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Breaking the AFP Barrier: CD166 as a Next-Generation Biomarker for Hepatocellular Carcinoma Detection

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a leading cause of morbidity and death among cirrhotic patients. The ability of several biomarkers, such as alpha-fetoprotein (AFP) and CD166, to differentiate HCC from cirrhosis has been investigated.

Aim of the work: The present case control study was to assess the diagnostic accuracy of AFP, CD166, and their combination in differentiation of HCC and liver cirrhosis.

Methods: A total of 120 persons divided into three equal groups: 1: cirrhotic only group, 2: cirrhotic and HCC group and 3: control healthy group. AFP and CD166 serum concentrations were assessed to all groups. Statistical measures like sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operating characteristic (ROC) curve analysis were used to assess the obtaining results.

Results: Group 2 had a substantially higher mean age (63.2 years) than Groups 1 and 3 (59.5 and 58.0 years respectively). Group 2's AFP levels (mean: 2830.2 ng/ml) were significantly higher than those of Groups 1 (199.5 ng/ml) and 3(17.68 /ml) ($p<0.001$). Likewise, Group 2 had greater CD166 levels (19.86 ng/ml) than Groups 1 (10.03 ng/ml) and 3(3.54 ng/ml) ($p<0.001$). With an area under the curve (AUC) of 0.868, sensitivity of 87.5%, and specificity of 82.5%, the combination of AFP and CD166 demonstrated the best diagnostic accuracy, surpassing the performance of either marker alone.

Conclusion: The combination of AFP and CD166 supports its promise as a non-invasive diagnostic tool for HCC by improving diagnostic accuracy in differentiating HCC from cirrhosis.

Keywords: Hepatocellular carcinoma, Cirrhosis, Alpha-Fetoprotein, CD166, biomarkers, Diagnostic accuracy.

INTRODUCTION

The most common type of primary liver cancer and a major public health concern worldwide is hepatocellular carcinoma (HCC). It is the third leading cause of cancer-related death globally and the sixth most frequently diagnosed cancer [(1)]. Regional variations exist in the incidence of HCC, with the highest rates seen in places with high prevalence of hepatitis B (HBV) and hepatitis C (HCV) infections. About 70% of liver malignancies in Egypt are HCC, which frequently develops as a serious consequence of chronic liver cirrhosis. Improved surveillance techniques, longer life times for cirrhotic patients and changing trends in viral hepatitis epidemiology have all been connected to the rising number of cases [(2)].

The most popular biomarker for tracking HCC is still alpha-fetoprotein (AFP), but its poor sensitivity and specificity point to the need for more diagnostic methods. Even while non-invasive blood-based markers are crucial for the early detection of HCC, particularly in high-risk patients, imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound are crucial for verifying the diagnosis [(3)].

Cluster of Differentiation 166, also known as activated leukocyte cell adhesion molecule (ALCAM), is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily. In addition to being engaged in several cellular processes like adhesion, migration, and tumor formation, CD166 has been connected to a number of malignancies, including colorectal, prostate, and breast cancers [(4)]. CD166 has a role in the formation of liver cancer by interacting with Yes-associated protein (YAP), a critical regulator of hepatocarcinogenesis [(5)]. The overexpression of CD166 in HCC has been suggested as a potential biomarker to distinguish HCC from benign liver diseases and cirrhosis [(6)].

In order to differentiate HCC in cirrhotic patients from healthy controls and cirrhotic patients without HCC, our study sought to assess the diagnostic accuracy of AFP, CD166, and their combination.

PATIENTS AND METHODS

The Hepatogastroenterology Unit at the Internal Medicine Department of the Faculty of Medicine at Menoufia University conducted this case-control study between April 1, 2023, and July 15, 2024. Under approval number 768/2022, the study was approved by Menoufia University's Faculty of Medicine's Research Ethics Committee.

A total of 120 people took part, split up into three groups: Group 1 consisted of 40 cirrhotic patients without HCC; Group 2 consisted of 40 cirrhotic patients with HCC who were diagnosed by ultrasonography and confirmed by dynamic contrast-enhanced MRI or triphasic computed tomography (CT); and Group 3 consisted of 40 individuals who seemed healthy and served as the control group.

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While Group 3 included healthy people without liver disease, Group 1 and 2's inclusion criteria required patients to be adults over 18 with cirrhosis, with or without HCC. The study excluded patients under the age of 18, pregnant women, and those with cancers other than HCC.

