# Role of Tie2, CD14, Angiopoietin as Angiogenetic Markers in Hepatocellular Carcinoma Complicating Hepatitis C Virus Infection

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## Abstract

**Background:** Identifying biomarkers for early detection of hepatocellular carcinoma [HCC] remains quite challenging. In this study we aimed to estimate the number of TIE2-expressing monocytes [TEMs] cells, which display pro-tumoral activities and are defined as CD14+, TIE2+, and angiopoietin-2; and its potential use as a possible diagnostic marker in HCC patients complicating HCV induced cirrhosis.

**Methods:** Current study was conducted on 112 patients. They were divided into two groups: Group I (78 patients) with HCC complicating HCV induced cirrhosis; and group II chronic hepatitis C patients (34 patients). Both groups were compared to [age and sex-matched] healthy persons as group III (38 persons).

**Result:** The number of the circulating TEMs: CD14+ and TIE2+ monocytes were significantly higher in the peripheral blood of HCC patients than HCV LC patients and healthy controls, sensitivity and specificity for HCC diagnosis were respectively: CD14 [89.7%, 83.3%], TIE 2 [76.9%, 83.3%], and Ang-2 [76.9%, 66.7%]. Moreover, analysis of the P-value and the odd's ratio showed that CD14 has the highest predictive value for HCC.

**Conclusion:** Our results suggest that TEMs and Ang-2can be used as diagnostic markers for HCC, especially among the high-risk group of patients.

## Introduction

Hepatocellular carcinoma [HCC] has an increasing incidence all over the world, being the second most common worldwide cancer among males and the sixth among females [1, 2]. It is also the second most common cause of cancer deaths [3, 4]. HCC has a very high mortality rate due to limited treatment options in the vast majority of cases [1]. In Egypt, liver cancer accounts for around 11.75% of gastrointestinal malignancies and 1.68% of the total malignancies. Also, HCC constitutes 70.48% of all hepatic malignancies among the Egyptian population [5]. The etiology of HCC is multifactorial, with many infectious and noninfectious health problems. These include hepatitis viral infections (hepatitis B virus [HBV] and hepatitis C virus [HCV]), alcoholic liver cirrhosis and non-alcoholic fatty liver disease [NAFLD]. However, the pathogenesis of HCC is not yet fully understood [6]. The limited current treatment options available, and treatment resistance, especially chemotherapy, remain the major challenges in the management of such cases. The cause of this is usually due to late diagnosis of HCC, and so there is an increasing need for identifying suitable biomarkers for early detection of HCC; aiming to improve the treatment outcomes [7]. Macrophages display several pro-tumorigenic functions that have important roles in cancer initiation and progression [8]. Tumor-associated macrophages [TAMs] are heterogeneous macrophage cells that can be subdivided into different populations according to either high or low expression of major histocompatibility complex [MHC] class II. TAMs remain poorly understood and might differ according to tumor type and localization [9]. Angiogenesis, or the formation of new blood vessels, is an essential process in the progression, extension, and distant metastasis of many tumors. This is a known dependent risk factor of hematological malignancies and was also proven for many solid tumors[10]. Angiopoietin regulators act on tyrosine kinase receptors[Tie] either Tie1 or Tie2, under both normal and pathologic conditions. Angiopoietin-1 [Ang-1] is a constitutive agonist for Tie2phosphorylation [p-Tie2] that contributes to vascular remodeling during the development and subsequent maintenance of vascular stability [11]. Angiopoietin-2 [Ang-2] competitively inhibits the action of Ang-1 on Tie2 and promotes vascular remodeling by suppression of Tie2

signaling. The over-expression of Ang-2 leads to embryonic lethality which is similar to what is caused by deletion of either Ang-1 or Tie2[12]. Tie2 expressed on proangiogenic macrophages are involved in tumor angiogenesis and intravasation of malignant cells and subsequent metastasis [13]. The angiopoietin Ang/Tie2 kinase signaling pathway is an important angiogenic signaling axis which subsequently ends with poor outcome and recurrence in cancer patients [14, 15].

CD14 levels are increased in some inflammatory conditions and may reflect disease severity [16]. It is also found to correlate with the degree of liver inflammation in non-alcoholic steatohepatitis [NASH] patients [17].

The study aimed to examine the number of TEM in the blood of HCC patients complicating HCV induced liver cirrhosis(TEM); defined as CD14+, TIE2+, and angiopoietin-2 in HCC cases and its value as a possible diagnostic marker in.

## Methods

This study was carried out on 112 Patients: Group I (78) newly diagnosed HCC patients on top of HCV induced cirrhosis according to typical characteristics of HCC seen in dynamic contrast enhancement CT, recruited between October 2016 and November 2017; and group II (34) chronic hepatitis C patients, compared to group III (38) healthy controls.

