壳酶蛋白（Fibro-CHI）

全新肝纤维化诊断及动态监测方法

无创精准 动态监测

学术文献汇编

杭州普望生物技术有限公司

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Hepatoma Research

Review

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Chitinase-3-like protein 1 as a predictor for the progression or regression of liver fibrosis

Biaoyang Lin[1,2,3], Shengjun Wu[4], Yunhua Liu[5], Longgen Liu[6], Saadiya Mushtaq[7]

[1]Zhejiang-California International Nanosystems Institute (ZCNI) Proprium Research Center, Zhejiang University, Hangzhou 310058, Zhejiang Province, China.
[2]Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310058, Zhejiang Province, China.
[3]University of Washington School of Medicine, Seattle, WA 98195, USA.
[4]Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310020, Zhejiang Province, China.
[5]Department of Liver Diseases, The Second Hospital of Yunnan Province, Kunming 650000, Yunnan Province, China.
[6]The Third People's Hospital hospital of Changzhou, Changzhou 213001, Jiangsu Province, China.
[7]Virology Department, Armed Forces Institute of Pathology, Block E-16, New Cob Lane, Javed Sultan Shaheed Colony, Rawalpindi Cantt, Rawalpindi, Pakistan.

Correspondence to: Dr. Biaoyang Lin, Zhejiang-California International Nanosystems Institute (ZCNI) Proprium Research Center, Zhejiang University, Hangzhou 310058, Zhejiang Province, China. E-mail: biaoylin@gmail.com

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Abstract

Liver fibrosis is a wound-healing response of liver cells to chronic injuries caused by viral infections, including hepatitis B virus (HBV), hepatitis C virus (HCV), toxins, and alcohol abuse. The ability to stage diseases for treatment naïve patients to initiate proper medical procedures and predict the clinical causes of the disease or the treatment response is important given the increased prevalence of liver fibrosis caused by HBV, HCV and fatty liver diseases. CHI3L1 (chitinase-3-like protein 1, also known as YKL-40), which belongs to the chitinase family but lacks chitinolytic activity and is highly expressed in the liver, seems to fulfill this role. CHI3L1 is a non-invasive staging marker for liver fibrosis caused by HBV, HCV and non-alcoholic fatty liver disease as well as a predictor of the clinical causes and fibrotic changes after treatments. CHI3L1 predicts histological progression of liver fibrosis and fibrosis progression rate (fibrosis unit/year), rapid fibrosis progression after liver transplantation and response to interferon and recent direct acting antiviral therapy in chronic HCV patients. CHI3L1 also predicts response to antiviral therapy in chronic HBV patients.

Keywords: CHI3L1, liver fibrosis, progression, regression, hepatitis B virus, hepatitis C virus, treatment response

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INTRODUCTION

Liver fibrosis is a wound-healing response of liver cells to chronic injuries caused by viral infections, toxins, alcohol abuse and other causes. Liver fibrosis is accompanied by a constant process of destruction and repair of the hepatic parenchyma that is caused by inflammation and often results in serious complications, including portal hypertension and liver failure. Liver fibrosis can also give rise to hepatocellular carcinoma. Liver fibrosis can lead to cirrhosis, which is defined as the end stage of liver fibrosis[1]. In China, hepatitis B is the major cause of inflammation leading to liver fibrosis and cirrhosis[2,3]. Cirrhosis is an important factor in the development of hepatocellular carcinoma (HCC) because the cumulative 5-year risk of developing HCC in patients with cirrhosis ranges from 5% to 30%, depending on several factors, including the presence and stage of underlying liver disease, ethnicity, age, sex and the duration of exposure to primary hepatotropic viruses. To reduce the burden of the end stage liver diseases (cirrhosis and HCC), it is critical to identify liver fibrosis at its early stage, predict the direction and speed of the progression, and finally to monitor and predict the treatments responses (antiviral or anti-fibrotic treatments).

Although many biomarkers (e.g., APRI, FIB4, fibrometer, fibrotest, *etc*.) and imaging methods (e.g., Fibroscan, ARFI, MRE) have been widely proposed for staging liver fibrosis, their abilities in predicting liver fibrosis progression are very limited. Given that fibrosis is a very slow process, it often takes years to progress or recede from one pathological stage to the next. Therefore, a biomarker that can fulfill this role is most desirable. A search for such a biomarker would require an understanding of the mechanism of liver fibrosis and the key molecules involved in the process.

CHI3L1 (also known as YKL-40) belongs to the chitinase family but lacks chitinolytic activity, which is highly enriched in the liver[4]. CHI3L1 acts as a growth factor for fibroblasts and is involved in matrix remodeling[5]. Serum CHI3L1 levels are associated with the severity of liver fibrosis caused by non-alcoholic fatty liver disease[6], schistosomiasis[7,8], hepatitis C virus (HCV)[9,10] and hepatitis B virus (HBV)[11].

CHI3L1 PREDICTS HISTOLOGICAL PROGRESSION OF LIVER FIBROSIS IN CHRONIC HCV PATIENTS

Fontana *et al*.[12] analyzed the association of serum fibrosis marker levels with the risk of clinical and histological disease progression in a large cohort of patients with chronic hepatitis C consisting of 462 prior non-responders to peg-interferon and ribavirin enrolled in the randomized phase of the Hepatitis C Antiviral Long-term Treatment against Cirrhosis (HALT-C) trial. They performed pretreatment liver biopsy and follow-up biopsies at years 2 and 4 and defined histological progression as a ≥ 2-point increase in the Ishak fibrosis score in patients without cirrhosis. Clinical outcomes included development of decompensation, hepatocellular cancer, death or an increase in the Child-Turcotte-Pugh score to ≥ 7. They collected and compared serial YKL-40 levels in patients who progressed clinically to the levels in patients who did not progress using random effects modeling. YKL-40 levels increased in both groups of patients over time ($P = 0.0026$) and were significantly increased in the progressors ($P < 0.0001$).

CHI3L1 PREDICTS RESPONSE TO INTERFERON THERAPY IN CHRONIC HCV PATIENTS

Saitou *et al*.[10] analyzed noninvasive markers as predictors of interferon responses with HCV-associated diseases. A total of 109 patients with HCV-associated liver disease were enrolled, and 88 patients underwent liver biopsy. In total, 67 of 109 patients received interferon therapy. YKL-40 was superior to other fibrosis markers for predicting severe fibrosis (F2-F4) from mild fibrosis (F0-F1) (YKL-40, AUC = 0.809; HA, AUC = 0.805). They also evaluated the changes of the levels of fibrosis markers before and after interferon (IFN) therapy. After IFN therapy, only the concentration of serum YKL-40 significantly decreased in the responder group and the non-responder group ($P = 0.03$). No changes were noted among type IV collagen, amino-terminal peptide

of type III procollagen, hyaluronic acid (HA). They concluded that YKL-40 might be a useful non-invasive serum marker to evaluate the efficacy of IFN therapies in patients with HCV-associated liver disease.

CHI3L1 PREDICTS RESPONSE TO ANTIVIRAL THERAPY IN CHRONIC HBV PATIENTS

Wang *et al.*[13] compared serum CHI3L1 levels with liver tissue collagen proportionate area (CPA) and liver stiffness measurement (LSM) in a cohort of 131 CHB patients before treatment and after receiving entecavir-based antiviral therapy for 78 weeks. Before treatment, correlation analysis revealed positive correlations between CHI3L1 levels and the CPA ($r = 0.351$, $P < 0.001$) and between CHI3L1 and LSM ($r = 0.412$, $P < 0.001$). After 78 weeks of treatment, serum CHI3L1 levels decreased compared with baseline (87.8 *vs.* 69.6 ng/mL, $P < 0.001$). Furthermore, the changes in CHI3L1 are correlated with changes in CPA ($r = 0.366$, $P < 0.001$) and the changes in LSM ($r = 0.438$, $P < 0.001$) before and after antiviral treatments. They concluded that CHI3L1 is a useful non-invasive marker for the assessment of liver fibrosis in CHB patients before treatment and a potential useful marker for monitoring the change in liver fibrosis during therapy. More interestingly, in many cases, CHI3L1 concentrations decreased after 78 weeks of antiviral therapies, whereas histological stages based on biopsy did not change. However, upon closer examination of the histological images, they found that many samples exhibited improvement in fibrosis as demonstrated by thinning of the septa and reduction in the numbers of the septa. However, the Ishak histological stage remains the same based on the classification standards (personal communication).

CHI3L1 PREDICTS FIBROSIS PROGRESSION RATE (FIBROSIS UNIT/YEAR) IN CHRONIC HCV PATIENTS

Kamal *et al.*[7] conducted serial liver biopsies in a 10-year longitudinal cohort study consisting of patients with HCV alone or HCV and schistosomiasis. Two liver biopsies were performed for patients at the time of acute HCV infection and at the end of the follow-up to calculate the fibrosis progression rate/year. In addition, CHI3L1 serum concentrations were measured yearly and at the end of the follow-up. The serum CHI3L1 change rate (difference between baseline and follow-up values) was compared with the fibrosis progression rate/year. Kamal *et al.*[7] reported that the CHI3L1 change rate had a very high linear correlation with the fibrosis progression rate/year ($r = 0.892$, $P < 0.001$). Furthermore, the CHI3L1 increase rate increases from years 4 to 8 compared with years 1 to 4 for HCV mono-infected patients, and the increase was noted at year 2 instead of at year 4 in HCV and schistosomiasis co-infected patients. Using data from the table of Kamal *et al.*[7], we generated a scatter plot of CHI3L1 concentration and the fibrosis progression rate per year (increase in histological stages per year) [Figure 1]. As noted, no fibrosis progression is noted when the CHI3L1 concentration is 53 ng/mL. As the CH3L1 concentration increases, the speed of fibrosis progression increases. When the CHI3L1 concentration is 110 ng/mL, the speed of fibrosis progression is at 0.8 histological stages per year [Figure 1].

