RESEARCH ARTICLE

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Potential Role of the Tie2/ Angiopoietin System in Hepatitis C Virus- Induced Hepatocellular Carcinoma

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Abstract

Background: The Tie2/Ang pathway was found to be involved in forming tumor blood vessels in various tumors. The goal of this study was to evaluate the value of Tie2/Ang pathway as a novel biomarkers for the early detection of chronic hepatitis C virus (CHC)-related hepatocellular carcinoma (HCC). And the possibility of their future application in HCC treatment. **Methods:** Flow cytometry was performed to identify and count Tie2 expressing monocytes (TEMs) in peripheral blood monocytes from HCC patients (n = 25), CHC cirrhotic patients (n = 25) and healthy volunteers (n = 25). In addition, Angiopoietin 1 and 2 (Ang) levels in the serum were determined by enzyme linked immunosorbent assay (ELIZA). **Results:** Percentage of TEMs in peripheral blood monocytes, serum Ang2 levels and Ang2/Ang1 ratio significantly increased in HCC patients compared with CHC patients and healthy controls ($P< 0.001$). However significant increase was only noticed in serum Ang1 levels in HCC group compared to the control group ($P <0.05$). **Conclusions:** TEMs may promote angiogenesis in HCC regarding the Ang2/Tie2 signal pathway. Percentage of TEMs in peripheral blood monocytes, Ang2 serum levels and Ang2/Ang1 ratio may be applied as a biomarkers for identifying CHC-related HCC. Moreover, inhibiting the proangiogenic functions of this pathway may represent a promising strategy to improve the efficacy of current treatments for HCC.

Keywords: Tie2/Ang pathway- TEMs- HCC

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Introduction

An important feature of chronic hepatitis C (CHC) progression is the persistence of hepatitis C virus (HCV) in the liver, which perpetuates the inflammatory response and deregulates other repairing processes, leading to angiogenesis, fibrosis, cirrhosis and Hepatocellular carcinoma (HCC) [1]. HCC is a highly vascular tumor in which angiogenesis plays a major role in tumor growth and spread. Tumor-induced angiogenesis is usually related to a complex interplay between multiple factors and pathways [2]. Most HCC patients present with advanced and symptomatic disease not amenable to curative surgery. Novel biomarkers for the early detection of HCC are greatly needed for high-risk populations which increase the effectiveness of surgical treatment of HCC.

Tumor-associated macrophages (TAM) are well known as a key player in the tumor microenvironment [3]. They are involved in crucial processes during tumor development. A lineage of monocytes characterized by the expression of monocyte/macrophage markers as well as the angiopoietin (Ang) receptor *Tie2* expression was identified as a subset of circulating and tumor-associated monocytes. They were found to be endowed with proangiogenic activity [4].

TIE-2 binds to angiopoietins produced during activation and dysfunction of the vascular endothelium in several infectious diseases. Ang-1 and Ang-2 are considered the two pivotal members of the Ang family [5]. In vitro, TEM migrated toward Ang-2 released from Weibel-Palade bodies in activated endothelial cells (EC) and angiogenic vessels, suggesting a homing mechanism for TEM to tumors [6]. However, in contrast to Ang-2 expression, Ang-1 upregulation did not found to be correlated with angiogenesis or tumor progression [7].

In this study we aimed to try to understand the Tie/Ang pathway in HCC-induced angiogenesis which may led to the emergence of novel biomarkers useful in diagnosing, predicting the prognosis, and future application in HCC treatment.

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Materials and Methods

Study design and participants

This study enrolled 50 patients (15 women, 35 men), with chronic liver disease admitted to gastroenterology and hepatology department,Theodor Bilharz Research Institute (TBRI), Giza, Egypt. All patients were positive for hepatitis C virus, negative for hepatitis B virus. The patients were classified into two groups, group I included patients with HCC owing to hepatitis C (CHC) virus infection induced cirrhosis, and group II included CHC induced cirrhotic patients without HCC (25 each). In addition to a healthy age and sex matched control group (25 individuals). Diagnosis of patients was based on thorough clinical examination, liver function tests and hepatitis markers. HCC patients were diagnosed by abdominal ultrasonography, computed tomography or magnetic resonance imaging. None of the patients had active variceal bleeding or encephalopathy at time of investigation. Patients with fever, overt infectious disease (septicemia, pneumonia, urinary tract infection) or renal insufficiency were excluded.

