



CÁC DẤU ẤN HIỆN TẠI VÀ MỚI LIÊN QUAN UNG THƯ BIỂU MÔ TẾ BÀO GAN- HCC. ỨNG DỤNG LÂM SÀNG



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8-2023

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NỘI DUNG

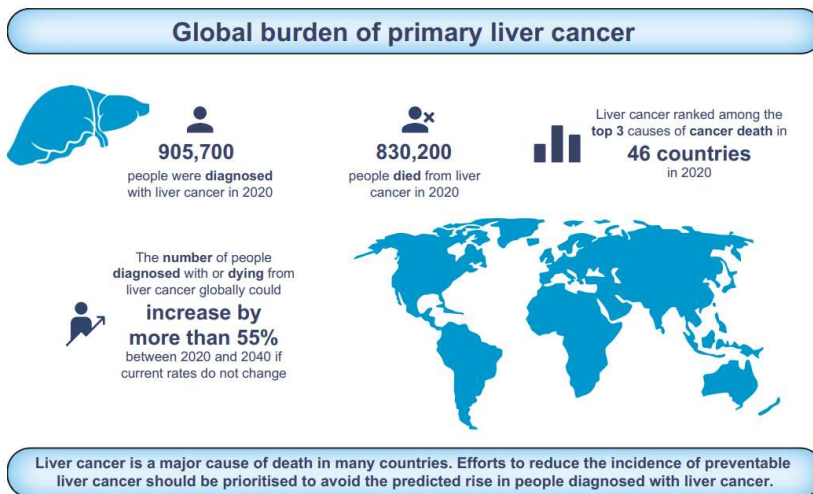
- I. Tại sao rất cần chẩn đoán sớm HCC.
- II. Chẩn đoán HCC.
 - A. Chẩn đoán hình ảnh.
 - B. Chỉ dấu xét nghiệm máu.
- III. Ứng dụng lâm sàng.
- IV. Kết luận.



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I. Tại sao rất cần chẩn đoán sớm HCC ?

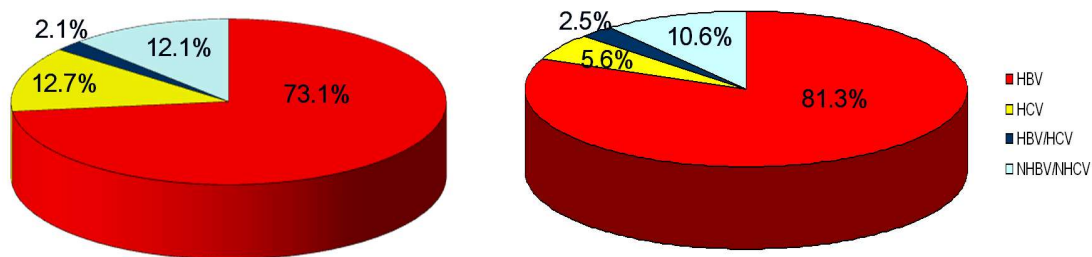
Global burden of primary liver cancer in 2020 and predictions to 2040



Harriet Rungay et al. *Journal of Hepatology* 2022 vol. 77 | 1598–1606

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HBV là nguyên nhân chủ yếu của HCC ở VIỆT NAM



Nguyen et al. ¹

Le et al. ²

1, Nguyen-Dinh SH et al. *Viruses* 2022, 14, 2571. <https://doi.org/10.3390/v14112571>

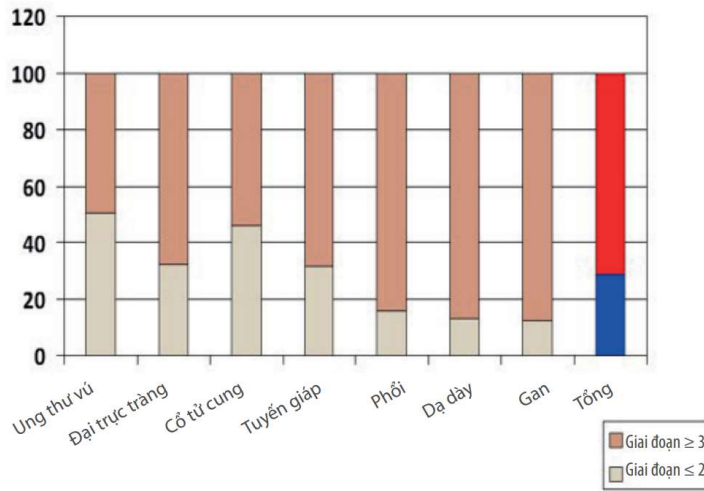
2, Le et al. *Cancer Control* Volume 26: 1-6, 2019.



Dhanasekaran R et al. *F1000Res* 2016

4

UNG THƯ GAN THƯỜNG PHÁT HIỆN TRỄ: DỰ HẬU XẤU



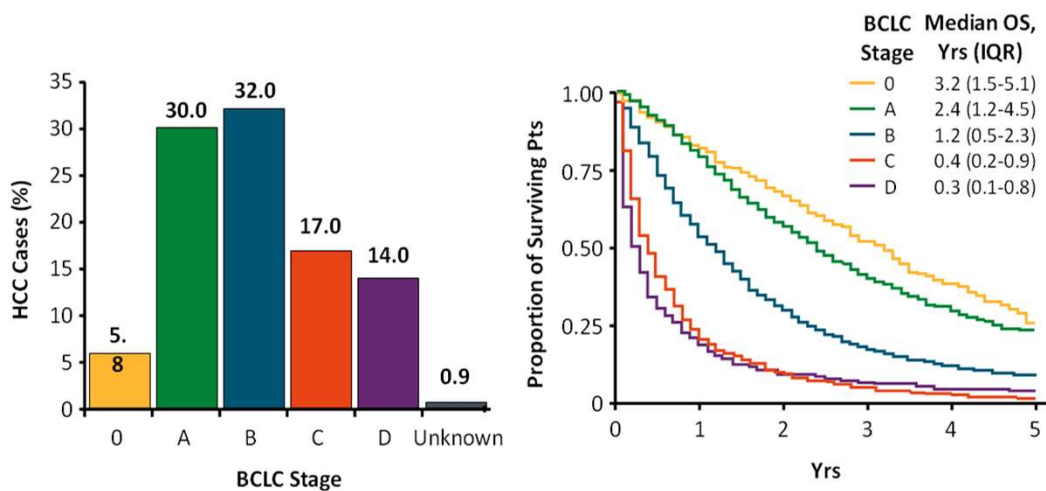
Dữ liệu từ 5 bệnh viện: Bệnh viện K, Bệnh viện Ung bướu Hà Nội, BV Bạch Mai, Việt Tiệp - Hải Phòng và BV Huế.

Phân bố các ca ung thư theo giai đoạn chẩn đoán ở 5 bệnh viện tại Việt Nam, 2009

TÌNH HÌNH BỆNH VIÊM GAN VI RÚT VÀ ĐÁP ỨNG Ở VIỆT NAM - WHO representative office for Viet Nam 2019

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Stage at Presentation and Survival Curves

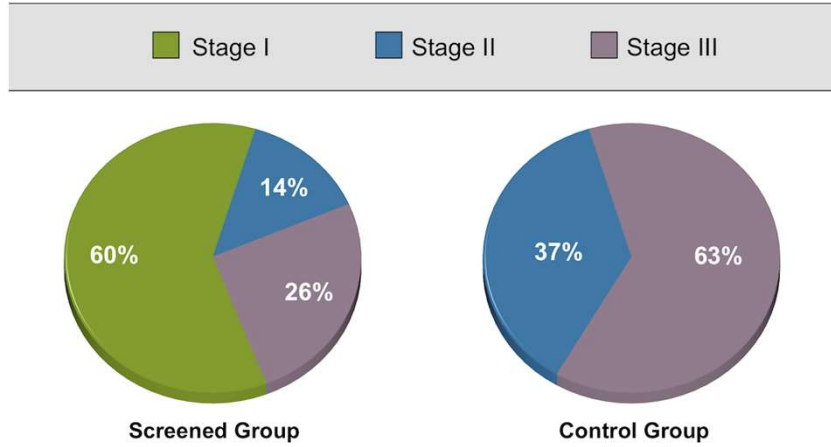


Serper. Gastroenterology. 2017;152:1954.

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Quan trọng là kiểm tra định kỳ: Phát hiện sớm HCC.

Impact of Screening on Stage of HCC at Time of Diagnosis



In a trial performed in Shanghai, China, more than 18,000 persons with chronic viral hepatitis (most of whom had chronic hepatitis B), were randomized to screening for HCC or no screening (control). As shown, individuals who received screening were more likely to have their HCC diagnosed at an earlier stage (Stage 1) than those who did not have screening.

Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130:417-22.

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II. Chẩn đoán HCC



- Tuổi.
- Yếu tố gia đình.
- Sắc tộc.
- Độ xơ hóa gan.
- Yếu tố bản thân.

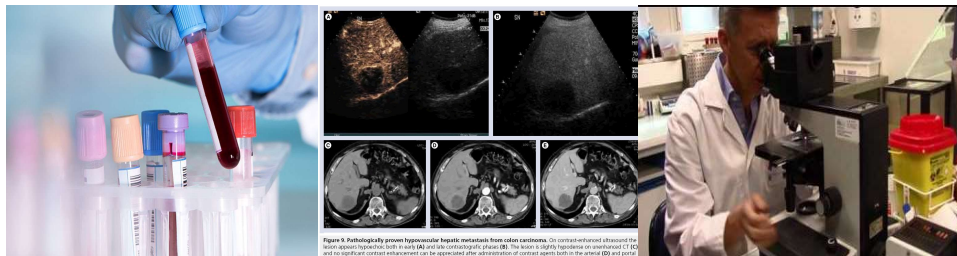
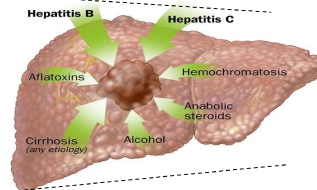


Figure 9. Pathologically proven hepatocellular hepatic metastasis from colon carcinoma. On contrast-enhanced ultrasound the lesion showed hyperechoic foci in early (A) and late (B) phases (C). The same is clearly dependent on conventional CT (D) and no significant contrast enhancement can be appreciated after administration of contrast agents both in the arterial (E) and portal (F) phases (G).

