Priority considerations in early laboratory diagnosis of Hepatocellular carcinoma

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Abstract
Currently, early diagnosis of hepatocellular carcinoma (HCC) is the most critical step in liver cancer (LC) management. Most imaging techniques help to discover LC after considerable time of onset of tumor. Also, in most instances, oncologists rely on alpha fetoprotein (AFP) as the most common and feasible marker for assessing LC in addition to imaging. This constitutes a marker which is not completely reliable marker in early LC prevention or therapy because of its low specificity and sensitivity. Liver biopsy is always considered as an invasive procedure, so chemical findings are still greatly appreciated. In the present review, a list of laboratory markers useful in diagnosing HCC, elicited either as specific RNAs or serum proteins is compiled. These include molecular markers such as: hepatoma specific alpha fetoprotein (HS-AFP) mRNA, hepatoma specific–gamma glutamyl transferase (HS-GGT) mRNA, transforming growth factor β1(TGF-β1) mRNA, insulin-like growth factor-II (IGF-II) mRNA, heat shock protein (HSP) and methylated apoptotic factors. Serum markers such as AFP, alpha-L-fucosidase (AFU), GGT, TGF-β1, IGF-II, anti-p53 antibodies and des-gamma-carboxy prothrombin (DCP) in addition to less common markers as r-glutamyl transpeptidase (r-GT), tumor necrosis factor alpha (TNF-α), pancreatitis-associated protein (PAP), serine-threonine kinase 15 (STK-15) and plasma glutamate carboxypeptidase (PGCP) were also collated. The use of both AFP, AFU and methylated p53-mRNA together, is suggested, to get a 100% early prediction of HCC development in risky subjects. This short panel of three markers is recommended to assure optimal HCC prediction with the highest priority to other studied markers.

Keywords: Alpha fetoprotein, gamma glutamyl transferase, alpha-L-fucosidase, transforming growth factor beta, insulin-like growth factor II.

INTRODUCTION
Hepatocellular carcinoma (HCC) is considered the third deadliest and fifth known cancer allover the world (Srivatanakul et al., 2004 and Peto, 2001). HCC is a high-grade malignancy showing a rapid infiltrating growth, early-stage metastasis, poor therapeutic response and disappointing prognosis even after successful curative resection surgery (Jiang et al., 2000 and Shi et al., 2004).

Early detection of HCC is the most critical step in the management process. A combination of both pathological features and biochemical markers with high sensitivity and specificity are still the most convenient policy in medical practice (Yao et al., 2007).

In addition, common imaging techniques as ultrasonography (USG), computer tomography (CT) and magnetic resonance imaging (MRI) are the most important tools for the surveillance of liver cirrhosis patients for detection of primary liver cancer (LC). To elevate the sensitivity, specificity and the predictive value of the imaging techniques, the estimation of several relevant serological markers is strongly recommended. This improves the diagnostic process of HCC (Madalinski et al., 2005).

The goal of the present review is to collect and discuss laboratory markers considering their sensitivity and specificity figures with a ranging
system, showing priority sequence in early detection of HCC.

Generally, HCC biomarkers can be classified into two major categories, sensitive molecular markers and serological markers.

**Sensitive molecular markers for HCC**

Hepatoma tissues can synthesize many tumor-related proteins, polypeptides and iso-enzymes. Circulating hepatoma-specific biomarkers are useful predictors for early diagnosis of HCC or monitoring metastasis or postoperative recurrence of the disease (Yao et al., 2007):

**Hepatoma specific alpha fetoprotein (AFP), HS-AFP and AFP-mRNA**

AFP is a 70 kD glycoprotein synthesized from fetal yolk, liver and intestinal tissues. Its half-life is 5-7 days (Fujiyama et al., 2002). Although, serum total AFP is extensively and frequently documented as a serological marker for detection of HCC, its sensitivity in the early detection is only around 60%. The remaining percentage constitutes either false-positive or false-negative results. In addition, AFP elevations may sometimes also be referred to non-neoplastic liver diseases, which further decreases AFP specificity (Yao et al., 2000). Recently, depending on proteomic profiling using surface enhanced laser desorption / ionization time of flight mass spectrometry (SELDI-TOFMS) enables the identification of biomarkers for cancer. Using the AFP cutoff of 20 ng/mL, the sensitivity was 73% and the specificity was 71%. Using the AFP-L3 cutoff of 10% yielded a sensitivity of 63% and a specificity of 94% (Zinkin et al, 2008). Thus, total serum AFP lacks both sensitivity and specificity in assessing HCC. This is why sub-forms of total AFP are studied to assign more sensitive fraction for detection of HCC. Three AFP glycoforms are classified according to their capacity in binding to lens culinaris agglutinin (LCA), namely AFP-L1, AFP-L2 and AFP-L3. HS-AFP, as LCA-bound fraction is the major glycoform in HCC patients (Cha et al., 2003).