### Exclusion Criteria

Known history of autoimmune diseases, chronic inflammatory diseases, history of other malignancies, and patients with chronic liver disease other than chronic HCV.

All patients were subjected to:

- History and Clinical Examination

- Laboratory Investigations

Complete blood count [CBC]; done by the use of cell dyne-3700 [Abbott Diagnostics, Dallas, USA]. Immunophenotyping [IPT] on peripheral blood monocytes CD14 fluoroisothiocyanate[FITC] and TIE2 phycoerythrin[PE]. Serum Ang-2 was assayed by enzyme-linked immunosorbent assay [ELISA] using commercially available kits [Elabscience systems Houston, Texas USA]. Other Lab Investigations: liver function, alpha-fetoprotein, prothrombin time and concentration, and hepatitis markers: HCV antibody and

HBV surface antigen.

*Determination of TIE2 Expression on CD14+ [FITC] Monocytes*

The steps involved in the cytoplasmic staining of phycoerythrin [PE] labeled monoclonal antibodies [MoAb]for detection of both TIE2 and Moab were performed in a biotechnology company [Becton Dickinson Bioscience in San Jose, California]. Peripheral blood mononuclear cells [PBMC] were isolated from whole blood by Ficolldensity centrifugation and were then stained with10 microliters of CD14 fluoroiso-thiocyanate [FITC] and incubated for 15 minutes at room temperature in the dark. This was then followed by incubation, red blood cell [RBC] lysis, washing with phosphate-buffered saline [PBS], and addition of BD Cytofix/Cytoperm solution to fix the cells, and incubation for 10 minutes. After incubation, the cells were washed with PBS, followed by the addition of permeabilizing solution and 10 microliters of TIE2, and incubated for 30 minutes at room temperature. Flow cytometric analysis was done by fluorescence-activated cell sorter [FACS] caliber Flow cytometer [Becton Dickinson Bioscience, San Jose, California]. Isotype-matched antibodies were applied with all the samples as controls. Forward and side scatter histograms were used to define the monocytes population [18].

Ang-2 measurement by ELISA: Serum levels of Ang-2 were assayed by ELISA using commercially available kits [Elabscience systems Houston, Texas, USA]. The serum obtained from plain blood sample tubes was kept at −80°C and Ang-2 levels in the serum were acquired by ELISA. Absorbance was recorded at 495 nm while Ang-2 concentrations were recorded according to the manufacturer's instructions.

*Statistical Analysis*

Data was analyzed using IBM SPSS Statistics for Windows version 24 and Medcalc version 18.1. Quantitative data were expressed as means ± standard deviation. Qualitative data were expressed as numbers and percentage. Quantitative data were tested for normality by Shapiro–Wilk test, Mann–Whitney U test, Wilcoxon Signed Ranks test, and Spearman's correlation were used for data which wasn't normally distributed. Independent Samples T-test and Pearson's correlation were used for normally distributed data. Chi-square (χ2)

test was used for comparison of qualitative variables as appropriate. Receiver operating characteristic [ROC] curve was constructed for CD 14+, TIE 2+& Ang-2 for optimum cut off point in predicting their levels in HCC patients and the area under the ROC curve value with 95% CI was calculated. Optimal cut off value was determined; sensitivity, specificity, positive predictive value, and negative predictive value were calculated. A p-value of less than 0.05 was considered significant in all statistical tests used in the study.

**Results**

A total of 112 patients were included in our study and 38 healthy controls classified into 3 groups.

Group I: 78 newly diagnosed hepatocellular carcinoma patients [55males and 23 females]; their ages ranged between 46 and 80 [mean 62.45±8.01] years;

Group II: 34 hepatitis C patients [19 males and 15 females]; their ages ranged between 48 and 79 [mean 63.77±8.23] years;

Group III: 38 healthy controls [25males and 13 females]; their ages ranged between 52 and 77 [mean 61.40±6.71] years.

Table [1] shows the comparison among studied groups with regards to age, sex, and laboratory parameters.

According to this table, highly significant decreased values of hemoglobin, platelets and prothrombin concentration [PC] were found in groups I and II as compared to the control group

A highly significant rise in AST and ALT were detected in HCC patients group[I], in comparison to the cirrhotic group[II]. Also, CD 14, TIE2, and angiopoietin 2, Alpha-fetoprotein were significantly increased in the HCC patients group[I] than in both groups [II] and [III].