Six milliliters of venous blood were drawn using sterile syringes in an aseptic manner. The samples were then subjected to full blood count (CBC), prothrombin time (PT), INR, and concentration assessment as well as serological investigations, which included kidney function tests (serum urea and creatinine), liver panel tests (serum ALT, AST, bilirubin (total and direct), and albumin), and serum alpha-fetoprotein (AFP) levels, which were ascertained by immunoassay. An ELISA kit (Human ALCAM/CD166 Picokine ELISA Kit, Sunred Biological Technology, China) was used to determine the levels of serum CD166.

Abdominal ultrasonography was one of the radiological evaluations performed on each subject. To confirm HCC, patients in Groups 1 and 2 had dynamic contrast-enhanced MRI or triphasic CT scanning. The American Association for the Study of Liver Diseases (AASLD) criteria, which call for either histological confirmation or distinctive imaging signs on triphasic CT or dynamic contrast-enhanced MRI, were used to diagnose HCC. If an HCC lesion was ≥1 cm in diameter and showed hypoenhancement (wash-out) in the portal venous or delayed phases after arterial phase hyperenhancement (wash-in), it was deemed positive.

Statistical Analysis

Quantitative data was displayed as means ± SD or medians with IQR, depending on distribution, and qualitative data as frequencies and percentages using SPSS version 28.0 for Windows. Fisher's exact test, often known as the Chi-square test, evaluated qualitative data, while the Independent-Samples t-test and Mann-Whitney U test were employed for group comparisons. The ROC curve was used to assess CD166's diagnostic ability to distinguish HCC from non-HCC.

Ethical Approval:

Prior to the commencement of the study, each participant completed a written consent that was authorized by National Liver Institute Menoufia university's Local Ethical Research Committee [under code no. 768/2022]. Additionally, the Institutional Review Board approval was obtained. The study was conducted in accordance with ethical standards, including the Declaration of Helsinki and its amendments.

RESULTS

The sex distribution among the three groups showed no significant difference (p = 0.147), with males representing 50.0% in Group 1, 67.5% in Group 2, and 47.5% in Group 3. Age differed significantly (p < 0.001), with Group 2 having the highest mean age of 66.23 ± 6.10 years, followed by Group 1 at 62.80 ± 5.50 years and Group 3 at 55.55 ± 9.40 years **(Table 1)**.

Table 1: Comparison between the three studied groups according to sociodemographic and clinical characteristics

Characteristics	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Test of significance	P-value	Significant between groups
Sex						
Male	20 (50.0%)	27 (67.5%)	19 (47.5%)	X^2= 3.838	0.147	>0.05, all groups
Female	20 (50.0%)	13 (32.5%)	21 (52.5%)			
Age (years)						
Min – Max	45.0 – 75.0	50.0 – 78.0	30.0 – 65.0	F= 8.245*	<0.001*	0.035*, 1 vs 2
Mean ± SD	62.80 ± 5.50	66.23 ± 6.10	55.55 ± 9.40			0.001*, 1 vs 3, 2 vs 3
Smoking				X^2= 9.801*	0.007*	0.002*, 1 vs 3, 2 vs 3
Yes	12 (30.0%)	20 (50.0%)	7 (17.5%)			
No	28 (70.0%)	20 (50.0%)	33 (82.5%)			

* Significant

The presence of ascites did not significantly differ between Group 1 (47.5%) and Group 2 (27.5%) (p = 0.065). Abdominal pain was exclusive to Group 2 (100.0%) and absent in Group 1 (p < 0.001). Splenomegaly was significantly higher in Group 1 (57.5%) than in Group 2 (30.0%) (p = 0.013) **(Table 2)**

Table 2: Comparison of the clinical data between the two studied groups according to clinical data

	Group 1 (n = 40)		Group 2 (n = 40)		P-value
	No.	%	No.	%	
Ascites					
No	21	52.5	29	72.5	0.065
Yes	19	47.5	11	27.5	
Abdominal pain	0	0.0	16	100.0	<0.001*
Loss of weight	17	42.5	18	45.0	0.822
Tumor no (single)	40	100.0	–	–	–
Liver consistency (firm)	40	100.0	40	100.0	–
Hepatic Encephalopathy	3	7.5	4	10.0	FEp=1.000
Splenomegaly	23	57.5	12	30.0	0.013*
Comorbidities					
DM	22	55.0	22	55.0	1.000
HTN	24	60.0	25	62.5	0.818
Child Pugh Class					
A	21	52.5	28	70.0	MCp= 0.176
B	16	40.0	8	20.0	
C	3	7.5	4	10.0	
Heart diseases	20	50.0	23	57.5	0.501
Jaundice	4	10.0	6	15.0	0.499
HbsAg antibody	9	22.5	15	37.5	0.143
HCV antibody	34	85.0	17	42.5	<0.001*

