

# **Significance of Angiopoietin-2 as a Serum Marker for Hepatocellular Carcinoma**

**Thesis**

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# ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and it is one of the major causes of death. HCC is now a rather common malignancy in Egypt which usually develops on top of liver cirrhosis secondary to viral infection.

**AIM:** The aim of this study was to investigate the potential role of Angiopoietin-2 (Ang-2) as a diagnostic non-invasive marker for HCC, in order to add a beneficial diagnostic value in patients with low levels of alpha-fetoprotein (AFP) and suspected to have HCC.

**METHODS:** This study was conducted on **30** patients with documented HCC using triphasic computerized tomography (CT) scan and histopathological assessment (when needed) and **30** cirrhotic patients with no evidence of HCC; as well as **30** healthy subjects who served as control group.

We determined the level of AFP and Ang-2 for all cases together with full clinical assessment, liver biochemical profile, viral markers, conventional ultrasound (US), abdominal triphasic CT scan and guided liver biopsy for HCC cases with atypical CT pattern.

**RESULTS:** We found that the diagnostic sensitivity of AFP at a cutoff of **200** ng/ml was **24%** and the specificity was **100%**. The cutoff level of Ang-2 for diagnosis of HCC in this study was **8100** pg/ml, with a sensitivity and specificity of **70%** and **80%** respectively. Serum Ang-2 was significantly elevated in the HCC group than cirrhosis and control groups and in HCC patients with portal vein thrombosis than those without. There was a significant positive correlation between the number of hepatic focal lesions and the serum level of Ang-2. The combined use of the two markers (AFP & Ang-2) led to an increase in the sensitivity of AFP from **53.3%** to **83.3%**.

**CONCLUSION:** Serum Ang-2 is elevated in patients with cirrhosis and further elevated in patients with HCC, so its use as an independent tumor marker in the diagnosis of HCC is to be considered. Simultaneous measurement of serum AFP and Ang-2 may enhance the sensitivity of HCC detection.

**Key words:**

- Hepatocellular carcinoma (HCC)
- Alpha-fetoprotein (AFP)
- Angiopoietin-2 (Ang-2)

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- **ARABIC SUMMARY**

# LIST OF ABBREVIATIONS

<b>AASLD</b>	American Association for the Study of Liver Disease
<b>Ab</b>	Antibody
<b>AFB1</b>	Aflatoxin B1
<b>AFP</b>	Alpha fetoprotein
<b>Ag</b>	Antigen
<b>AIH</b>	Autoimmune hepatitis
<b>AJCC</b>	American Joint Committee on Cancer
<b>ALP</b>	Alkaline phosphatase
<b>ALT</b>	Alanine transaminase
<b>Ang-1</b>	Angiopoietin-1
<b>Ang-2</b>	Angiopoietin-2
<b>AR</b>	Acyclic retinoid
<b>AST</b>	Aspartate transaminase
<b>BCLC</b>	Barcelona Clinic Liver Cancer
<b>bFGF</b>	basic fibroblast growth factor
<b>CEA</b>	Carcinoembryonic antigen
<b>CLD</b>	Chronic liver disease
<b>CLIP</b>	Cancer of the Liver Italian Program
<b>CRG –L2</b>	Cancer related gene-Liver 2
<b>CT</b>	Computerized tomography
<b>CTA</b>	Computerized tomography angiography
<b>CTAP</b>	Computerized tomography angioportography
<b>CTHA</b>	Computerized tomography hepatic angiography
<b>DCP</b>	Des- $\gamma$ -carboxy prothrombin
<b>EGF</b>	Endothelial growth factor
<b>ELISA</b>	Enzyme linked immune sorbent assay
<b>EMEA</b>	European Medicine agency
<b>ETs</b>	Endothelins
<b>FDA</b>	Food & Drug Administration
<b>FLC</b>	Fibrolamellar carcinoma
<b>GGT</b>	Gamma glutamyl transpeptidase
<b>GPC3</b>	Glypican-3
<b>GST-<math>\pi</math></b>	Glutathione-S-transferase- $\pi$

<b>HAP</b>	Hepatic arterial phase
<b>HBV</b>	Hepatitis B virus
<b>HCC</b>	Hepatocellular carcinoma
<b>HCV</b>	Hepatitis C virus
<b>HFLs</b>	Hepatic focal lesions
<b>HGF</b>	Hepatocyte growth factor
<b>HGV</b>	Hepatitis G virus
<b>HIF</b>	Hypoxia inducible factor
<b>HIFU</b>	High intensity focused ultrasound
<b>IFN-<math>\alpha</math></b>	Interferon- $\alpha$
<b>IGF-<math>\beta</math></b>	Insulin-like growth factor $\beta$
<b>ILP</b>	Interstitial laser photocoagulation
<b>INR</b>	International normalization ratio
<b>IRS-1</b>	Intracellular receptor substrate-1
<b>LC</b>	Liver cirrhosis
<b>LCA</b>	Lens-culinaris agglutinins
<b>LDH</b>	Lactic dehydrogenase
<b>LH</b>	Local hyperthermia
<b>LTAE</b>	Lipiodol trans arterial embolization
<b>LTx</b>	Liver transplantation
<b>M</b>	Mean
<b>MRI</b>	Magnetic resonance imaging
<b>msAFP</b>	Monosialyted alpha fetoprotein
<b>NAFLD</b>	Non-alcoholic fatty liver disease
<b>NASH</b>	Non-alcoholic steatohepatitis
<b>PAI</b>	Percutaneous acetic acid injection
<b>PBC</b>	Primary biliary cirrhosis
<b>PC</b>	Prothrombin concentration
<b>PCR</b>	Polymerase chain reaction
<b>PDGF</b>	Platelet derived growth factor
<b>PEI</b>	Percutaneous ethanol injection
<b>PET</b>	Positron emission tomography
<b>PIVKA II</b>	Prothrombin induced by vitamin K absence II
<b>PLGF</b>	Placental growth factor
<b>PLTs</b>	Platelets

