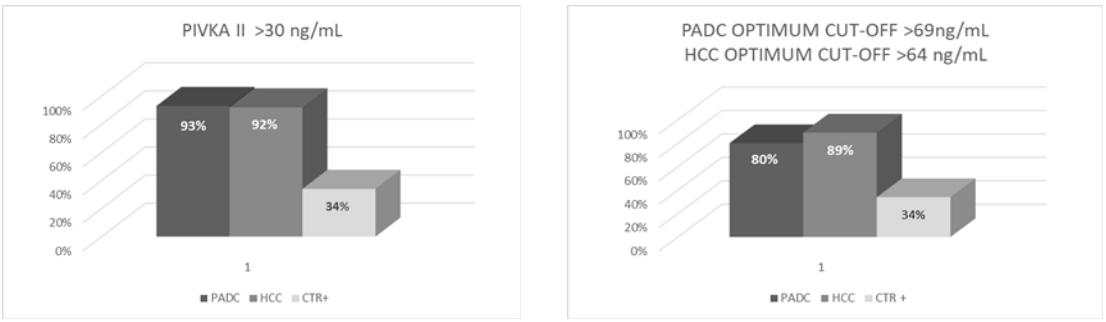
Table 3. 2x2 Contingency Evaluation Comparing Sensitivity of PIVKA II vs Gold Standard

HCC	Disease presence (positive)	Disease absence (negative)	Total
Positive test	82	0	82
Negative test	10	60	70
Total	92	60	152

Sensibility: 89%

PDAC	Disease presence (positive)	Disease absence (negative)	Total
Positive test	73	0	73
Negative test	18	60	78
Total	91	60	151

Sensibility: 80%



A **B**

Figure 2. Comparison of PIVKA II Positivity Using Different Thresholds. (a) PIVKA II percentage of positivity using standard cut-off. (b) PIVKA II percentage of positivity by using new calculated cut-off.

PIVKA II diagnostic performance also in PDAC. Results were then compared with those obtained using the new cut-off applied to our study population. With the new decision thresholds previously identified through the ROC curve (69 and 64 ng/mL for PDAC and HCC patients respectively), we have observed a lower percentage of positive patients for PIVKA II in both group 1 and group 2. Specifically, in PDAC patients, we observed a variation of 14 percent, compared to the 4 percent observed in HCC patients. In the group of healthy controls, however, we did not observe any variation (Figure 2a, Figure 2b). Therefore, a statistically significant difference was observed in the group of PDAC and HCC patients compared to the control group, both when considering the reference cut-off from the literature and the one identified in our study ($p<0.0001$). The new cut-off reduced false positives, improving diagnostic accuracy.

Discussion

Our study aimed to identify an optimal cut-off of PIVKA II as a novel tumor marker for the diagnosis of PDAC. This neoplasm is characterized by an aggressive clinical course and severe prognosis: despite its low incidence, it is one of the leading causes of death from cancer in industrialized countries [14]. The unfavorable clinical outcome is due both to the aggressiveness of the pancreatic cancer cells and to the lack of early diagnosis: the latter is due to the absence of effective screening methods, because PDAC symptoms are often nonspecific and circulating biomarkers with high analytical sensitivity and specificity are not currently available [14]. In fact, CA 19.9, although considered the gold-standard has a low specificity since an increase in blood levels is observed in other malignant and benign[9]. Thus, a minimal-invasive, accurate, and real-time monitoring method for diagnosing PDAC remains a major unmet medical need and there is ongoing research aimed at identifying circulating biomarkers with better analytical performance. In this direction, the study of PIVKA II is finding particular importance. It is currently already in use in the diagnosis of hepatocellular carcinoma (HCC), and numerous studies have demonstrated how this marker increases the diagnostic sensitivity, even in the early stages, in comparison to alpha-fetoprotein [15].

In terms of diagnostic and prognostic implications, recent evidence highlights that PIVKA II values are significantly higher in PDAC demonstrating good diagnostic performance compared to reference tumor markers (CA 19.9 CEA and Ca 242)[8]. These preliminary findings are further supported by the presence of the PIVKA II protein in pancreatic biopsies of PDAC. Indeed in vitro study using EMT biomarkers Snail and Vimentin, it has been observed that the release of PIVKA II occurs concomitantly with the activation of epithelial-mesenchymal transition (EMT), suggesting that PIVKA II may represent an early signal of cancer progression, thus conferring a new significance to this molecule in tumor progression [10, 11, 16]. Nevertheless, to date, no strong data are available on which should be the optimal PIVKA II cut-off in PDAC assay.