* Significant, HTN: hypertension, DM: Diabetes Miletus, HCV: Hepatitis C Virus, HbsAg: Hepatitis B surface antigen,

AFP levels varied significantly among the three groups ($p < 0.001$), with the highest median in Group 2 (1155 ng/ml), followed by Group 1 (36.0 ng/ml), and the lowest in Group 3 (13.0 ng/ml). The mean AFP level was 2830.2 ± 3402.0 ng/ml in Group 2, compared to 199.5 ± 216.2 ng/ml in Group 1 and 17.68 ± 8.27 ng/ml in Group 3 **(Table 3).**

Table 3: Comparison between the three studied groups according to AFP and CD166

	Group 1 (n = 40)	Group 2 (n = 40)	Group 3 (n = 40)	H	P-value	Sig. Bet. Grps.		
						1 vs 2	1 vs 3	2 vs 3
AFP (ng/ml)								
Min. – Max.	4.0 – 672.0	5.0 – 13558.0	2.0 – 29.0	27.226*	<0.001*	0.003*	0.028*	<0.001*
Mean ± SD.	199.5 ± 216.2	2830.2 ± 3402.0	17.68 ± 8.27					
CD166								
Min. – Max.	0.40 – 30.51	0.20 – 34.30	0.18 – 24.43	39.411*	<0.001*	0.024*	<0.001*	<0.001*
Mean ± SD.	10.03 ± 5.59	19.86 ± 11.65	3.54 ± 4.06					

AFP at a cutoff of >39 ng/ml showed a sensitivity of 72.5% and specificity of 55.0% in distinguishing HCC from cirrhosis, with a PPV of 61.7% and an NPV of 66.7%. CD166 at a cutoff of >10.94 demonstrated the same sensitivity (72.5%) but higher specificity (70.0%), achieving a PPV of 70.7% and an NPV of 71.8%. The combination of AFP and CD166 improved diagnostic performance, reaching a sensitivity of 87.5% and specificity of 82.5% **(Table 4).**

Table 4: Agreement (sensitivity, specificity) for AFP (ng/ml), CD166 to diagnose HCC patients (n = 40) from Cirrhosis (n = 40)

	AUC	P-value	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
AFP (ng/ml)	0.728	<0.001*	0.613 – 0.843	>39	72.50	55.0	61.7	66.7
CD166	0.738	<0.001*	0.612 – 0.864	>10.94	72.50	70.0	70.7	71.8
Combination AFP& CD166	0.868	<0.001*	0.770 – 0.966		87.50	82.50	83.3	86.5

For distinguishing cirrhotic patients without HCC from controls, AFP at a cutoff of >23 ng/ml had a sensitivity of 75.0% and specificity of 70.0%, while CD166 at >4.78 showed similar specificity (70.0%) but slightly lower sensitivity (72.0%). Combining AFP and CD166 improved accuracy, achieving a sensitivity of 92.5% and specificity of 90.0%. When differentiating cirrhotic patients with HCC from controls, AFP at >15 ng/ml had a sensitivity of 62.5% and specificity of 60.0%, whereas CD166 at >5.37 showed higher sensitivity (82.5%) and specificity (80.0%). **(Table 5, Figure 1).**

Table 5: Agreement (sensitivity, specificity) for AFP, CD166 and Combination AFP& CD166 to diagnose Cirrhotic patients without HCC (n = 40) from control (n = 40)

	AUC	P-value	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Cirrhotic patients without HCC from control								
AFP (ng/ml)	0.803	<0.001*	0.688 – 0.917	>23	75.0	70.0	71.4	73.7
CD166	0.810	<0.001*	0.707 – 0.913	>4.78	72.0	70.0	70.7	70.3
Combination AFP& CD166	0.935	<0.001*	0.865 – 1.0		92.50	90.0	90.2	92.3
Cirrhotic patients with HCC from control								
AFP (ng/ml)	0.676	0.007*	0.539 – 0.812	>15	62.50	60.0	61.0	61.5
CD166	0.848	<0.001*	0.748 – 0.947	>5.37	82.50	80.0	80.5	82.1
Combination AFP& CD166	0.937	<0.001*	0.874 – 1.0		92.50	82.50	84.1	91.7

