Serum angiopoietin-2 as a noninvasive diagnostic marker of stages of liver fibrosis in chronic hepatitis C patients
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Received 1 September 2016
Accepted 25 October 2016

The Egyptian Journal of Internal Medicine 2016, 28:140–148

Background
The process of fibrogenesis is associated with the development of disease-specific markers. The management of chronic hepatitis C (CHC) depends on the staging of liver fibrosis. Use of noninvasive methods is preferable in diagnosis and follow-up.

Objective
The aim of this study is to evaluate serum angiopoietin-2 (Ang-2) as a noninvasive marker in the diagnosis of different stages of liver fibrosis in CHC patients.

Materials and methods
A total of 90 individuals were included. They were divided into a patient group (75 patients) and a control group (15 normal individuals). Serum Ang-2 was measured using enzyme-linked immunosorbent assay. Pretreatment liver biopsy was performed for the patients. The METAVIR score was used in the staging of liver fibrosis. A comparison of Ang-2 was performed between patients and controls, and between different stages of liver fibrosis. A receiver operating characteristic curve analysis was carried out to determine the best cutoff values of Ang-2 in the differentiation of different stages of fibrosis.

Results
Ang-2 serum levels were significantly higher in advanced stages of liver fibrosis. The cutoff points 869.3, 2226, and 7205 pg/ml were the best for differentiating fibrosis stages >F1; >F2; and >F3, respectively. Ang-2, international normalized ratio, α-fetoprotein, and albumin were found to be independent predictors of liver fibrosis using univariate analysis.

Conclusion
Ang-2 correlated significantly with liver fibrosis stage. It can aid noninvasive differentiation between different stages of liver fibrosis in patients with CHC.

Keywords:
angiogenesis, angiopoietin, hepatitis C virus, liver fibrosis

Introduction
Hepatitis C virus (HCV) infection is a major global health problem [1–3]. Patients with detectable levels of HCV-RNA have an increased risk of hepatic and extrahepatic disease [4]. The persistence of inflammatory responses and cellular damage of HCV promote disease progression toward fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [5]. Hepatic fibrosis is the deposition of excess extracellular matrix. The major effectors of fibrosis are the hepatic stellate cells and the portal fibroblasts, which are activated by soluble mediators produced by activated hepatic resident cells [6]. The lack of easy-to-use methods for the assessment of liver fibrosis has been a major limitation for clinical management and research [7]. Although liver biopsy (LB) is considered the standard for determination of the stage of fibrosis, it has disadvantages such as invasiveness, expensiveness, risk of complications, and potential for sampling errors. Noninvasive markers such as serum angiopoietin-2 (Ang-2) are being sought as an alternative [8]. Angiopoietins are a family of vascular growth factors that play a role in the physiological angiogenesis process [9]. Ang-2 is produced by the endothelial cells [10,11]. It has been shown that Ang-2 is overexpressed in fibrotic liver tissues, and therefore, play a pathophysiological role in chronic liver disease (CLD) [12]. Ang-2 is believed to be involved in both the angiogenesis and the inflammatory pathways in pathological situations. Ang-2 may result in leaky vasculatures that facilitate the extravasation of lymphocytes and promote the adhesion of rolling leukocytes to blood vessels [10].

The aim of the current study is to evaluate the role of serum Ang-2 as a noninvasive marker in the diagnosis of different stages of liver fibrosis in patients with chronic hepatitis C (CHC) infection.
Materials and methods

This current study was carried out at the National Hepatology and Tropical Medicine Research Institute Cairo together with Ain Shams University Hospital, Internal Medicine, Gastrointestinal and Hepatology outpatient clinics. The study included 90 individuals. They were divided into two groups: group A, which included 75 patients in whom antiviral therapy was planned for CHC, and group B, which included 15 normal controls. Group A included 37 (49.3%) men and 38 (50.7%) women with BMI (mean±SD = 31.07±8.55) and age (40.45±12.04). Group B included seven (46.7%) men and eight (53.3%) women with BMI (33±10.62) and age (39.40 ±15.18). Age, sex, and BMI were matched.

According to the METAVIR score for the classification of hepatic fibrosis [13], patients of group A were divided into five subgroups according to the stage of liver fibrosis: nine (10%) patients with stage F0, 16 (17%) patients with stage F1, 26 (28.8%) patients with stage F2, 18 (20%) patients with stage F3, and six (6.7%) patients with stage F4. The individuals in the control group (group B) were found to have no fibrosis by Fibroscan.