Clinical and laboratory assessment

The studied patients were subjected to full history taking and laboratory investigations, including complete blood count (ACT differential Beckman Coulter), liver function tests and hepatitis markers (Cobas 8000 auto-analyzer, supplied by Roche Diagnostics), and immunophenotyping characterization for identification of TEMs (CD14+CD16+*Tie-2*+ cells) using anti-CD14, anti-CD16, and anti-CD202b antibodies by flow cytometry and performed on Becton Dickinson (Los Angeles, California, USA). In addition, Ang1 and Ang 2 serum levels were measured using ELISA technique (R&D systems, USA)

Specimen collection

Six milliliters of venous blood samples were collected under complete aseptic conditions from all patients and control subjects.

Samples were divided into

- two milliliter EDTA blood was used for complete blood counts using electronic cell counter, and flow cytometric assay of CD14, CD16 and *Tie2*.

- four milliliters of blood was delivered into a clean tube and left to clot. Serum was separated by centrifugation at 3000 rpm for 15 min. Serum samples were used for assay of liver function tests {Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)} using autoanalyzer. Also, serum samples were used for assay of hepatitis markers (HBs-Ag and HCV-ab) using ELISA technique. In addition, serum samples were used for quantitative assay of serum Ang1 and Ang2 using ELISA technique.

Immunophenotypic analysis

The panel of monoclonal antibodies labeled with fluorescein isothiocyanate (FITC) CD14+, phycoerythrin (PE) CD16+, and peridinin-chlorophyll-protein complex (PerCP) *Tie-2*+ were used for each sample supplied by Thermo Fisher Scientific, performed on Becton Dickinson.

Statistical analysis

Statistical analysis was done using software version SPSS (Statistical Package for the Social Sciences), version 21 (Chicago, Illinois, United States). Data were presented, and suitable analysis was done according to the type of data obtained for each parameter. Qualitative data were presented as numbers and percentages; quantitative data were presented as mean, SD, and ranges; and nonparametric distribution data were presented as a median, with interquartile range. To compare parametric quantitative variables between two groups, Student t test was applied. For comparison of nonparametric quantitative variables between two groups, Mann–Whitney test was used.

Results

The demographic and clinical characteristics of the studied participants are shown in Table 1. Laboratory parameters in different studied groups are illustrated in Table 2. Percentage of TEMs in peripheral blood monocytes in different groups

Results revealed highly significant expression of *Tie-2* receptor on non classical monocyte subset (CD14+/CD16+) compared to the classical subset (CD14+/CD16-) (Table 3).

Circulating TEMs were detectable in all studied subjects. The percentage of TEMs in peripheral blood monocytes was significantly higher in HCC patients (52.5±14.9%) compared to patients with HCV (24.3±13.4%) and controls (7.5±4.1%) as shown in (Table 4).

Serum Ang1 and Ang2 concentration

The results of ELISA demonstrated that the mean serum Ang2 showed highly significant increase in HCC patients compared to both controls and HCV patients ($p<0.001$). Moreover, significant difference in Ang2 levels was also detected between HCV patients and the control group ($p<0.001$). Significant difference in Ang1 levels were only detected between controls and HCC patients. Calculating the Ang2/Ang1 ratio revealed highly significant increase in HCC group compared to the HCV and control groups ($p<0.001$) (Table 5)

Subsequently, receiver operating curve (ROC) analyses were performed to demonstrate the HCC diagnostic validity of each individual studied markers or combined markers (Table 6 & 7) (Figure 1, 2)

ROC curve analysis was constructed to examine the diagnostic performance of studied parameters frequency in differentiating HCC from control. We found that the AUC of Ang1 was 0.707 and sensitivity was 76% and specificity was 64 (p value: 0.012).

Our results revealed that the AUC of Ang2 was 1, with sensitivity 100% and specificity 100% (p value: <0.0001**). The diagnostic performance of Ang2 frequency in combination with AFP , the AUC was 0.983, with sensitivity 96% and specificity 80% (p value: <0.0001**).

