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VIEWPOINT

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The Changing Face of the Diagnosis of Chronic and Malignant Liver Diseases: Potential New Biomarkers

Vania Cruz-Ramón,* Paulina Chinchilla-López,*
Oscar Ramírez-Pérez,* Xingshun Qi,** Nahum Méndez-Sánchez*

* Liver Research Unit, Medica Sur Clinic & Foundation, Departments of Gastroenterology & Hepatology, Mexico City, Mexico.
** Liver Cirrhosis Study Group, Department of Gastroenterology, General Hospital of Shenyang Military Area, Shenyang, China.

ABSTRACT

The early diagnosis of primary sclerosing cholangitis, hepatocellular carcinoma, and cholangiocarcinoma is often challenging. In a recent study in 134 patients (Arbelaiz, *Hepatology* 2017; 66: 1125-43), it was reported that specific proteins found in serum extracellular vesicles of patients with primary sclerosing cholangitis, hepatocellular carcinoma, or cholangiocarcinoma may be useful as noninvasive diagnostic and prognostic tools. This current article critically appraises this study.

Key words. Noninvasive methods. Primary Sclerosing Cholangitis. Hepatocellular Carcinoma. Cholangiocarcinoma. Biomarkers.

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are the most frequently occurring types of primary liver cancer worldwide and account, respectively, for 80% and 15% of primary liver cancer.[1,2] Recent studies have reported that the worldwide incidence of these liver and biliary tract diseases is increasing. The reported overall rate for primary sclerosing cholangitis (PSC) is 0.77 per 100,000 person-years,[3] 0.71-85 per 100,000 inhabitants for intrahepatic cholangiocarcinoma[4,5] and for HCC range from 1.4-20 per 100,000 individuals.[6] Surprisingly, the increased incidence rates of HCC and intrahepatic cholangiocarcinoma are higher in western countries, mainly in those countries whose population has a high risk for developing these disorders.[7]

Environmental and cultural factors are important determinants of the incidence of HCC. Nonalcoholic liver disease is a potential key player in the increasing incidence of HCC in the Hispanic population. For example, the trend for HCC incidence in Mexico has been projected to increase by 73% from 2005 to 2050, especially in people aged between 35 and 55 years.[8] A study from a general hospital in Mexico City reported a prevalence of 0.59% (n = 73) in people with a median age of 65 years; the age at death of these patients was 25-90 years.[9,10] Our group has analyzed data on national mortality from death certificates reported for the years 2000-2006 by the Health Ministry of Mexico, and we found that the rates were markedly higher in men than in women over the 6-year period.[11]

The highest incidence rates of HCC occur in highly endemic areas for hepatitis virus B and hepatitis virus C as well as in countries with elevated rates of alcohol abuse and nonalcoholic liver disease.[12,13] By contrast, the incidence of intrahepatic cholangiocarcinoma is higher in countries where liver fluke infection is endemic.[5] The incidence of extrahepatic cholangiocarcinoma is 0.72 per 100,000 individuals.[14] However, it was recently reported that global rates seem to be stable or to be decreasing slightly.[15-18] HCC causes 250,000-1,000,000 deaths globally per year.[19-21] The mortality rates for extrahepatic cholangiocarcinoma are 6.9 for males and 5.1 for females, but the rate for intrahepatic cholangiocarcinomais 6.3 per 100,000 per year for both males and females.[22,23] PSC, a chronic cholestatic disease of unknown origin, is a risk factor for CCA, and the prevalence of CCA in patients with PSC is 7-13%.[24] The global prevalence of this disease is 0-16.2 per 100,000 inhabitants per year, and its mortality rate is about 8.3%.[25]

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Unfortunately, the early diagnosis of these diseases remains difficult and, in most cases, the disease is diagnosed late because of the absence of symptoms in the early stages.[26-28] At present, the most accurate diagnostic methods for these disorders, such as endoscopic retrograde cholangiopancreatography-guided bile duct biopsy, percutaneous transhepatic cholangiography, and endoscopic ultrasonography-guided fine needle aspiration, are invasive. However, in most cases, these invasive methods have a similar or lower sensitivity/specificity compared with noninvasive methods and are not useful for detecting these disorders in the early stages. Therefore, they are not recommended as a first-line diagnostic method.[29-31] In this setting, it is necessary to find new noninvasive tools.

There is much interest in the development of noninvasive diagnostic methods for these disorders. One trend in biomarker discovery is the use of high-throughput proteomics-based approaches because these methods can be used to examine thousands of hypothetical candidates in very large study groups and, when coupled with bioinformatics analysis, allow researchers to pinpoint accurately the global and local discrepancies in protein profiles between different populations.[32] Because of the mechanistic complexity and heterogeneity of these disorders, these features may provide huge benefits, particularly for the identification of cancer biomarkers.

Recently, there has been remarkable progress in applying proteomics to the identification of potential biomarkers for HCC, CCA, and PSC. In this context, using a mass spectroscopic approach, Yin, *et al*.[33] screened the serum of patients with various liver diseases for core-fucosylated proteins. They found that 3 core-fucosylated peptides of fibronectin at site 1,007 could discriminate HCC from cirrhosis in patients with alcohol liver diseases; the sensitivity was 85.7% and specificity was 92.9%. These results suggested that the core-fucosylated peptides may also have a role as biomarkers for the detection and diagnosis of HCC.