Table [2] shows correlation between angiogenetic markers CD14 [%], TIE2 [%], and Ang-2 [pg/ml], and laboratory results in group [I] HCC [N= 79]

There was significant positive correlation between the age of the patients and CD14 [%] [r = 0.232; p = 0.041], there was negative significant correlation between CD 14 and WBC count [r = -0.254; p = 0.025], there was significant positive correlation between haemoglobin concentration of the patients and CD14 [%] [r = 0.294; p = 0.009],

There was highly significant negative correlation

| | Group 1 HCC (n= 78) | Group II Hepatitis cirrhosis (n= 34) | Group III Controls (n= 38) | ANOVA*/ Chi square** | P value |
|---|---|---|---|---|---|
| Age | | 62.45±8.01 | 63.77±8.23 | 61.40±6.71 | 0.839* | 0.434(NS) |
| Sex | Male | 55(70.5%) | 19(55.9%) | 25(65.8%) | 0.918** | 0.632(NS) |
| | Female | 23(29.5%) | 15(44.1%) | 13(34.2%) | | |
| WBCs | | 7.00±3.20 | 5.93±1.89 | 6.34±1.69 | 2.210* | 0.113(NS) |
| HB | | 10.05±1.71 | 12.79±2.25 | 13.58±1.41 | 60.467* | <0.001(HS) |
| PLT | | 110.03±54.84 | 191.21±69.12 | 253.61±55.26 | 81.577* | <0.001(HS) |
| PT | | 16.29±2.09 | 12.59±1.10 | - | 94.935* | <0.001(HS) |
| PC | | 56.86±14.26% | 77.47±8.11% | - | 62.080* | <0.001(HS) |
| AST | | 119.62±119.74 | 40.74±14.86 | - | 14.583* | <0.001(HS) |
| ALT | | 74.41±87.32 | 43.03±14.44 | - | 4.318* | 0.040(S) |
| CD14 % | | 31.74±16.58 | 13.35±5.42 | 6.61±2.16 | 60.110* | <0.001(HS) |
| TIE 2 % | | 7.32±4.80 | 2.65±0.89 | 2.02±1.10 | 37.680* | <0.001(HS) |
| Ang-2 (pg/ml) | | 3216.53±2427.59 | 1932.00±1090.12 | 801.18±416.98 | 22.879* | <0.001(HS) |
| AFP | | 1233.3±1746.8 | 75.15±50.58 | 62.61±39.79 | 15.560 | <0.001(HS) |

Table 1. Comparison among studied groups with regards to age, sex, and laboratory parameters.

between TIE2 [%]and Plts [r = -0.470; p = 0.001], there was significant positive correlation between TIE [%]and total bilirubin [r = 0.273; p = 0.015]

While there was significant negative correlation between Angiopoietin2 [pg/ml] and Plts [r = -0.286; p = 0.011], there was positive significant correlation between Ang-2 and serum AFP [r=0.263; p=0.020] while there was no significant correlation between angiogenetic markers [CD14 [%], TIE2 [%] and Angiopoietin2 [pg/ml] and laboratory results in group [I].

Figures [1] show dot plot CD14+ monocytes CD14 FTIC vs TIE2 PE co-expression in three different participants; one from each group.

In table [3], using univariate regression analysis for possible predicting factors for HCC [compared to both positive and negative controls], we included all the demographics and laboratory data of the study groups in univariate regression analysis, with the output result considered to be HCC. This table showed that CD14+,

TIE2+, and Ang-2 could be considered predicting factors for HCC.

In table [4] multivariate regression analysis for possible independent predicting factors for HCC among routing investigations, CD14, TIE 2 and Ang-2

Using the items which showed significant results in table [3] in a multivariate regression analysis showed that only HB is significant from routine investigations, while CD14, TIE2, and Ang-2 could be considered as independent predicting factors for HCC. Moreover, analysis of the p-value and the odd's ratio showed that CD14 has higher predictive power for HCC.

Analysis of the ROC curve [Figure 2] showed that the cut-off point for CD14+is14.25, with a sensitivity of 89.7% and a specificity of 83.3%. Cut off point for TIE2+ is 3.45, with a sensitivity of 76.9% and a specificity of 83.3%. The cut-off point for Ang-2 was 1240, with a sensitivity of 76.9% and a specificity of 66.7%. The cut-off value for AFP was 89.5, with a sensitivity of 66.7% and a specificity of 77.8%