Table 1. Demographic and Clinical Characteristics in Different Studied Groups

Demographic& clinical data	Group I HCC n: 25	Group II CHC n: 25
Age (Mean ±SD)	55.7±7.3	57.2±5.3
Male (%)	17 (68%)	18 (72%)
Female (%)	8 (32%)	7 (28%)
Ascites, n (%)		
No	14 (56%)	9 (36%)
Mild	10 (40%)	12 (48%)
Moderate	1 (4%)	4 (16%)
Hepatic Encephalopathy, n (%)		
No	21 (84%)	25 (100%)
Grade 1-2	4 (16%)	0
Grade 3-4	0	0
Child-Pugh grade, n (%)		
A	0	7 (28%)
B	18 (72%)	5 (20%)
C	7 (28%)	13 (52%)
Clinical status as regard the HCV		
HCV Antibody	25 (100%)	25 (100%)
HCV-RNA PCR +VE	23 (92%)	25 (100%)

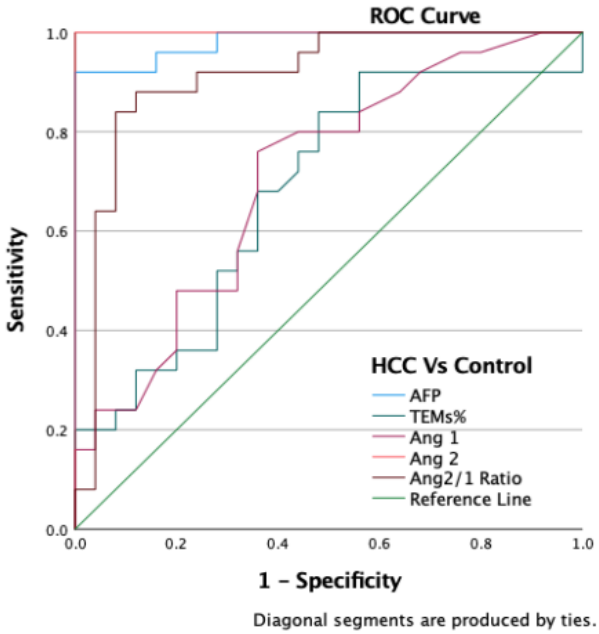

Figure 1a. The HCC Diagnostic Performance of the Studied Parameters

ROC curve analysis for the Ang 2/Ang1Ratio had an AUC of 0.910 with sensitivity 88% and specificity 88% (p value: <0.0001**). On the other hand, combination of Ang 2/Ang1Ratio and AFP appeared to have be more sensitive in differentiating HCC patients with an AUC of 0.953, a sensitivity of 92% and a specificity of 80% (p value: <0.0001**).

