Type I insulin-like growth factor as a liver reserve assessment tool in hepatocellular carcinoma

Abstract: Chronic liver diseases (CLDs) encompass a wide range of illnesses, including nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and viral hepatitis. Deterioration of liver capacity, with subsequent progression into cirrhosis and hepatocellular carcinoma (HCC), ultimately leads to a further decrease in the hepatic reserve. The Child–Turcotte–Pugh scoring system is the standard tool for assessing underlying liver reserve capacity in routine practice and in clinical trials of CLD and HCC. In this review, we highlight the clinical significance of insulin-like growth factor-I (IGF-I) and the growth hormone (GH) signaling pathway in HCC. IGF-I could be a marker for liver reserve capacity in CLDs and HCC in clinical practice. This approach could improve the risk assessment and stratifications of patients on the basis of their underlying liver reserve, either before active treatment in routine practice or before they are enrolled in clinical trials.

Keywords: IGF-I, growth hormone, chronic liver disease

Insulin-like growth factors and binding proteins

Insulin-like growth factors (IGFs) were first described by Salmon and Daughaday in 1957.1 The IGF axis includes several molecules: two ligands (IGF-I [somatomedin C] and IGF-II [somatomedin A]), two transmembrane receptors (IGF-I receptor [IGF-IR] and IGF-II receptor [IGF-IIR]), and eight high-affinity IGF binding proteins (IGFBPs) (IGFBP-1 to -6, along with the lesser characterized IGFBP-7 and -8) (Table 1).2–9 These factors stimulate musculoskeletal growth and differentiation, particularly during prenatal growth.1,10–12 Under normal physiological conditions, all IGF axis molecules work together in a harmonized manner to maintain cellular homeostasis.

IGF-I is a hormone with a small molecular weight; it contains 70 amino acids, and unlike other peptides, 99% of it is protein bound. As the biochemical structure of IGF-IR is similar to that of the insulin receptor, the free IGF-I possesses a high affinity to bind with IGF-IR compared to that of insulin receptor, inducing cell proliferation and inhibiting apoptosis. It also binds with a high affinity to hybrid receptors, which contain an alpha–beta IGF half-receptor paired with an alpha–beta insulin half-receptor. The physiologic significance of hybrid receptors is not well defined, but they may mediate the insulin-like actions of IGF-I (Figure 1). These effects can be primarily inhibited by IGFBP-3, which binds to and prevents IGF-I from binding to IGF-IR.5,7,13

Mechanism and sources of IGF-I synthesis

Growth hormone (GH) is secreted, with diurnal variation, from the anterior pituitary gland in a pulsatile manner. This occurs under hypothalamic control through the
influence of hypothalamic neuropeptides, GH-releasing hormone, and GH-inhibitory hormone (somatostatin); in addition to the influence of both IGF-I and ghrelin (a gastric hormone).\textsuperscript{14,15}

Released GH produced from the pituitary gland is transported by GH binding protein to bind to its receptors in different tissues, including the liver, which is considered the main target of GH. This binding upregulates IGF-I synthesis through stimulation of IGF-I gene transcription.\textsuperscript{8,16} Approximately 75\% of circulating IGF synthesized by the liver is believed to perform an “endocrine” function as it is typically used remotely.\textsuperscript{17–19} In contrast, approximately 25\% of IGF-I that is synthesized in the bones, cartilage, central nervous system, kidneys, ovaries, and erythroid cell precursors executes autocrine and paracrine functions (Figures 1 and 2).\textsuperscript{20–25}