Inclusion criteria included patients with CHC diagnosed by countable HCV-RNA in their serum by PCR, and for whom oral antiviral therapy was planned. Exclusion criteria included patients with HIV coinfection, hepatitis B virus coinfection, patients with autoimmune liver disease, HCC, decompensated liver cirrhosis, and patients with hepatic or extrahepatic malignancies.

Informed written consent was obtained from patients and controls before inclusion. The study protocol was approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University, according to the ethical guidelines of the 1975 Declaration of Helsinki.

All the following were performed for recruited patients and controls.

1. Complete assessment of history together with a full clinical examination.
2. Laboratory investigations including complete blood count; liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, international normalized ratio (INR), prothrombin time, serum albumin, and kidney function tests by standard laboratory tests.
3. Radiological examination including pelviabdominal ultrasound with examination of liver size, echogenicity, splenomegaly, presence of ascites, portal vein diameter and patency, presence of any hepatic focal lesions, or any abdominal malignancy, and a detailed examination of the kidneys (equipment used: Hitachi, EUB-5500; Hitachi Medical Systems America, Inc.; Twinsburg, OH 44087, USA).

4. LB was performed only in the patient group for histopathological examination, which was the standard for determining the stage of fibrosis according to the METAVIR score [13]. Exclusion of liver fibrosis in the control group was performed using Fibroscan.

Transient elastography (Fibroscan) was performed for the patients in group B to confirm the absence of any fibrosis using Fibroscan 502 equipment (ECHOSENS Company, Paris, France). The patients lay on their back with their arm raised behind their head. The physician applied a water-based gel to the skin, placed the probe with a slight pressure, and examines the right lobe of the liver through the intercostal space. The examination included 10 consecutive measurements performed on the same location. The result was obtained at the end of the examination in the form of a number in kilopascals (kPa).

Liver histology

Liver biopsies from patients were obtained by percutaneous needle extraction under ultrasound guidance and were embedded in paraffin for routine histopathological examination. The median length of liver biopsies was 1.9 mm. The METAVIR scoring system was used for staging liver fibrosis [13]. Liver fibrosis was divided into five stages from F0 to F4. F0 indicated the absence of fibrosis, F1 indicated the presence of portal fibrosis without septa, F2 indicated the presence of numerous septa without cirrhosis, and F4 indicated is the presence of cirrhosis.

Angiopoietin-2 concentrations in the serum of chronic hepatitis C patients

Circulating Ang-2 levels were measured in the serum samples from CHC patients on the same day that they underwent LB, before initiation of antiviral combination therapy, using commercially available Human Angiopoietin-2 ELISA kits following the manufacturer's instructions (Boster Immunoleader; Biological Technology Company Ltd, Pleasanton, California, USA). Blood samples (5 ml) were obtained from both groups A and B by a trained, professional nurse using sterile, disposable equipment. After centrifuging the blood samples, serum samples were separated in serum separator tubes and were stored horizontally at –20°C. Serum samples were mixed with diluents buffer samples
in a ratio of 1 : 2. Serum levels of human Ang-2 were estimated by enzyme-linked immunosorbent assay and measured in pg/ml with levels ranging from 156 to 10 000 pg/ml.

Serological indices of fibrosis were calculated for the patients and presented in the results. Comparisons of the diagnostic values of Ang-2, aspartate aminotransferase-platelet ratio index (APRI), and Fibrosis-4 (FIB4) were performed.

APRI: It was calculated as: (AST/upper limit of normal range)/[platelet count (10⁹/l)]×100 [14].

The FIB4 score: this score was calculated as: age (years)×AST (IU/l)/platelet count (10⁹/l)×ALT (IU/l)¹/² [15].

AAR (ALT/AST ratio): this score was calculated as: AST (IU/l)/ALT (IU/l) [16].

Statistical analysis
The statistical package for the social science (SPSS Inc., IBM Company, Chicago, USA), version 17 for Microsoft Windows, was used for data analysis. Quantitative variables were expressed as mean±SD, whereas qualitative variables were expressed in percentage or number. The correlation between Ang-2 serum levels and other variables was analyzed by the Pearson correlation coefficient. A univariate regression analysis was carried out. Significant factors were then subjected to a multivariate analysis. Comparison of quantitative variables was carried out using the Student t-test, whereas comparison between more than two groups was carried out using the analysis of variance test (ANOVA). The area under the receiver operating characteristic curve (AUROC=AUC) was used to assess the discriminatory ability of the test under study to predict the stages of liver fibrosis among CHC patients. Significance level (P) value: P ≤ 0.05 is significant (S). P < 0.01 is highly significant (HS). P > 0.05 is insignificant (NS).

