Biomarkers of Liver Cancer

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Hepatocellular Carcinoma (HCC)

- Sixth most common cancer and third leading cause of cancer-related death worldwide
- More common in men (2-4 times higher incidence than in women)
- Prognosis is poor in all regions of the world. As a result, incidence and mortality rates are roughly equivalent
- Median survival of patients with early HCC is > 5 years, but < 1 year when detected at an advanced stage
- Most HCC cases are detected at late stages and not when the tumor is localized and treatment options are more effective
- Less than 20% of at-risk patients receive surveillance
- Current surveillance strategies have limited sensitivity and specificity for early HCC detection
Cirrhosis is the single most important risk factor for HCC, being present in more than 80% of the cases.

One third of cirrhotic patients will develop HCC during their lifetime.

HBV is the leading cause of HCC worldwide and with HCV accounts for 71% of the cases, although there has been a risk reduction, but not elimination, with antiviral therapy.

Lancet 2018; 392: 1789-898
HCC Surveillance

Major goals:

- Detect HCC at early stage
- Implement treatment options
- Increase patient survival
## High-Risk Populations Recommended for HCC Surveillance

<table>
<thead>
<tr>
<th>Population</th>
<th>Annual incidence of HCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis of any etiology HBV related cirrhosis</td>
<td>3-8%</td>
</tr>
<tr>
<td>Asian male hepatitis B carriers over age 40</td>
<td>0.4-0.6%</td>
</tr>
<tr>
<td>Asian female hepatitis B carriers over age 50</td>
<td>0.3-0.6%</td>
</tr>
<tr>
<td>Africans and African-Americans with chronic hepatitis B over age 20</td>
<td>0.3-0.6%</td>
</tr>
<tr>
<td>Hepatitis B carriers with family history of HCC</td>
<td>Higher than without family history</td>
</tr>
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</table>

Marrero et al., AASLD Practice Guidance, Hepatology 2018
HCC Surveillance and Diagnostic Tests

- Serum biomarkers

- Imaging
  - Ultrasound
  - Computed tomography (CT)
  - Magnetic resonance imaging (MRI)
Biomarkers are molecules detected in the blood, urine, or other body fluids that indicate the presence of cancer or predict the risk of cancer development.

Ideally, biomarkers should:
- Allow early detection of cancer by screening healthy or high-risk populations
- Help to confirm the diagnosis or identify a specific type of cancer
- Predict prognosis
- Monitor treatment response
- Detect early recurrence
| Phase 1 — Preclinical Exploratory Studies | To identify promising biomarker candidates |
| Phase 2 — Clinical Assay and Validation | To detect the disease versus controls (e.g., distinguish HCC from non-HCC) |
| Phase 3 — Retrospective Longitudinal Repository Studies | To detect preclinical disease by retrospective analysis |
| Phase 4 — Prospective Screening Studies | To determine the detection rate of the assay (sensitivity and specificity) |
| Phase 5 — Cancer Control Studies | To assess the impact of screening on reducing the disease burden in the target population |

Biomarkers for HCC Diagnosis and Monitoring

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<th>Serum biomarkers in phase 2</th>
<th>More advanced serum biomarkers</th>
<th>Genetic and cellular biomarkers: “Liquid biopsy”</th>
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<td>• Osteopontin</td>
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<td>• Dikkopf-1</td>
<td>• DCP (phase 2/3)</td>
<td>• MicroRNA (miRNA)</td>
</tr>
<tr>
<td>• Glypican-3</td>
<td></td>
<td>• Long-noncoding RNA (lncRNA)</td>
</tr>
<tr>
<td>• Alpha-1 fucosidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Golgi Protein 73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• SCCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Others</td>
<td></td>
<td></td>
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</table>
Alpha-Fetoprotein (AFP)

- AFP is the best characterized and most widely used serum biomarker for HCC surveillance. However, not all HCCs secrete AFP.

- There is little debate that AFP should not be used alone in HCC surveillance.

- There is great debate on whether AFP should be included in HCC surveillance due to its suboptimal sensitivity (39% to 65%) and specificity (76% to 97%).

- However, most studies showed a benefit of the combination of AFP with ultrasound in HCC surveillance.
### Guidelines for HCC Surveillance in High-Risk Populations

<table>
<thead>
<tr>
<th>Society/Institution</th>
<th>Guidelines</th>
</tr>
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</table>
| **AASLD**<sup>1</sup>  
American Association for the Study of Liver Diseases | US every 6 months with or without AFP |
| **EASL**<sup>2</sup>  
European Association for the Study of the Liver | US every 6 months |
| **APASL**<sup>3</sup>  
Asian-Pacific Association for the Study of the Liver | US every 6 months with AFP |
| **JSH-HCC**<sup>4</sup>  
Japan Society of Hepatology | High-risk: US every 6 months + AFP/DCP/AFP-L3 every 6 months  
Very High-risk: US every 3-4 months + AFP/DCP/AFP-L3 every 3-4 months + CT/MRI (optional) every 6-12 months |

AFP = alpha-fetoprotein; AFP-L3 = *Lens culinaris* agglutin-reactive fraction of AFP; CT = computerized tomography; DCP = des-γ-carboxyprothrombin; MRI = magnetic resonance imaging; US = ultrasound.

Its sensitivity for detecting HCC at an early stage is highly variable, ranging from 21% to 89%.