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Summary of current international guidelines on HCC surveillance

Guidelines	Surveillance population	Surveillance modality	Surveillance interval
AASLD 2018 ⁴⁰	All patients with liver cirrhosis except patients with Child–Pugh stage C cirrhosis unless on transplant waiting list	US ± AFP	6 months
APASL 2017 ⁴¹	All patients with cirrhosis Chronic HBV carriers without cirrhosis <ul style="list-style-type: none"> • Asian females >50 years • Asian males >40 years • Africans >20 years • Family history of HCC 	US + AFP	6 months
EASL 2018 ²⁴	Cirrhosis Child–Pugh stage A and B Cirrhosis Child–Pugh stage C awaiting liver transplant Chronic HBV without cirrhosis at intermediate (10–17) or high risk (≥18) of HCC according to PAGE-B score Non-cirrhotic patients with Metavir F3 fibrosis regardless of etiology	US	6 months
ESMO 2018 ⁴²	All patients with cirrhosis as long as liver function and comorbidities allow curative or palliative treatment Chronic HBV and HCV carriers with Metavir F3 fibrosis Asian chronic HBV carriers with serum HBV-DNA above 10,000 copies/mL	US ± AFP	6 months

AASLD, American Association for the Study of Liver Disease; AFP, alpha fetoprotein; APASL, Asian Pacific Association for the Study of the Liver; EASL, European Association for the Study of the Liver; ESMO, European Society for Medical Oncology; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PAGE-B score, Platelets, Age, Gender, Hepatitis B; US, ultrasound.

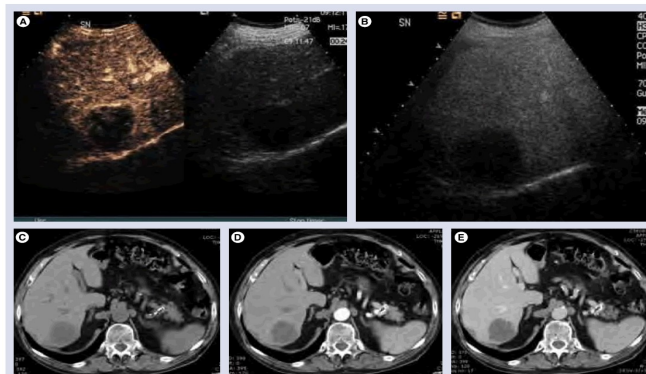
Geh et al. Journal of Hepatocellular Carcinoma 2019;6

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A. CÁC KỸ THUẬT CHẨN ĐOÁN HÌNH ẢNH.

Chẩn đoán hình ảnh giữ 1 vai trò không thể thiếu trong chẩn đoán HCC: Từ bệnh cảnh lâm sàng, triệu chứng cũng như xét nghiệm đều hướng đến HCC nhưng hình ảnh học không xác định được u ở đâu thì cũng không thể chẩn đoán là HCC.

Kỹ thuật chẩn đoán hình ảnh giúp chẩn đoán vị trí u, kích thước, số lượng u, giai đoạn, mức độ cũng như giữ vai trò tầm soát bệnh.



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ĐẶC ĐIỂM CỦA CÁC PHƯƠNG TIỆN CHẨN ĐOÁN HÌNH ẢNH PHÁT HIỆN HCC

PHƯƠNG TIỆN HÌNH ẢNH	THUẬN LỢI	KHÔNG THUẬN LỢI
Siêu âm	Không xâm lấn Có sẵn ở mọi nơi Chi phí thấp	Kết quả tùy thuộc vào kinh nghiệm và kỹ năng của bác sĩ siêu âm Béo phì Đánh giá mô mềm Độ nhạy thấp
MSCT bụng	Độ nhạy cao (80%)	Nguy cơ nhiễm xạ Chi phí cao
MRI bụng	Độ nhạy cao (86%) Độ phân giải cao	Sử dụng hạn chế Chi phí cao

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B. VAI TRÒ CÁC CHỈ DẤU XÉT NGHIỆM MÁU TRONG CHẨN ĐOÁN UNG THƯ GAN.

- Alpha-fetoprotein (AFP)
- AFP-L3%
- Des-gamma carboxyprothrombin (DCP)
- Thuật toán
- Artificial Intelligence and HCC
- Ct DNA
- HBcrAg
- M2BPGi

Debes JD et al. Cancers 2021

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1. Alpha-fetoprotein (AFP)

- Glucoprotein AFP được sản xuất bởi:
 - ✓ Tế bào gan bình thường của thai nhi.
 - ✓ Tế bào gan tăng sinh bình thường.
 - ✓ Một số HCC.
 - ✓ Ung thư tinh hoàn.
- Độ nhạy và độ đặc hiệu tổng thể thấp.
- AFP > 400-500 ng /ml được xem là chẩn đoán HCC.
- AFP không có trong khuyến cáo AASLD hiện tại cho việc theo dõi HCC.

Sherman M. Clin Liver Dis. 2011; 15: 323-334

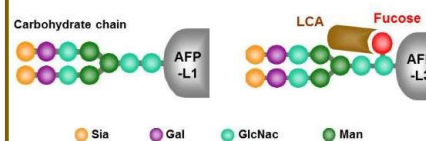
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2. HCC Surveillance Biomarker: Alpha-Fetoprotein-L3 (AFP-L3)

- AFP-L3 is a fucosylated isoform of AFP.
- AFP-L3 binds to lectin Lens culinaris (lentil) agglutinin (LCA) which interacts with AFP-L3 but not AFP-L1 (majority of AFP).
- Relevance of AFP-L3 to HCC:
 - AFP-L3 has been shown to be elevated in patients with HCC. Elevation of L3 occurs early in HCC
 - AFP-L3 (%) is highly specific for HCC

$$\text{AFP-L3 (\%)} = \frac{\text{AFP-L3 (ng/mL)}}{\text{Total AFP (ng/mL)}} \times 100$$

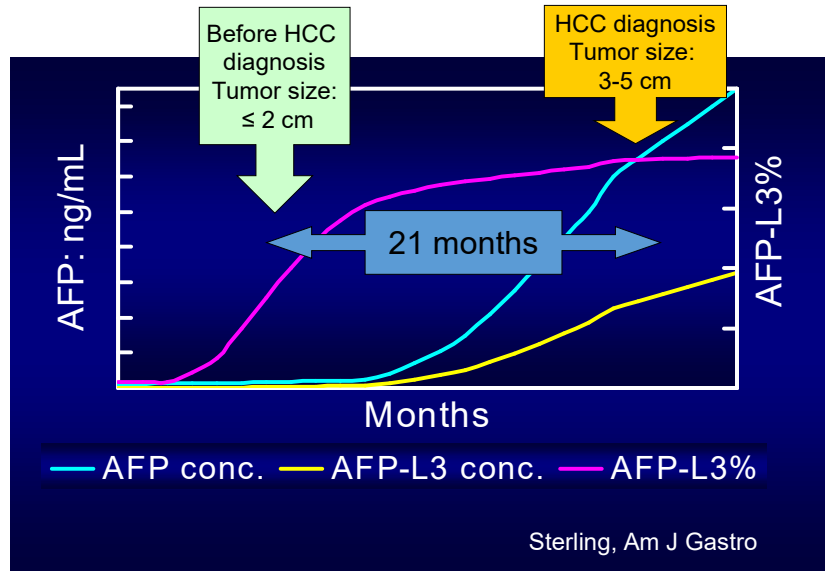
Cut-off Point: 10% (Intended Use)



Sato Y, et al. *N Engl J Med.* 1993;328:1802-6; Makuuchi M, et al. *Hepato Res.* 2008;38:37-51.

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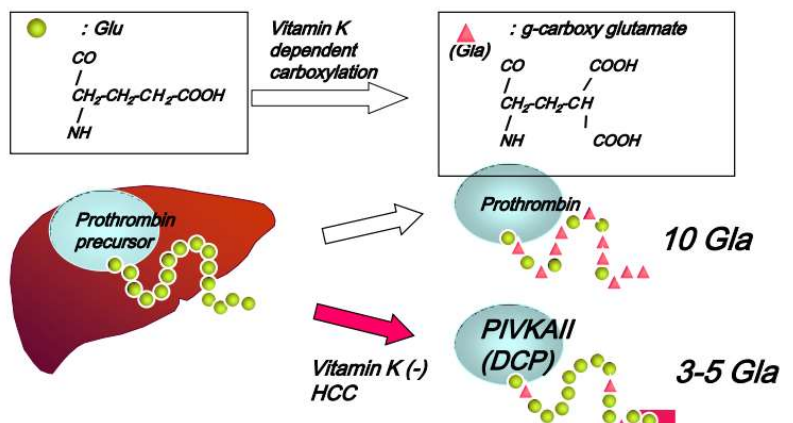
AFP L3% rises before AFP in typical course of HCC occurrence case



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3. PIVKA II (DCP)

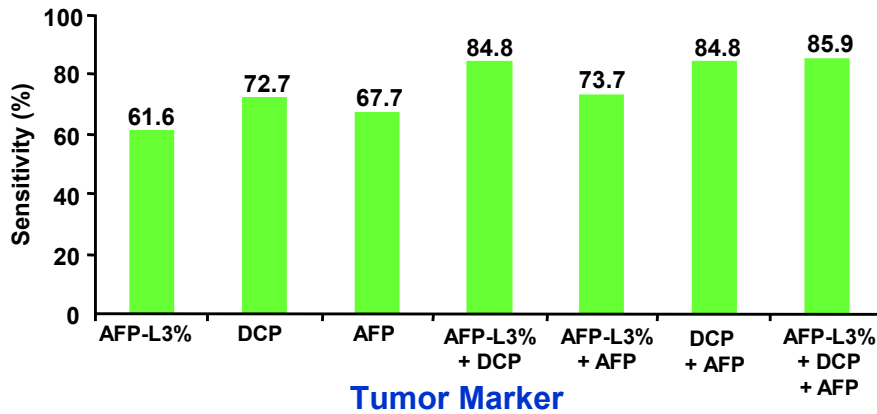
- PIVKA II (DCP) là một dạng bất thường của prothrombin tăng trong huyết thanh của bệnh nhân mắc bệnh HCC.