Results
There was a statistically significant higher mean of Ang-2 in higher stages of liver fibrosis (P<0.001) according to the ANOVA test (Table 1 and Fig. 1).

On comparing Ang-2 serum levels between different subgroups (Table 2).

1. There was a statistically significantly higher mean of Ang-2 in stages F2, F3, and F4 of liver fibrosis in comparison with stage F0 (P<0.05).
2. There was a statistically significantly higher mean of Ang-2 in stages F3 and F4 of liver fibrosis in comparison with stage F1 (P<0.05).
3. There was a statistically significantly higher mean of Ang-2 in stages F3 and F4 of liver fibrosis in comparison with stage F2 (P<0.05).
4. There was a statistically significantly higher mean of Ang-2 in stage F4 of liver fibrosis in comparison with stage F3 (P<0.05).
5. There was a statistically significantly lower mean of Ang-2 in normal cases in comparison with stages F2, F3, and F4 of liver fibrosis (P<0.05).

On comparing between patients with liver fibrosis (n=66, F1–F4) and those without (n=9, F0) using the ANOVA test, there was a statistically significantly higher mean Ang-2 serum levels (Table 3 and Fig. 2), α-fetoprotein (AFP), and INR in cases with liver fibrosis than in those without fibrosis (P<0.05). However, there was a statistically significantly lower mean albumin in cases with liver fibrosis in comparison with cases without fibrosis (P<0.05).

Analysis of the relationship between liver fibrosis and different variables by univariate regression analysis (Table 4) indicated that:

1. Ang-2 serum levels showed an independent significant positive relation with liver fibrosis (coefficient: 0.000023, P<0.05).
2. AFP showed an independent significant positive relation with liver fibrosis (coefficient: 0.05848, P<0.05).
3. Albumin showed an independent significant negative relation with liver fibrosis (coefficient: -0.09636, P<0.05).
4. INR showed an independent significant positive relation with liver fibrosis (coefficient: 0.2451, P<0.05).

An analysis of the relationship between different variables and liver fibrosis was carried out using

| Stages of fibrosis | Normal (control) | F0     | F1     | F2     | F3     | F4     | P value |
|--------------------|------------------|--------|--------|--------|--------|--------|---------|
| Angiopoietin-2 (pg/ml) Mean | 202.44 | 367.40 | 739.90 | 1578.16 | 3831.66 | 13095  | <0.001  |
| SD     | 21.58            | 83.10  | 105.047| 453.455| 1386.09 | 2622.29|         |

Table 1 Comparison of angiopoietin-2 between normal controls and patients with different stages of liver fibrosis by the analysis of variance test
multivariate regression analysis (Table 4). Among the total effects of all variables, only BMI showed a significant positive relation with liver fibrosis (coefficient: 0.01100, \(P = 0.0463\)).

Pearson correlation analysis was used to find the correlation between Ang-2 and other variables in cases with CHC (\(n = 75\)) (Table 5). There was a statistically significantly positive correlation between Ang-2 serum levels and AFP, FIB4, AST, APRI score, HCV-PCR, ALT, and total bilirubin (\(P < 0.05\)). There was a statistically significantly negative correlation between Ang-2 and serum albumin (\(P < 0.05\)).

Receiver operating characteristic curve (ROC) showed that the FIB4 score (Table 6) had a significant diagnostic value for liver fibrosis stages (F\(>2\)) at a cutoff more than 2.5 pg/ml (\(P = 0.032\)) and F\(>3\) at a cutoff more than 2.5 pg/ml (\(P = 0.048\)). FIB4 showed 32% sensitivity and 93.7% specificity in the diagnosis of significant fibrosis (F\(>1\)) (Fig. 3).

The ROC curve showed that the APRI score (Table 6) had significant diagnostic value for liver fibrosis stages (F\(>2\)) at a cutoff more than 0.96 (\(P = 0.046\)) and F\(>3\) at a cutoff more than 1.06 (\(P = 0.011\)). It showed 52% sensitivity and 75% specificity in differentiating patients with significant fibrosis (F\(>1\)) at a cutoff value more than 0.67 (Fig. 4).