Regarding the diagnostic performance of TEMs

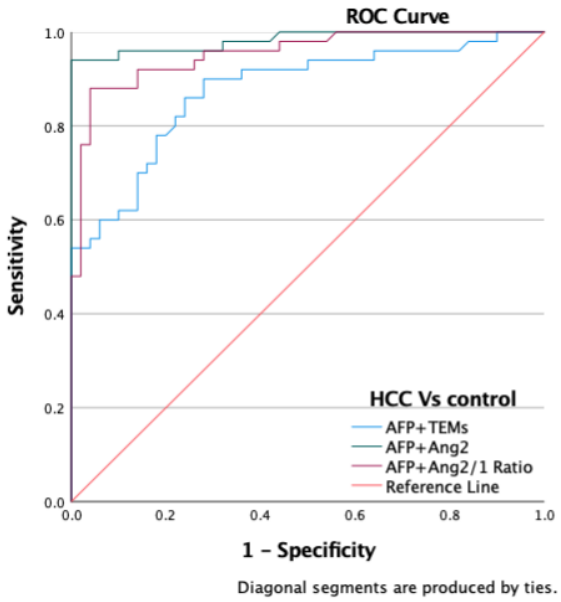

Figure 1b: The HCC Diagnostic Performance of the Studied Parameters

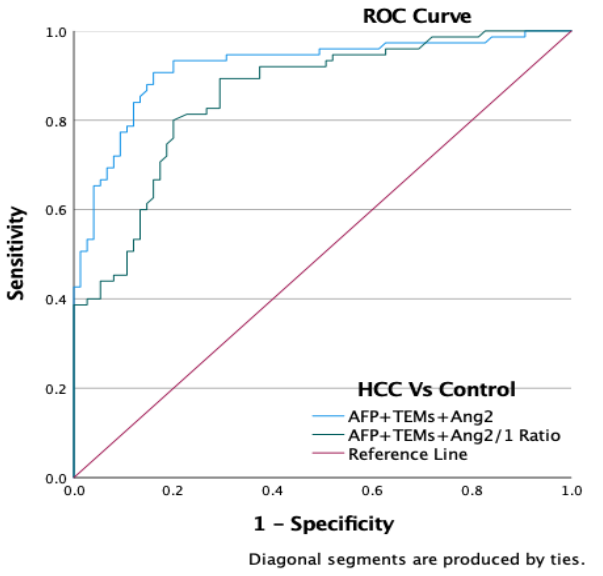

Figure 1c. The HCC Diagnostic Performance of the Studied Parameters

frequency for differentiating HCC patients from healthy subjects, ROC curve indicates that the sensitivity and specificity was 68 and 60 respectively with AUC 0.681 (p value : 0.028). ROC curves for the combination of TEMs and AFP in the diagnosis of HCC with sensitivity 80%, specificity 78% with AUC 0.876 % (p value: <0.0001**).

ROC curve analysis for the comparison of HCC patients with HCV patients, revealed that the AUC of Ang1show sensitivity 76% and specificity 52% and AUC was 0.604 (p value: 0.207).

ROC curve analysis for Ang2 was 0.886, with sensitivity 84% and specificity 72%. The diagnostic performance of Ang2 frequency in combination with AFP, the AUC was 0.829, with sensitivity 76% and specificity 64% (p value: <0.0001**). In addition, Ang 2/Ang1Ratio

Table 2. Laboratory Parameters in Different Studied Groups

Laboratory parameters		Group I HCC (n: 25)	Group II CHC (n: 25)	Control group (n:25)	P- Value
TLC ($\times 10^9$/L)	Median	8	5.3	8.1	0.053
	(Range)	(3.3-15.3)	(2.1-14.3)	(4.7-11.1)	
Hemoglobin (g/dl)	Median	9.4#†	11.5▫	13.4	0.001*
	(Range)	(5.9-14.3)	(8.1-14.1)	(11.1-15.3)	
Platelets ($\times 10^9$/L)	Median	127#†	131▫	233	0.001*
	(Range)	(29-426)	(75-232)	(152-393)	
INR	Median	1.33#†	1.9▫	1	0.001*
	(Range)	(1-2.6)	(1-3.1)	(1-1.3)	
Total bilirubin (mg/dl)	Mean ±SD	3.2±2.5#†	1.6±0.9▫	0.5±0.15	0.000*
	(Range)	(1.3-14.1)	(0.6-2.6)	(0.3-0.8)	
Direct bilirubin (mg/dl)	Mean ±SD	1.6±1.3#†	0.8±0.5▫	0.17±0.07	0.000*
	(Range)	(0.5-6.5)	(0.3-4.1)	(0-0.3)	
ALT (IU/L)	Mean ±SD	131.5±120.6#†	62.64±33.9▫	28.8±6.8	0.000*
	(Range)	(63-642)	(15-163)	(16-40)	
AST (IU/L)	Mean ±SD	77.48±98.9#	57.2±28.7▫	25.9±4.6	0.011*
	(Range)	(25-527)	(18-141)	(19-35)	
Total protein (g/dl)	Mean ±SD	6.34±0.68#†	6.1±0.42▫	7.24±0.61	0.000*
	(Range)	(5-7.8)	(5.9-7.1)	(6.2-8.2)	
Albumin (g/dl)	Mean ±SD	2.8±0.48#†	3±0.5▫	3.81±0.49	0.000*
	(Range)	(1.9-3.7)	(2-4.5)	(3-4.8)	
AFP (ng/ml)	Median	433#†	11.1	5	0.001*
	(Range)	(7-3810)	(4.2-293)	(2.1-9.3)	

Angiopoietin 1and 2 are represented as Median (Interquartile rang); the data were analyzed by Kruskal-Wallis H test; ‡, p. value is significant in comparison with control group; #p. value is significant in comparison with CHCgroup; *p. value <0.05 is significant, ** p. value <0.01 is highly significant

had an AUC of 0.694 with sensitivity 72% and specificity 60% (p value: <0.018*). Essentially, combination of Ang 2/Ang1Ratio and AFP displayed more accuracy in discriminating HCC patients with an AUC of 0.829, a sensitivity of 74% and a specificity of 78% (p value: <0.0001**).