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Current Surveillance Tests Are Not Sufficiently Sensitive

- Prospective analysis of 99 patients with histologically proven, unresectable HCC



AFP –L3 Lens culinaris fraction of AFP

Carr BI, et al. Dig Dis Sci. 2007;52:776-782.

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Inclusion of AFP-L3 & DCP Improves Early Detection

	Primary Tool	Sensitivity	Specificity	Conclusions
Colli et al (2006) ¹	Ultrasound	60% (95% CI 44-76%)	97% (95% CI 95-98%)	<i>“Ultrasound is...insufficiently sensitive to detect HCC in many cirrhotics or to support an effective surveillance program.”</i>
Singal et al (2012) ²	Ultrasound	43.9%	91.5%	Ultrasound is suboptimal when used alone
Volk et al (2007) ³	AFP, AFP-L3 & DCP	88%	91%	Combined use of AFP, AFP-L3 and DCP results in early detection of HCC with minimal false positives
Hann et al (2013) ⁴	AFP, AFP-L3 & DCP	83%	>90%	

Colli A, et al. *Am J Gastroenterol.* 2006;101:513-23; Singal, et al. *Effectiveness of Hepatocellular Carcinoma Surveillance in Patients with Cirrhosis.* *Cancer Epidemiol Biomarkers Prev.* May 2012 21; 793; Volk, et al. *Cancer Bio* 3. 2007, 79-87. Hann, et al. Potential usefulness of highly sensitive AFP-L3% and DCP in risk assessment in surveillance of patient at risk for HCC with total AFP in reference range. *Gastroenterology.* Vol. 144, Issue 5, Supplement 1, Page S-1040.

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4. Thuật toán

GALAD score: A valuable tool to diagnose HCC?

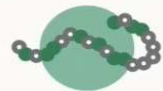
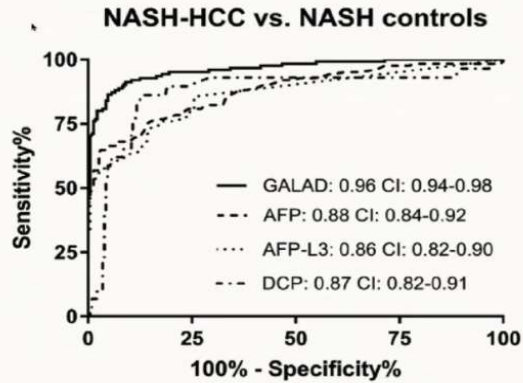
Clinical parameters

Gender
Age



Biomarker

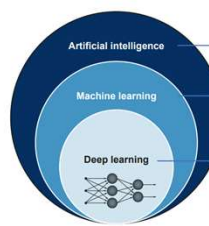
AFP-L3
AFP
DCP (PIVKA)

Best J, et al. Clinical Gastroenterology and Hepatology 2020



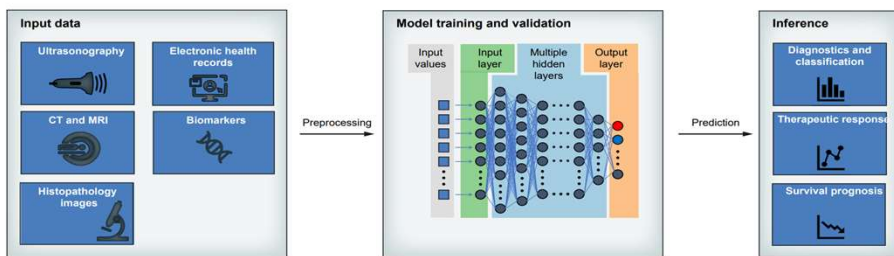
5. Artificial Intelligence and HCC



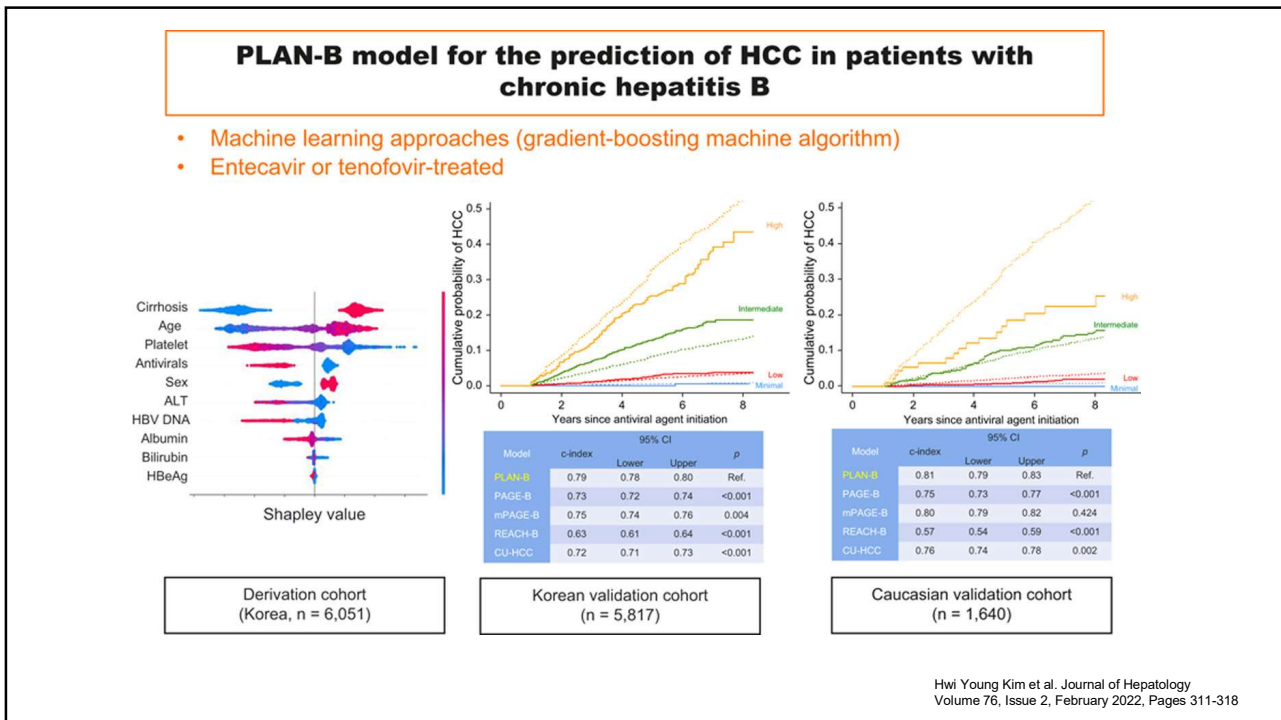
Artificial Intelligence (AI):
Computer programs designed to mimic human intelligence and/or behavior, including learning, adapting and problem-solving

Machine learning (ML):
Subtype of AI, in which computer programs are enabled to "learn" from data and improve with experience

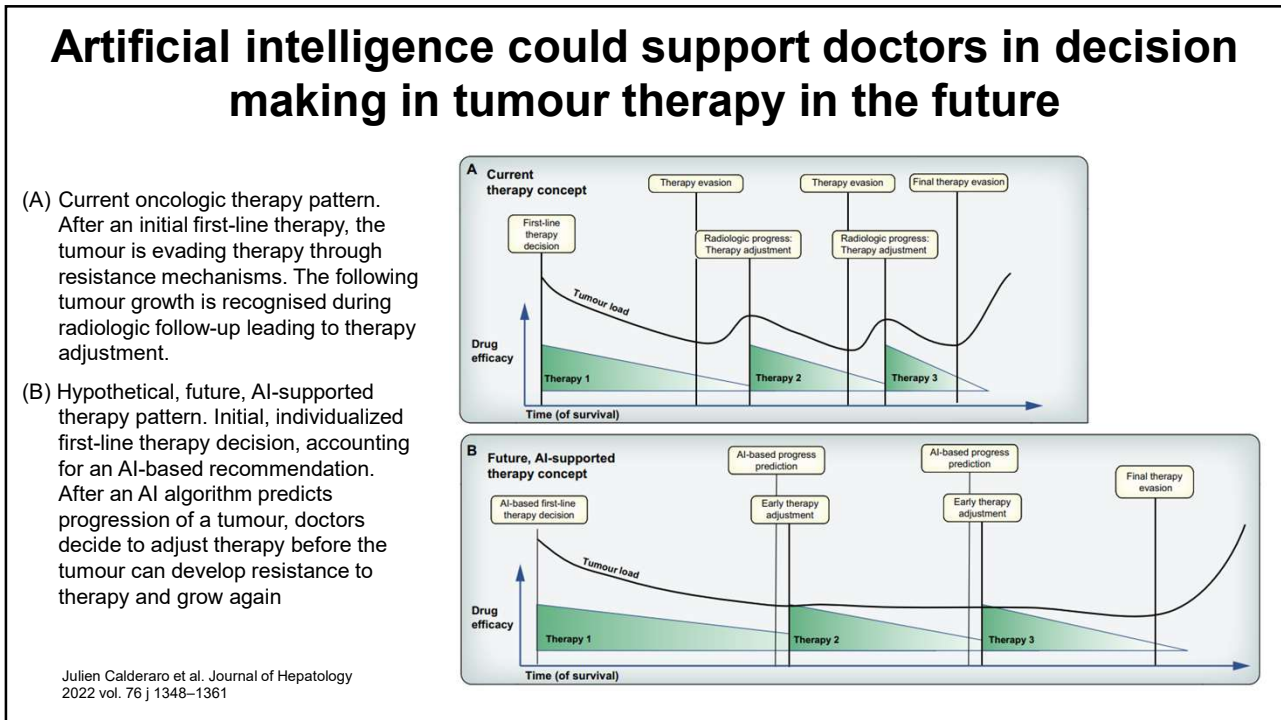
Deep learning (DL):
Subtype of ML inspired by the human brain, in which programs utilize the complex architecture of multi-layered neural networks to analyze large amounts of data



Julien Calderaro et al. Journal of Hepatology 2022 vol. 76 | 1348-1361



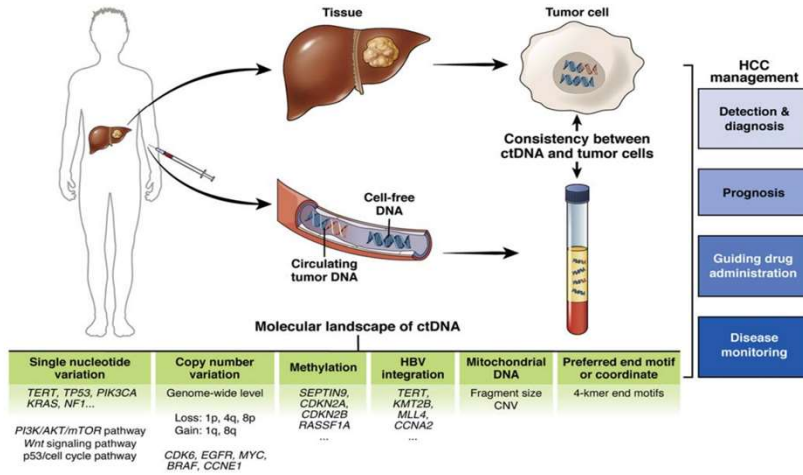
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6. CtDNA

An overview of the molecular landscape of ctDNA and its relevance in the clinical management of HCC.