AUC-ROC analysis of the diagnostic value of Ang-2 showed 100% sensitivity and 100% specificity in differentiating fibrosis stages (F\(1\), F\(2\), F\(3\)) at cutoff values more than 869.3, 2226, and 7205 pg/ml, respectively, as shown in Table 6 and Fig. 5.

**Discussion**

CHC viral infection represents a serious health problem for nearly 200 million infected individuals worldwide. Morbidity and mortality rates of chronic HCV infection have been increasing since 2007 [17]. Egypt has the highest prevalence of HCV worldwide (15%) [18] and the highest prevalence of HCV genotype 4, which is responsible for almost 90% of HCV infections [19]. Patients have different clinical outcomes, ranging from acute resolving hepatitis to CLDs including liver cirrhosis or HCC [20]. Accurate evaluation of hepatic

**Table 2** Pairwise comparisons of the mean angiopoietin-2 between different stages of liver fibrosis by the analysis of variance

| Fibrosis stages | Mean difference | \(P\) value | 95% confidence interval         |
|-----------------|-----------------|-------------|---------------------------------|
| F0              |                 |             |                                 |
| F1              | −372.59         | 1.0000      | −1541.4734–796.2859             |
| F2              | −1210.76        | 0.0170      | −2295.7089 to −125.8219         |
| F3              | −3464.26        | <0.0001     | −4609.5301 to −2319.0032       |
| F4              | −12727.6        | <0.0001     | −14206.128 to −11249.0712      |
| Normal          | 164.95          | 1.0000      | −1017.8697 to −1347.7763       |
| F1              |                 |             |                                 |
| F2              | −838.17         | 0.0846      | −1729.5437 to −53.2004         |
| F3              | −3091.67        | <0.0001     | −4055.5557 to −2127.7901       |
| F4              | −12355          | <0.0001     | −13697.946 to −11012.0658      |
| Normal          | 537.54          | 1.0000      | −470.6751 to −1545.7693        |
| F2              |                 |             |                                 |
| F3              | −2253.5         | <0.0001     | −3113.6716 to −1393.3310       |
| F4              | −11516.8        | <0.0001     | −12787.390 to −10246.2789      |
| Normal          | 1375.7          | 0.0002      | 466.1392 to −2285.2982         |
| F3              |                 |             |                                 |
| F4              | −9263.3         | <0.0001     | −10585.7697 to −7940.8970      |
| Normal          | 3629.2          | <0.0001     | 2648.4750 to −4609.9650        |
| F4              |                 |             |                                 |
| Normal          | 12892.5         | <0.0001     | 11537.4593 to −14247.6473      |

![Figure 1](image-url)
fibrosis has become the primary goal in managing the progression of CHC because its morbidity and mortality are linked to cirrhosis and its complications. In addition, the decision of physicians to administer antiviral therapy depends on the stage of fibrosis [21]. LB is considered the standard method for the diagnosis and staging of fibrosis on the basis of its value in assessing the stage of fibrosis and necroinflammatory grade [22]. However, LB can overestimate or underestimate the degree of fibrosis [23]. LB is not reasonable for the repetitive assessment of liver fibrosis during the long-term follow-up of patients, necessitating noninvasive markers that accurately diagnose the progression of CLDs as CHC before, during, and after treatment [24].

In the current study, using univariate regression analysis, there was a significant relation between liver fibrosis on the one hand and Ang-2, AFP, INR, and albumin on the other. INR and AFP were found to have an independent significant positive relation, whereas serum albumin was found to have a significant negative relation with liver fibrosis. Also, statistically significantly higher means of INR and AFP, and lower mean of serum albumin were found in cases with liver fibrosis in comparison with cases without fibrosis. This is in agreement with Hernández-Bartolomé et al. [25]. In a study carried out by Sebastián and Alberti [26], age, platelet count, INR, AST, and GGT were found to be independent variables linked to liver fibrosis.

There was no statistically significant relation between liver fibrosis and the main demographic features of CHC patients in the present study. Hernández-Bartolomé et al. [25], found a statistically significant relation by univariate regression analysis between liver fibrosis and age. Poynard et al. [27] found a correlation between a higher grades of fibrosis and male patients. In a study carried out by Wong et al. [28], age at the time of infection was found to be one of the most important host-related factors in the progression of liver fibrosis. Costa et al. [29] reported that patients who acquired HCV after the age of 40 years had a higher rate of fibrosis progression and reached the stage of liver cirrhosis within a period of time that was four to five times shorter than that observed for patients who were infected at a younger age.