Table 2. Correlation between angiogenetic markers CD14 [%], TIE2 [%], and Ang-2[pg/ml], and laboratory results in group [I] HCC [N= 79]

| | | CD 14% | TIE2 (%) | Ang-2 (pg/ml) |
|---|---|---|---|---|
| Age | Pearson Correlation | **0.232** | -0.051 | -0.106 |
| | P value | **0.041 (S)** | 0.660 | 0.354 |
| WBCs | Pearson Correlation | **-0.254** | 0.024 | 0.079 |
| | P value | **0.025 (S)** | 0.832 | 0.490 |
| HB | Pearson Correlation | **0.294** | -0.022 | -0.064 |
| | P value | **0.009 (S)** | 0.849 | 0.580 |
| PLT | Pearson Correlation | -0.102 | **-0.470** | **-0.286** |
| | P value | 0.372 | **<0.001(HS)** | **0.011 (S)** |
| PT | Pearson Correlation | 0.076 | 0.164 | 0.156 |
| | P value | 0.509 | 0.150 | 0.172 |
| PC | Pearson Correlation | -0.171 | -0.157 | 0.192 |
| | P value | 0.135 | 0.171 | 0.093 |
| AST | Pearson Correlation | 0.022 | 0.019 | -0.168 |
| | P value | 0.850 | 0.868 | 0.141 |
| ALT | Pearson Correlation | -0.205 | -0.201 | -0.129 |
| | P value | 0.071 | 0.077 | 0.262 |
| Total Bilirubin | Pearson Correlation | -0.007 | **0.273** | -0.065 |
| | P value | 0.948 | **0.015** | 0.569 |
| Direct Bilirubin | Pearson Correlation | 0.039 | 0.156 | -0.095 |
| | P value | 0.735 | 0.172 | 0.410 |
| Albumin | Pearson Correlation | 0.149 | 0.210 | 0.036 |
| | P value | 0.193 | 0.065 | 0.757 |
| AFP | Pearson Correlation | -0.188 | 0.001 | **0.263** |
| | P value | 0.099 | 0.991 | **0.020** |

S: Significant, HS: highly significant

Table 3. Univariate Regression Analysis for possible predicting factors for HCC [compared to both positive and negative controls]

| Item | P value | Odd's ratio | CI of Odd's ratio |
|---|---|---|---|
| **Age** | 0.959 (NS) | 0.999 | 0.958-1.041 |
| **Male sex** | 0.226 (NS) | 1.522 | 0.772-3.001 |
| **WBCs** | 0.051 (NS) | 1.136 | 1.000-1.292 |
| **HB** | **<0.001 (HS)** | 0.407 | 0.307-0.539 |
| **PLT** | **<0.001 (HS)** | 0.973 | 0.964-0.981 |
| **PT** | **<0.001 (HS)** | 4.847 | 2.438-9.638 |
| **PC** | **<0.001 (HS)** | 0.836 | 0.779-0.898 |
| **AST** | **<0.001 (HS)** | 1.045 | 1.020-1.069 |
| **ALT** | **0.038 (S)** | 1.016 | 1.001-1.032 |
| **CD14** | **<0.001 (HS)** | 1.275 | 1.181-1.376 |
| **TIE 2** | **<0.001 (HS)** | 2.321 | 1.724 -3.125 |
| **Ang-2** | **<0.001 (HS)** | 1.001 | 1.000-1.001 |
| **AFP** | **0.002(S)** | 1.006 | 1.002-1.010 |

Table 4. Multivariate Regression Analysis for possible independent predicting factors for HCC among routing investigations, CD14, TIE 2 and Ang-2.

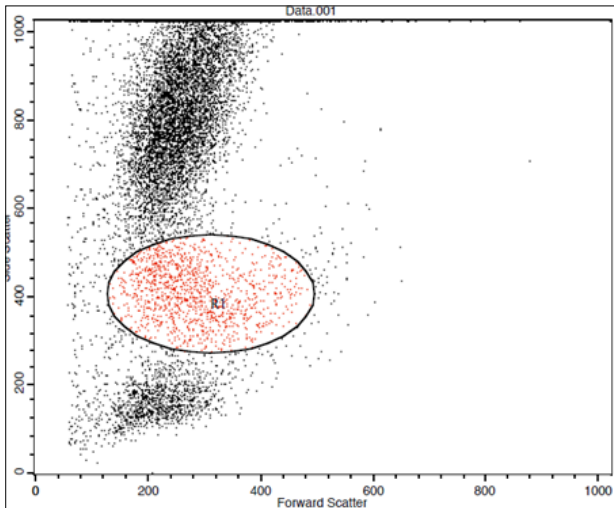| Item | P value | Odd's ratio | CI of Odd's ratio |
|---|---|---|---|
| **CD14** | **<0.001 (HS)** | 1.203 | 1.106-1.309 |
| **TIE 2** | **0.019 (S)** | 1.661 | 1.087-2.539 |
| **Ang-2** | **0.003 (S)** | 1.001 | 1.000-1.001 |
| **HB** | **0.039 (S)** | 0.375 | 0.148-0.953 |
| **PLT** | 0.374 (NS) | 0.975 | 0.922-1.031 |
| **PT** | 0.557 (NS) | 1.777 | 0.261-12.077 |
| **PC** | 0.195 (NS) | 0.685 | 0.386-1.214 |
| **AST** | 0.087 (NS) | 1.123 | 0.983-1.283 |
| **ALT** | 0.234 (NS) | 0.946 | 0.863-1.037 |