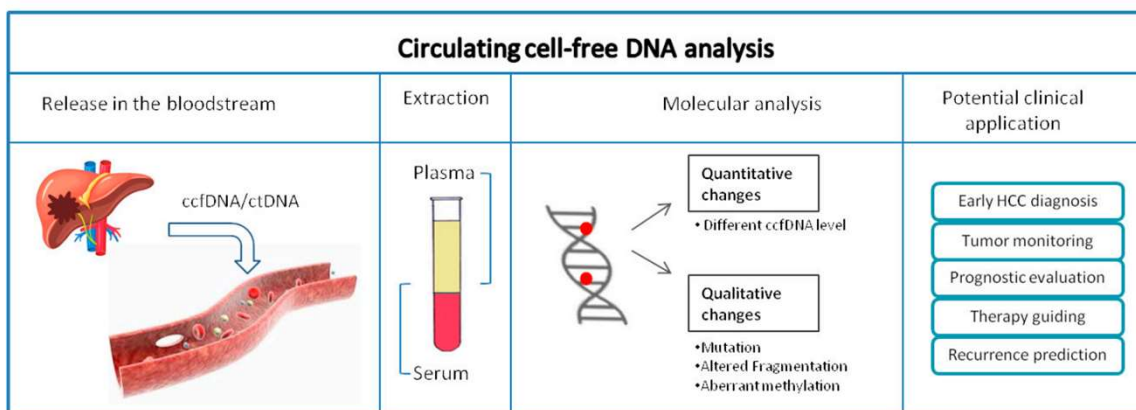


ctDNA originates from the tumor tissue and carries the same genetic aberrations as the tumor cells. With the consistency between ctDNA and tumor cells, ctDNA as a form of liquid biopsy could potentially be used for different aspects in the clinical management of HCC.

Lyu et al. Cellular and Molecular Gastroenterology and Hepatology Vol. 13, No. 6. 2022, Pages 1611-1624.

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Schematic overview of circulating cell-free DNA analysis and its potential clinical application in hepatocellular carcinoma (HCC) setting.

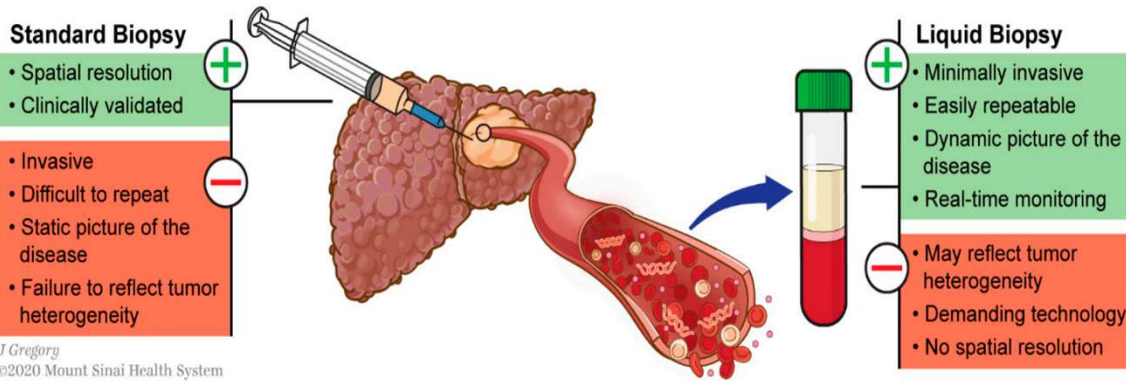


Circulating cell-free DNA (ccfDNA) can be released in the bloodstream from a variety of different cells under physiological and pathophysiological conditions. In cancer patients, a fraction of ccfDNA comprises circulating tumor DNA (ctDNA). ccfDNA can enter systemic circulation where can be isolated from serum or plasma. ccfDNA can undergo both quantitative (i.e., monitoring of changes in the ccfDNA concentration) and qualitative (somatic mutational profile, altered fragmentation and aberrant methylation pattern) analysis. The evaluation of the ccfDNA and its tumoral fraction, ctDNA, can improve the management of HCC patients permitting an early diagnosis, a better tumor monitoring (i.e., recurrence prediction, supervision of the dynamic tumor evolution) and an improved therapy outcome prediction that finally help clinicians in the treatment decision making.

Silvia Mezzalana et al. Int. J. Mol. Sci. 2019, 20, 5498

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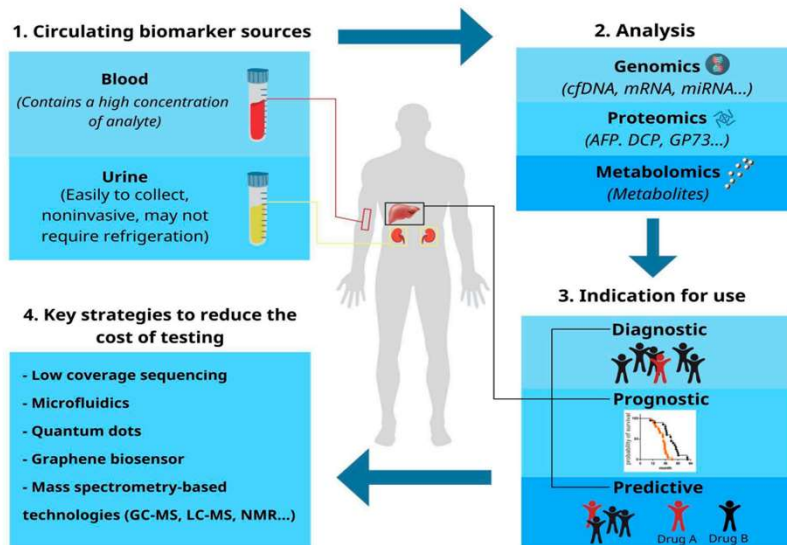
Pros and cons of liquid biopsy versus standard biopsy.



Ismail Labgaa et al. Cancers Cancers2021 2021, , 1313, 659

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Circulating biomarkers of HCC



Shown are two types of circulating biomarkers (blood and urine), the proposed strategies to reduce the cost of testing, and their potential applications in LRS for HCC screening, diagnosis, and management.

Annabelle Pan et al. Diagnostics 2023, 13, 676. <https://doi.org/10.3390/diagnostics13040676>

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Combined methylated DNA and protein markers: an accurate blood-based test for early-stage detection of hepatocellular carcinoma

Aim:

To identify a panel of blood-based biomarkers with high sensitivity for early-stage detection of hepatocellular carcinoma

Methods:

- Multi-center, case-control study
- Patient population: 135 HCC cases; 305 age- and liver disease etiology-matched controls
- Whole blood collected at clinical sites and shipped to central lab for processing; samples blinded upon delivery
- 10 methylated DNA markers (MDMs) and multiple proteins evaluated via logistic regression algorithm to classify samples as positive or negative for HCC

Main Findings:

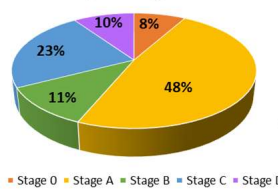
At 90% specificity, a panel of 4 MDMs (DAB2IP, EMX1, HOXA1, TSPYL5) and 2 proteins (AFP, AFP-L3) detected 71% of early-stage HCC.

Conclusions:

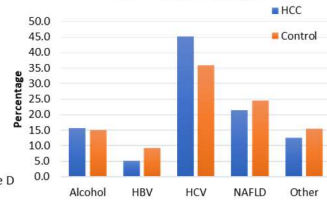
We identified a panel of 6 biomarkers with significantly higher sensitivity for early-stage HCC compared to AFP with or without AFP-L3.

Chalasanani N, et al., Abstract 209

Distribution of HCC Cases by BCLC Stage



Distribution of Liver Disease Etiologies



Biomarker Panel	Early Stage* Sensitivity (95% CI)	All Stage Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
Exact (4 MDM + 2 Protein)	71% (60-81%)	80% (72-86%)	90% (86-93%)	0.912 (89-94%)
AFP (20 ng/mL)	21% (13-32%)	43% (35-52%)	98% (95-99%)	0.706 (66-76%)
AFP (100 ng/mL)	6.6% (2-15%)	27% (20-36%)	100% (99-100%)	0.637 (58-69%)
AFP (5 ng/mL) + AFP-L3 (4%)	37% (26-49%)	55% (46-63%)	94% (90-96%)	0.795 (75-84%)

*Early Stage = BCLC Stage 0 and A



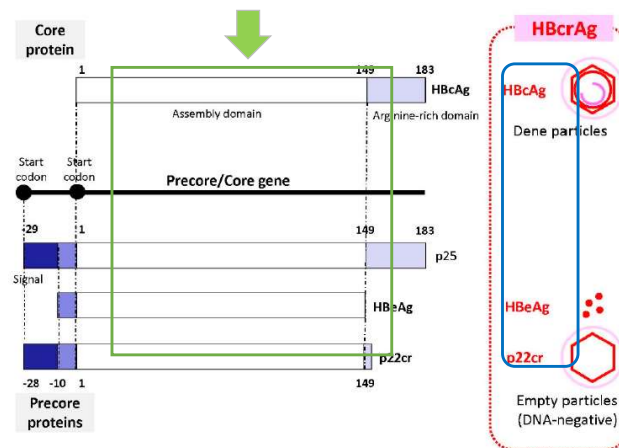
THE BEST OF THE LIVER MEETING® 2019 | LIVER AND BILIARY CANCER | 5
© 2019 American Association for the Study of Liver Diseases. Not for Commercial Use



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7. GIỚI THIỆU XÉT NGHIỆM HBcrAg

- Xét nghiệm HBcrAg phát hiện đồng thời 3 thành phần: HBcAg, HBeAg và protein p22cr trên cùng gen C của vi rút viêm gan B.
- Cả 3 kháng nguyên này có cùng vị trí nhận diện kháng nguyên 149 acid amin.