In the current study, univariate regression analysis showed no significant relation between liver fibrosis and liver enzymes. These parameters were found to

| Variables | No fibrosis (n=9) | CHC (mean±SD) | Fibrosis (n=66) | P value |
|-----------|------------------|---------------|-----------------|---------|
| Angiopoietin-2 (pg/ml) | 367.4±83.10 | 3036.54±3574.37 | 0.029 |
| Age (years) | 37.9±13.10 | 40.8±11.96 | 0.500 |
| Albumin (g/dl) | 3.9±0.49 | 3.13±0.95 | 0.015 |
| ALT (UI) | 32.2±13.79 | 49.04±49.31 | 0.315 |
| AFP (ng/ml) | 0.39±0.15 | 1.28±1.32 | 0.049 |
| APRI | 0.47±0.19 | 0.88±0.97 | 0.162 |
| AST (UI) | 35.4±12.83 | 61.48±58.27 | 0.188 |
| AAR | 1.2±0.57 | 1.40±0.66 | 0.467 |
| BMI (kg/m²) | 28.6±8.29 | 31.41±8.59 | 0.360 |
| FIB4 | 1.28±0.65 | 2.07±1.64 | 0.178 |
| HCV-PCR (copies/ml) | 306049±66164.34 | 298783.22±97574.66 | 0.830 |
| INR | 1.17±0.13 | 1.40±0.32 | 0.039 |
| Platelets (10⁹/l) | 197.7±55.51 | 200.28±65.26 | 0.913 |
| PT (s) | 13.15±1.72 | 13.08±2.22 | 0.921 |
| TB (mg/dl) | 0.52±0.17 | 0.65±0.23 | 0.125 |

AAR, aspartate aminotransferase/alanine aminotransferase ratio; AFP, α-fetoprotein; ALT, alanine aminotransferase; APRI, the AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB4, Fibrosis-4; HCV, hepatitis C virus; INR, international normalized ratio; PT, prothrombin time; TB, total bilirubin. The Bold values P ≤ 0.05 are significant (S).
have positive significant relations with liver fibrosis in the study by Hernández-Bartolomé et al. [25]. In studies carried out by Adinolfi et al. [30] and Zechini et al. [31], there was a correlation between liver fibrosis and serum ALT levels. It is well known that ALT is released by direct virus-related cytopathic activity and/or by an immune-mediated process [32]. In a study carried out by Liu et al. [33], serum ALT levels were not found to be a useful parameter to assess liver damage in the patients with CHC.

In the present study, the univariate regression analysis found no statistically significant relation between liver fibrosis and total bilirubin, which is different from the results of Hernández-Bartolomé et al. [25].

The ROC curve analysis in this study showed that the FIB4 score had significant diagnostic value in differentiating patients with fibrosis stages (F>2) at a cutoff more than 2.5 and patients with F>3 at a cutoff more than 2.5. It showed low sensitivity and high specificity in differentiating patients with significant fibrosis (those with >F1). In previous studies, the use of this index correctly classified 87% of patients with FIB4 values beyond 1.45–3.25 and biopsy could be avoided in 71% of the patients in the validation set with an AUC of 0.765, a sensitivity of 70%, and a specificity of 97% for differentiating fibrosis stages of Ishak score 0–3 from 4 to 6 [15].

In the present study, the APRI score had significant diagnostic value in the stages F>2 at a cutoff more than 0.96 and F>3 at a cutoff more than 1.06 using ROC curve analysis. It showed 52% sensitivity and 75% specificity in the diagnosis of significant hepatic fibrosis (stages>F1). This is in agreement with a recent meta-analysis study carried out by Lin et al. [34], who suggested that the APRI score can identify hepatitis C-related fibrosis with only a moderate degree of accuracy. In the study by Giannini et al.