<b>PSI</b>	Percutaneous hot saline injection
<b>PT</b>	Prothrombin time
<b>PVP</b>	Portal venous phase
<b>RFA</b>	Radiofrequency ablation
<b>ROC</b>	Receiver operating curve
<b>SBP</b>	Spontaneous bacterial peritonitis
<b>SD</b>	Standard deviation
<b>TACE</b>	Trans arterial chemoembolization
<b>TAE</b>	Trans arterial embolization
<b>TGF</b>	Transforming growth factor
<b>THI</b>	Tissue harmonic imaging
<b>TKRs</b>	Tyrosine kinase receptors
<b>TNM</b>	Tumor- nodes-metastasis
<b>TSH</b>	Thyroid stimulating hormone
<b>UCSF</b>	University of California at San Francisco
<b>US</b>	Ultrasound
<b>VEGF</b>	Vascular endothelial growth factor



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# INTRODUCTION

**H**epatocellular carcinoma (HCC) is the fifth most common cancer in men and the eighth in women (*Parkin et al., 1999*). HCC commonly develops in cirrhotic livers whatever the etiology, so that liver cirrhosis by itself represents the strongest risk factor (*Colombo, 1999 & Giannelli et al., 2002*).

Both hepatitis C virus (HCV) and hepatitis B virus (HBV) infections increased the risk of HCC in Egyptian patients (*Badawy & Micheal, 1999*). Over a decade (1993-2002), there was nearly a twofold increase of the proportion of HCC among chronic liver disease (CLD) patients in Egypt with a significant decline of HBV and slight increase of HCV as risk factors (*El-Zayadi et al., 2005*).

Many surveillance programs aimed at detecting early stage HCC have been widely recommended. These programs are based on the use of ultrasound and alphafetoprotein (AFP). The reliability of imaging techniques has greatly improved in the last years but such diagnostic procedures are expensive and subject to mis-interpretation. On the other hand, the only diagnostic serologic test currently available in clinical practice is AFP. Receiver operating curve (ROC) analysis of AFP suggests that a value of about 20 ng/ml provides the optimal balance between sensitivity and specificity. However, at this level the sensitivity is only 60%, i.e., AFP surveillance would miss 40% of HCC if a value of 20 ng/ml is used as the trigger for further investigation. This is inadequately sensitive for general use. If a higher cut-off is used a progressively smaller proportion of HCCs will be detected. If the AFP cut-off is raised to, e.g., 200ng/ml the sensitivity drops to 22% (*Trevisani et al., 2001*). An elevated concentration may be

found in certain benign liver diseases. Furthermore, 20% to 30% of patients with HCC had a negative AFP result, while some cases of chronic hepatitis and cirrhosis showed a positive AFP result (*Chen & Sung, 1997*). In addition, the serum AFP level does not always correspond to the clinical stage of HCC (*Nakagawa et al., 1999*). Thus, AFP appears to have limited utility as a screening test (*Bruix & Sherman, 2005*).

It is to be mentioned that the mortality rate due to HCC has not improved over the last 20 years. This is in part due to the poor performance of available tumor markers leading to delay in diagnosis. Therefore, new and more specific markers for HCC are critically needed (*El-Serag & Manson, 1999*).

Angiogenesis is the process of formation of new capillaries from preexisting blood vessels (*Risau, 1997*), it represents an essential component of embryogenesis, normal physiological growth, repair, and tumor expansion. Although a variety of factors can modulate endothelial cell responses in vitro and blood vessel growth in vivo, only vascular endothelial growth factor (VEGF) family members and the angiopoietins are believed to act almost exclusively on vascular endothelium (*Loughna & Sato, 2001*).

Angiopoietins 1 and 2 (Ang1 and Ang2) were originally identified in tissue culture experiments as agonist and antagonist, respectively. Ang1 was shown to support endothelial cells survival and to promote endothelium integrity, whereas Ang2 (a 66 kDa protein consisting of 496 amino acid residues) had the opposite effect promoting blood vessel destabilization and regression (*Maisonpierre et al., 1997*).

Induction of Ang-2 was proposed to be a survival signal to thwart the tumor's growth process. However, VEGF up regulation coincident with Ang-2 expression induces angiogenesis at the margins of the tumor,

allowing the tumor to thrive. Late expression of tumor-derived VEGF may serve to repress the signal for vessel regression by Ang-2, which is consistent with the observation that VEGF is necessary for promoting tumor vessel survival (*Holash et al., 1999*).

High serum Ang-2 values are found in patients with inflammatory conditions as chronic HCV infection (*Salcedo et al., 2005*), inflammatory bowel disease (*Koutroubakis et al., 2006*) & sepsis (*Parikh et al., 2006*); also production of Ang-2 has been implicated in tumor development in human gastric cancers (*Etoh et al., 2001*), human prostate carcinoma (*Wurmbach et al., 2000*), and human breast cancers (*Currie et al., 2001*).

Ang-2 is overexpressed in HCC-as measured by immunohistochemistry-especially of the highly vascular type (*Sugimachi et al., 2003*); Ang-2 expression is associated with portal infiltration, microvessel density, recurrence of HCC & decreased survival (*Wada et al., 2006*). Recent studies reported high serum Ang-2 values in patients with HCC suggesting that Ang-2 might represent a useful marker for HCC and a complementary diagnostic tool (*Scholz et al., 2007*).