- **Ngưỡng phân tích:** 3,0-7,0 LogU/mL (1,0-10.000,0 kU/mL)

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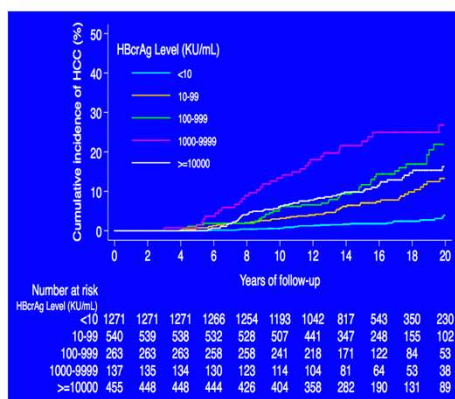
Ý NGHĨA HBcrAg TRONG THỰC HÀNH.

1. Theo dõi các phase trong HBV.
2. Liên quan HBV DNA.
3. Tương quan cccDNA.
4. Đánh giá hiệu quả điều trị. Quyết định ngưng điều trị.
5. Phát hiện HBV tiềm ẩn.
6. Dự đoán xơ gan, HCC.
7. Dự đoán tái hoạt HBV.

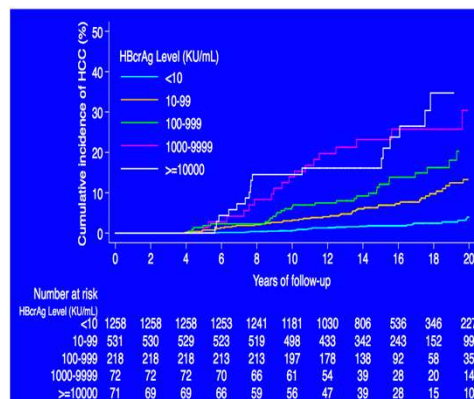
29

Mối tương quan giữa HBcrAg và HCC

Tất cả bệnh nhân



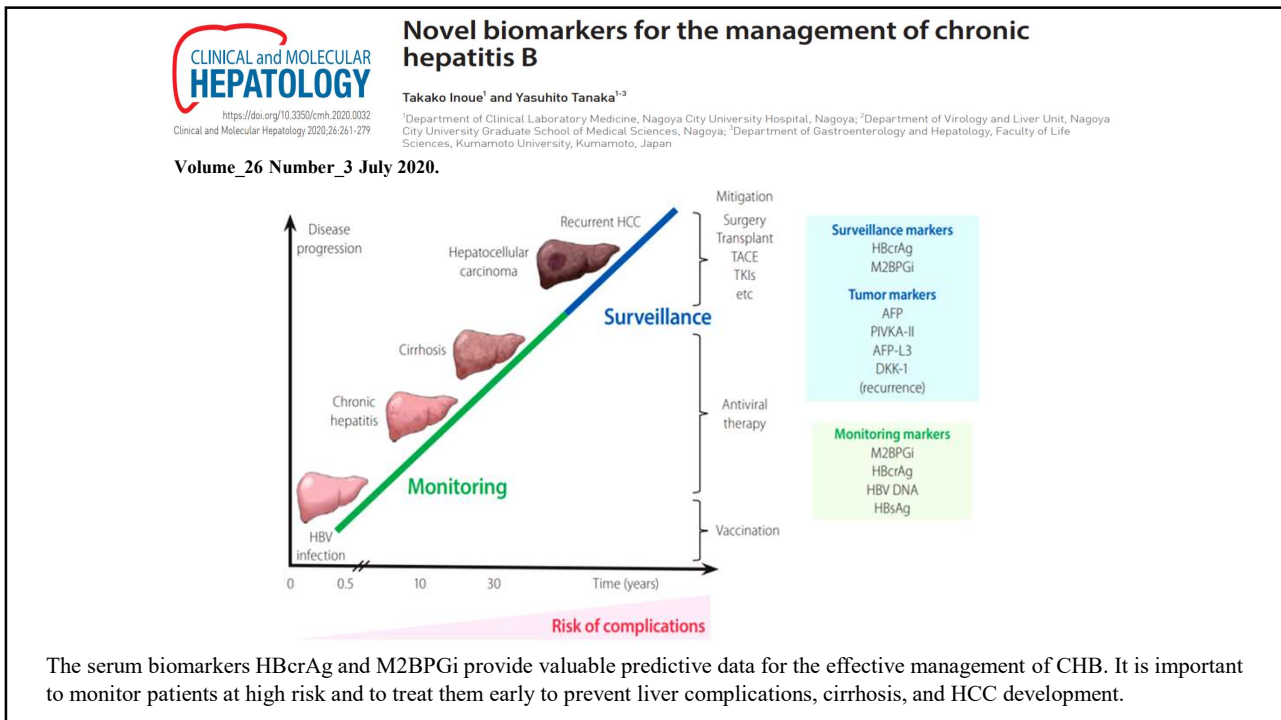
Bệnh nhân HBeAg-negative



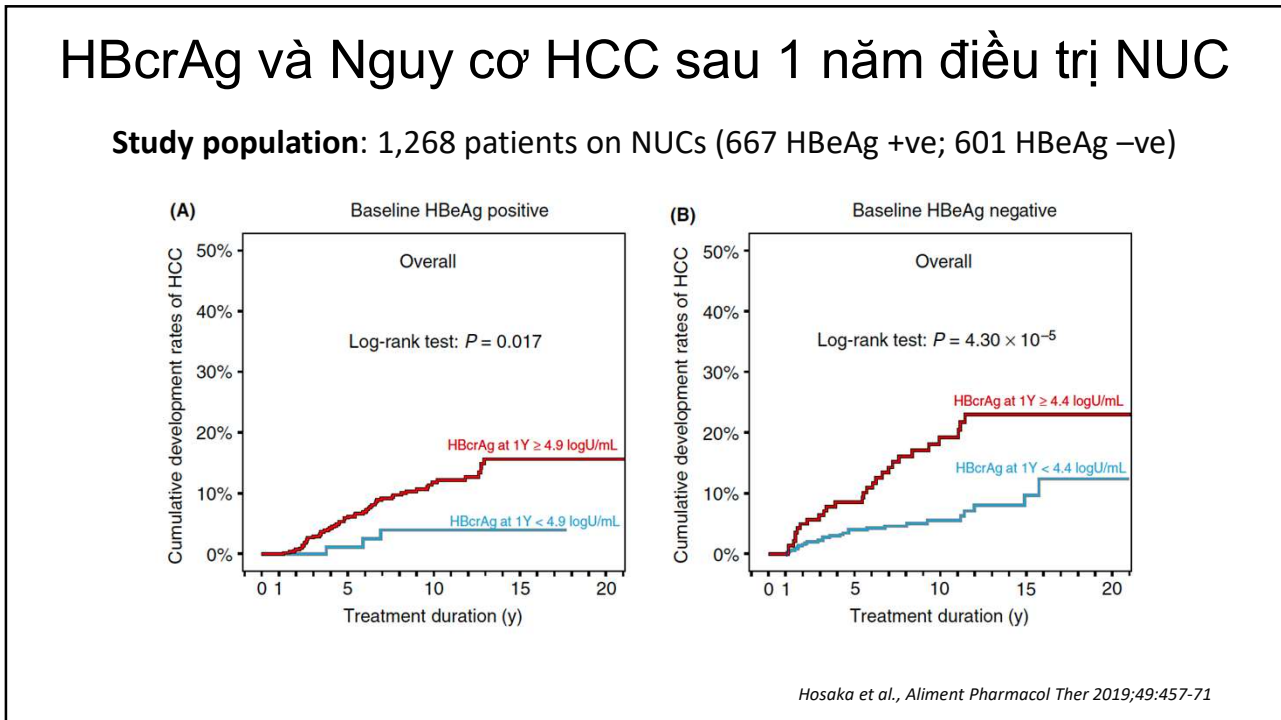
Ngưỡng cắt của HBcrAg: 10KU/ml hay 4 LogU/ml, có nguy cơ HCC rất cao.

Tseng and Kao, Gastroenterology 2012

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8. Vai Trò M2BPGi Trong Đánh Giá Xơ Hóa Gan / HCC

Collaboration between RCMG of AIST and RCHI of NCGM



Prof. Narimatsu H.
National Institute of
Advanced Industrial Science
and Technology, Japan



Prof. Mizokami M.
National Center for
Global Health and
Medicine, Japan.

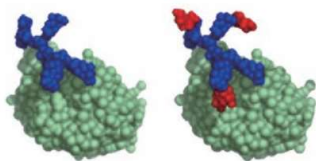
**New
Technologies**



**Material
with clinical data**

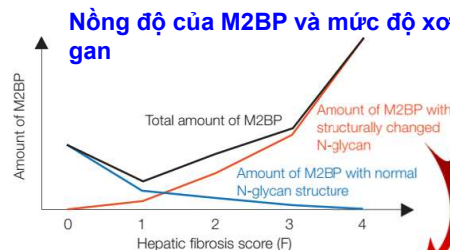
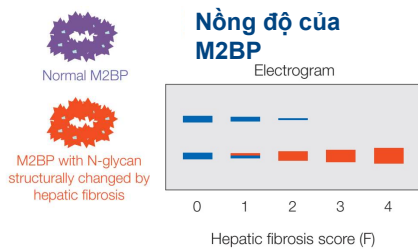
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Sự thay đổi cấu trúc chuỗi đường của M2BP cùng với sự phát triển của xơ gan



Tiến triển của xơ gan

- Cấu trúc chuỗi trên M2BP sẽ bị thay đổi theo quá trình xơ hóa gan mặc dù cấu trúc của protein không thay đổi.