Table 4 Relation between liver fibrosis and angiopoietin-2 and other variables using univariate and multivariate regression analyses among chronic hepatitis C cases (n=75)

| Variables                      | Univariate regression analysis |                      | Multivariate regression analysis |                      |
|--------------------------------|--------------------------------|----------------------|---------------------------------|----------------------|
|                                | Coefficient | P value            | Coefficient | P value            |
| Ang-2 (pg/ml)                  | 0.000023    | 0.029              | 0.0000678 | 0.245              |
| Age (years)                    | 0.002148    | 0.499              | −0.004439 | 0.506              |
| Sex                            | 0.22314     | 0.754              | 0.042888 | 0.621              |
| BMI (kg/m²)                    | 0.004101    | 0.360              | 0.01100  | 0.046              |
| AFP (ng/ml)                    | 0.05848     | 0.049              | −0.1233  | 0.407              |
| Albumin (g/dl)                 | −0.00636    | 0.015              | −0.06096 | 0.236              |
| ALT (UI/l)                     | 0.0008232   | 0.314              | 0.001273 | 0.712              |
| AST (UI/l)                     | 0.0009070   | 0.188              | −0.002137| 0.619              |
| AAR                            | 0.04272     | 0.466              | 0.03844  | 0.767              |
| INR                            | 0.2451      | 0.038              | 0.2519   | 0.070              |
| Platelets (10⁹/l)              | 0.000065    | 0.912              | 0.000864 | 0.467              |
| PT (s)                         | −0.001768   | 0.920              | −0.007385| 0.707              |
| TB (mg/dl)                     | 0.2486      | 0.125              | −0.04796 | 0.811              |
| TLC (x10³/mm³)                 | 0.01781     | 0.546              | 0.04284  | 0.187              |
| HCV-PCR (copies/ml)            | −0.00000008 | 0.829              | −0.0000033| 0.481              |
| APRI                           | 1.72090     | 0.162              | 0.04742  | 0.875              |
| FIB4                           | 0.54212     | 0.178              | 0.06034  | 0.595              |

AAR, aspartate aminotransferase/alanine aminotransferase ratio; AFP, α-fetoprotein; ALT, alanine aminotransferase; Ang-2, angiopoietin-2; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB4, Fibrosis-4; HCV, hepatitis C virus; INR, international normalized ratio; PT, prothrombin time; R, correlation coefficient; TB, total bilirubin; TLC, total leukocyte count. The Bold value P ≤ 0.05 is significant.

Table 5 Pearson correlation between angiopoietin-2 and other variables in patients with chronic hepatitis C (n=75)

| Variables                      | Angiopoietin-2 (R) | P value |
|--------------------------------|--------------------|---------|
| AFP (ng/ml)                    | 0.9724             | <0.0001 |
| FIB4                           | 0.3508             | 0.0020  |
| AST (UI/l)                     | 0.4677             | <0.0001 |
| APRI                           | 0.4020             | 0.0004  |
| HCV-PCR (copies/ml)            | 0.2957             | 0.0100  |
| ALT (UI/l)                     | 0.484              | <0.0001 |
| Albumin (g/dl)                 | −0.489             | <0.0001 |
| ALT/AST                        | −0.106             | 0.3648  |
| INR                            | 0.011              | 0.9287  |
| Platelets (10⁹/l)              | −0.053             | 0.6489  |
| PT (s)                         | −0.042             | 0.7175  |
| TB (mg/dl)                     | 0.456              | <0.0001 |
| TLC (x10³/mm³)                 | 0.039              | 0.7395  |

AFP, α-fetoprotein; ALT, alanine aminotransferase; Ang-2, angiopoietin-2; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB4, Fibrosis-4; HCV, hepatitis C virus; INR, international normalized ratio; PT, prothrombin time; R, correlation coefficient; TB, total bilirubin; TLC, total leukocyte count. The Bold values P ≤ 0.05 are significant.
it was found that APRI scores in patients with CHC showed a rather good diagnostic performance and reproducibility, particularly for cirrhosis.

In this study, statistically significantly higher mean Ang-2 levels were found in CHC patients compared with normal individuals. Also, higher levels of Ang-2 were present in higher stages of liver fibrosis. A significant positive correlation was found between Ang-2 and AFP, FIB4, AST, APRI score, HCV-PCR, ALT, and total bilirubin, whereas there was a statistically significant negative correlation between Ang-2 serum levels and serum albumin.

Ang-2 is one of the regulators of neovascularization, vascular remodeling, and maturation in CHC patients through agonistic and antagonistic autophosphorylation of its common tyrosine kinase receptor, Tie2 [35]. Also, it is one of the most significant signaling pathways in pathological angiogenesis and HCC [36].