Factors affecting plasma levels of GH/IGF-I

GH/IGF secretion can be stimulated directly by the “push effect” or indirectly by the “pull effect” by reducing the negative feedback inhibitory effect.\textsuperscript{26} Normally, the circulating IGF level changes with age. During childhood, increase in the production of sex steroid hormones results in increased pro-
duction of GH and subsequently IGF-I in both sexes. However, the relationship between steroid hormones and GH/IGF is affected by sex. Several studies have shown that in men, regardless of age, testosterone centrally increases the GH level, followed by IGF-I production through the push effect. Studies were performed to determine whether testosterone enhances IGF-I directly or as a costimulatory factor to GH; testosterone alone had a very limited or no effect on circulating IGF-I levels except in the presence of GH. On the contrary, there is a debate concerning the relationship between estrogen and GH/IGF-I. Researchers have found that during menstruation, GH levels increase in response to an estrogen peak, with higher GH levels in premenopausal women than in postmenopausal women. Notably, unilateral nephrectomy induces compensatory growth of the contralateral kidney, with a subsequent increase in IGF-I expression.

Mechanism of action of IGF-I

Synthesized IGF-I is cleaved by protease enzymes before being released into the circulation. IGFBPs, which are present in all extracellular fluids, transport IGF-I by binding to approximately 99% of it with a higher affinity than IGF-IR. The bound form of IGF-I is mainly synthesized in peripheral tissues. IGFBPs are present in all extracellular fluids, which transport IGF-I by binding to approximately 99% of it with a higher affinity than IGF-IR. The bound form of IGF-I is mainly synthesized in peripheral tissues.
in the liver, while the free form, which is produced by other tissues, has a low affinity to IGFBPs and is responsible for its autocrine and paracrine effects.16,17

Notably, elevated serum levels of IGF-I induce a negative feedback effect on GH secretion, either directly through a local inhibitory effect on the pituitary gland or indirectly by stimulating somatostatin release. Thus, IGF-I and GH work cooperatively as IGF-I regulates GH effects, which in turn control the release of IGF-I.60–67

The role of GH receptor (GHR) in harmonizing the association between GH elevation68 and IGF-I suppression69 has been reported in previous studies. Chang et al70 studied the correlation between these changes and GHRs, which are present on hepatocyte cell membranes. They determined the presence of GHR in human HCC, cirrhosis, and normal tissue samples using radio-receptor assays and discovered that GHR was absent in both cirrhotic and HCC samples, which explains the persistent decrease in the serum level of IGF-I with elevated levels of GH.

Normally, both GH and IGF-I have an anabolic effect, promoting lipolysis and protein synthesis by stimulating amino acid uptake, stimulating cell growth and differentiation, increasing muscle mass through sarcomere hyperplasia, and stimulating the immune system by restoring a normal nitrogen balance and causing a 25% increase in GFR. IGF-I also decreases blood glucose levels, improves insulin resistance, decreases reactive oxygen species, and has an antifibrotic effect.71–73

**Molecular role of IGF-I in cancer development**

In 1990, the role of IGF-I in the process of tumorigenesis was revealed. Since then, a major research focus has been to better understand the nature and role of the IGF axis in the pathogenesis of various neoplasms.11

Recently, there has been renewed interest in the roles of GH/IGF-I in cancer development because of an increase in cancer incidence, including breast, thyroid, colon, and prostate cancers, in acromegalic cancer patients with elevated serum IGF-I secondary to GH-producing pituitary tumors.74 Elevated serum IGF-I and GH levels were reported in non-acromegalic cancer patients. GH enhances cancer development through several pathways;75–81 1) it binds to GHR and activates several intracellular signal pathways; 2) it stimulates IGF-I production from the liver; and 3) it induces peripheral tissue insulin resistance, with subsequent elevation of serum insulin levels.

Binding of IGF-I to the alpha subunit of IGF-IR leads to auto-activation of tyrosine kinase and the auto-phosphorylation of tyrosines, with subsequent

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**Figure 3** Roles of insulin-like growth factor-I (IGF-I) in cancer development.