In the present research, through statistical processing (ROC curve and Youden Index) we identified 69 ng/mL as the decisional threshold of PIVKA II for PDAC.

We also observed a similarity of the analytical performance found for PIVKA II in patients affected by pancreatic adenocarcinoma compared to HCC. For the latter neoplasm, the clinical-diagnostic validity of this biomarker has already been proven, and therefore could be considered as a reference group. A plausible explanation of this observation could be related to the common embryonic origin of the pancreas and the liver, as it is known, both derive from the same embryonic layer. It was therefore reasonable to hypothesize that PIVKA II expression, which is characteristic of HCC, may also be present in pancreatic cancer.

Finally, the comparison between the positivity rates for PIVKA II detected in PDAC, HCC patients, and the control group has shown lower sensitivity compared to the standard cut-off. We can speculate that interfering factors present in each biological condition may be reduced with the new cut-off, thus providing greater accuracy. Thus, the new threshold value is able to exclude patients with borderline levels which are often misclassified.

The identification of circulating biomarkers with robust analytical performance would indeed facilitate the detection of initial tumor lesions, thereby potentially reducing mortality rates. Laboratory medicine plays

a central role in the clinical management of patients, particularly in oncology, where it is crucial for prevention, diagnosis, prognosis, and follow-up. The identification of circulating biomarkers with optimal analytical performance remains a continual task in the scientific scenario [17, 18]. Some limitations need to be acknowledged for the present study, including the single center and a design with a relatively small sample size, resulting in limited statistical power: larger prospective studies are needed to strengthen these results.

In the present study, the optimal cut-off value for PIVKA II in a population of Italian PDAC patients was described: a threshold value > 69 ng/mL provided the best sensitivity and specificity for the discrimination of subjects with PDAC and those without tumor. PIVKA II decisional cut-off for HCC and PDAC are similar, thus enhancing the validation of the positivity of this new biomarker in PDAC patients. To the best of our knowledge, our report is the first to describe the cut-off values of PIVKA II for PDAC diagnosis. The results obtained by our working group enhance this new line of research in pancreatic oncology, representing a step forward for the management of such an aggressive and lethal neoplasm. Additional research should possibly determine PIVKA II different thresholds according to patients' individual biological characteristics, practical application conditions, taking into account local epidemiology and the various methods adopted for biomarker determination. Additionally, efforts should be made to standardize assay protocols and methodologies for PIVKA II testing to ensure consistency and reproducibility across different clinical laboratories. By establishing a consensus on cut-off values and assay procedures, researchers and clinicians can improve the reliability and clinical utility of PIVKA II as a biomarker for PDAC diagnosis.

This study identified a novel cut-off for PIVKA II in PDAC diagnosis, demonstrating comparable diagnostic performance to HCC. The findings align with prior evidence supporting PIVKA II as a biomarker for pancreatic cancer, particularly in the head region.

The shared embryonic origin of the pancreas and liver may explain the similarity in PIVKA II thresholds for PDAC and HCC. Our results underscore the importance of optimizing biomarker thresholds to improve diagnostic precision, minimizing misclassification.

Despite promising findings, limitations include the single-center design and relatively small sample size. Future multicenter studies are needed to validate these results and explore PIVKA II thresholds across diverse populations.

In conclusion, a PIVKA II threshold of 69 ng/mL offers a robust diagnostic tool for PDAC, with sensitivity and specificity comparable to HCC. This study lays the groundwork for integrating PIVKA II into clinical practice, though further standardization and validation are needed.

Author Contribution Statement

The authors confirm contribution to the paper as follows: study conception and design: IO, EA; data collection: IO, VV; analysis and interpretation of results: EA, VV, ST, AF; draft manuscript preparation: ST, AF, EA; critical revision: ST, EA, AF, AA. All authors reviewed the results and approved the final version of the manuscript.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are available to the corresponding author upon reasonable request.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Sapienza University of Rome (protocol code ME-3-PIvka) for studies involving humans.

Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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