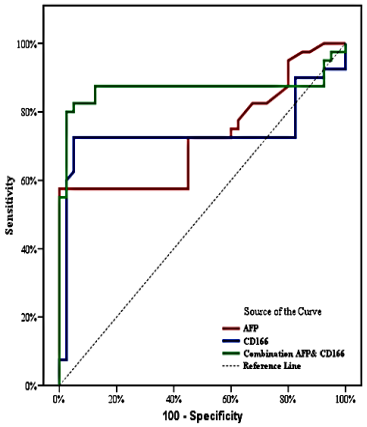

Figure (1): ROC curve for AFP, CD166 to diagnose HCC patients (n = 40) from Cirrhotic patients without HCC (n = 40).

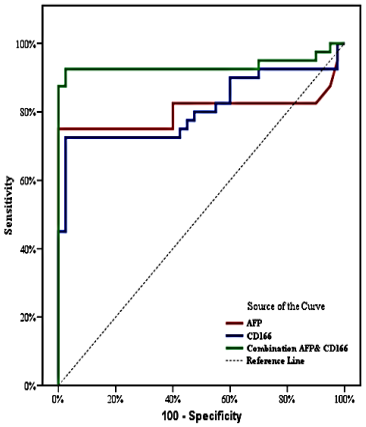

Figure (2): ROC curve for AFP, CD166 and Combination AFP& CD166 to diagnose Cirrhotic patients without HCC (n = 40) from control (n = 40)

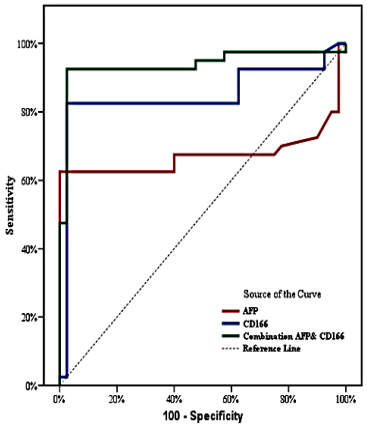

Figure (3): ROC curve for AFP, CD166 and Combination AFP& CD166 to diagnose Cirrhotic with HCC (n = 40) from control (n = 40)

DISCUSSION

Liver cirrhosis and HCC represent major global health concerns with substantial morbidity and mortality. The diagnosis of HCC often relies on biomarkers such as AFP, though the specificity and sensitivity of this marker can be limited. CD166 has recently emerged as a potential biomarker in HCC detection [(7)]. Our study aims to evaluate the diagnostic value of both AFP and CD166 biomarkers, individually or in combination, in patients with cirrhosis and HCC.

Our study demonstrated that AFP >39 ng/ml gave 72.5% sensitivity and 55% specificity in differentiating HCC from cirrhosis. The findings are in agreement with previous research stressing the limited diagnostic utility of AFP. For instance, **Marrero *et al.*** [(8)] discovered that a cutoff of AFP >20 ng/ml had a sensitivity of 61% and specificity of 81% in detecting HCC, suggesting that AFP, while not definitive, is helpful when included as part of the diagnostic evaluation. Similarly, **Tzartzeva *et al.*** [(9)] also reported comparable results, with AFP >20 ng/ml showing 60% sensitivity and 80% specificity, once again corroborating the fact that although AFP alone may not be conclusive, it remains a useful biomarker when taken in conjunction with other diagnostic tools.

By contrast, though, some studies have shown much lower sensitivity for AFP, particularly for higher cutoffs. For example, **Zang *et al.*** [(10)] found that an AFP cutoff of >200 ng/ml had a sensitivity of only 40–50%, meaning poorer performance in some cases. This

discrepancy could be explained due to differences in patient populations; Yang et al. largely treated patients with late-stage HCC, in whom AFP levels would be more heterogeneous, while our population included significantly more patients with early-stage disease, in whom AFP elevation would be more consistent.

In addition, **Galle *et al.*** [11] have raised doubts regarding the use of AFP in diagnosing HCC at an early stage and suggested imaging-based modalities such as MRI and CT scans instead. Although the above view may appear contradictory to our findings, our study is in support of complementary use of AFP in the diagnostic process. Rather than relying solely on AFP, we recommend a multimodal approach an approach that finally aligns with EASL's emphasis on the integration of biomarkers with advanced imaging to enhance diagnostic accuracy.