**Abbreviations:** IGFBP, IGF binding protein; IGF-IR, IGF-I receptor; MMP-9, matrix metallopeptidase-9; uPAR, urokinase plasminogen activator receptor; ERK, extracellular signal-regulated kinases; ECM, extracellular matrix; SOS, son of sevenless; GRB2, Growth factor receptor-bound protein 2; SHC, src homology/α-collagen related protein; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase.
phosphorylation of insulin receptor substrate-1 (IRS-1) and insulin receptor substrate 2 (IRS-II). IRS-1 stimulates several kinase pathways, such as phosphatidylinositol 3 kinase (PI3K), SHC, and Src. Through the SHC pathway, Grb-2 forms a complex that activates son of sevenless (SOS) protein. This complex activates p21 Ras, which is a mitogen-activated protein kinase pathway. Activation of this pathway is important for stimulating cell growth. IRS-1 also activates the PI3K/mitogen-activated protein kinase (mTOR) pathway, which is important for stimulating protein synthesis, glucose transportation, cell motility, and apoptosis inhibition.

IGF-I plays an important role in cancer development by regulating angiogenesis, lymphangiogenesis, degradation of the extracellular matrix (ECM), tumor invasion into both the ECM and blood vessels, and maintenance of tumor cell survival and proliferation.

Several basic science studies showed that IGF-I regulates angiogenesis and lymphangiogenesis by activating vascular endothelial growth factor and stimulating the expression of hypoxia-inducible factor 1 via the PI3K/Akt and Ras/mTOR pathways. IGF-I is transported across the vascular endothelial cell lining through a paracellular route where it binds to the subendothelial ECM to stimulate the migration and morphological differentiation of endothelial cells. Subsequently, IGF-I activates matrix metalloproteinase-9, which is a type IV collagenase. IGF-I also increases the binding of single-chain urokinase-type plasminogen activator (uPA) to the cell-surface uPA receptor (uPAR). This combination converts serum plasminogen to plasmin, which is a broad-spectrum serum protease enzyme. Both metalloproteinase-9 and uPAR/uPA are major molecular mediators that play a significant role in ECM proteolysis and degradation, followed by tumor invasion (Figure 3).

**GH/IGF-I as an indicator of hepatic reserve**

A previous article reported an IGF-I deficiency in CLDs such as nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, viral hepatitis, cirrhosis, and HCC; it occurs through several mechanisms, including insulin resistance, oxidative stress, mitochondrial dysfunction, and the inflammatory cascade.

The correlation between GH and IGF-I levels in liver cirrhosis has been previously evaluated; elevated plasma levels of GH were found in cirrhosis patients, with a pulse frequency and plasma half-life that were more than twice those in the control group; this was partially explained by the associated hyperglycemia.

In 1993, Buzzelli et al studied changes in the GH/IGF-I circadian rhythm in cirrhosis patients, regardless of the presence or absence of associated HCC. They concluded that, compared to the control group, patients with cirrhosis had lower serum IGF-I levels and higher GH levels. These changes remained stable for 24 hours, resulting in loss of GH/IGF-I circadian rhythm. This phenomenon was explained by the decrease in GHRs and their binding capacity in damaged liver tissue compared with normal liver tissue.

A low serum IGF-I level leads to several metabolic changes induced by reduced peripheral glucose and lipid uptake, increased liver glucose production, increased stored triglyceride hydrolysis, and subsequently elevated circulating glucose and free fatty acid levels (Figure 2). Furthermore, a few studies showed that, under normal conditions, IGF-I stimulates hepatocyte growth factor (HGF) production from hepatic stellate cells, and that administration of human recombinant HGF suppressed the onset of liver fibrosis/cirrhosis in animal models.

Thus, low levels of free IGF-I lead to a loss of antifibrotic effects. Reactive oxygen species, different cytokines, and inflammatory mediators can easily activate hepatic stellate cells and induce fibrosis.

Further studies showed a lower rate of IGF-I expression in patients diagnosed with either nonalcoholic fatty liver disease or nonalcoholic steatohepatitis (Table 1).