TEMs had an AUC of 0.664 for diagnosing HCC patients, with sensitivity 64% and specificity 76% (p value: 0.047*). Importantly, combination of TEMs and AFP displayed an AUC of 0.842, a sensitivity of 76% and a specificity of 80% (p value: <0.0001**).

Discussion

Angiogenesis is deemed to be a critical step in the development and progression of HCC. TEMs have been detected in multiple solid and hematological human tumors and represent the main population of TAM in peripheral circulation. TEMs pro-angiogenic activity and recruitment to tumor is critically controlled by Tie/Ang axis [8].

Ang1, Ang2, and *Tie2* play different roles in mediating vascular quiescence and inflammation. Ang2 has a

Table 3. *Tie-2* Receptors Expression on CD16 (positive and negative) Monocytes in the Studied Subjects.

		CD14+/CD16- Monocytes	CD14+/CD16+ Monocytes	P- value
Tie-2 expression%	Mean ±SE	2.8±0.43	19.1±1.7	0.0001*
	(Range)	(0.7-8.1)	(11-61.8)	

P>0.05= not significant; *p<0.05=: Significance; SE, Standard error of mean.

Table 4.Percentage of TEMs in PB Monocytes among Studied Groups

		Group I HCC n: 25	Group II CHC n: 25	Control group n: 25	P-Value
TEMs%	Mean ±SD	52.5±14.9#†	24.3±13.4#	7.5±4.1	0.0001*
	(Range)	(21-90)	(9-56)	(4-20)	

P>0.05= not significant; *p<0.05=: Significance; #Significance differences in comparison with control group; †Significance differences in comparison with group1I

Table 5. Serum Levels of Ang1 and Ang 2 in Different Studied Groups

		Group I HCC n: 25	Group II CHC n: 25	Control group n: 25	P-Value
Ang1 (ng/ml)	Mean ±SD	3.1 (2.35- 4.65)‡	2.4 (1.3- 4.25)		0.038*
	(Range)			2.1 (0.85- 3.4)	
Ang2 (ng/ml)	Mean ±SD	50.6 (40.5- 62.75)‡#	28.6 (15.55- 35)‡	7.2 (5.25- 12.35)	<0.001**
	(Range)				
Ang2/Ang1 ratio	Mean ±SD	16.3 (10.8- 22.5)‡#	10.3 (6.6- 15.6)‡	4.0 (2.6- 6.8)	<0.001**
	(Range)				

‡p. value is significant in comparison with control group; #p. value is significant in comparison with CHC group; *p. value <0.05 is significant, ** p. value <0.01 is highly significant

Table 6. The Diagnostic Performance of the Studied Parameters for Differentiating HCC from Control

	Studied Variable	Cut-off	Sn.	Sp.	AUC	S.E	Asymptotic 95% C.I		P. value
							Lower Bound	Upper Bound	
HCC Vs Control	AFP	7.95	96	84	0.982	0.015	0.954	1	<0.0001
	TEMs	21.7	68	60	0.681	0.077	0.53	0.832	0.028
	Ang1	2.35	76	64	0.707	0.073	0.563	0.851	0.012
	Ang 2	19	100	100	1	0.001	1	1	<0.0001
	Ang 2/Ang1Ratio	8	88	88	0.91	0.045	0.822	0.998	<0.0001
	AFP + TEMs	-	80	78	0.876	0.035	0.807	0.944	<0.0001
	AFP + Ang2	-	96	80	0.983	0.011	0.961	1	<0.0001
	AFP + Ang2/Ang1 ratio	-	92	80	0.953	0.02	0.915	0.992	<0.0001
	AFP + TEMs + Ang2	-	90	84	0.919	0.023	0.873	0.965	<0.0001
	AFP + TEMs + Ang2/Ang1 ratio	-	89	71	0.856	0.03	0.796	0.915	<0.0001