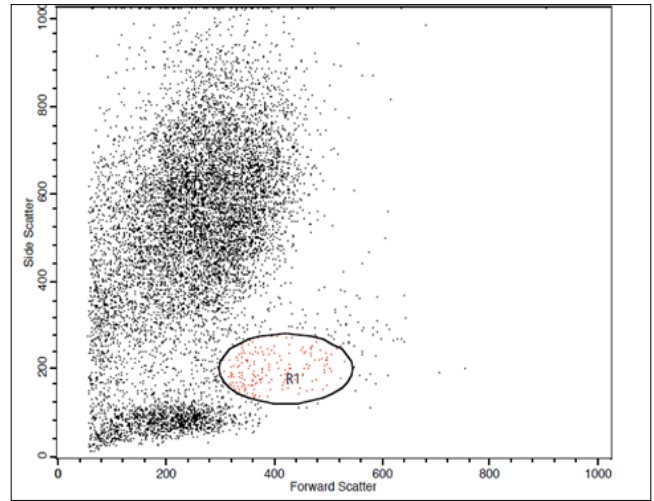Figure 1. A. Forward Scattering (Normal control)
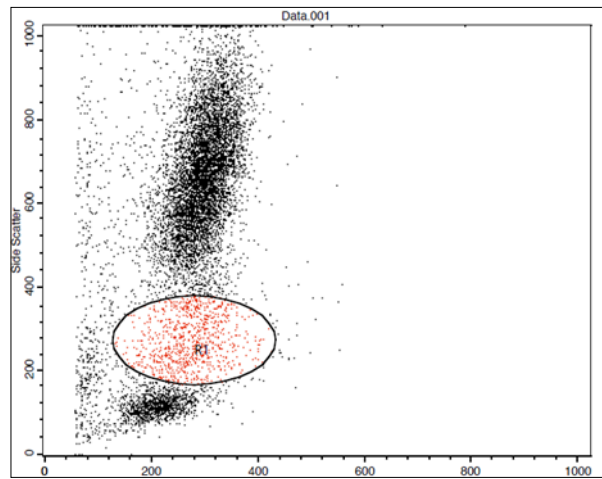


Figure 1. B. Forward Scattering (LC Patient)
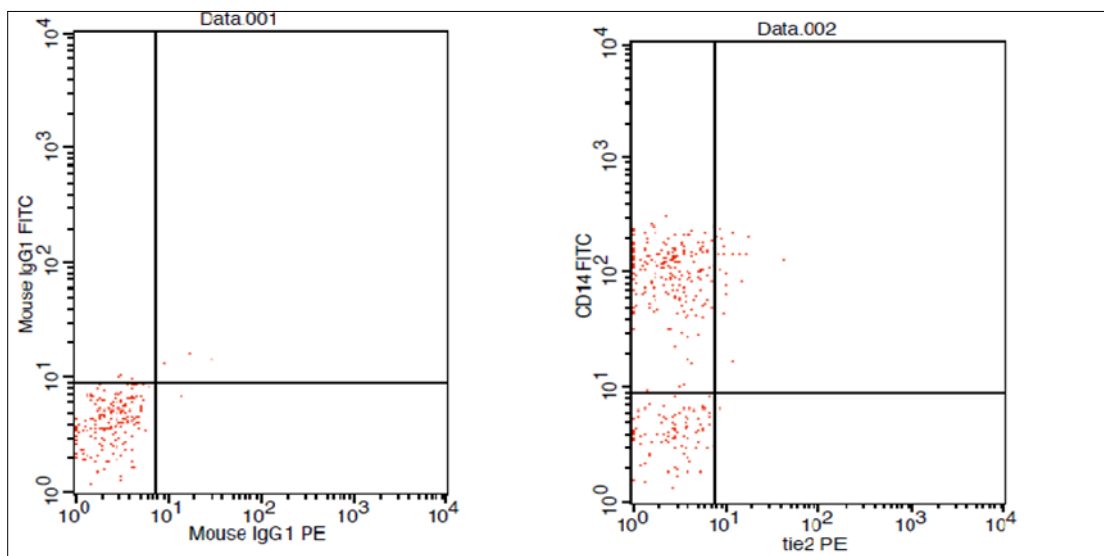