Serum vascular endothelial growth factor (VEGF), Ang-2, and Tie2 are increased in viral hepatitis and their concentrations could be valuable markers of hepatic inflammation, disease progression, and response to therapy. Treatment of CHC patients with pegylated interferon and ribavirin was found to reduce the inflammatory process and therefore could decrease VEGF and Ang-2 concentration [37].

In the current study, ROC curve analysis showed that the Ang-2 cutoff value more than 869.3 pg/ml was the best in differentiating patients with significant fibrosis (>F1) [sensitivity=100%, specificity=100%, 95% confidence interval (CI)=0.94–1, and AUC=1].

Table 6 Diagnostic value of each of the Fibrosis-4 score, the aspartate aminotransferase–platelet ratio index score, and angiopoietin-2 in discriminating different stages of liver fibrosis using receiver operating characteristic curve analysis (n=66)

| Parameters     | FIB4     |      |      | APRI     |      |      | Angiopoietin-2 |
|----------------|----------|------|------|----------|------|------|----------------|
|                | F>1      | F>2  | F>3  | F>1      | F>2  | F>3  | F>1            |
| AUROC          | 0.57     | 0.65 | 0.77 | 0.58     | 0.65 | 0.81 | 1              |
| SE             | 0.07     | 0.07 | 0.14 | 0.07     | 0.07 | 0.12 | 0.00           |
| 95% CI         | 0.44–0.6 | 0.52–0.7 | 0.66–0.8 | 0.45–0.70 | 0.52–0.76 | 0.69–0.89 | 0.94–1         |
| P value        | 0.31     | 0.03 | 0.04 | 0.28     | 0.046| 0.011| <0.0001         |
| Cutoff         | >2.39    | >2.5 | >2.5 | >0.67    | >0.96| >1.06| >869.3         |
| Sensitivity (%)| 32       | 45.8 | 83.3 | 52       | 50   | 83.3 | 100            |
| Specificity (%)| 93.7     | 88.1 | 81.6 | 75       | 85.7 | 83.3 | 100            |
| PPV (%)        | 94.1     | 68.7 | 31.2 | 86.7     | 66.7 | 33.3 | 100            |
| NPV (%)        | 30.6     | 74   | 98   | 33.3     | 75   | 98   | 100            |

APRI, aspartate aminotransferase-to-platelet ratio index; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; FIB4, Fibrosis-4; NPV, negative predictive value; PPV, positive predictive value. The Bold values $P \leq 0.05$ are significant.
The Ang-2 cutoff value more than 2226 pg/ml was the best in differentiating patients with advanced fibrosis (F>2) \[P<0.0001\], positive predictive value=100%, negative predictive value=100%, sensitivity=100%, specificity=100%, 95% CI=0.94–1, SE=0, and AUC=1].

The cutoff value more than 7205 pg/ml was the best in differentiating patients with cirrhosis (F>3) \[P<0.0001\], positive predictive value=100%, negative predictive value=100%, sensitivity=100%, specificity=100%, 95% CI=0.94–1, SE=0, and AUC=1].

These findings are in agreement with Hernández-Bartolomé et al. [25], who found that Ang-2 serum levels increased progressively with the stages of fibrosis. Hernández-Bartolomé et al. [25] found that Ang-2 was accurate in differentiating between different fibrosis stages (F1, F2, and F3) in 107 CHC patients. In their study, AUC values were 0.886 for F1, 0.920 for F2, and 0.923 for F3. Their cutoffs had a sensitivity and specificity of ~80% or higher and accuracy above 80% for all stages (F1, F2, and F3) [25].

**Conclusion**

Ang-2 correlated significantly with liver fibrosis stage. It can aid noninvasive differentiation between different stages of liver fibrosis in patients with CHC.