HS-AFP is significantly correlated to HCC differentiation, metastasis and relapse (Yoshida et al., 2002). Circulating cancer cells are considered as important source for HCC metastasis to extrahepatic tissues (Plebani and D'Amico, 2005). Detection of hepatocyte-specific mRNA in peripheral blood mononuclear cells by RT-PCR is reported to predict hematogenous metastasis (Yao et al., 2000).

**Hepatoma specific –gamma glutamyl transferase (HS-GGT) isoenzyme**

Gamma glutamyl transferase (GGT) is a membrane-bound enzyme which catalyzes glutathione degradation (hydrolysis) into its monomeric amino acids. Its expression is tissue specific and greatly affected by different physiologic and pathologic conditions including carcinogenesis (Yao et al., 2004).

Total GGT activity is increased in both hepatic and extrahepatic tumors. This mostly decreases both sensitivity and specificity of this enzyme in detecting HCC. Evaluation of GGT isoforms improves the specificity to HCC. HS-GGT can be separated from patient sera by electrophoresis. This isoenzyme of GGT is only found in HCC patient's sera, its expression starts early in HCC development and analysis of this isoenzyme significantly increases both specificity and sensitivity in early diagnosis (Jian et al., 2007).

**Transforming growth factor β1 (TGF-β1) and TGF-β1- mRNA**

All body cells, including epithelial, endothelial, hematopoietic, neuronal and connective tissues, produce TGF-β and all have specific receptors for it. TGF-β1 is an isoform of five TGF-β isoforms It arrests cell cycle in the G1 phase and can be considered as an apoptotic factor (Pasche, 2001). TGF-β family are regulatory proteins controlling cellular growth, differentiation, extracellular matrix formation and immunosuppression (Dong et al., 2007). TGF-β1 is produced by normal liver cells (Kupffer's, fat-storing and endothelial cells, all constitute non-parenchymal cells). Despite being growth inhibitor, TGF-β1 is over-expressed in HCC tissues and it is considered as a marker for progression and prognosis of HCC (Lu et al., 2008). Both sensitivity and specificity of TGF-β1 and its mRNA are more than 90% in the circulation of HCC patients, although AFP levels are not significantly correlated to TGF-β1 expression (Kim et al., 2003). Thus, both circulating TGF-β1 and its messenger RNA can be used as sensitive markers for early prediction of HCC in viral-infected patients. Addition of AFP to serum TGF-β1 may raise the sensitivity up to 97% (Dong et al., 2006).
Insulin-like growth factor-II (IGF-II) and its mRNA

It is a polypeptide closely related to insulin. It can also potentiate the function of vascular endothelial growth factor (VEGF) in the development of new vascularization, which is a must in hepatocellular carcinogenesis. Thus, IGF-II is extensively expressed during HCC and it is frequently speculated to be a mitogenic factor (Cantarini et al, 2006) and Wang et al., 2003). The circulating IGF-II and its messenger RNA can be used as useful markers in detection of HCC, differentiation of extrahepatic metastasis and monitoring post-operative recurrence (Dong et al., 2005).

Heat shock protein (HSP)

HSPs is a family of numerous proteins produced inside the cell under the effect of various chemical and physical stimuli, including carcinogenesis. Its primary function is the regulation of target proteins and protein transport in cell organelles participating in protein folding and extension, in addition to the assembly of polypeptide complexes (Meng et al., 2002).

Being an inhibitor of apoptosis, HSP is over-expressed in HCC development and prognosis (Wang et al., 2005). HSP70 expression can contribute to hepatocarcinogenesis through promotion of tumor cell proliferation (Qin and Tang, 2004).

Methylated apoptotic factors

Methylation specific PCR can be used to investigate methylated genes responsible for expression of apoptotic factors as p53. The detection of blood mononuclear methylated p53 genes in combination with serum AFP is reported to introduce 100% confidence in early detection of HCC (Yanhong et al., 2007).

Serological markers for HCC

The next markers will be discussed in regard to being either traditional, cost-effective and/or common parameters, although having variable degrees of specificity and sensitivity. These parameters, being time-effective, can be considered as first line diagnostic tools in the clinical laboratory. However, these markers are known to increase in cancer patients, in comparison to healthy subjects and their concentration is proportional to the number of neoplastic cells. Finally, the level of these markers decreases after decreasing cancer size or suppressing its development by different therapeutic modalities (Kulpa and Rychlick, 2004).