Figure 1. C. Forward Scattering (HCC Patient)



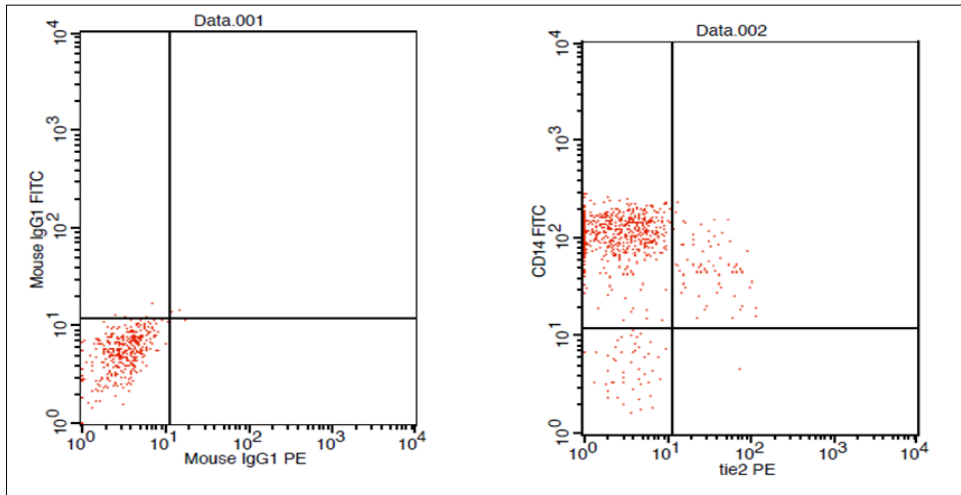Figure 1. D. Normal control CD 14 & Tie2 co expression 2% & isotopic control.

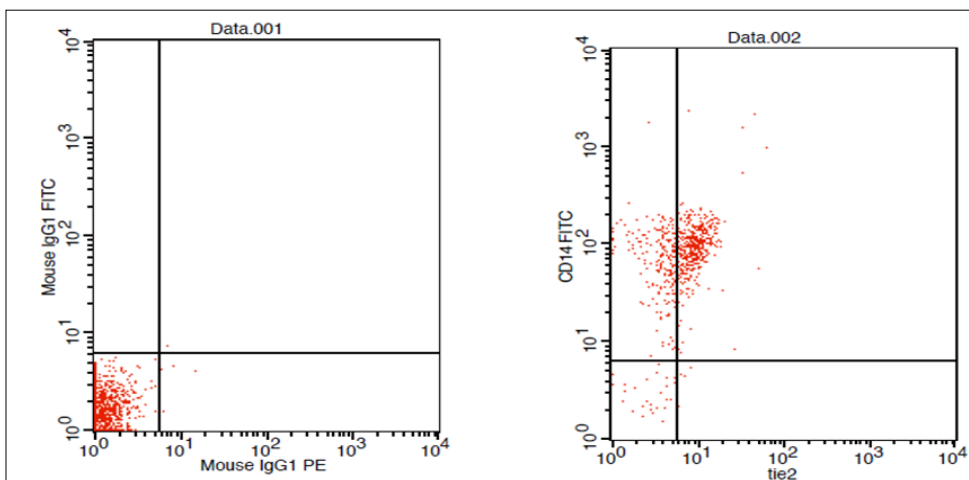Figure 1. E. LC Patent CD 14 & Tie2 coexpression 4% & isotopic control