**Acknowledgements**

Author contributions: Mohamed M Makhlouf and Mahmoud A Osman contributed equally to this work; Mohamed M Makhlouf, Mahmoud A Osman, Shereen A Saleh and Wael A Yousry designed the research; Mohamed Lotfy and Fayrouz Wahba performed the research; Mohamed M Makhlouf, Mahmoud A Osman, Shereen A Saleh, Wael Yousry contributed analytic tools; Wahid Doss, Mohamed Lotfy and Fayrouz Wahba analyzed the data; Shereen A Saleh and Fayrouz Wahba wrote the paper. The manuscript has been read and approved by all authors. Each author believes that the manuscript represents honest work.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Seeff LB. Natural history of chronic hepatitis C. Hepatology 2002; 36: S35–S46.
2. Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, Alomair A, et al. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int 2011; 31(Suppl 2):61–80.
3. Doss W, Esmat G, El Serafy M, Sayed MHE, Hassany M, Yousry A, et al. Interim analysis for sofosbuvir national treatment program in Egypt National Liver Institute, Menoufia, Egypt. J Viral Hepat 2015; 22:13–15.
4. Lee MH, Yang HI, Chen CH. Long-term health outcomes of chronic hepatitis C patients: a review of findings of REVEAL-HCV cohort study. BioMedicine 2012; 2:99–107.
5. Zhang DY, Friedman SL. Fibrosis-dependent mechanisms of hepatocarcinogenesis. Hepatology 2012; 56:769–775.
6. Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology 2008; 134:1655–1669.
7. Baranova A, Lal P, Birerdinc A, Younossi ZM. Non-invasive markers for hepatic fibrosis. BMC Gastroenterol 2011; 11:91.
8. Castera L. Noninvasive methods to assess liver disease in patients with hepatitis B or C. Gastroenterology 2010; 142:1293–1302.
9. Barton WA, Tzvetkova D, Nikolov DB. Structure of the angiopoietin-2 receptor binding domain and identification of surfaces involved in Tie2 recognition. Structure 2005; 13:825–832.
10. Fiedler U, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, et al. The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. Blood 2004; 103:4150–4156.
11. Hashizume H, Falcon BL, Kuroda T, Baluk P, Coxon A, Yu D, et al. Complementary actions of inhibitors of angiopoietin-2 and VEGF on tumor angiogenesis and growth. Cancer Res, 2010; 70:2213–2223.
12. Tugues S, Fernandez-Varo G, Muñoz-Luque J, Ros J, Arroyo V, Rodés J, et al. Antiangiogenic treatment with sunitinib ameliorates inflammatory infiltrate, fibrosis, and portal pressure in cirrhotic rats. Hepatology 2007; 46:1919–1926.
13. Bedossa P, Poynad T An algorithm for the grading of activity in chronic hepatitis C. The METAIRV Cooperative Study Group. Hepatology 1996; 24:269–293.
14. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjevaram HS, Lòk AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003; 38:518–526.
15. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, et al. APRICOT Clinical Investigators: development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006; 43:1317–1325.
16. Williams AL, Hooymange JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. Gastroenterology 1998; 95:734–739.
Kanwal F, Hoang T, Kramer JR, Asch SM, Goetz MB, Zeringue A et al. Increasing prevalence of HCC and cirrhosis in patients with chronic hepatitis C virus infection. Gastroenterology 2011; 140:1182–1188.

Mahmoud YA, Muntaz DR, Riome S, Miller D, Abu-Raddad L. The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. BMC Infect Dis 2013; 13:288.

Abdel-Aziz F, Habib M, Mohamed MK, Abdel-Hamid M, Gamli F, Madkour S, et al. Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. Hepatology 2000; 32:111–115.

Lee YS, Yoon SK, Chung ES, Bae SH, Choi JY, Han JY, et al. The relationship of histologic activity to serum ALT, HCV genotype and HCV RNA titers in chronic hepatitis C. J Korean Med Sci 2001; 16:585–591.

Kim YW, Kwon JH, Jang JH, Kim MJ, OH BS, Chung KW, et al. Diagnostic usefulness of real-time elastography for liver fibrosis in chronic viral hepatitis B and C. Gastroenterol Res Pract 2014; 2014:210407.

Lee YS, Yoon SK, Chung ES, Bae SH, Choi JY, Han JY, et al. The relationship of histologic activity to serum ALT, HCV genotype and HCV RNA titers in chronic hepatitis C. J Korean Med Sci 2001; 16:585–591.

Rockey DC, Bissell DM. Noninvasive measures of liver fibrosis. Hepatology 2006; 43:S113–S120.

Augustin HG, Koh GY, Thurston G, Alitalo K. Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat Rev Mol Cell Biol 2009; 10:165–177.

Salcedo X, Medina J, Sanz-Cameno P, Garcia-Buey L, Martin-Vilchez S, Borque MJ, et al. The potential of angiogenesis soluble markers in chronic hepatitis C. Hepatology 2005; 42:696–701.