CHI3L1 PREDICTS RAPID FIBROSIS PROGRESSION AFTER LIVER TRANSPLANTATION FOR HCV PATIENTS

Pungpapong *et al.*[14] obtained serum and liver biopsy samples from 46 liver transplantation (LT) recipients at two time points: time point 1, means of 5 ± 2 (biopsy 1) months; time point 2, means of 39 ± 6 (biopsy 2) months post-LT. Rapid fibrosis progression (RFP) was defined as an increase in the fibrosis score ≥ 2 from biopsy 1 to biopsy 2 (a mean interval of 33 ± 6 months). They analyzed the ability of parameters, including serum CHI3L1 and hyaluronic acid (HA), histological assessment, and hepatic stellate cell activity (HSCA) at biopsy 1, to predict RFP. They found that serum HA and YKL-40 performed significantly better than conventional parameters and HSCA in predicting RFP post-LT. Furthermore, CHI3L1 (cutoff ≥ 200 μg/L) exhibited 96% accuracy and performed better than serum HA (cutoff ≥ 90 μg/L) in predicting RFP at biopsy 1 with 80% accuracy.

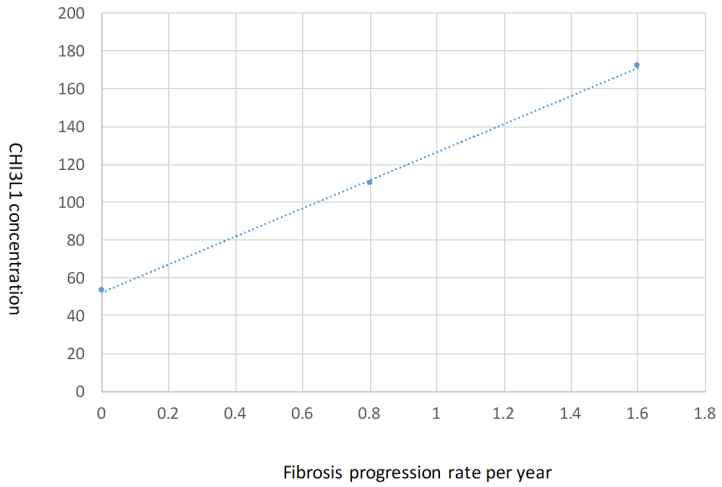

Figure 1. Scatter plot demonstrating the slope of CHI3L1 concentration and fibrosis progression rate per year

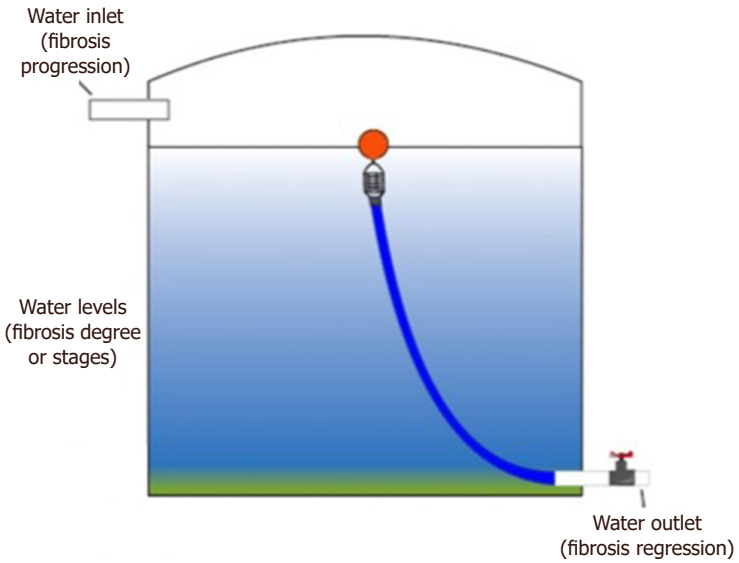

Figure 2. A water tank model to describe the relationship between the progression or regression of liver fibrosis and CHI3L1

CONCLUSION

CHI3L1 is not only a staging marker for fibrosis in treatment naïve HBV- or HCV-infected patients and NAFLD patients. CHI3L1 is also predictive of progression or regression of fibrosis. These abilities are likely due to the fact that CHI3L1 is actively involved in the process of liver fibrosis. Johansen *et al.*[15] used immunohistochemical analysis to demonstrate that CHI3L1 is expressed in areas with fibrosis, particularly leading edges/areas with active fibrogenesis. CHI3L1 staining was not observed in hepatocytes but was expressed in Kupffer cells[6] and potentially hepatic stellate cells (HSC)[15]. He *et al.*[16] demonstrated that CHI3L1 binds to interleukin-13 receptor α2 (IL-13Rα2), activates MAPK (macrophage mitogen-activated protein kinase), protein kinase B/AKT, and Wnt/β-catenin signaling, and regulates TGF-β1 production via IL-13Rα2-dependent mechanisms. CHI3L1 also promotes HSC activation and proliferation[4].

Here, we present a water tank model [Figure 2] to explain the relationship between the progression or regression of liver fibrosis and the concentration and increasing speed of CHI3L1. The inlet of water represents the parameters of CHI3L1, and the girth of the inlet pipe represents the absolute concentration of

CHI3L1. The water pressure (inlet water speed) represents the speed of the increase of CHI3L1 concentration in liver. The outlet represents the natural ability of the liver to repair the fibrosis damage (e.g., degradation of the extracellular matrix). The height of the water tank represents the degree (stages) of liver fibrosis. For example, if the water intake is greater than the water outflow, then the height of the water tank (degree of the fibrosis) would increase after a period of time, thus representing a model of chronic liver fibrosis similar to that observed in chronic HBV patients. If treatment, such as antiviral treatment of HBV, was initiated, the water intake would decrease (measured by a reduction in CHI3L1 concentration). Thus, over time, the height of the water tank (degree of fibrosis) would decrease due to natural recovery properties of the liver.

DECLARATIONS

Authors' contributions

Drafted the manuscript: Lin B
Edited and approved the manuscript: Wu S, Liu Y, Liu L, Saadiya M

Availability of data and materials

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Conflicts of interest

The author declares that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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·指南·

肝硬化肝性脑病诊疗指南

中华医学会肝病学分会

通信作者：徐小元，Email：xiaoyuanxu6@163.com，100034 北京大学第一医院感染疾病科；

段钟平，Email：duan2517@163.com，100069 首都医科大学附属北京佑安医院人工肝中心

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【关键词】 肝硬化； 肝性脑病； 诊断； 治疗

Guidelines for the diagnosis and management of hepatic encephalopathy in cirrhosis *Chinese Society of Hepatology, Chinese Medical Association*

Corresponding author: Xu Xiaoyuan, Email: xiaoyuanxu6@163.com, Department of Infectious Diseases, Peking University First Hospital, Beijing 100034, China

Co-corresponding author: Duan Zhongping, Email: duan2517@163.com, Artificial Liver Centre, Beijing You-An Hospital, Capital Medical University, Beijing 100069, China

【Abstract】 Current guideline developed by the Chinese Society of Hepatology on the management of hepatic encephalopathy in cirrhosis is grounded on the published evidences and panelists' consensus. This guideline presents recommendations for diagnosis and management of covert and overt hepatic encephalopathy, and underline the importance of screening minimal hepatic encephalopathy in patients with end-stage liver diseases. In addition, it also stresses that early identification and timely treatments are the means to know the prognosis. The principles of treatment are primary and secondary prevention, prompt removal of the cause, and recovery of acute neuropsychiatric abnormalities to baseline status.

【Key words】 Liver cirrhosis; Hepatic encephalopathy; Diagnosis; Therapy

一、前言

肝性脑病（hepatic encephalopathy，HE）是由急、慢性肝功能严重障碍或各种门静脉-体循环分流（以下简称门-体分流）异常所致的、以代谢紊乱为基础、轻重程度不同的神经精神异常综合征。

为了促进HE临床诊疗的规范化，一些国际胃肠和肝病学会陆续发布了HE的指南或共识，对HE的定义及诊疗提出了建议。1998年维也纳第11届世界胃肠病大会（World Congresses of Gastroenterology，WCOG）成立HE工作小组，并于2002年制定了《肝性脑病的定义、命名、诊断及定量分析》；美国胃肠病学会（American Gastroenterological Association，AGA）实践标准委员会、国际肝性脑病和氮代谢学会（International Society for Hepatic Encephalopathy and NitrogenMetabolism，ISHEN）、美国肝病学会（American Association for the Study of Liver Diseases，AASLD）和欧洲肝病学会（European Association for the Study of the Liver，EASL）等先后制定了多部指南或共识，从HE的发病机制、自然史、流行病学、诊断评价和治疗等方面提出了推荐意见。对HE的实验模型、神经生理研究、神经生理学和影像学检测及临床试验设计等方面也进行了阐述[1-3]。

中华医学会消化病学分会和肝病学分会于2013年制订了《中国肝性脑病诊治共识意见（2013年，重庆）》[4]。近年来，随着基础和临床研究的进展，人们对HE，尤其是轻微肝性脑病（minimal hepatic encephalopathy，MHE）有了进一步的认识。中华医学会肝病学分会组织肝病、感染、消化、外科、中医、介入、肿瘤、药理、护理和临床研究方法学等领域的专家共同编写了本指南，旨在为HE的临床诊断和治疗提供指导。本指南不是强制性标准，不可能包括或解决HE诊治中的所有问题，因此，临床医生在面对某一患者时，应遵循本指南的原则，充分了解患者的病情，认真考虑患者的观点和意愿，并结合当地的医疗资源和实践经验，制订全面合理的个体化诊疗方案。