Sn, Sensitivity; Sp, Specificity; AUC Area under curve and C.I: 95% Confidence Interval; * p value <0.05 is significant, ** p value <0.01 is highly significant.

similar *Tie2* receptor affinity as Ang1, thus competitively inhibits Ang1 binding ability to *Tie2* [9]. Ang1 promotes vessel stability, suppresses inflammation, and promotes endothelial cell survival by activating the *Tie2* receptor complex [10]. whereas Ang2 destabilizes blood vessels, potentiates inflammation, and promotes proangiogenic effects, which result in vascular leakage and organ dysfunction by initially blocking the *Tie2* receptor [11].

In our study, the frequency of *Tie 2* expression was compared in non-classical (CD14+/CD16+) and classical (CD14+/CD16–) monocytes. We observed a higher *Tie-2* expression in the non classical monocytes subset. Our finding was supported by other preliminary studies by Murdoch et al. [11], Venneri et al. [6] and Hristov et al. [12].

Our results showed a significantly higher frequency of TEMs among HCC patients compared to HCV-infected patients and healthy subjects .In agrement with our finding, Matsubara et al. [13] found the frequency of TEMs were significantly higher in HCC than HCV-infected patients or healthy subjects. No difference was found among HCC patients at different stages of the disease, thus establishing

Table 7. The Diagnostic Performance of the Studied Parameters for Differentiating HCC from HCV

	Studied Variable	Cut-off	Sn.	Sp.	AUC	S.E	Asymptotic 95% C.I		P. value
							Lower Bound	Upper Bound	
HCC Vs HCV	AFP	12.9	92	68	0.936	0.034	0.868	1	<0.0001**
	TEMs	26.5	64	76	0.664	0.078	0.51	0.818	0.047*
	Ang1	2.45	76	52	0.604	0.081	0.445	0.763	0.207
	Ang 2	34.5	84	72	0.886	0.046	0.796	0.975	<0.0001**
	Ang 2/Ang1Ratio	11.05	72	60	0.694	0.075	0.547	0.842	0.018*
	AFP + TEMs	-	76	80	0.842	0.041	0.762	0.922	<0.0001**
	AFP + Ang2	-	76	64	0.829	0.041	0.749	0.908	<0.0001**
	AFP + Ang2/Ang1 ratio	-	74	78	0.829	0.041	0.749	0.908	<0.0001**
	AFP + TEMs + Ang2	-	81	71	0.841	0.033	0.776	0.905	<0.0001**
	AFP + TEMs + Ang2/Ang1 ratio	-	70	66	0.769	0.038	0.694	0.844	<0.0001**

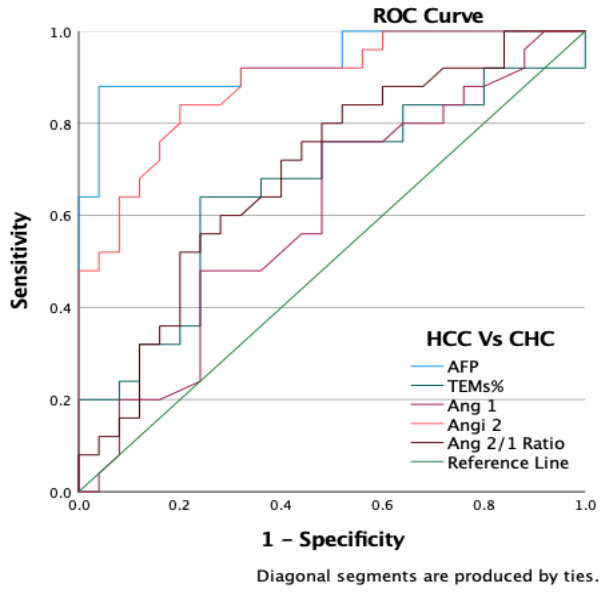

Figure 2a. The Diagnostic Performance of the Studied Parameters for Differentiating HCC from HCV