AFP

It is one of the first registered markers for detection of HCC both in humans and experimental animals (Bergstrand and Czar, 1956; Abelev et al., 1967). Both specificity and predictive value of AFP in HCC decline because some cases do not secrete diagnostically significant amounts (Giardina et al., 1998). Thus, to increase the sensitivity of AFP, AFP-mRNA can be detected by RT-PCR in peripheral blood mononuclears (Matsumura et al., 1999) and AFP-L3 fraction in patient serum., However, there is no available commercial kits for the later test (Yuen and Lai, 2005). The estimation of AFP-L3 increased AFP sensitivity from around 60% to about 90% in discriminating HCC from liver cirrhosis in viral hepatitis-patients (Wawrzynowicz-Syczewska et al., 2003).

Alpha-L-fucosidase (AFU)

It is a lyzosomal enzyme present in all mammalian cells. Its natural substrates are L-fucose-containing sugar residues. Like AFP, it increases in some physiologic states as pregnancy and in neonates, but falls into baseline after delivery and adulthood, respectively (Sztefko et al., 1998). AFU activity is reported to be a valuable marker in HCC detection (Giardina et al., 1992). It significantly rises 6-9 months before ultrasonographic (USG) imaging can depict the case, this is why it is a reliable early marker. It shows about 85% sensitivity and 91% specificity for HCC (Ishizuka et al., 1999; Ma et al., 2000). These authors recommended that cirrhotic patients with increased AFU activity should have frequent USG examinations on the liver every three months to monitor cancer development and to achieve better prognosis.

Anti-p53 antibodies

p53 is a tumor suppressor protein, produced by p53 gene, which is recognized as genome guardian or cancer suppressor gene (Soussi et al., 1994). p53 gene mutations are the most frequent changes in cancer. Thus, neoplastic cells have high concentration of p53 mutated protein, which has a greater survival time (longer half-life). This long-term mutated protein initiates the
formation of anti-p53 auto-antibodies, which could be detected in blood of cancer patients (Schlichtholz et al., 1994). Due to the importance of p53 antibodies determination, commercial kits are available. Unfortunately, the anti-p53 test is not sensitive for HCC, as it is only positive in 20-25% of liver cancer patients. However, it is 100% positive in cholangiocarcinoma, as well as in gastric, pancreatic, esophageal and colon cancers (Yuen and Lai, 2005).

**Serum des-gamma-carboxy prothrombin (DCP)**

It is a prothrombin precursor with no coagulation activity. It is synthesized by the liver depending on the presence of vitamin K-dependent $\gamma$-glutamyl carboxylase (Suzuki et al., 2005). To be converted into prothrombin, DCP terminal residue, which contains 10 molecules of glutamine, should be converted into the corresponding glutamate by $\gamma$-glutamyl carboxylase. In HCC, both vitamin K and $\gamma$-glutamyl carboxylase are improperly available, so there is a decrease in the conversion of DCP into prothrombin. This consequently results in a tendency of bleeding (Furukawa et al., 1992; Huisse et al., 1994; Okuda et al., 1987).

Serum DCP is used as a clinical parameter for the development of portal venous invasion and cell proliferation in HCC (Suehiro et al., 1995, Koike et al., 2001 and Tamano et al., 2002). Thus, serum DCP is considered as a sensitive marker for early diagnosis (62%) and prognosis of HCC after treatment (Ikoma et al., 2002). DCP is alternatively known as a protein induced by vitamin K absence (PIVKA) and is estimated by ELISA (Chawla et al., 2007).

**Supportive serum markers for early detection of HCC**

Less frequently, some other markers can be added to the panel of HCC early markers. These include r-glutamyl transpeptidase (r-GT), tumor necrosis factor alpha (TNF-\(\alpha\)), pancreatitis-associated protein (PAP), serine-threonine kinase 15 (STK-15) and plasma glutamate carboxypeptidase (PGCP) (Yuen and Lai, 2005; Ma et al., 2000).

These markers can be considered as supportive markers for AFP or AFU and increase both sensitivity and specificity in HCC early assessment. However, since both AFP and AFU, are synthesized by different liver cells, co-determination may greatly elevate predictability of HCC development among patients with risk factors for this common and fatal malignancy (Madalinski et al., 2005).

**CONCLUSION**

From the previous surveillance of HCC markers, it is concluded that patients with risk factors for HCC should undergo frequent periodical laboratory investigations every 6 months to predict early development. The most sensitive panel of laboratory markers is a simultaneous determination of AFP, AFU and methylated p53 gene. This provides for a 100% sensitivity and specificity in HCC early assessment.

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