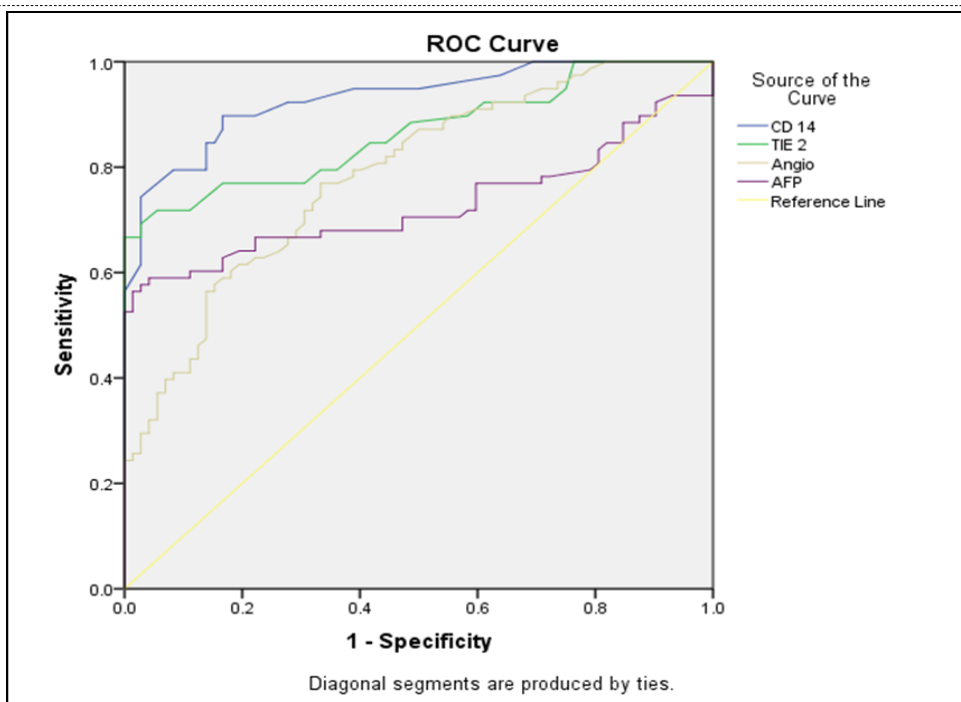Figure 1. F. HCC Patient CD 14 & Tie2 coexpression 10% & isotopic control.



Figure 2. ROC curve analysis to differentiate cases from controls [both positive and negative controls].

## Discussion

Due to the increasing incidences of many liver disorders that can develop HCC and due to the limited treatment options and the poor efficacy of most of them in later stages of the disease course; it is mandatory to find suitable screening methods for early detection of HCC in order to improve the outcome of this major health hazard [7]. Tumor-associated macrophages (TAMs) differ from monocytes by being able to secrete many substances including cytokines, growth factors necessary for tumor growth and angiogenic factors as a vascular endothelial growth factor (VEGF) which promoting tumor angiogenesis [19].

We found the following in our data analysis, hemoglobin level and platelet count were significantly lower in the HCC group compared with HCV related LC and control group [p≤0.001], this was agreed by Pratt and Kaplan who stated that thrombocytopenia was highly due to portal hypertension with attendant congestive splenomegaly [20]. We found that the prothrombin concentration was statistically lower in patients with HCC and patients with cirrhosis [p≤0.001] compared to controls which could be explained by the decreased synthesis of coagulation factors by the diseased liver. This agreed with what was reported by Youssef et al [21] &Tripod et al [22] who noticed defective utilization of vitamin K in advanced liver disease.

Concerning Ang-2 levels in different groups: serum levels were significantly higher in HCC patients [3216.53±2427.59] than in HCVLC patients [1932.00±1090.12] and healthy controls [801.18±416.98] [p≤0.001]. This was agreed by Youssef et al, who reported that the serum Ang-2 in the HCC group was [11142.5±6222.8 pg/ml] while in the cirrhosis group it was [6230.3±3053.4 pg/ml] and in the control group, it was [546.8±199.5pg/ml] [p≤0.001]. This is following our results. Hunter et al [23] also reported serum Ang-2 in the HCC group [10855 ± 5321.92 pg/ml] when compared with the cirrhosis group [5578.33±2928.21 pg/ml] and the control group [480.67±202.3 pg/ml] [p< 0.001]. Badran et al [24] also reported elevated angiopoietin-2 levels in HCC patients compared to LC and healthy controls [1671.6±284.2 vs1416.6±502.7 and 194.97±76.9 ug/ml respectively [P < 0.0001].

Concerning the correlation between laboratory data, there was no significant correlation with serum Ang-2 levels in HCC groups, except a negative significant correlation between platelet counts and serum Ang-2. Also, we found a significant and positive correlation between serum Ang-2 and serum AFP in HCC patients group. This was in discordance with Scholz et al [25] and Youssef et al [21] who failed to find any correlation between both markers among patients with HCC. Concerning the percentage of the circulating TEMs: CD14+ and TIE2+ monocytes were significantly increased in the peripheral blood of HCC patients than HCV LC patients and healthy controls, in agreement with Matsubara et al [26] who found a higher percentage of circulating TEMs in HCC on top of HCV patients compared to HCV patients or healthy controls. To assess the significance of TEM levels as a diagnostic marker of HCC, we did a correlation between TEM level and HCC clinical marker alpha-fetoprotein [AFP] and found no significant correlation which was in agreement with Matsubara et al [26]. There was a higher level of CD14+in HCC patients than in LC group, Guo et al agreed with this in spite of deference of detection method of CD14+ as he also found it to be superior to alpha-fetoprotein in the detection of HCC [27]. ROC curve analysis was done in this study for serum Ang-2, CD14+, and TIE2+ to differentiate cases from controls which revealed a cut-off point for CD14+ of 14.25, with a sensitivity of 89.7% and a specificity of 83.3%. Cut off point for TIE2+ of 3.45, with a sensitivity of 76.9% and a specificity of 83.3%. Cut off point for Ang-2 of 1240, with a sensitivity of 76.9% and a specificity of 66.7%. All with highly statistically significant analyses, TEM frequency [CD14+ and TIE2+] was superior to Ang-2 levels as a diagnostic marker for HCC and these results are consistent with Matsubara et al [26] who found that ROC curve analysis revealed that TEM was superior to both AFP and Ang-2 levels as a diagnostic marker for HCC. We used the binary logistic regression analysis to identify predictors of an unfavorable outcome and we found that CD14+, TIE2+, and-2 could be used as predictive of such.