- Operator dependent
  - Based on skill of the sonographer

- Influenced by patient characteristics
  - Obesity
  - Liver nodularity
  - Ascites
Various Factors May Influence the Performance of AFP as HCC Biomarker

- Patient demographics
- Etiology of underlying liver disease
- Severity of liver disease (cirrhosis, chronic hepatitis, ALT values)
- Antiviral treatment
- Tumor stage
- Tumor biology
What is the Best Strategy for Early HCC Detection?
Meta-analysis of 32 studies, comprising 13,367 patients, compared the performance of ultrasound alone versus ultrasound plus AFP for the early detection of HCC in patients with cirrhosis.
Ultrasound alone detected early-stage HCC with a sensitivity of 45% compared to 63% when ultrasound was combined with AFP. The improved sensitivity was associated with a decrease in specificity (84% vs 92%).

Addition of AFP to ultrasound significantly increases the sensitivity of early HCC detection, suggesting this may be the preferred surveillance strategy for patients with cirrhosis.
Strategies to Improve the Performance of HCC Biomarkers

AFP

- Single time-point vs. longitudinal analysis
- Tailoring cut-off according to:
  - Liver disease etiology
  - Severity
  - Antiviral treatment
Longitudinal Determinations Can Improve the Performance of AFP

Lee et al. Clin Gastro Hep 2013
Longitudinal Assessment of Three Serum Biomarkers to Detect Very Early-Stage Hepatocellular Carcinoma

Jonggi Choi, Gi-Ae Kim, Seungbong Han, Woochang Lee, Sail Chun, and Young-Suk Lim

- Phase-3 biomarker study to evaluate the surveillance performance of AFP, lectin-reactive AFP (AFP-L3), des-γ-carboxy prothrombin (DCP), and their combinations for the early detection of HCC in prospectively collected longitudinal samples.

- Nested case–control study in which serum was analyzed at 0, 6, and 12 months prior to the diagnosis of HCC in 42 cases and 168 matched controls.

- The majority (79%) was chronically infected with HBV; all were virally suppressed (HBV DNA <2,000 IU/mL), and 28 (85%) had undetectable serum HBV DNA (<15 IU/mL).

- The majority (86%) also had normal ALT levels; 39 (93%) had cirrhosis, and 31/42 (74%) had very early-stage HCC (single <2 cm).
Longitudinal Analysis of Three Serum Biomarkers in HCC Cases and Matched Controls

Choi et al., Hepatology, VOL. 69, NO. 5, 2019
Combination of AFP and AFP-L3 at Diagnosis Differentiates Early-Stage HCC from Cirrhosis Better than Individual Biomarkers

AFP cut-off: >5 ng/mL

Choi et al., Hepatology, VOL . 69, NO. 5, 2019
### Sensitivity and Specificity of Ultrasound (US) Alone or in Combination with Biomarkers

**HCC cases: n=35**  
**Matched controls: n=168**

<table>
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<tr>
<th>Surveillance test at the time of diagnosis</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>US alone</td>
<td>48.6%</td>
<td>96.4%</td>
</tr>
<tr>
<td>US + AFP</td>
<td>88.6%</td>
<td>82.7%</td>
</tr>
<tr>
<td>US + AFP + AFP-L3</td>
<td>94.3%</td>
<td>82.7%</td>
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Adding AFP to US increased sensitivity to 88.6%, and adding AFP + AFP-L3 to US increased sensitivity to 94.3%

Choi et al., Hepatology, VOL. 69, NO. 5, 2019
## GALAD Score

**Gender, Age, AFP-L3, AFP, DCP**

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<th>GALAD Model</th>
<th>Early HCC (within Milan criteria)</th>
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<tr>
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<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
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<tr>
<td>UK</td>
<td>80.2</td>
<td>89.7</td>
</tr>
<tr>
<td>Japan</td>
<td>82.1</td>
<td>81.6</td>
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Berhane et al. Clinical Gastroenterology and Hepatology 2016;14:875–886
GALAD Score

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GALAD model improved ROC curves compared to individuals biomarkers for early HCC

Berhane et al. Clinical Gastroenterology and Hepatology 2016;14:875–886
GALAD Score for the Detection of Early-Stage HCC Performs Well in Patients with Different Etiologies

United States: NCI Multicenter EDRN cohort (phase-2 study)

- 233 consecutive early-stage HCC
- 412 patients with cirrhosis

Yang J D et al., Cancer Epidemiology, Biomarkers & Prevention, 2019
The addition of ultrasound to GALAD (GALADUS Score) further enhanced the performance, although the clinical benefit remains to be established.

Studies are under way to evaluate the performance of GALADUS versus GALAD in comparison to ultrasound alone in a large multicenter phase-3 biomarker study (HEDS) in the United States.
# Biomarkers for HCC Diagnosis and Monitoring

**Serum biomarkers in phase 2**
- Osteopontin
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- Alpha-1 fucosidase
- Golgi Protein 73
- SCCA
- Others

**More advanced serum biomarkers**
- AFP (phase 5)
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- DCP (phase 2/3)

**Genetic and cellular biomarkers: “Liquid biopsy”**
- Circulating tumor cells (CTC)
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- MicroRNA (miRNA)
- Long-noncoding RNA (IncRNA)

**Biomarkers for HCC Diagnosis and Monitoring**

More advanced serum biomarkers
Biomarkers in Liver Cancer: Summary

- The addition of AFP to ultrasound imaging significantly improves the early detection of HCC, although the results are still suboptimal.

- Longitudinal determination of AFP appears to increase the sensitivity and specificity for HCC surveillance.

- Additional studies are necessary to establish the best cut-off values for AFP and other biomarkers for HCC surveillance in HBV-suppressed patients with minimal hepatic inflammation.

- Given the high degree of heterogeneity of HCC, combination of AFP with other biomarkers and clinical parameters appears to improve the sensitivity and specificity of surveillance for the early detection of HCC.

- The recent expansion of the landscape of HCC biomarkers holds promise for the future and may pave the way for tailoring surveillance using a personalized approach based on individual risk factors.