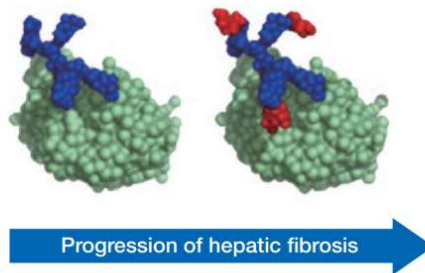
M2BP Glycosylation isomer (M2BPGi)

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Ứng dụng lâm sàng của M2BPGi

1. Là marker phản ánh hoạt động của HSCs, các HSCs đóng vai trò chủ chốt trong tiến triển xơ hóa gan.
2. **Tiên lượng phát triển của HCC.**
3. Có các cutoffs khác nhau, tùy theo nguyên nhân dẫn đến xơ hóa gan.

Figure 2. Structure change of sugar chains of glycoprotein following the progression of hepatic fibrosis



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M2BPGi tiên đoán- theo dõi HCC

Reference	Etiology	Treatment status	Threshold of M2BPGi for HCC risk	HR (95% CI)
Yamasaki, <i>et al.</i> [31]	HCV		≥ 4	8.3 (1.8–38)
Tamaki, <i>et al.</i> [32]	HCV		≥ 4.2	4.1 (1.1–15)
			≥ 0.3 increase/yr	5.5 (1.5–19)
Inoue, <i>et al.</i> [36]	HCV		≥ 4 (mortality risk)	
Sasaki, <i>et al.</i> [48]	HCV SVR		≥ 2.0	5.7 (1.7–20)
Nagata, <i>et al.</i> [49]	HCV SVR		≥ 1.8	2.0 (1.4–2.4)
Yasui, <i>et al.</i> [50]	HCV SVR		≥ 1.75	6.0 (1.8–19)
Akuta, <i>et al.</i> [51]	HCV SVR		≥ 1.0	4.9 (1.4–18)
Ichikawa, <i>et al.</i> [53]	HBV	Naive	≥ 0.71	8.3 (1.0–67)
Jun, <i>et al.</i> [58]	HBV	Naive	Each 1 increase	1.1 (1.05–1.18)
Liu, <i>et al.</i> [62]	HBV	Naive	≥ 2.0 (1–2 yr HCC)	7.4 (2.4–23)
Kim, <i>et al.</i> [63]	HBV	Naive	≥ 1.8	1.5 (1.1–2.1)
Mak, <i>et al.</i> [64]	HBV	NA treatment	≥ 1.15 before NA treatment	1.2 (1.04–1.5)
Kawaguchi, <i>et al.</i> [65]	HBV	NA treatment	≥ 1.2 after NA treatment	10.5 (3.0–38)
Shinkai, <i>et al.</i> [66]	HBV	NA treatment	≥ 1.2 after NA treatment	5.0 (1.7–15)
Su, <i>et al.</i> [67]	HBV	NA treatment	Each 1 increase after NA treatment	1.6 (1.2–2.1)
Heo, <i>et al.</i> [68]	HBV	Naive/NA treatment	≥ 1.8	11.5 (1.4–97)
Mak, <i>et al.</i> [69]	HBV	Naive/NA treatment	≥ 0.68	4.7 (1.3–17)
Kawanaka, <i>et al.</i> [79]	NAFLD		≥ 1.255	1.7 (1.1–2.3)

Nobuharu Tamaki et al. Annals of Laboratory Medicine 2021

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III. Ứng dụng lâm sàng



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NGHIÊN CỨU GIÁ TRỊ CỦA AFP, AFP-L3, PIVKA II TRONG CHẨN ĐOÁN UNG THƯ BIỂU MÔ TẾ BÀO GAN

- Nghiên cứu hồi cứu 224 trường hợp viêm gan mạn/xơ gan tại Khoa Gan – Trung tâm Y khoa Medic từ 01/2016 đến 06/2018.
- Có 103 trường hợp chẩn đoán xác định HCC và 121 trường hợp không có HCC qua CT bụng và/hoặc MRI bụng.
- Nồng độ của AFP, AFP-L3, PIVKA II trong nhóm HCC đều cao hơn có ý nghĩa thống kê so với nhóm không HCC với $p < 0,001$.
- Trong 3 dấu ấn chỉ có nồng độ PIVKA II là có tương quan tuyến tính với kích thước khối u với hệ số tương quan $r = 0,553$.
- PIVKA II có giá trị chẩn đoán cao nhất trong 3 dấu ấn.
- Kết hợp 3 dấu ấn cho kết quả chẩn đoán chính xác nhất.

Phùng Huy Hoàng. (2018). Luận văn bác sĩ nội trú, chuyên ngành nội khoa. Trường đại học y khoa Phạm Ngọc Thạch.

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VALUES OF AFP, AFP-L3, PIVKA II MARKERS IN EARLY DETECTING HEPATOCELLULAR CARCINOMA IN CHRONIC HEPATITIS PATIENTS

POSTER NUMBER: 1138
 PRESENTING AUTHOR: Thuy Pham
 CO-AUTHORS: Dat Ho, Thuy Pham, Hoang Phung
 CATEGORY: Hepatobiliary Neoplasia

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BACKGROUND & AIMS
 In Asia-Pacific regions, hepatocellular carcinoma (HCC) is the third leading cause of cancer death, and Vietnam is a country with the most prevalence of liver cancer, especially in HBV and HCV patients. The aim of this study:
 - To define the values of AFP, AFP-L3 and PIVKA II in early detection of HCC.
 - To define and compare AUROC, receiver characteristics of markers and combine of these markers in screening HCC in chronic hepatitis and cirrhotic patients.

METHODS
 Retrospective study with a control group. 224 patients with chronic hepatitis or cirrhosis was enrolled in this study. Patients took screening tests with AFP, AFP-L3 and PIVKA II to diagnose HCC. This study was to define similar characteristics of markers and the value of markers in HCC diagnosis, to compare AUROC between HCC group and non HCC group and define cutoff characteristics of markers.

RESULTS
 224 patients enrolled in this study, after MRI diagnosis they were divided into two group, one with 183 HCC patients and the other with 41 non HCC patients.
 - Combination of markers in HCC group was higher than that of non HCC group. It was significant (p=0.001).
 - Among the three markers, PIVKA II was lower corrected AUC with the tumor size, with a close correlation (r=0.533).
 - All markers had high values to diagnose HCC with AUROC higher than 0.8 and that of PIVKA II was highest with 0.885 (95%CI: 0.848-0.912).
 - PIVKA II had higher value among the 3 markers, addition of AFP and/or AFP-L3 didn't increase the diagnostic value with statistical significance.
 - Combination of the three markers increased the sensitivity compared with only one marker, but decreased the specificity respectively.
 - Cut off values using Youden index of AFP was 4.7 ng/mL, AFP-L3 was 1.81%, and PIVKA II was 25 AU/mL, and Youden index are 0.59, 0.55 and 0.79 respectively. The sensitivity and the values of negative predicting were all higher than the recommended cut off values.

CONCLUSION
 In the screening and prognosis of hepatitis B virus and hepatitis C virus, the rate of HCC is high. So early detection of HCC is very important. PIVKA II is the best marker among the 3 markers. The combination of these three markers is an excellent means for early HCC screening in chronic hepatitis and cirrhotic patients.

CHARACTERISTICS OF THE PATIENTS

Characteristics	Hepatocellular carcinoma (n=183)	Non-hepatocellular carcinoma (n=41)	P
Gender			
Male	173 (97.8)	37 (90.2)	0.043
Female	10 (5.5)	4 (9.8)	
Age	50.27 ± 11.12	48.84 ± 12.17	<0.1
Liver disease			
P1, P2, P3	82 (78.8)	35 (78.5)	0.87
P4	21 (20.2)	6 (21.4)	
Liver fibrosis			
Hepatitis B	15 (8.2)	4 (9.8)	<0.1
Hepatitis C	29 (28.1)	14 (41.0)	
Hepatitis B + C	1 (0.5)	2 (5.0)	
Others	13 (7.1)	6 (14.6)	

METHODS AND PERCENTILES OF MARKERS

Markers	Hepatocellular carcinoma	Non-hepatocellular carcinoma	P
AFP (ng/ml)	61.81 (54.5)	23.3 (6)	<0.001
AFP-L3 (%)	81.2 (20.2)	13.6 (7)	<0.001
PIVKA II (AU/mL)	20.95 (26.6)	9.14 (4)	<0.001

THE SENSITIVITY, THE SPECIFICITY, PPV, NPV FOLLOW CUT OFF VALUES USING YOUDEN INDEX

Markers	AUROC	Sensitivity	Specificity	PPV	NPV	Youden index
AFP	0.8619	0.7607	0.8719	<0.0001	<0.0001	0.6326
AFP-L3	0.8608	0.8457	0.8314	<0.0001	<0.0001	0.6771
PIVKA II	0.8617	0.8147	0.8007	<0.0001	<0.0001	0.6154
AFP + PIVKA II	0.9042	0.8649	0.8443	<0.0001	<0.0001	0.7092
AFP + AFP-L3 + PIVKA II	0.8689	0.8725	0.8473	<0.0001	<0.0001	0.7198
AFP + AFP-L3 + PIVKA II	0.9120	0.8751	0.8408	<0.0001	<0.0001	0.7159

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Circulating Tumor DNA (ctDNA) Methylation Profile Improves the Accuracy of Serum Biomarkers (AFP, AFP-L3, and DCP) for the Detection of Early-Stage Hepatocellular Carcinoma

AASLD The Liver Meeting Nov. 4-8, 2022

Medical Genetics Institute

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Background
 Late detection of hepatocellular carcinoma (HCC) limits its overall 5-year survival rate to <15%. Patients at high risk for HCC include those with hepatitis B virus (HBV) infection, non-alcoholic fatty liver disease, and other cirrhotic disorders are recommended for biannual liver ultrasonography (LUS) in the United States and Europe¹. However, one meta-analysis showed that the performance of LUS is suboptimal for early cancer detection, with a sensitivity of merely 63% for early HCC². Most recently, a commercially available diagnostic assessment of serum levels of AFP, AFP-L3, and des-gamma-carboxy prothrombin (DCP), on a microfluidics-based assay named μ TASWako³ demonstrated up to 78% sensitivity and 62% specificity for early HCC⁴. Moreover, incorporation of those three markers into an HCC risk-scoring system, GALAD further increased both sensitivity and specificity⁵. It is thought that multiple biomarkers might be required to improve the early detection rate of HCC⁶. The use of circulating tumor DNA (ctDNA) as a non-invasive and highly sensitive marker for HCC detection has now been evaluated by several studies. Indeed, plasma ctDNA could be distinguished from other non-tumor cell-free DNA (cfDNA) via several features, including aberrant structure, genetic anomalies, and epigenetic modifications. Of those features, "signature" changes in DNA methylation patterns, an epigenetic mark, occur early in carcinogenesis and thus represent promising markers for early cancer.