Notably, liver cirrhosis, which is a chronic disease in which the liver tissue is irreversibly replaced by fibrous tissue, necrosis, and regenerating nodules, leads to the deterioration of normal hepatic function. Systematically, cirrhosis patients experience several clinical manifestations of their decreased metabolic liver capacity and subsequent IGF-I deficiency and GH elevation.

Several studies reported decreased serum IGF-I levels in patients with diseased liver compared to normal population. This suggests that circulating levels of IGF-I are a surrogate marker for assessment of liver dysfunction (Table 1).

Collectively, these findings support the hypothesis that plasma IGF-I levels reflect hepatic synthetic function and hence should be considered a surrogate marker for determining the hepatic reserve.

**IGF-I as an assessment tool for liver reserve capacity in HCC**

Currently, surgical resection and liver transplantation are the only curative treatments for HCC. Unfortunately, most patients are not surgical candidates because of an advanced tumor stage at presentation or advanced under-
lying CLDs.\textsuperscript{122,123} These factors have a significant effect on treatment decisions and outcomes (including overall survival [OS]) and prognostic stratification for clinical trial enrollment.

Several HCC prognostic systems are used to assess underlying CLD status, predict treatment outcome and OS, and stratify patients in clinical trials. However, the standard system for assessing hepatic reserve in HCC staging systems is the Child-Turcotte-Pugh (CTP) score, which depends on two subjective parameters (encephalopathy and ascites) and three objective parameters (serum albumin, serum bilirubin, and prothrombin time or the international normalized ratio).\textsuperscript{107,111,113,116,117,119} Despite its limitations, the CTP score has remained the standard tool for predicting the degree of underlying CLD in HCC patients before active therapy or trial entry, using CTP class A (CTP-A) as the standard treatable