Using a similar approach, Zinkin, *et al*.[34] used surface-enhanced laser desorption/ionization time of flight mass spectrometry to create an 11-peak algorithm based on their analysis of serum proteins. Their algorithm was shown to be more accurate than conventional biomarkers in identifying small HCC tumors. For diagnosing CCA, protein expression of biomarkers appears to be promising, such as matrix metalloproteinase-7 (75% sensitivity, 78% specificity), tumor type M2 pyruvate kinase (84.2% sensitivity, 90% specificity), serum mucin 5AC (71% sensitivity, 90% specificity), interleukin-6 level (73% sensitivity, 92% specificity), and carbohydrate antigen 19-9 (72% diagnostic sensitivity, 84% specificity). However, these biomarkers are useful for discriminating between benign and malignant biliary conditions only in patients who have undergone bile drainage.[35,36]

Stinton, *et al*.[37] found that the presence of proteinase 3-antineutrophil cytoplasmic antibodies may be a useful biomarker for differentiating PSC from other liver disease. They detected proteinase 3-antineutrophil cytoplasmic antibodies using a chemiluminescence immunoassay in 94 of 244 patients with PSC. This finding suggests a potential role of proteinase 3-antineutrophil cytoplasmic antibodies in the diagnosis of PSC in patients with higher concentration of liver enzymes.

Arbelaiz, *et al*.[38] published in the 4th issue of *Hepatology* in 2017 their research of other new biomarkers that may be useful for the early diagnosis of HCC, CCA, and PSC. These investigators isolated serum extracellular vesicles from the blood of patients with CCA (n = 43), PSC (n = 30), HCC (n = 29), and healthy controls (n = 32), and they characterized the protein contents. Extracellular vesicles have emerged as important mediators of intercellular communication because of their involvement in the transmission of biological signals and function as vehicles for transfer between cells of membrane and cytosolic proteins, lipids, and RNA. The pathophysiological roles of these vesicles are beginning to be recognized in diseases including cancer, infectious diseases, and neurodegenerative disorders, which suggests that they have potential as new targets for recognizing various disorders. Extracellular vesicles are generally referred to as microvesicles, ectosomes, shedding vesicles, or microparticles.[39] In the late 1990s, Zitvogel, *et al*. discovered that exosomes are released by B Lymphocytes and dendritic cells through a similar route.[40] In particular, exosomes have been isolated from diverse body fluids, including semen, blood, urine, saliva, breast milk, amniotic fluid, ascites fluid, cerebrospinal fluid, and bile.[41]

Arbelaiz, *et al*. used nanoparticle tracking analysis to measure the serum extracellular vesicle size and concentration as well as the transmission electron microscopy to study the morphology of these vesicles. They reported particle diameters of ~180 nm using nanoparticle tracking analysis and the presence of markers CD9, CD63, and CD81 by immunoblot analysis. They also compared the most highly expressed proteins between patient groups. Interestingly, they found a total of 95 proteins (exosomes) that were expressed in CCA patients *vs*. controls, 161 in PSC patients *vs*. controls, 50 in CCA patients *vs*. PSC patients, and 98 in HCC patients *vs*. controls. Gene ontology analysis demonstrated that the differentially expressed proteins are related to the response to wounding, defense to infections, inflammatory responses, and immune activation.

It is likely that there is a relationship between the protein expression and the pathophysiology of each disorder. Arbelaiz, *et al*. reported those biomarkers with the best diagnostic capacity. For example, their comparison between

CCA patients *vs*. healthy control identified aminopeptidase N, pantetheines, and polymeric immunoglobulin receptor with area-under-the-curve values of 0.878, 0.876, and 0.844, respectively. Comparison of serum extracellular vesicles from PSC patients *vs*. healthy controls identified Aminopeptidase N, Ficolin-1, and Neprilysin as having the best diagnostic capacity, with area-under-the-curve values of 0.789, 0.771, and 0.761, respectively. Surprisingly, the area-under-the-curve values in the patient groups seem to be in the range of a nonspecific serum tumor biomarker (CA 19-9) that is used in the diagnosis of CCA. Differential diagnosis between CCA and PSC is complicated, and Arbelaiz, *et al*. also compared these patient groups. They found that fibrinogen gamma chain, alpha-1-acid glycoprotein, and S100A8 protein showed the best differential diagnostic capacity, with area-under-the-curve values of 0.796, 0.794, and 0.759, respectively. The inclusion of the best biomarker candidates for the differential diagnosis of CCA *vs*. PSC improved their diagnostic values when comparing early stage of CCA (I–II) *vs*. PSC.

In summary, the study by Arbelaiz, *et al*. provides more accurate and valuable information for the future diagnosis of CCA, HCC, and PSC in individual patients. The use of proteomic technology facilitates the simultaneous measurement and analysis of several protein markers. This approach can be used to produce a protein profile signature from appropriate biological samples that can help to identify and diagnose CCA, HCC, and PSC. Many biomarkers discovered to date seem to be useful for distinguishing these disorders from normal or malignant pathologies and may be useful for providing an early differential diagnosis of CCA from PSC. The incorporation of these new promising markers should provide greater diagnostic value than the use of single markers alone. Importantly, researchers will continue to search specific and sensitive molecular markers for population screening for these diseases, which may lead to early diagnosis and improved outcomes.

ABBREVIATIONS

- **CCA:** cholangiocarcinoma.
- **HCC:** hepatocellular carcinoma.
- **PSC:** primary sclerosing cholangitis.

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Correspondence and reprint request:
Prof. Nahum Méndez-Sánchez, M.D., MSc, PhD.
Liver ResearchUnit, Medica Sur Clinic & Foundation,
Puente de Piedra No. 150, Col. Toriello Guerra, Z.P. 14050,
Mexico City, Mexico.
E-mail: nmendez@medicasur.org.mx