本指南推荐意见的证据级别和推荐强度按照推荐意见分级评估和制定及评价（GRADE）系统进行分级（表1）。

依据基础肝病的类型，HE分为A、B、C 3型。A型HE发生在急性肝衰竭基础上，进展较为迅速，其重要的病理生理学特征之一是脑水肿和颅内高压。B型HE是门-体分流所致，无明显肝功能障碍，肝活组织病理学检查（肝活检）提示肝组织学结构正常。C型则是指发生于肝硬化等慢性肝损伤基础上的HE（表2）。

本指南主要针对由肝硬化引起的HE即C型HE，不包括急性肝衰竭以及其他原因门-体分流所致的A/B型HE。

二、流行病学

肝硬化HE的发生率国内外报道不一，可能是因为临床医生对HE诊断标准不统一及对MHE的认知存在差异。多数肝硬化患者在病程的某一时期会发生一定程度的MHE，其在整个肝硬化病程中发生率为30%～84%[5]。

万方数据

表1 推荐意见的证据级别和推荐强度

级别	详细说明
证据级别	
A	高质量，进一步研究不可能改变对该疗效评估结果的可信度
B	中等质量，进一步研究有可能影响该疗效评估结果的可信度，且可能改变该评估结果
C	低或非常低质量，进一步研究很有可能影响该疗效评估结果的可信度，且很可能改变该评估结果
推荐强度	
1	强推荐，明确显示干预措施利大于弊或者弊大于利
2	弱推荐，利弊不确定或无论质量高低的证据均显示利弊相当

表2 1998年第11届世界胃肠病大会推荐的肝性脑病分类

肝性脑病类型	定义	亚类	亚型
A型	急性肝功能衰竭相关肝性脑病	无	无
B型	门静脉-体循环分流相关性肝性脑病，无肝细胞损伤相关肝病	无	无
C型	肝硬化相关肝性脑病，伴门静脉高压或门静脉-体循环分流	发作型肝性脑病	伴诱因

近年来，我国学者对HE包括MHE的流行病学进行的多中心研究显示，在住院的肝硬化患者中约40%有MHE；30%～45%的肝硬化患者和10%～50%的经颈静脉肝内门-体分流术（TIPS）后患者发生过显性肝性脑病（overt hepatic encephalopathy，OHE）[6]。据国外资料报道，肝硬化患者伴HE的发生率为30%～45%，在疾病进展期发生率可能更高。北美终末期肝病研究联盟(NACSELD)证实，HE与肝硬化患者死亡具有独立相关性[7]。

三、病理生理学与发病机制

目前，我国肝硬化的主要病因是慢性乙型肝炎和慢性丙型肝炎，其次是酒精性或药物性肝病；自身免疫性肝病尤其是原发性胆汁性肝硬化（PBC）在临床上也逐渐增多。在长江流域，血吸虫病也曾是肝硬化的主要病因。MHE的发生与病因无明显相关性，但其发生率随着肝硬化失代偿程度的加重而增加，即使Child-Pugh A级肝硬化患者中，MHE的发生率也可高达24.8%[8]。

（一）发病机制与病理生理学

肝硬化门静脉高压时，肝细胞功能障碍对氨等毒性物质的解毒功能降低，同时门-体循环分流（即门静脉与腔静脉间侧支循环形成），使大量肠道吸收入血的氨等有毒性物质经门静脉，绕过肝脏直接流入体循环并进入脑组织，这是肝硬化HE的主要病理生理特点。

HE的发病机制至今尚未完全阐明，目前仍以氨中毒学说为核心，同时炎症介质学说及其他毒性物质的作用也日益受到重视[9]。

1. 氨中毒学说：氨中毒学说是HE的主要发病机制之一。饮食中的蛋白质在肠道经细菌分解产氨增加，以及肠壁通透性增加可导致氨进入门静脉增多，肝功能不全导致血氨不能经鸟氨酸循环有效解毒[10]；同时门-体分流致含有血氨的门静脉血流直接进入体循环。血氨进入脑组织使星状胶质细胞合成谷氨酰胺增加，导致细胞变性、肿胀及退行性变，引发急性神经认知功能障碍。氨还可直接导致兴奋性和抑制性神经递质比例失调，产生临床症状，并损害颅内血流的自动调节功能。

2. 炎症反应损伤：目前认为，高氨血症与炎症介质相互作用促进HE的发生发展。炎症可导致血脑屏障破坏，从而使氨等有毒物质及炎性细胞因子进入脑组织，引起脑实质改变和脑功能障碍。同时，高血氨能够诱导中性粒细胞功能障碍，释放活性氧，促进机体产生氧化应激和炎症反应，造成恶性循环。另一方面，炎症过程所产生的细胞因子又反过来加重肝损伤，增加HE发生率。此外，HE发生还与机体发生感染有关。研究结果显示，肝硬化患者最为常见的感染为腹膜炎、尿路感染、肺炎等[11-12]。

3. 其他学说：

（1）氨基酸失衡学说和假性神经递质学说：肝硬化肝功能障碍时，降解芳香族氨基酸的能力降低，使血中苯丙氨酸和酪氨酸增多，从而抑制正常神经递质生成。增多的苯丙氨酸和酪氨酸生成苯乙醇胺和羟苯乙醇胺即假性递质，大量假性神经递质代替正常神经递质，导致HE的发生[13]。

（2）γ-氨基丁酸／苯二氮䓬复合受体假说：γ-氨基丁酸是中枢神经系统特有的、最主要的抑制性递质，在脑内与苯二氮䓬类受体以复合受体的形式存在。HE时血γ-氨基丁酸含量升高，且通过血脑屏障量增加，脑内内源性苯二氮䓬水平升高。实验研究证实，给肝硬化动物服用可激活γ-氨基丁酸／苯二氮䓬复合受体的药物如苯巴比妥、地西泮，可诱导或加重HE；而给予苯二氮䓬类受体拮抗剂如氟马西尼，可减少HE的发作[14]。

（3）锰中毒学说：有研究发现，部分肝硬化患者血和脑中锰含量比正常人高2～7倍。当锰进入神经细胞后，低价锰离子被氧化成高价锰离子，通过锰对线粒体特有的亲和力，蓄积在线粒体内。同时，锰离子在价态转变过程中可产生大量自由基，进一步导致脑黑质和纹状体中脑细胞线粒体呼吸链关键酶的活性降低，从而影响脑细胞的功能[15]。

（4）脑干网状系统功能紊乱：严重肝硬化患者的脑干网状系统及黑质-纹状体系统的神经元活性受到不同程度的损害，导致HE发生，产生扑翼样震颤、肌张力改变；且脑干网状系统受损程度与HE病情严重程度一致[16]。

（二）诱发因素

HE最常见的诱发因素是感染（包括腹腔、肠道、尿路和呼吸道等感染，尤以腹腔感染最为重要）。其次是消化道

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出血、电解质和酸碱平衡紊乱、大量放腹水、高蛋白饮食、低血容量、利尿、腹泻、呕吐、便秘，以及使用苯二氮䓬类药物和麻醉剂等。TIPS后HE的发生率增加，TIPS后HE的发生与术前肝功储备状态、有无HE病史及支架类型及直径等因素有关[17]。研究发现，质子泵抑制剂（PPI）可能导致小肠细菌过度生长，从而增加肝硬化患者发生HE的风险，且风险随用药量和疗程增加而增加[18]。

在肝硬化患者存在高血氨的状态下，如果出现以上诱因，可进一步加重脑水肿和氧化应激，导致认知功能的快速恶化。

四、临床表现和诊断

（一）临床症状与体征

HE是一个从认知功能正常、意识完整到昏迷的连续性表现。目前国内外应用最广泛的仍是West-Haven HE分级标准，它将HE分为0～4级[19]。该分类标准主要缺陷为对于0级（可能是MHE）及1级判别的主观性很强。MHE为没有能觉察的人格或行为异常变化，神经系统体征正常，但神经心理测试异常。而1级HE临床表现中，欣快或抑郁或注意时间缩短等征象难以识别，只有了解患者性格的细心亲属才能洞悉患者轻度认知功能异常变化，在临床实践及多中心研究中重复性和可操作性较差。

在近年ISHEN提出的肝硬化神经认知功能变化谱(Spectrum of Neuro-cognitive Impairment in Cirrhosis)分级标准中，将MHE和West-Haven分类0、1级HE统称为隐匿性肝性脑病（covert hepatic encephalopathy，CHE）；若出现性格行为改变等精神异常、昏迷等神经异常，属于West-Haven分类2～4级HE，称为OHE[2, 4]。需要注意的是，1级HE患者存在轻微认知功能障碍，少数扑翼样震颤阳性的患者按SONIC标准属于OHE。

过去，临床上曾经用“亚临床肝性脑病”、“早期肝性脑病”等词语描述肝硬化0级HE患者，也就是无精神、神经异常表现的患者。1998年，第11届世界胃肠病大会一致通过将其命名为MHE[1]。MHE是HE发病过程中的一个非常隐匿的阶段，其定义为肝硬化患者出现神经心理学／神经生理学异常而无定向力障碍、无扑翼样震颤等，即认知功能正常[3, 20]；其发病率高达25%～39.9%[8, 21]，发病率的高低与年龄、性别、吸烟及受教育程度无关，而与Child-Pugh分级有明确关系。MHE尽管无明显的临床症状和体征，但其临床预后及生活质量均较肝硬化神经心理测试正常者差[22]。在临床随访中，MHE 3年累计发生OHE占56%，且其他并发症发生率和病死率显著增加。OHE恢复后，MHE可能持续存在[23]。另一方面，这些患者的健康相关的整体生活质量、驾驶安全性、工作效率及社会经济地位显著降低。如果没有得到有效治疗，部分患者可进展成为OHE。因此，临床的重点是在肝硬化等终末期肝病患者中筛查MHE，故本指南应用MHE和HE 1～4级修订的分级标准（表3，4）。对于意识显著改变的患者可进一步采用格拉斯哥（Glasgow）昏迷量表评分进行评估和描述患者的意识状态（附件1）。