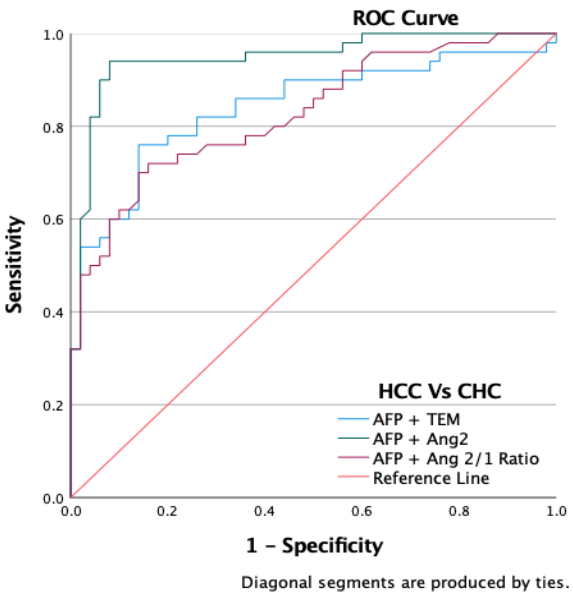

Figure 2b. The Diagnostic Performance of the Studied Parameters for Differentiating HCC from HCV

TEMs as a stage-independent diagnostic biomarker for HCC.Furthermore, Germano and Daniele, [14] found that a higher TEM infiltration correlated with increased micro-vessel density in HCC.

Contrary to our findings, a study by Rodriguez-Munoz et al. [15] showed that circulating and intrahepatic TEMs are significantly increased in HCV-infected patients without HCC, compared to healthy subjects. *Tie2* in non-classical monocytes. Although Rodriguez-Munoz et al. [15] analyzed a relatively small cohort of HCV-infected patients, their data raise the concern that mobilization/ expansion of TEMs may not be strictly HCC driven, but more generally associated with chronic liver infection [16].

We also assessed the diagnostic performance of

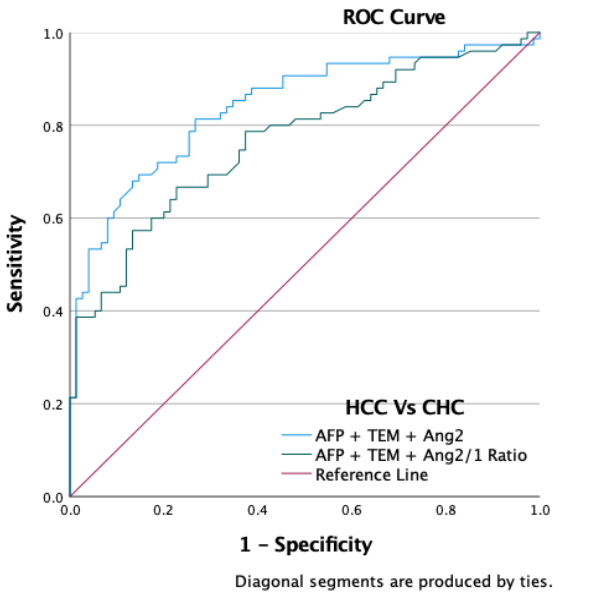

Figure 2c. The Diagnostic Performance of the Studied Parameters for Differentiating HCC from HCV

TEMs frequency for differentiating HCC patients from HCV-infected patients,a cut off point of 26.5% with 64% sensitivity and 76% specificity was reported. In comparison to our results, He et al. [16] stated a cut off value of 28.9% to discriminate HCC from HBV-infected patients. This cut off provides sensitivity and specificity of 76.2 and 76.2, respectively.Additionally, we proposed a cut-off point of 21.7% for differentiating HCC patients from healthy subjects with 68% sensitivity and 60% specificity. In this series, He et al. [16] also indicated a cut off value of 26.2% with 81% sensitivity and 60.9% specificity .

The diagnostic performance of combination of TEMs and AFP was also assessed with 76% sensitivity and 80% specificity in accordance with Mao et al., 2017 who reported that combination of TEMs, plasma Dickkopf-1 and AFP could significantly increase the AUC for HCC diagnosis than that when any of the biomarkers was used alone.