### Table 1: Clinical studies of circulating IGF-I in CLDs

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Type of disease</th>
<th>Study design</th>
<th>Sample size</th>
<th>Correlation with liver dysfunction</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaseb et al\textsuperscript{136,137}</td>
<td>2011</td>
<td>USA</td>
<td>CLDs and HCC</td>
<td>Prospective cohort</td>
<td>288 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Rehem and El-Shikh\textsuperscript{115}</td>
<td>2011</td>
<td>Egypt</td>
<td></td>
<td>Case-control</td>
<td>20 HCC</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Su et al\textsuperscript{118}</td>
<td>2010</td>
<td>Taiwan</td>
<td></td>
<td>Case-control</td>
<td>65 cases</td>
<td>+</td>
<td>↓ IGF-I (P&lt;0.001)</td>
</tr>
<tr>
<td>Lorenzo-Zúñiga et al\textsuperscript{112}</td>
<td>2007</td>
<td>Spain</td>
<td></td>
<td>Cohort</td>
<td>40 HCV</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Elsammak et al\textsuperscript{110}</td>
<td>2006</td>
<td>Egypt</td>
<td></td>
<td>Case-control</td>
<td>30 HCC</td>
<td>+</td>
<td>↓ IGF-I (P&lt;0.001)</td>
</tr>
<tr>
<td>Stuver et al\textsuperscript{138}</td>
<td>2000</td>
<td>USA</td>
<td></td>
<td>Case-control</td>
<td>73 HCC</td>
<td>+</td>
<td>Serum IGF-I (P&lt;0.0001)</td>
</tr>
<tr>
<td>Arturi et al\textsuperscript{101}</td>
<td>2011</td>
<td>Italy</td>
<td>Steatosis and NASH</td>
<td>Case-control</td>
<td>308 nondiabetic cases</td>
<td>+</td>
<td>↓ IGF-I (P=0.001)</td>
</tr>
<tr>
<td>Yölzke et al\textsuperscript{105}</td>
<td>2009</td>
<td>Germany</td>
<td></td>
<td>Cohort</td>
<td>3,863 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Ronsoni et al\textsuperscript{116}</td>
<td>2013</td>
<td>Brazil</td>
<td>Cirrhosis</td>
<td>Cross-sectional</td>
<td>74 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Castro et al\textsuperscript{111}</td>
<td>2013</td>
<td>Brazil</td>
<td></td>
<td>Case-control</td>
<td>25 cases</td>
<td>+</td>
<td>↓ IGF-I (P&lt;0.05)</td>
</tr>
<tr>
<td>Dehghani et al\textsuperscript{108}</td>
<td>2012</td>
<td>Iran</td>
<td></td>
<td>Case-control</td>
<td>45 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Sandahl et al\textsuperscript{117}</td>
<td>2011</td>
<td>Denmark</td>
<td></td>
<td>Case-control</td>
<td>38 controls</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Jeyaratanganthan et al\textsuperscript{111}</td>
<td>2010</td>
<td>Denmark</td>
<td></td>
<td>Case-control</td>
<td>43 cases</td>
<td>+</td>
<td>↓ IGF-I (P&lt;0.05)</td>
</tr>
<tr>
<td>Assy et al\textsuperscript{105}</td>
<td>2008</td>
<td>Israel</td>
<td></td>
<td>Case-control</td>
<td>53 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Wu et al\textsuperscript{119}</td>
<td>2004</td>
<td>People’s Republic of China</td>
<td>Case-control</td>
<td>44 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
<td></td>
</tr>
<tr>
<td>Vyzantiadis et al\textsuperscript{118}</td>
<td>2003</td>
<td>Greece</td>
<td></td>
<td>Case-control</td>
<td>40 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
<tr>
<td>Mazzotti et al\textsuperscript{113}</td>
<td>2002</td>
<td>Italy</td>
<td></td>
<td>Prospective cohort</td>
<td>114 HCV cases</td>
<td>+</td>
<td>↓ IGF-I in patients developed HCC</td>
</tr>
<tr>
<td>Donaghy et al\textsuperscript{129}</td>
<td>2002</td>
<td>UK</td>
<td></td>
<td>Cohort</td>
<td>50 cases</td>
<td>+</td>
<td>↓ IGF-I/GH</td>
</tr>
<tr>
<td>Assy et al\textsuperscript{104}</td>
<td>1998</td>
<td>Israel</td>
<td></td>
<td>Cohort</td>
<td>15 cases</td>
<td>+</td>
<td>↓ IGF-I and ↑ after rhGH</td>
</tr>
<tr>
<td>Caregaro et al\textsuperscript{106}</td>
<td>1997</td>
<td>Italy</td>
<td></td>
<td>Cohort</td>
<td>64 cases</td>
<td>+</td>
<td>↓ IGF-I (P&lt;0.01)</td>
</tr>
<tr>
<td>Möller et al\textsuperscript{114}</td>
<td>1993</td>
<td>Denmark</td>
<td></td>
<td>Case-control</td>
<td>36 cases</td>
<td>+</td>
<td>↓ IGF-I</td>
</tr>
</tbody>
</table>

**Abbreviations:** CLDs, chronic liver diseases; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; SHF, schistosomal hepatic fibrosis; IGF, insulin-like growth factor; GH, growth hormone; rhGH, recombinant human growth hormone.
patient population. Several studies have concluded that patients classified as CTP-B or CTP-C have significantly shorter survival duration than those classified as CTP-A because of deterioration in their hepatic function. Therefore, CTP-A patients are the only patients who are eligible for active treatment and clinical trials and are the only population approved by the US Food and Drug Administration (FDA) for sorafenib therapy on the basis of the results of the first international, randomized, double-blind, placebo-controlled, multicenter Phase III study. The CTP-A group is heterogeneous (especially nonsurgical patients, who constitute the main pool in clinical practice and clinical trials, as described at the most recent international expert consensus conference), and post-therapeutic decline in liver functions is still a major challenge to outcome prediction. Furthermore, the survival benefit of sorafenib was associated with only a 2.3% objective response rate, as defined by Response Evaluation Criteria in Solid Tumors. Sorafenib is an expensive treatment; it is not affordable to many patients and may increase health care costs, especially in low- and middle-income countries. National and international guidelines recommend sorafenib only in patients with CTP-A to avoid potential severe adverse effects and death due to hepatic failure. However, hepatic failure and sorafenib intolerance still occur in patients with CTP-A. Thus, there is a critical and immediate need for more sensitive tools than the CTP score to predict survival duration in HCC patients undergoing treatment and in selected patients who are not eligible for enrollment in clinical trials.