Our study identified CD166 as a potential biomarker for HCC with a cut-off value of >10.94 ng/ml showing sensitivity of 72.5% and specificity of 70%. It shows that CD166 can offer improved diagnostic performance over AFP and confirms its growing recognition in recent literature as a novel marker for HCC. Specifically, **Ma *et al.*** [12] have reported that CD166 is overexpressed in HCC tissue and its expression is positively associated with tumorigenesis, pointing out its diagnostic as well as prognostic significance. Likewise, **Ma *et al.*** [12] have reported that higher serum levels of CD166 were associated with poorer survival in patients with HCC, pointing to its clinical relevance beyond diagnosis per se, potentially with guidance to therapeutic management and follow-up planning.

However, few reports agree with the specificity of CD166 in liver disease. Some of them have noted its elevation in non-malignant conditions with the possibility of false-positive results. For example, **Lu *et al.*** [14] documented an elevation of the levels of CD166 in advanced cirrhosis that may compromise its specificity in distinguishing early HCC. This discrepancy could be due to the difference in study design and patient recruitment. Our study particularly excluded patients with decompensated cirrhosis and acute liver injury, thereby limiting confounding factors that could elevate CD166 in non-cancerous liver diseases. However, **Lu *et al.*** [14] group included patients who had severe liver dysfunction, which may have affected the observed overlap of CD166.

The combined use of AFP and CD166 in this study significantly improved diagnostic precision for hepatocellular carcinoma (HCC), with 87.5% sensitivity and 82.5% specificity, decisively better than either marker alone. This result is in line with increasing agreement that multi-marker approaches are better for early detection of HCC. For instance, **Ma *et al.*** [12] emphasized that panels of markers—e.g., AFP, DCP, and the GALAD score—have better sensitivity than individual markers. In the same vein, **El-Bagory *et al.*** [15] demonstrated how the incorporation of protein biomarkers with imaging modalities significantly improved overall diagnostic performance. Such research informs the basis for combining CD166 and AFP to enhance detection of HCC, especially in the early stages when intervention is most beneficial.

Despite such progress, some research has challenged the utility of protein biomarkers. Emerging modalities such as ctDNA, exosomal markers, and microRNAs have shown promise in early studies. For example, **Attia *et al.*** [16] reported that ctDNA offers enhanced sensitivity for the detection of early HCC compared to AFP-based panels. Nevertheless, despite such future technologies providing superior analysis capacity, currently, they are expensive, technically sophisticated, and unavailable at a mass level, particularly to resource-poor communities. The AFP + CD166 combination, however, presents an affordable and pragmatist alternative solution, therefore making it relatively feasible for routine screening to be exercised in communities without strong healthcare infrastructures.

According to these findings, our study attains great clinical relevance. In the first place, CD166 alone was more specific (70%) than AFP (55%), which can reduce false positivity, and unnecessary biopsies or scans. In the second place, the combination of AFP with CD166 gave a high diagnostic sensitivity, making this a prime target for screening high-risk individuals, especially patients with cirrhosis. Thirdly, this panel potentially holds the key for the early detection of liver disease beyond HCC. Interestingly, we found that AFP >23 ng/ml and CD166 >4.78 ng/ml could separate cirrhosis from healthy controls with an AUC of 0.935, which suggests potential value in monitoring liver disease.

Of note, this approach would be able to reduce the employment of imaging modalities like MRI or CT, which could be unavailable or out of reach in large parts of the globe. A sensitive serum test would serve as a first-line screening test with imaging reserved for definitive diagnosis or therapeutic planning. However, there are a number of limitations to be overcome. Our study utilized a relatively modest number of subjects (40 per arm), and the population was predominantly based on cirrhotic patients with HCV-related disease. Outcomes could be varied in patients with HBV or NAFLD-related liver disease, making it a need for larger, multicenter trials in a broad population base. Moreover, longitudinal studies are required to assess how AFP and CD166 levels vary over time and whether they can predict prognosis or response to treatment.

CONCLUSION

In conclusion, our findings point to CD166 as a putative biomarker of HCC alone or in conjunction with

AFP. While AFP remains widely used, its limitations necessitate further diagnostic methods. The two-marker panel consisting of AFP and CD166 not only improves diagnostic efficacy but also aligns with global patterns of multi-marker strategies for the detection of early cancer. Although some of the newer biomarkers like ctDNA will ultimately be more sensitive, the availability and ease of CD166 + AFP make it a strong contender in the current clinical practice. Further validation in diverse populations and longer follow-up studies will be needed before widespread clinical application.

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