（二）血液检查

1. 生物化学指标：检测患者的肝生物化学指标，如胆红素、丙氨酸氨基转移酶（ALT）、天冬氨酸氨基转移酶（AST），白蛋白、凝血酶原活动度等是否有明显异常。肾功能和血常规，在疑诊HE时均作为常规检查。

2. 血氨：血氨升高对HE的诊断有较高的价值。多个研究表明，HE特别是门-体分流性HE患者血氨多数增高，但血氨的升高水平与病情的严重程度不完全一致[24-25]。血氨正常的患者亦不能排除HE。止血带压迫时间过长、采血后较长时间才检测、高温下运送，均可能引起血氨假性升高。应在室温下采静脉血后立即低温送检，30 min内完成测定，或离心后4℃冷藏，2 h内完成检测 。

3. 其他：血清壳多糖酶3样蛋白1（chitinase-3-like protein 1，CHI3L1）为糖基水解酶家族成员之一。它可以结合壳多糖，但没有壳多糖酶的活性，在炎症和组织重塑中起重要作用。是肝脏分泌到胞外基质的蛋白，在肝硬化、肝纤维化时表达明显增高，CHI3L1表达水平反映了肝硬化、肝纤维化的程度[26]。

高尔基体蛋白73（Golgi protein 73，GP73）是一种位

表3 修订的HE分级标准

传统West-Haven标准	0级		HE1级	HE2级	HE3级	HE4级
建议修订的HE分级标准	无HE	MHE	HE1级	HE2级	HE3级	HE4级

注：HE为肝性脑病；MHE为轻微肝性脑病

表4 HE的分级及症状、体征

修订的HE分级标准	神经精神学症状（即认知功能表现）	神经系统体征
无HE	正常	神经系统体征正常，神经心理测试正常
MHE	潜在HE，没有能觉察的人格或行为变化	神经系统体征正常，但神经心理测试异常
HE1级	存在轻微临床征象，如轻微认知障碍，注意力减弱，睡眠障碍（失眠、睡眠倒错），欣快或抑郁	扑翼样震颤可引出，神经心理测试异常
HE2级	明显的行为和性格变化；嗜睡或冷漠，轻微的定向力异常（时间、定向），计算能力下降，运动障碍，言语不清	扑翼样震颤易引出，不需要做神经心理测试
HE3级	明显定向力障碍（时间、空间定向），行为异常，半昏迷到昏迷，有应答	扑翼样震颤通常无法引出，踝阵挛、肌张力增高、腱反射亢进，不需要做神经心理测试
HE4级	昏迷（对言语和外界刺激无反应）	肌张力增高或中枢神经系统阳性体征，不需要做神经心理测试

注：HE为肝性脑病；MHE为轻微肝性脑病

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于高尔基体的跨膜糖蛋白。GP73主要在胆管上皮细胞中表达，很少在肝细胞中表达；但在各种原因引起的进展期肝病中，GP73在肝细胞中的表达水平升高[27]。最近研究发现，肝细胞癌（HCC）患者中GP73水平升高主要与肝硬化有关，而与HCC本身无关。

（三）神经心理学测试

神经心理学测试是临床筛查及早期诊断MHE及1级HE最简便的方法，神经心理学测试方法被多国HE指南推荐作为MHE的筛查或早期诊断的重要方法，每个试验均需结合其他检查（表5）。

1. 传统纸-笔神经心理学测试：HE心理学评分（psychometric hepatic encephalopathy score, PHES），包括数字连接试验（number connection test, NCT）A、B、数字符号试验（digit symbol test, DST）、轨迹描绘试验、系列打点试验5个子测试试验（附件2）。目前常用NCT-A、DST均阳性，或5个子试验中任何2项异常，即可诊断为MHE。但值得注意的是，尽管PHES的灵敏度和特异度较高，但结果可受患者的年龄、受教育程度、合作程度、学习效果等多种因素影响[28-29]。

国内有学者采用年龄、受教育程度矫正的NCT、DST，显示了更高的准确性和应用价值[30-31]。总之，NCT、DST简单易行，可操作性强，适合MHE流行病学调查。近年来，开发了电子数字连接试验（eNCT）等计算机软件辅助的工具，用于肝硬化患者自身认知功能障碍的监测与筛查，具有更好的重复性和可靠性[32]。

2. 可重复性成套神经心理状态测验（repeatable battery for the assessment of neuropsychological status, RBANS）：是ISHEN指南推荐的两个神经心理测查工具之一；测查内容包括即时记忆、延迟记忆、注意、视觉空间能力和语言能力，已用于阿尔茨海默病、精神分裂症和创伤性脑损伤，并有部分研究用于等待肝移植患者，但不是专门用于HE的检测工具。

3. Stroop及Encephal APP测试：Stroop（附件3）是通过记录识别彩色字段和书写颜色名称之间的干扰反应时间来评估精神运动速度和认知灵活性，被认为是反映认知调控和干扰控制效应最有效、最直接的测试工具。近期，开发出基于该测试的移动应用软件工具——Encephal APP，显示出较好的区分肝硬化认知功能障碍的辨别能力和应用前景[33]。需要注意的是，有色盲的患者无法使用该项测试工具。

4. 控制抑制试验（inhibitory control test, ICT）：在肝硬化相关的神经功能障碍中，低级别的认知功能障碍如警惕性和注意力改变是最敏感的指标。ICT通过计算机技术在50 ms周期内显示一些字母，测试患者的反应抑制、注意力和工作记忆，可以用于MHE的检测。有研究证明，ICT诊断MHE的灵敏度可达88%，是诊断MHE的简易方法。

5. 临界闪烁频率（critical flicker frequency, CFF）检测：CFF是能引起闪光融合感觉的最小刺激频率。可以反映大脑神经传导功能障碍，研究显示其在诊断MHE时灵

表5 临床常用的神经心理／生理学测试方法注解

测试方法	测试目的	时间	备注
心理测试			
HE心理学评分（PHES）	是测定肝硬化患者认知功能障碍和诊断MHE的重要方法	包括数字连接试验A/B，数字符号试验、系列打点试验、轨迹描绘试验5个子测试试验	纸、笔 临床诊断至少需要2个试验阳性
数字连接试验A	持续型注意力，精神运动速度，可用于门诊MHE快速筛查	30～120 s	年龄及受教育程度校正后具有更好的准确性
数字连接试验B	持续型注意力，精神运动速度，分配型注意力，可用于门诊MHE快速筛查	1～3 min	需要心理学专家 比数字连接试验A更加复杂
数字符号试验	持续型注意力，精神运动速度，可用于门诊MHE快速筛查	2 min	需要心理学专家
Stroop智能手机应用（EncephalApp）	注意力，可用于门诊MHE快速筛查	3～5 min	可靠、容易使用
可重复性成套神经心理状态测验	顺应性和工作记忆，视觉空间能力、语言、认知处理速度	25 min	纸、笔 需要心理学专家 ISHEN推荐作为HE心理测量评分的替代指标
抑制控制测试	注意力、反应抑制、工作记忆	15 min	计算机处理 需要患者配合、在测试前需要患者学习
神经生理学测试			
闪光融合频率	视觉辨别，可用于门诊2级以下HE，辅助诊断价值小	10 min	在测试前需要患者学习
脑电图	广义脑活动，适用于儿童	变化	需要神经学专家和专业工具
诱发性电位	测试电刺激和反应之间的时间差	变化	听觉P300已被用于MHE的诊断

注：HE为肝性脑病；MHE为轻微肝性脑病；ISHEN为国际肝性脑病和氮代谢学会

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敏度适中、特异度较高，且易于解读，可作为辅助检查手段[34-35]。当阈值在39 Hz时，MHE患者和正常人并无差异，而2级HE与1级以下差异较大，故该检测更适用于区分2级HE[36]。CFF < 39 Hz的肝硬化患者达到5年生存期比例显著小于CFF ≥ 39 Hz者，高龄、CFF < 39 Hz和终末期肝病模型（MELD）评分均与随访期内生存独立相关[37]。

6. 扫描测试（SCAN）：是一种计算机化的测试，可以测量速度和准确度，用以完成复杂性增加的数字识别记忆任务。SCAN已被证明具有预后的预测价值；但其临床应用受教育背景影响较大。

7. 新的神经心理学测试方法：包括动物命名测试[38]（animal naming test，ANT），姿势控制及稳定性测试[39]，多感官组合（multi-sensory integration）测试[40]。

（四）神经生理学检查

1. 脑电图检查：脑电图可以反映大脑皮质功能，不需要患者的合作，也没有学习效应的风险。虽然脑电图早已被临床广泛研究和应用，但只有在严重HE患者中才能检测出典型的脑电图改变，故临床上基本不用于HE的早期诊断，仅用于儿童HE的辅助诊断。脑电图的异常主要表现为节律变慢，而该变化并非HE的特异性改变，亦可见于低钠血症、尿毒症性脑病等其他代谢性脑病[41]。

2. 诱发电位检测：诱发电位包括视觉诱发电位、听觉诱发电位和躯体诱发电位，以内源性时间相关诱发电位P300诊断的灵敏性最好。MHE患者可表现为潜伏期延长、振幅降低。

神经生理学检测的优点是结果相对特异，没有学习效应，但缺点是灵敏度差，需要专业设备、人员，与神经心理学测试结果一致性差。

（五）影像学检查

1. 肝脏及颅脑CT：肝脏增强CT血管重建，可以观察是否存在明显的门-体分流。颅脑CT检测本身不能用于HE的诊断或分级，但可发现脑水肿，并排除脑血管意外及颅内肿瘤等[42-43]。

2. 核磁共振成像（MRI）：

（1）脑结构损伤或改变：弥散张量成像（DTI），是一种描述大脑结构的新方法。可以显示脑白质结构损伤程度及范围。研究显示，肝硬化及HE患者MRI表现正常的脑白质区，平均弥散度（mean diffusivity，MD）仍可显著增加，且与HE分期、血氨及神经生理、神经心理改变程度相关[44]。