Integrating IGF-I levels into HCC management

Since the liver produces >75% of circulating IGF-I, and IGF-I’s role is documented in other CLDs, several studies have investigated whether IGF-I levels can be used to assess hepatic capacity in HCC patients and detect its correlation with HCC prognosis and survival outcome (Table 1). The decline in serum levels of IGF-I in HCC is likely mediated through the decreased synthetic capacity of normal liver cells, which have been replaced by tumor cells. We recently reported the utility of plasma IGF-I as a molecular biomarker for assessing liver reserve in HCC patients. In addition, two recent studies reported that low pretreatment IGF-I levels independently correlated with poor outcome in the form of a shorter TTP and OS in patients with HCC who underwent TACE.

On the basis of the widely adopted American Association for the Study of Liver Diseases guidelines, HCC can be diagnosed using a noninvasive imaging approach. There is a critical need to develop a blood-based biomarker strategy to assess hepatic reserve and predict patients’ survival and treatment outcomes. This approach will improve the personalization of HCC treatment by allowing us to select the best candidates for specific therapeutic modalities and avoid unnecessary harm and health care expenses.

Developing the IGF-I score

The CTP score is the standard tool currently used for assessing hepatic reserve in HCC staging systems. Recently, our research group studied the value of incorporating IGF-I into the CTP system to replace the two subjective parameters, ascites and encephalopathy (Table 2). Our results indicated that the IGF-CTP score significantly improved OS prediction and patient risk stratification compared to the CTP score in both the training and validation cohorts (P<0.003 and P<0.005, respectively, when measured by the C-index) (Table 3).
Differences between the C-indices were not large but were statistically significant as the C-index computes the ability to predict OS for all patients in the cohort, including those whose CTP and IGF-CTP scores are different and those whose scores are the same. Interestingly, patients with CTP-A that was reclassified as IGF-CTP-B had significantly shorter OS than did patients whose IGF-CTP-A classification remained unchanged in both the training and validation cohorts (P=0.03 and P<0.001, respectively) (Figure 4 and Table 4).

**Conclusion**

Classification of the degree of liver reserve is critical to HCC management and for selecting patients for clinical trials. CTP is the most commonly used clinical tool to assess hepatic reserve, but it has multiple limitations, including the use of two subjective variables (ascites and encephalopathy) that are difficult to assess and may change daily under the influence of medications, nutritional status, and comorbidities. In addition, these subjective variables and their arbitrary cutoff points have been randomly selected. Emerging data about the GH/IGF-I axis in HCC by our research group and others indicate that plasma IGF-I should be incorporated in assessment of the liver reserve capacity. In our recent studies, we incorporated plasma IGF-I into the objective parameters of CTP to create an exclusively objective blood-based score and reported the results from two independent cohorts at our institution. The score is currently undergoing independent multicenter and international validation. We anticipate that IGF-I use will enhance the accuracy of selecting appropriate patients for active therapy in routine practice and for enrollment in clinical trials. Importantly, a rigorous analysis of the interactions and correlations between the GH/IGF-I axis in HCC will help advance our current understanding of the complex pathogenesis of HCC development and progression. The emerging data on upregulating GH in patients with cirrhosis and HCC is intriguing, given the potential to target this pathway for HCC prevention and treatment. Future validation studies of this approach are warranted.

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The authors report no conflicts of interest in this work.

References