## Conclusion

Proangiogenic markers TEM and serum Ang-2 levels were elevated more in HCC patients than in patients with liver cirrhosis and in normal control and

they were superior to AFP in the detection of HCC. We can consider a cut-off point for CD14+of 14.25, with a sensitivity of 89.7% and a specificity of 83.3%. Cut off point for TIE2+ of 3.45, with a sensitivity of 76.9% and a specificity of 83.3%. Cut off point for Ang-2 was 1240, with a sensitivity of 76.9% and a specificity of 66.7%. Also, we can conclude that CD14+, TIE2+, and Ang-2 could be used as predictive of bad outcome

## Ethical Approval

All procedures performed in the current study were following the ethical standards of the Ethics Committee, Faculty of Medicine, Sohag University, Egypt and with the 1964 Helsinki declaration and its later amendments and comparable ethical standards.

## Consent

Consent was taken from the participant after discussion of the study details.

## Conflict of Interest

All authors declare no conflict of interest.

## Abbreviation

AFP: alpha-fetoprotein

ALB: albumin

ALT: alanine aminotransferase

Ang-1: angiopoietin-1

Ang-2: angiopoietin-2

AST: aspartate aminotransferase

CBC: complete blood count

ELISA: enzyme-linked immunosorbent assay

FACS: fluorescence-activated cell sorter

FITC: fluoroisothiocyanate

HBV: hepatitis B virus

HCC: hepatocellular carcinoma

HCV: hepatitis C virus

LC: liver cirrhosis

MHC: major histocompatibility complex

Moab: monoclonal antibodies

NAFLD: non-alcoholic fatty liver disease

NASH: non-alcoholic steatohepatitis

PBMC: peripheral blood mononuclear cells

PBS: phosphate-buffered saline

PC: prothrombin concentration

PE: phycoerythrin

p-Tie2: Tie2 phosphorylation

Plts: platelets

RBC: red blood cell

ROC: receiver operating characteristic

RTKs: receptor tyrosine kinases

TAMs: tumor-associated macrophages

TEMs: TIE2-expressing monocytes

Tie: tyrosine kinase receptor

Tie2: tyrosine kinase receptor-2

VEGF: vascular endothelial growth factor.

VEGFR-1: vascular endothelial growth factor receptor-1

$\chi^2$: Chi square

## Declaration

Ethics approval and consent to participate: Approval for this work was obtained from the Ethics Committee, Faculty of Medicine, Sohag University, Egypt. Samples were taken after obtaining a consent from the patients and controls

## Consent for Publication

Not applicable.

Availability of data and materials: All data generated or analyzed during the present study are included in this published article.

## Authors' Contributions

**1-El-Zahraa M. Meghezel:** data collection, patients' selection, analysis, manuscript preparation and revision.

**2-Heba A Ahmed:** idea generation, data collection, analysis, manuscript preparation, and laboratory work, revision

**3-Ashraf A Askar:** data collection, analysis, and manuscript preparation

**4-Emad Eldin Nabil:** data collection, and manuscript preparation, and revision

**5-Ashraf Elyamany:** data collection, manuscript preparation, revision, and submission

**Disclosure**

This paper was presented at the 2018 ESMO Asia Conference as a poster presentation. The poster's abstract was published *Annals of Oncology (2018) 29 (suppl_9): ix46-ix66. 10.1093/annonc/mdy432*: *Hyperlink with DOI* https://oncologypro.esmo.org/Meeting-Resources/ESMO-Asia-2018-Congress/Role-of-Tie2-CD14-angiopoietin-as-angiogenetic-markers-in-hepatocellular-carcinoma-complicating-hepatitis-C-virus-infection-in-Egyptian-patients

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