（2）血流灌注改变：动脉自旋标记（arterial spin labeling，ASL）采用磁化标记的水质子做示踪剂，通过获取脑血容量、脑血流量、氧代谢率等多个灌注参数，可无创检测脑血流灌注变化。有研究显示，MHE患者比无MHE的患者脑灰质脑血流灌注增加，且这种改变与神经心理学评分有一定相关性[45]。但是否可作为MHE的诊断标志物之一，尚需大规模临床验证。

3. 功能性核磁共振成像（fMRI）：近年来，国内外在应用fMRI技术研究大脑认知、感觉等功能定位及病理生理机制取得了很大进步。多位学者[46-48]采用静息态fMRI研究发现HE患者的基底节-丘脑-皮层回路受损，功能连接的改变与HE患者认知功能的改变有关。采用ReHo分析的静息态fMRI可作为一种无创性检查方法，用于揭示有关肝硬化患者认知改变具有重要价值。

由于MHE患者预后差，发生OHE、安全风险及其他肝硬化门静脉高压症并发症的风险高，因此，临床医生应恰当利用目前的检测技术与方法，高度重视MHE的筛查与早期诊断。

（六）诊断与鉴别诊断

1. OHE：依据临床表现和体征，按照West-Haven分级标准，OHE诊断并不困难[49-50]，一般不需要做神经心理学、神经生理学及影像学等检查。诊断要点：（1）有引起HE的基础疾病，严重肝病和/或广泛门体侧支循环分流；（2）有临床可识别的神经精神症状及体征；（3）排除其他导致神经精神异常的疾病，如代谢性脑病、中毒性脑病、神经系统疾病（如颅内出血、颅内感染及颅内占位）、精神疾病等情况；（4）特别注意寻找引起HE（C型、B型）的诱因，如感染、上消化道出血、大量放腹水等；（5）血氨升高。

2. MHE：由于患者无明显的认知功能异常表现，常常需要借助特殊检查才能明确诊断，是临床关注的重点[51-53]。符合以下主要诊断要点（1）、（2）及（3～6）中任意一条或以上，即可诊断为MHE。主要诊断要点：（1）有引起HE的基础疾病，严重肝病和/或广泛门体侧支循环分流；（2）传统神经心理学测试指标中至少2项异常；（3）新的神经心理学测试方法中（ANT、姿势控制及稳定性测试、多感官整合测试）至少1项异常；（4）CFF检测异常；（5）脑电图、视觉诱发电位（VEP）、脑干听觉诱发电位（BAEP）异常；（6）fMRI异常。

3. 鉴别诊断要点：HE需与以下疾病鉴别：（1）精神障碍：以精神症状如性格改变或行为异常、失眠等为唯一突出表现的HE易被误诊为精神障碍。因此，凡遇有严重肝脏疾病或有门-体分流病史的患者出现神经、精神异常，应警惕HE的可能。（2）颅内病变：包括蛛网膜下腔、硬膜外或脑内出血，脑梗死，脑肿瘤，颅内感染，癫痫等。通过检查神经系统定位体征或脑膜刺激等体格检查，结合CT、腰穿刺、动脉造影、脑电图、病毒学检测等做出相应诊断。（3）其他代谢性脑病：包括酮症酸中毒、低血糖症、低钠血症、肾性脑病、肺性脑病等。可通过相应的原发疾病及其血液生物化学分析特点，做出鉴别诊断。（4）韦尼克脑病：多见于严重酒精性肝病患者，维生素B1缺乏导致，补充维生素B1后患者症状可显著改善[54]。（5）中毒性脑病，包括酒精性脑病、急性中毒、戒断综合征、重金属（汞、锰等）脑病，以及精神药物或水杨酸盐药物毒性反应等。通过追寻相应病史和/或相应毒理学检测进行鉴别诊断。（6）肝硬化相关帕金森病。（7）肝性脊髓病：多发生在肝硬化基础上，以皮质脊髓侧束对称性脱髓鞘为特征性病理改变，临床表现为肢体缓慢进行性对称性痉挛性瘫痪，肌力减退，肌张力增高，痉挛性强直，腱反射亢进，常有病理反射阳性，部分患者有血氨升高。（8）获得性肝脑变性：少见且大部分为不可逆性神经功能损害，

是慢性肝病引起的一种不可逆性锥体外系综合征。表现为帕金森综合征、共济失调、意向性震颤、舞蹈症等运动障碍以及精神行为异常和智能障碍等神经心理学改变，fMRI有较好鉴别价值。

推荐意见1：HE是程度和范围较广的神经精神异常，结合临床表现、神经心理测试方法和鉴别诊断，肝硬化HE可分为MHE和HE1～4级（C1）。

推荐意见2：HE是一个连续的临床过程，在严重肝病的基础上，HE1～级依据临床表现可以做出诊断，不推荐做神经心理学、神经生理学及影像学等检查（B1）。

推荐意见3：MHE为没有能觉察的认知功能障碍，神经系统体征正常，但神经心理测试异常，诊断MHE需要特殊的神经心理学或脑功能影像学检查（B1）。

推荐意见4：传统纸笔PHES及计算机辅助PHES是目前广泛应用于MHE的筛查与诊断的方法（A1），应用年龄和受教育程度矫正的PHES可提高MHE诊断的准确性(B1)。

推荐意见5：MHE在肝硬化患者中常见，特别是Child-Pugh C级肝硬化及TIPS术后患者，可影响患者预后，需要重点筛查（A1）；从事驾驶等安全性要求高的肝硬化患者，应该常规筛查MHE（B1）。

推荐意见6：血氨检测需注意质控，止血带压迫时间过长、采血后较长时间才检测、高温下运送，均可能引起血氨假性升高。应室温下采静脉血后立即送检，30 min内完成测定，或离心后4℃冷藏，2 h内完成检测（B1）。

推荐意见7：血氨升高不作为病情轻重、预后及HE分级的指标（C1）。

五、HE的治疗

HE是终末期肝病患者主要死因之一，早期识别、及时治疗是改善HE预后的关键。HE的治疗依赖于其严重程度分层管理（图1）。治疗原则包括及时清除诱因、尽快将急性神经精神异常恢复到基线状态、一级预防及二级预防[55-57]。

（一）去除MHE/HE的诱因

临床上，90%以上MHE/HE存在诱发因素，去除MHE/HE的诱因是治疗的重要措施。

对于肝硬化HE患者，感染是最常见的诱发因素，应积极寻找感染源，即使没有明显感染灶，但由于肠道细菌易位、内毒素水平等升高，存在潜在的炎症状态，而抗菌药物治疗可减少这种炎症状态。因此，应尽早开始经验性抗菌药物治疗。

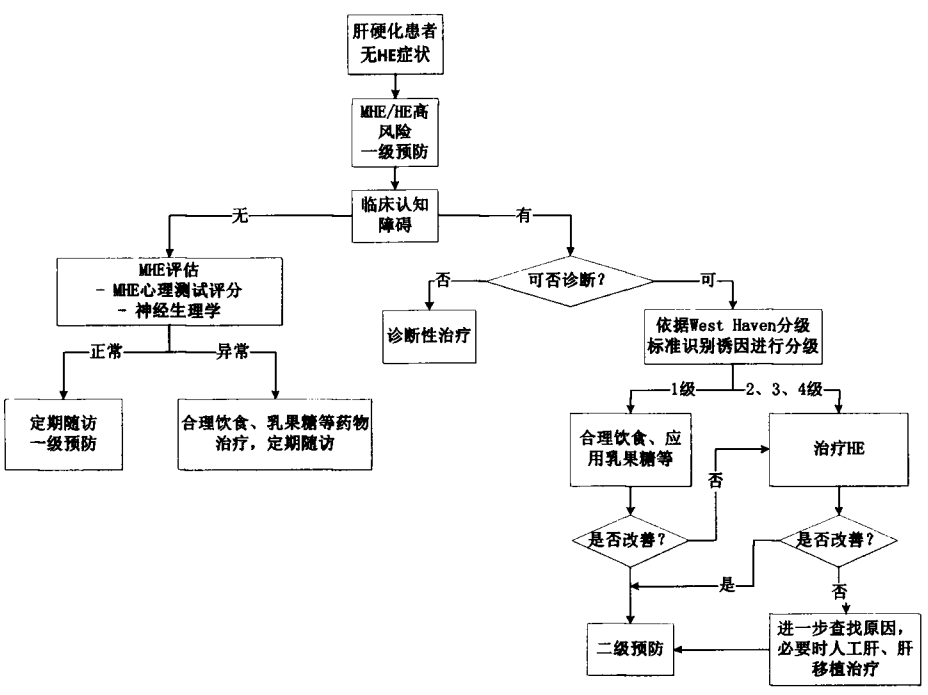
消化道出血也是HE的常见诱发因素，出血当天或其后几天，均易诱发HE；隐匿性消化道出血也可诱发HE。应尽快止血，并清除胃肠道内积血。

过度利尿引起的容量不足性碱中毒和电解质紊乱会诱发HE。此时应暂停利尿剂、补充液体及白蛋白；纠正电解质紊乱（低钾或高钾血症，低钠或高钠血症）。低血容量性低钠血症（特别是血钠低于110 mmol/L），应静脉补充生理盐水；而对于高血容量或等容量低钠血症患者，可使用选择性血管加压素2型受体(V2)拮抗剂。对于3～4级HE患者，积极控制脑水肿，20%甘露醇（250～1 000 ml/d，2～6次/d）或联合呋塞米（40～80 mg/d）[58-59]。