In our study, we found that serum Ang-2 levels in patients were significantly higher than in controls. Statistically significant increase was also observed in Ang2/Ang1 ratio. However, only slight significant increase in HCC patients from controls was observed regarding Ang-1 levels. On evaluation the diagnostic performance value of the studied parameters. We found that The Ang2 was the most sensitive and specific for HCC diagnosis compared to control. Although,it is not so sensitive and specific in early detection of HCC in HCV patients. Of importance, we found that AFP was the most sensitive marker for early detection of HCC development on top of HCV but the combination of AFP &TEMs was the most spesific.

In agreement of our results Saharinen et al . [17] found that Statistically significant differences were observed in Ang-2 and Flt-1 levels in CHC patients. also, Pocino et al. [18] stated that in cirrhotic patients, Ang-1 and Ang-2 correlate with MELD (Model for End-Stage Liver

Disease" (MELD) score and Fibrosis Index (FI) [19].

Consistent with our results, a retrospective study by Hernández-Bartolomé et al. [1] analyzing 179 patients had demonstrated that although Ang-1 was decreased in cirrhotic compared with non-cirrhotic patients, Ang-2 was significantly increased as the stage of liver disease progressed. Moreover, these authors demonstrated that the ratio of serum Ang- 2 to Ang-1 displayed notable accuracy for the diagnosis of cirrhosis at the optimal cut-off. In accordance to our results, Scholz et al. [20] demonstrated that Ang-2 levels were significantly higher in HCC patients than in cirrhotic patients.

In addition, Roberts et al. [21] Observed in their study that plasma Ang-1 levels were significantly lower in cirrhotic patients than in healthy controls and in HCC patients and plasma Ang-2 levels were significantly lower in healthy controls than in cirrhotic and in HCC patients.

In accordance with our results, Thurston and Daly, [22] and Liu et al. [23] approved that Ang2 was found to be over-expressed in a wide range of inflammatory conditions, including HCC, which is a typical inflammation-associated cancer. Vanderborght et al. [5] also demonstrated that Ang2 over-expression promotes rapid HCC development and worsens prognosis.

Environmental signals associated with tumor pathogenesis such as hypoxia, VEGF upregulation and increased inflammatory cytokines such as tumor necrosis factor (TNF) can stimulate the release and production of Ang2 in endothelial cells. Ang2 was found to be over-expressed in a wide range of inflammatory conditions, including HCC, which is a typical inflammation-associated cancer [23, 24]. It was also demonstrated that Ang2 over-expression promotes rapid HCC development and worsens prognosis [5].

The angiogenic function of TEMs in hypoxic tumor sites may be related to its secretion of VEGFA, MMP-9. Coffelt et al. [25] also demonstrated that Ang-2 meanwhile stimulates TEMs to express interleukin-10 and chemokine (C-C motif) ligand 17 (CCL17) in vitro. The cytokines may reduce T cells proliferation, increase the ratio of CD4+ T cells to CD8+ T cells, and accelerate the amplification of regulatory T cells (CD4+CD25+ FOXP3+).

Targeting *Tie-2*, the receptor for Ang-1 and Ang-2, promote microvascular stability and decrease angiogenesis, and a selective Ang-2 inhibitor has been shown to reduce vascular growth by 46% and tumor size by 62% over a period of 26 days in preclinical models, demonstrating the role of this signaling pathway in HCC progression and further establishing angiopoietin as a potential target for therapy development.

In summary, we have demonstrated that circulating levels of the proangiogenic cytokines Ang-1, Ang-2 and Percentage of TEMs in peripheral blood monocytes may be applied as a complementary diagnostic cellular biomarker for identifying CHC-related HCC along with AFP. Independent validation studies with serial plasma measurements are needed to further characterize the significance of plasma angiopoietins levels, not only as prognostic markers, but even more critical, as predictors of response or resistance to therapies, particularly in the era of immunotherapy and antiangiogenesis-directed HCC therapy

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

Ethical issues

This study was performed in accordance with the Helsinki Declaration. The protocol was designed according to the study objectives and approved by the Theodor bilharz Research Institute Ethical Committee (ID:23/2019-TBRI). All participants included were informed about the study plan and the potential benefits and hazards and provided their written informed consent to participate in this study.

Conflict of Interest

I have no conflicts of interest to disclose.

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