Results
 1) Patients with early-stage HCC display aberrant methylation changes in cell-free circulating DNA
 • HCC patients showed higher DNA methylation levels in multiple regions, with 261450 differentially methylated regions (DMRs) while there were 140 DMRs distinguishing HCC patients from high-risk patients (Fig 1A).
 • 80 regions were shared between the two pairwise comparisons (Fig 1B).
 • Pathway enrichment analysis was performed for the set of genes mapped to these DMRs using gProfiler, and one of identified significantly enriched pathways was found to regulate the expression of non-coding RNAs, involved in Wnt signaling in HCC reflective of methylation profiles in liver (Fig 1C).
 2) Construction of machine-learning models for distinguishing HCC patients from non-HCC patients
 • We examined the predictive performance of three different machine learning algorithms (logistic regression – LR, support vector machines – SVM, and extreme gradient boosting – XGB) after selecting significant features (Fig 2A). The classification performance of each algorithm with selected hyperparameters was tested using leave-one-out cross validation. The three tested models yielded comparable AUC (Fig 2B).
 • Although not statistically significant, SMV showed a slightly higher accuracy for discriminating early-stage HCC patients from high-risk patients. Therefore, we selected this model for subsequent validation studies. The assay achieved a sensitivity of 92.9% (Fig 2C).
 3) Methylation-based assay enhances the accuracy of the μ TASWako³ assay in a cohort of at-risk patients
 • In an external validation cohort, the assay demonstrated consistent performance, with AUC of 0.84, 0.86 and 0.81 for discriminating HCC from non-HCC (Fig 3A), healthy subjects (Fig 3B) and high-risk patients (Fig 3C).
 • Interestingly, the combination of methylation-based assay with the GALAD scores incorporating μ TASWako serum biomarkers with patients' age and gender provided additive diagnostic performance with AUC of 0.87 (Fig 3C).
 • Compared to GALAD model alone, this combination showed comparable specificity of 97.4%, but higher sensitivity (69.6% versus 56.6%) for classification of HCC patients from those with their controls.

Conclusions
 • Liver cancer-specific methylation signatures detected in ctDNA could be exploited to build machine learning classifiers for screening early-stage HCC patients from healthy or at-risk (cirrhotic or hepatitis) individuals.
 • Our study also provides rationale for using ctDNA methylation signatures in combination with serum biomarkers to maximize the sensitivity and specificity of early-stage HCC screening for at-risk individuals, which remains a major clinical challenge.

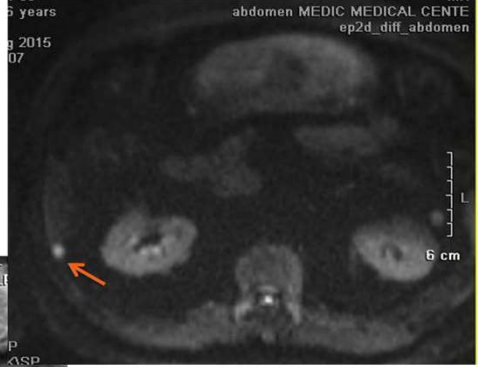

References
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 2. Tsuchiya N, et al., Biomarkers for the early diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2015;21(37):10573-83
 3. Singal A, et al., Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. *Aliment Pharmacol Ther* 2009;30(1):37-47
 4. Marzeto JA, et al., Alpha-fetoprotein, des-gamma carboxyprothrombin, and beta2-microglobulin alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009;137(1):110-8
 5. Roy D, Tinkelman M. Diagnostic Power of DNA Methylation Classifiers for Early Detection of

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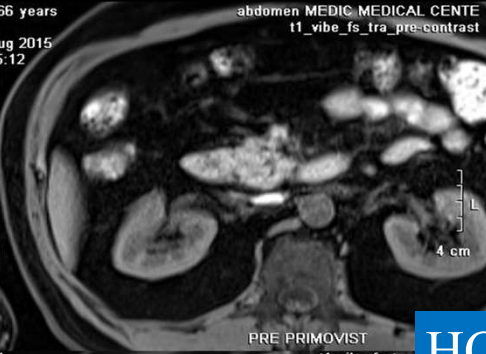


TRƯỜNG HỢP LÂM SÀNG 1

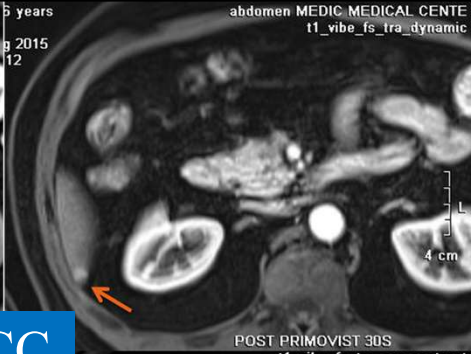
Bệnh nhân 66T. Nam. TPHCM.
 Kiểm tra sức khỏe định kỳ.
 HBV (-), HCV (-).
 Siêu âm bụng: bình thường.
 XN: Wako test (HCC risk): AFP=33.6ng/ml;
AFP-L3=62.4%; DCP=21mAU/ml.


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PRE PRIMOVIST




POST PRIMOVIST 30S



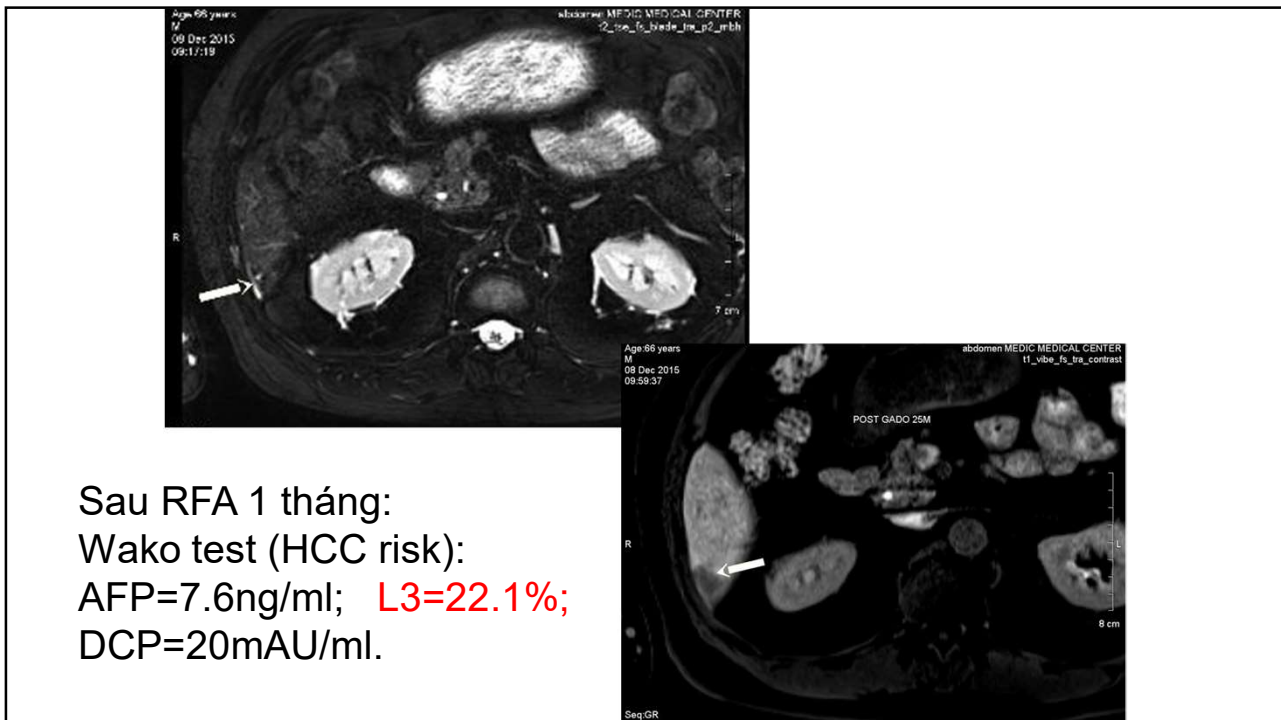


POST PRIMOVIST 60S



POST PRIMOVIST 20M

42



43

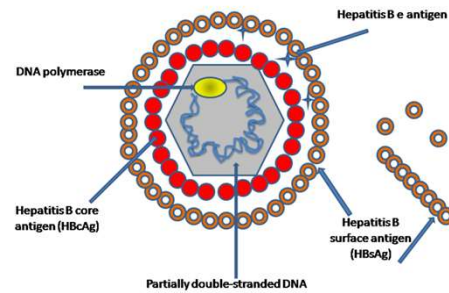
Trường hợp lâm sàng 2 : Dự đoán HCC

- Bệnh nhân nam , 57 tuổi, Bến Tre.
- Uống rượu rất nhiều.
- Mẹ: HBVI.
- Nhiễm HBV từ nhỏ, không điều trị.
- Không đồng nhiễm HCV.
- Không tiền căn phẫu thuật hay truyền máu.
- Mệt, chán ăn ---→ Khám Khoa Gan-Medic.

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Trường hợp lâm sàng 2 : Dự đoán HCC

- HBsAg : dương tính.
- HBeAg : âm tính.
- AntiHBcIgM: âm tính.
- AntiHCV: Negative.
- HBV DNA: 7.250.000 IU/mL.