（二）药物治疗

1. 降氨治疗：高血氨是HE发生的重要因素之一，因此降低氨的生成和吸收非常重要。降低血氨的主要药物有：

（1）乳果糖：是由半乳糖与果糖组成的二糖，在自然界中并不存在。其不良反应少，对于有糖尿病或乳糖不耐受的患者也可以应用。乳果糖在结肠中被消化道菌群转化成低分子量有机酸，导致肠道内pH值下降；并通过保留水分，增加粪便体积，刺激结肠蠕动，保持大便通畅，缓解便秘，发挥导泻作用，同时恢复结肠的生理节律。在HE时，乳果糖



注：HE为肝性脑病；MHE为轻微肝性脑病

图1　肝硬化肝性脑病临床诊治流程

促进肠道嗜酸菌（如乳酸杆菌）的生长，抑制蛋白分解菌，使氨转变为离子状态；乳果糖还减少肠道细菌易位，防治自发性细菌性腹膜炎。多项随机对照试验结果显示：乳果糖不仅可以改善MHE患者神经心理测验结果，提高生活质量，还可以阻止MHE进展，预防HE复发。常用剂量为每次口服15～30 ml，2～3次/d（根据患者反应调整剂量），以每天2～3次软便为宜。必要时可配合保留灌肠治疗。对乳果糖不耐受的患者可应用乳糖醇或其他降血氨药物，乳糖醇和乳果糖在灌肠时疗效相似[60-63]。

（2）拉克替醇：为肠道不吸收的双糖，能清洁、酸化肠道，减少氨的吸收，调节肠道微生态，有效降低内毒素[64]。拉克替醇治疗HE的疗效与乳果糖相当，同时起效速度快，腹胀发生率低，甜度较低，糖尿病患者可正常应用[65]。对行TIPS的肝硬化患者临床随机对照研究发现，拉克替醇组和乳果糖组，在治疗期间，两组HE的发生率及相关参数（精神状态、脑电图、扑翼样震颤、数字连接试验和血氨）改变差异无统计学意义，提示拉克替醇可有效长期预防TIPS的肝硬化患者HE的发作。推荐的初始剂量为0.6 g/kg，分3次于餐时服用。以每日排软便2次为标准来增减服用剂量[66]。

（3）L-鸟氨酸L-门冬氨酸（L-ornithine L-aspartate，LOLA）：可作为替代治疗或用于常规治疗无反应的患者。剂量为10～40 g/d，静脉滴注，对OHE和MHE均有治疗作用，LOLA可单药或联合乳果糖，亦有口服制剂。LOLA通过促进肝脏鸟氨酸循环和谷氨酰胺合成减少氨的水平，可明显降低患者空腹血氨和餐后血氨，改善HE的分级及神经心理测试结果，缩短住院时间，提高生活质量[67]。

（4）α晶型利福昔明：是利福霉素的合成衍生物，吸收率低。理论上讲，口服肠道不吸收抗菌药物，可以抑制肠道细菌过度繁殖，减少产氨细菌的数量，减少肠道NH3的产生与吸收，从而减轻HE症状，预防HE的发生，但对B型HE无明显效果。常用剂量为800～1 200 mg/d，分3～4次口服，疗程有待进一步研究。

（5）其他抗菌药物：新霉素、甲硝唑、万古霉素、巴龙霉素等，过去曾采用上述药物治疗，因不良反应及疗效不佳目前较少应用。

（6）微生态制剂：包括益生菌、益生元和合生元等，可以促进对宿主有益的细菌菌株的生长，并抑制有害菌群如产脲酶菌的繁殖；改善肠上皮细胞的营养状态、降低肠黏膜通透性，减少细菌易位，减轻内毒素血症并改善高动力循环；还可减轻肝细胞的炎症和氧化应激，从而增加肝脏的氨清除。多项随机对照试验结果显示益生菌和乳果糖在改善MHE试验的结果方面疗效相似[68-69]。

（7）其他治疗药物：①精氨酸：盐酸精氨酸，因含有盐酸，偏酸性，所以可用于治疗伴代谢性碱中毒的HE。在应用过程中应注意检测血气分析，警惕过量引起酸中毒。盐酸精氨酸在HE治疗中的效果有限，临床不常规应用。②谷氨酰胺：近年来认为，谷氨酸盐只能暂时降低血氨，不能透过血脑屏障，不能降低脑组织中的氨，且可诱发代谢性碱中毒，反而加重HE；另外，脑内过多的谷氨酰胺产生高渗效应，参与脑水肿的形成，不利于HE的恢复，目前临床上不常规应用。③阿卡波糖：最初用于治疗糖尿病，在HE中的确切机制不明，可能与抑制小肠刷状缘的α葡萄糖苷酶有关。阿卡波糖300 mg/d，可降低伴有2型糖尿病和1～2级HE患者的临床症状。不良反应有腹痛、胀气和腹泻。④清除幽门螺旋杆菌（Hp）药物：研究发现HE和MHE与肝硬化无HE患者发生Hp感染率差异有统计学意义，Hp感染与肝硬化HE可能有关，根治Hp可有利于临床预防及治疗肝硬化HE[70-72]。

2. 镇静药物的应用：HE与γ-氨基丁酸神经抑制受体和N-甲基-D-天冬氨酸-谷氨酸兴奋性受体的上调有关，导致抑制性和兴奋性信号的失衡。理论上应用氟马西尼、溴隐亭、左旋多巴和乙酰胆碱酯酶（AChE）抑制剂均是可行的。对于有苯二氮䓬类或阿片类药物诱因的HE昏迷患者，可试用氟马西尼或纳洛酮。溴隐亭、左旋多巴治疗HE有效的证据较少，还需进行仔细评估，一般不推荐使用。

（1）纳洛酮：血浆β内啡肽（β-EP）与HE的发生关系密切，一方面β-EP干扰脑细胞ATP的代谢过程，导致细胞膜稳定性下降及功能障碍，另一方面，β-EP与大脑内阿片受体结合，抑制大脑皮质血液循环，脑组织血供不足，进一步加重脑细胞功能障碍。Meta分析发现，LOLA联合纳洛酮治疗HE，治疗后血氨、总胆红素水平低于对照组，意识转清醒时间缩短，NCT、DST显著改善，无明显不良反应发生。有研究显示纳络酮单用或与乳果糖等药物联合，具有促进患者清醒的作用，但这些研究样本量均较小，且设计上存在一定缺陷[73-74]。

（2）丙泊酚：有研究比较了丙泊酚在40例有狂躁症的HE患者临床疗效及不良反应，与地西泮比较，丙泊酚更安全、更有效地控制HE的狂躁症状[75]。与咪唑安定相比，丙泊酚组恢复时间更短，认知功能恢复更快。

（3）苯二氮䓬类镇静药：由于肝硬化患者焦虑、抑郁、疼痛性疾病的发生率较高，扰乱睡眠-觉醒周期，因此这些患者常有镇静催眠或止痛药物使用史，这些药物可以诱发HE。氟马西尼是一种苯二氮䓬拮抗剂，一项随机双盲对照试验显示氟马西尼疗效优于安慰剂，且无受试者死亡[76]。对于严重精神异常，如躁狂、危及他人安全及不能配合医生诊疗者，向患者家属告知风险后，可使用苯二氮䓬类镇静药首先控制症状，药物应减量静脉缓慢注射。

3. 中医中药：中医认为HE是由于肝肾亏虚、感受湿热疫毒之邪，加之内伤七情，或饮食不节、嗜酒无度等，导致热毒炽盛、热入心包、痰浊内盛、痰迷心窍而发病。故急则治标，采用醒脑开窍法进行治疗，可选用安宫牛黄丸等中成药或汤剂辨证施治，予以开窍醒脑、化痰清热解毒[77]。另外，针对HE的氨中毒学说和肠源性内毒素学说，中医的“通腑开窍”理论亦被广泛应用于HE的防治[78-80]，其中最具代表性的是中药煎剂保留灌肠，如承气汤类、含大黄煎剂、生地黄制剂等。多个临床研究显示使用含大黄煎剂保留灌肠治疗HE均取得了良好效果，在通便、促进肠道毒性物质排出、降低血氨水平、缩短昏迷时间等方面

均有一定作用。

病缓则治本，扶正化淤片（胶囊）、安络化纤丸和复方鳖甲软肝片等因其扶正补虚、活血化淤等功效，具有抗肝纤维化／肝硬化、改善肝功能、改善免疫功能、减轻肝脏血液循环障碍、降低门静脉高压等作用[81-84]，对于肝硬化HE的预防可能有一定价值。

（三）营养支持治疗

传统观点对于HE患者采取的是严格的限蛋白质饮食。近年发现80.3%肝硬化患者存在营养不良，且长时间过度限制蛋白质饮食可造成肌肉群减少，更容易出现HE。正确评估患者的营养状态，早期进行营养干预，可改善患者生存质量、降低并发症的发生率、延长患者生存时间。

1. 能量摄入及模式：肝脏糖原的合成和储存减少，导致静息能量消耗增加，使机体产生类似于健康人体极度饥饿情况下发生的禁食反应。目前认为，每日理想的能量摄入为35～40 kcal/kg（1 kcal = 4.184 kJ）。应鼓励患者少食多餐，每日均匀分配小餐，睡前加餐（至少包含复合碳水化合物50 g），白天禁食时间不应超过3～6 h。进食早餐可提高MHE患者的注意力及操作能力。

2. 蛋白质：欧洲肠外营养学会指南推荐，每日蛋白质摄入量为1.2～1.5 g/kg来维持氮平衡，肥胖或超重的肝硬化患者日常膳食蛋白摄入量维持在2 g/kg，对于HE患者是安全的。因为植物蛋白含硫氨基酸的蛋氨酸和半胱氨酸少，不易诱发HE，含鸟氨酸和精氨酸较多，可通过尿素循环促进氨的清除。故复发性／持久性HE患者可以每日摄入30～40 g植物蛋白。HE患者蛋白质补充遵循以下原则：3～4级HE患者应禁止从肠道补充蛋白质；MHE、1～2级HE患者开始数日应限制蛋白质，控制在20 g/d，随着症状的改善，每2～3 d可增加10～20 g蛋白；植物蛋白优于动物蛋白；静脉补充白蛋白安全；慢性HE患者，鼓励少食多餐，掺入蛋白宜个体化，逐渐增加蛋白总量。

3. 支链氨基酸（BCAA）：3～4级HE患者应补充富含BCAA（缬氨酸、亮氨酸和异亮氨酸）的肠外营养制剂。尽管多项研究显示，BCAA不能降低HE患者病死率，但可耐受正常蛋白饮食或长期补充BCAA患者，可从营养状态改善中长期获益。另外，BCAA不仅支持大脑和肌肉合成谷氨酰胺，促进氨的解毒代谢，而且还可以减少过多的芳香族氨基酸进入大脑[85-86]。

4. 其他微量营养素：HE所致的精神症状可能与缺乏微量元素、水溶性维生素，特别是硫胺素有关，低锌可导致氨水平升高。对失代偿期肝硬化或有营养不良风险的应给予复合维生素或锌补充剂治疗[87]。

（四）人工肝治疗

肝衰竭合并HE时，在内科治疗基础上，可针对HE采用一些可改善HE的人工肝模式，能在一定程度上清除部分炎症因子、内毒素、血氨、胆红素等。常用于改善HE的人工肝模式有血液灌流、血液滤过、血浆滤过透析、分子吸附再循环系统（MARS）、双重血浆分子吸附系统（DPMAS）或血浆置换联合血液灌流等[88-89]。

（五）肝移植

对内科治疗效果不理想，反复发作的难治性HE伴有肝衰竭，是肝移植的指征[90]。

（六）HE护理

三防三护，“三防”指防走失、防伤人、防自残。“三护”指床档、约束带（家属签知情同意书后）、乒乓球手套。应密切观察HE患者性格和行为，意识和神志，神经精神症状及体征改变；观察患者饮食结构尤其是每日蛋白质摄入量并认真记录出入量，观察大小便颜色、性状、次数；观察生命体征、昏迷患者瞳孔大小变化、对光反射情况，痰液情况；观察静脉输液通路是否通畅、有无外渗、穿刺点及周围皮肤情况等。

推荐意见8：积极寻找及去除HE诱因（如感染、消化道出血及电解质紊乱等）(A1)。

推荐意见9：乳果糖可有效改善HE/MHE肝硬化患者的生活质量及生存率。推荐剂量为15～30 ml，2～3次/d，以每天2～3次软便为宜（A1）。

推荐意见10：拉克替醇能酸化肠道，调节肠道微生态，减少氨的吸收，有效降低内毒素，改善HE/MHE临床症状/指标。推荐初始剂量为0.6 g/kg，分3次于餐时服用（B1）。

推荐意见11：门冬氨酸鸟氨酸可降低HE患者的血氨水平、缩短住院时间，对HE具有治疗作用（B1）。

推荐意见12：BCAA可作为替代治疗或长期营养干预治疗（B2）。利福昔明对C型HE有一定治疗作用，800～1 200 mg/d，口服，每日2～4次（B2）。不推荐利福昔明用于B型HE（A1）。

推荐意见13：对于严重精神异常，如躁狂、危及他人安全及不能配合医生诊疗者，向患者家属告知风险后，可使用苯二氮䓬类镇静药或丙泊酚控制症状，药物应减量静脉缓慢注射（B1）。

推荐意见14：合并代谢性碱中毒的肝硬化HE患者可使用盐酸精氨酸等药物治疗（C2）。

推荐意见15：合理饮食及营养补充（每日进食早餐，给予适量蛋白），有助于提高患者生活质量，避免MHE/HE复发（B1）。

推荐意见16：血液灌流、血液滤过及MARS等能降低血氨、炎症因子、胆红素等，可改善肝衰竭患者HE临床症状（B1）。

推荐意见17：难控制的反复发作HE，伴肝衰竭者，应优先考虑肝移植（B1）。

推荐意见18：中药对HE/MHE有一定的防治作用（B2）。

六、预防

（一）一级预防

HE一级预防是指患者有发生HE的风险，但尚未发生HE，其目标是预防MHE/OHE发生、减少OHE相关住院、改善生活质量、提高生存率。对肝硬化、肝衰竭、TIPS术后患者，除了密切观察患者病情变化外，还应定期对患者进行神经生理学、神经心理学、影像学等MHE筛查，一旦诊断MHE，需要立即治疗，以免进展至OHE。

万方数据

一级预防的重点是治疗肝脏原发疾病及营养干预。病因治疗可减轻肝脏炎症损伤及肝纤维化，降低门静脉压力，阻止或逆转肝硬化的进展，对预防和控制 HE 及其他并发症的发生有重要意义。积极预防及治疗感染、消化道出血、电解质紊乱、酸碱平衡失调、便秘等 HE 的诱发因素，避免大量放腹水或利尿，少食多餐，避免摄入过量高蛋白饮食。

（二）二级预防

在第一次 OHE 发作后，患者反复发生 HE 的风险高，为了改善患者生活质量、提高生存率，推荐二级预防。二级预防的重点是患者及其家属健康教育、控制血氨升高及调节肠道微生态。加强对患者及家属健康教育，告知其 HE 特别是 MHE 的潜在危害，并使其了解 HE 的诱因。患者应在医生指导下根据肝功能损伤的情况，合理调整饮食结构，HE 发作期间避免一次性摄入大量高蛋白质饮食。乳果糖、拉克替醇等可作为预防用药。逐步引导患者自我健康管理，并指导家属注意观察患者的行为、性格变化，考察患者有无注意力、记忆力、定向力的减退，尽可能做到 HE 的早发现、早诊断、早治疗。

推荐意见 19：如 MHE 或 OHE 发生风险高，需进行一级预防（B1）。针对病因及营养干预是 MHE/OHE 一级预防的重点（C1）。

推荐意见 20：OHE 控制后，需进行二级预防（A1），乳果糖、拉克替醇等可作为一线药物（A1）。

推荐意见 21：二级预防重点是对患者及家属进行相关健康教育，加强适当营养支持，可明显减少 OHE 反复发作（B1）。睡眠障碍及注意力下降是 OHE 最早表现，指导家属密切观察（C1）。

七、需解决的问题

1. 神经影像组学生物标志物及 fMRI APP 在 HE 诊断中的研究与应用。

2. MHE 早期诊断血清生物标志物、新神经心理学测试方法的研究与应用。

3. HE 新的治疗方法的研究：包括粪便移植预防治疗 HE、干细胞治疗 HE 的研究、HE 新治疗靶点的研究。

专家组成员名单：

（按姓氏汉语拼音排序）

安纪红（内蒙古自治区医院感染科）
蔡大川（重庆医科大学附属第二医院感染科）
陈国凤（解放军第三〇二医院肝纤维化诊疗中心）
陈红松（北京大学人民医院肝病研究所）
陈京龙（首都医科大学附属北京地坛医院肝病中心）
陈　煜（首都医科大学附属北京佑安医院人工肝中心）
成　军（首都医科大学附属北京地坛医院肝病中心）
丁惠国（首都医科大学附属北京佑安医院肝病消化中心）
窦晓光（中国医科大学附属盛京医院感染科）
段钟平（首都医科大学附属北京佑安医院人工肝中心）
冯志杰（河北医科大学第二医院消化内科）
高　慧（北京大学第一医院感染科）
高沿航（吉林大学第一医院肝胆胰内科）
耿嘉蔚（云南省第一人民医院消化内科）
郭会敏（首都医科大学附属北京佑安医院人工肝中心）
郭武华（福建医科大学孟超肝胆医院介入科）
韩　涛（天津市第三中心医院肝内科）
韩　英（空军军医大学西京医院消化科）
黄　缘（清华大学附属北京清华长庚医院肝胆胰内科）
贾继东（首都医科大学附属北京友谊医院肝病中心）
江建宁（广西医科大学第一附属医院感染科）
江应安（湖北省人民医院感染科）
孔媛媛（首都医科大学附属北京友谊医院医学统计室）
李沧友（河北省沧州市传染病医院感染科）
李广明（郑州市第六人民医院肿瘤诊疗中心）
李　杰（北京大学医学部病原生物学系）
李树臣（哈尔滨医科大学附属第二医院感染内科）
李太生（中国医学科学院　北京协和医学院
北京协和医院感染内科）
李文刚（解放军第三〇二医院肿瘤放射治疗中心
肝脏肿瘤科）
李玉芳（宁夏医科大学总医院感染科）
令狐恩强（解放军总医院消化科）
刘景丰（福建医科大学孟超肝胆医院肝胆外科）
刘晓清（中国医学科学院　北京协和医学院
北京协和医院感染内科）
刘迎娣（解放军总医院消化内科）
刘玉兰（北京大学人民医院消化内科）
陆伦根（上海交通大学附属第一人民医院消化科）
罗新华（贵州省人民医院感染科）
马安林（中日友好医院感染疾病科）
马　雄（上海交通大学附属仁济医院消化科）
毛　青（陆军军医大学附属第一医院感染科）
茅益民（上海交通大学附属仁济医院消化科）
南月敏（河北医科大学第三医院中西医结合肝病科）
聂玉强（广州市第一人民医院消化科）
牛俊奇（吉林大学第一医院感染科）
任　红（重庆医科大学附属第二医院感染科）
任万华（山东省立医院感染科）
尚　佳（河南省人民医院感染科）
王　磊（山东大学第二医院感染科）
王宇明（陆军军医大学西南医院感染科）
汪晓军（首都医科大学附属北京佑安医院
中西医结合科）
魏　来（北京大学人民医院肝病研究所）
吴　静（首都医科大学世纪坛医院消化科）
谢渭芬（海军军医大学附属长征医院消化内科）
谢　雯（首都医科大学附属北京地坛医院肝病中心）
辛绍杰（解放军第三〇二医院肝衰竭诊疗与研究中心）
邢卉春（首都医科大学附属北京地坛医院肝病中心）
许建明（安徽医科大学第一附属医院消化科）