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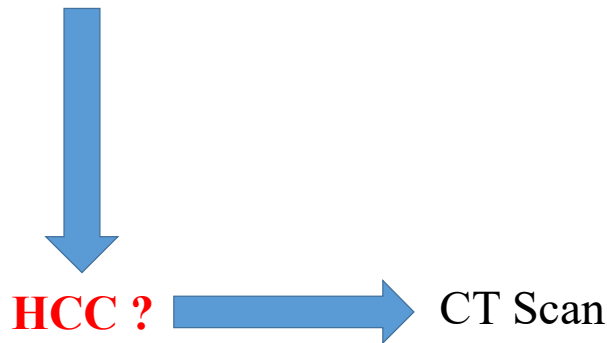
Trường hợp lâm sàng 2 : Dự đoán HCC

Các thông số CLS	Tháng 2/2020	Tháng 4/2020	Ghi chú
AST(U/L)	61	25	
ALT (U/L)	35	15	
GGT (U/L)	350	220	
AFP (ng/mL)	8	7	
Tiểu cầu (10 ⁹ /L)	88	101	
Bilirubin (mg/dL)	1.07	0.9	
Prothrombin time (%)	67	72	HBV DNA: 1200 IU/mL
Albumin (g/dL)	3.2	3,5	
HBcrAg	4.5	5.9	
Ultrasound : Doppler	Xơ gan, lách to	Xơ gan, lách to	
Elastography: FibroScan(kPa)	19,5	18,5	

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Trường hợp lâm sàng 2 : Dự đoán HCC

- AFP-L3: 37.2%
- PIVKAI: 1253 mAU/mL



U hạ phân thù VI , kích thước =1,1 cm nghi HCC

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Trường hợp lâm sàng 3: M2BPGi tiên đoán HCC

- Bệnh nhân nam 59t, Long An.
- Hút thuốc, uống rượu hơn 30 năm.
- Không tiền căn phẫu thuật hay truyền máu.
- Trong gia đình không ai bệnh gan.
- CHA > 10 năm.
- Phát hiện và điều trị viêm gan B mạn 2016, TDF (BHYT).
- Tháng 2/2021-----covid, bỏ điều trị.
- 12/2021: Mệt, khó ngủ , sụt cân-----Khoa Gan, Medic.

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Trường hợp lâm sàng 3: M2BPGi tiên đoán HCC

- HBsAg (+).
- AntiHBcAgIGM (-).
- HBeAg (-).
- AntiHBe (-).
- AntiHCV (-).
- HBV DNA: 1372 IU/mL.
- Creatinin: 1,07 mg%.
- eGFR: 76mL/phút/1,73 m².
- Bilirubin: 1,07 mg%.
- AST: 62 U/L.
- ALT: 22 U/L.
- GGT: 253 U/L.
- Albumin: 3,1 g/dL
- Tiểu cầu: 94 x 10⁹/L. PT: 82%

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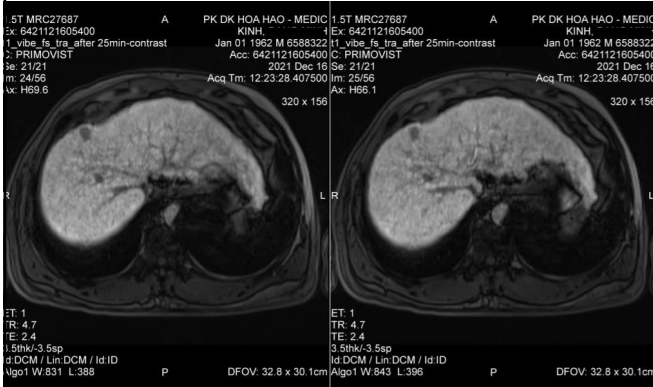
Trường hợp lâm sàng 3: M2BPGi tiên đoán HCC

- AFP: 4ng/mL.
- M2BPGi: 4,5.
- US Bụng: Xơ gan, lách to
- FibroScan: 40,8 kPa.

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Trường hợp lâm sàng 3: M2BPGi tiên đoán HCC

- M2BPGi: 4,5.
- FibroScan: 40,8 kPa.
- MRI Primovist: Nhân HCC hạ phân thùy IV 12x11 mm, xơ gan , lách to.



: MRI BỤNG

Không, sau đó tiêm tương phản

: KỸ THUẬT

Hình chụp vùng gan với máy cộng hưởng từ 1,5tesla, có tiêm thuốc tương phản PRIMOVIST 10ML, chuỗi xung Axial và coronal T2WI fatsat, TIGRE, các thông số kỹ thuật được in ở góc trái mỗi hình.

MÔ TẢ

Nhu mô gan thô, có tín hiệu không đồng nhất trên T2WI, bắt thuốc tương phản không đồng nhất thì trễ. Nốt thương tổn nhỏ ở gan hạ phân thùy IV, kích thước khoảng 12 x 11mm, tín hiệu cao nhẹ so với mô gan trên T2WI và trên T1WI, có hạn chế khuếch tán, bắt thuốc tương phản thì đồng mạch, thải thuốc chậm, sau 6 phút và 25 phút có tín hiệu thấp hơn nhu mô gan.

Đường mật trong và ngoài gan không giãn.

Không thấy túi mật.

Không thấy huyết khối tĩnh mạch cửa

Ổng mật chủ không giãn, không sỏi

Lách to 134x80mm, cường độ tín hiệu trong giới hạn bình thường.

Tụy không thương tổn.

Hai thận bình thường, không chướng nước.

Không dịch ổ bụng.

*** KẾT LUẬN:

THEO DỐI NHÂN HCC HẠ PHÂN THỤY IV / XƠ GAN - LÁCH TO.

Tp. Hồ Chí Minh, ngày 16/12/2021 13:01

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THE FUTURE IS IN OUR HANDS

XU HƯỚNG TƯƠNG LAI CÁC CHỈ DẤU XÉT NGHIỆM MÁU TRONG CHẨN ĐOÁN UNG THƯ GAN.

Current clinical diagnosis with ...



... conventional protein biomarkers

AFP
AFP-L3
DCP
HSP70
Glypican-3 (GPC3)
Glutamine synthetase (GS)

towards

Future clinical diagnosis with ...



... promising new protein biomarkers

Cytokeratin 19
Golgi protein 73
Annexin 2
Osteopontin (OPN)
Midkine
Dickkopf-1 (DKK-1)
Squamous Cell Carcinoma Antigen (SCCA)
Alpha-L-fucosidase (AFU)

Biomarker potential:
Diagnosis, Monitoring, Prognosis

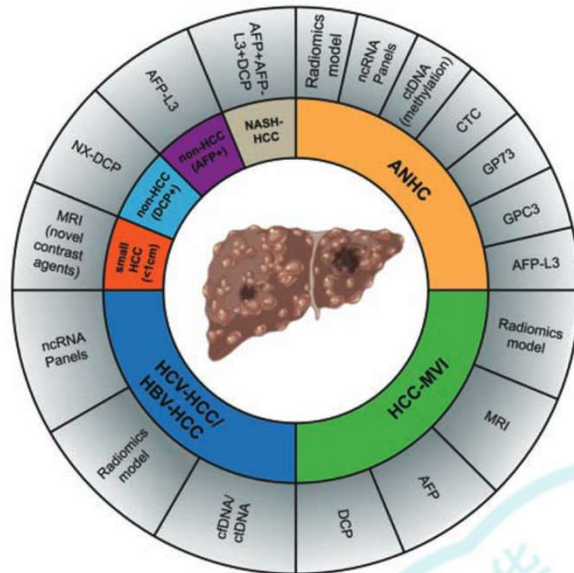
Advantages:
good stability, rapid analysis on a routine basis, advanced phase of biomarker development (phase 5)

Disadvantages:
limited diagnostic accuracy of AFP, early phase of biomarker development for new protein biomarkers

Schlosser et al. Frontiers in Oncology. 28 November 2022, Volume 12- | <https://doi.org/10.3389/fonc.2022.1016952>

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Precision diagnostic methods of different types of HCC



AFP : Alpha-fetoprotein; AFP-L3: Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; ANHC: AFP-negative HCC; cfDNA: Cell-free DNA; ctDNA: Circulating tumor DNA; CTC: Circulating tumor cell; DCP: Des-gamma-carboxy prothrombin; GP 73: Golgi protein 73; GPC3: Phosphatidyl alcohol proteoglycan-3; HBV-HCC: Hepatitis B virus-related hepatocellular carcinoma; HCC: Hepatocellular carcinoma; HCC-MVI: Hepatocellular carcinoma related microvascular invasion; HCV-HCC: Hepatitis C virus-related hepatocellular carcinoma; MRI: Magnetic resonance imaging; NASH-HCC: Non-alcoholic steatohepatitis related hepatocellular carcinoma; ncRNA Panels: Non-coding RNA panels; non-HCC (AFP+): Non-hepatocellular carcinoma (Alpha-fetoprotein positive); non-HCC (DCP+): Non-hepatocellular carcinoma (Des-gamma-carboxy prothrombin positive); NX-DCP: Novel des-gamma-carboxy prothrombin; small HCC (<1 cm): small hepatocellular carcinoma (tumor size <1 cm)

Zhenxiao Wang et al. Precision diagnosis of hepatocellular carcinoma. Chinese Medical Journal 2023;136(10)

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IV. Kết luận

- ☞ HCC là 1 ung thư khó điều trị và tiên lượng nặng nếu phát hiện trễ.
- ☞ Chẩn đoán sớm và chính xác ung thư biểu mô tế bào gan góp phần quan trọng trong lựa chọn phương pháp điều trị cũng như tiên lượng thời gian sống còn của bệnh nhân.
- ☞ Trong khi tỉ lệ HCC tại Việt Nam vẫn còn cao nên nhiệm vụ đặt ra cho người thầy thuốc phải biết vận dụng uyển chuyển tất cả các phương tiện chẩn đoán có trong tay để làm sao phát hiện được HCC càng sớm càng tốt.
- ☞ Trong tương lai sẽ có nhiều dấu ấn chẩn đoán sớm HCC hiệu quả.
- ☞ Quan trọng: Người dân phải biết HCC không triệu chứng rõ rệt ở giai đoạn sớm = điều trị hiệu quả cao & thăm khám định kỳ là chìa khóa quan trọng nhất.



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