万方数据

胥 婕（北京大学第三医院感染科）
徐京杭（北京大学第一医院感染科）
徐小元（北京大学第一医院感染科）
徐有青（首都医科大学天坛医院消化科）
阎 明（山东大学齐鲁医院消化科）
杨东亮（华中科技大学同济医学院附属协和医院感染科）
杨积明（天津市传染病医院感染科）
杨晋辉（昆明医科大学第二附属医院消化内科）
杨永峰（南京市第二医院肝病科）
杨永平（解放军第三〇二医院感染疾病诊疗与研究中心）
尤 红（首都医科大学附属北京友谊医院肝病中心）
于岩岩（北京大学第一医院感染科）
翟所迪（北京大学第三医院药剂科）
张春清（山东省立医院消化科）
张大志（重庆医科大学附属第二医院感染病科）
张岭漪（兰州大学第二医院肝病科）
张伦理（南昌大学附属第一医院感染科）
张文宏（复旦大学附属华山医院感染科）
赵景民（解放军第三〇二医院病理科）
赵 平（解放军第三〇二医院国际肝病科）
赵守松（蚌埠医学院附属医院感染科）
朱 萱（南昌大学附属第一医院消化科）
庄 辉（北京大学医学部病原生物学系）
左维泽（石河子大学医学院第一附属医院传染科）

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执 笔：徐小元 丁惠国 李文刚 贾继东 魏 来 段钟平 刘玉兰 令狐恩强 庄 辉

学术秘书：

韩 莹（首都医科大学附属北京佑安医院肝病消化中心）
亢 倩（北京大学第一医院感染科）
罗 皓（北京大学第一医院感染科）
谭 宁（北京大学第一医院感染科）

中英文缩略词表

AGA（American Gastroenterological Association）美国胃肠病学会
AASLD（American Association for the Study of Liver Diseases）美国肝病学会
ANT（animal naming test）动物命名测试
ASL（arterial spin labeling）动脉自旋标记
AChE（acetylcholine esterase）乙酰胆碱酯酶
ATP(adenosine-triphosphate) 腺嘌呤核苷三磷酸
BCAA（branched-chain amino acid）支链氨基酸
BAEP（brainstem auditory evoked potential）脑干听觉诱发电位
BEAM（brain electrical activity mapping）脑电图仪
Child-Pugh 肝功能分级
CHE（covert hepatic encephalopathy）隐匿性肝性脑病
CHI3L1（chitinase-3-like protein 1）壳多糖酶3样蛋白1
CFF（critical flicker frequency）临界闪烁频率
CT（computed Tomography）X线计算机断层摄影
DST（digit symbol test）数字符号试验
DTI（diffusion tensor imaging）磁共振弥散张量成像
EASL（European Association for the Study of the Liver）欧洲肝病学会
eNCT（electronic number connection test）电子数字连接试验
EEG（electroencephalogram）脑电图
fMRI（fuctional magnetic resonance imaging）功能性核磁共振成像
GRADE（grading of recommendations assessment development and evaluation）推荐分级的评估，制定与评价
GP73（Golgi protein 73）高尔基体蛋白73
HE（hepatic encephalopathy）肝性脑病
Hp（Helicobacter pylori）幽门螺旋杆菌
ICT（inhibitory control test）控制抑制试验
ISHEN（International Society for Hepatic Encephalopathy and NitrogenMetabolism）国际肝性脑病和氮代谢学会
LOLA（L-ornithine L-aspartate）L-鸟氨酸L-门冬氨酸
MHE（minimal hepatic encephalopathy）轻微肝性脑病
MELD (model for end-stage liver disease) 终末期肝病模型
Multi-sensory Intergration 多感官组合测试
MRI（magnetic resonance imaging）磁共振成像
MD（mean diffusivity）平均弥散度
MARS（molecular adsorbent recirculating system）分子吸附再循环系统
NACSELD（North American Consortium for the Study of End-Stage Liver Disease）北美终末期肝病研究联盟
NCT（number connection test）数字连接试验
OHE（overt hepatic encephalopathy）显性肝性脑病
PBC（primary biliary cirrhosis）原发性胆汁性肝硬化
PPI（proton pump inhibitors）质子泵抑制剂
PHES（psychometric hepatic encephalopathy score）肝性脑病心理学评分
RBANS（repeatable battery for the assessment of

neuropsychological status, RBANS) 可重复性成套神经心理状态测验

ReHo (Regional Homogeneity) 局部一致性

TIPS (transjugular intrahepatic potorsystemic shunt) 经颈静脉肝内门体静脉分流术

TBIL (total bilirubin) 总胆红素

VEP (visual evoked potential) 视觉诱发电位

WCOG (World Congresses of Gastroenterology) 世界胃肠病大会

β-EP (β-endorphin) β 内啡肽

附件1 格拉斯哥 (Glasgow) 昏迷量表

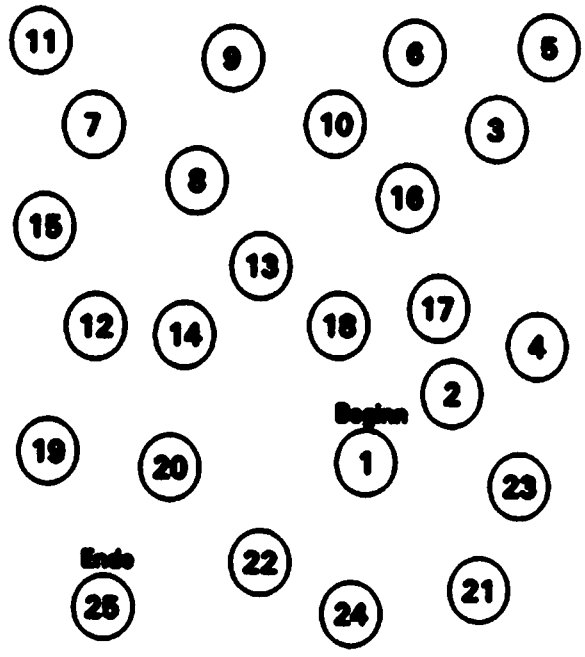
检查项目	表现	分数
眼球运动	有自主反应	4
	呼喊有反应	3
	对疼痛刺激有反应	2
	没有反应	1
运动反应	按命令运动	6
	能对疼痛刺激做出定位反应	5
	对疼痛的屈曲回避动作	4
	疼痛刺激下屈曲运动(去皮层强直)	3
	疼痛刺激下伸展运动(去大脑强直)	2
	无运动反应	1
语言反应	清楚	5
	言语混乱	4
	表达不确切	3
	难以理解	2
	无反应	1

注：该量表最高分是15分，最低分是3分。<12分为严重肝性脑病

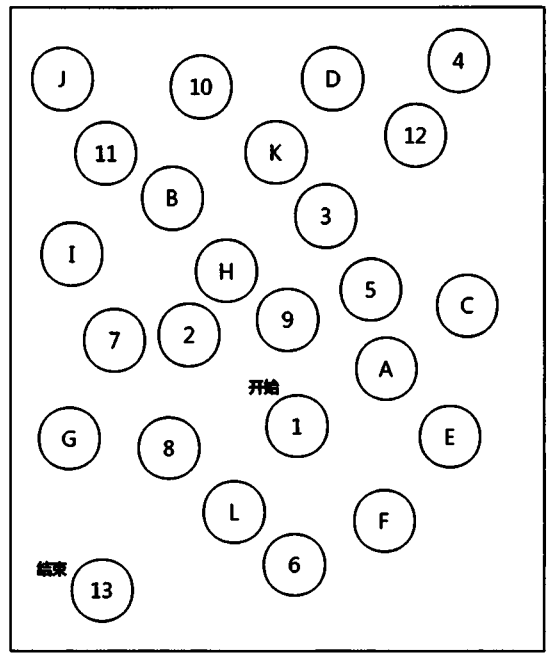
附件2 心理测量的肝性脑病评分 (PHES)

1. 数字连接试验 (number connection test, NCT)：其分为 A、B 两型。

NCT-A：将 1 ～ 25 的数字随机分布在纸上，要求受试者用笔将 1 ～ 25 按顺序连接起来。异常值（正常人均值＋2 倍标准差）：年龄＜35 岁，用时＞34.3s；35 ～ 44 岁，用时＞45.7 s；45 ～ 54 岁，用时＞52.8 s；55 ～ 64 岁，用时＞61.9 s。

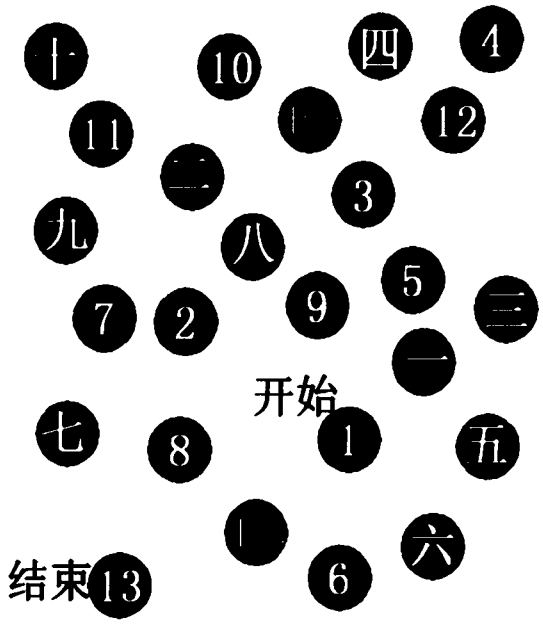


NCT-B：将 1 至 13 和 A 至 L 按 1-A、2-B ……对等顺序连接，如在连接过程中出现错误，要立即纠正并从纠正处继续下去。记录所需的时间，包括纠正错误所花的时间。

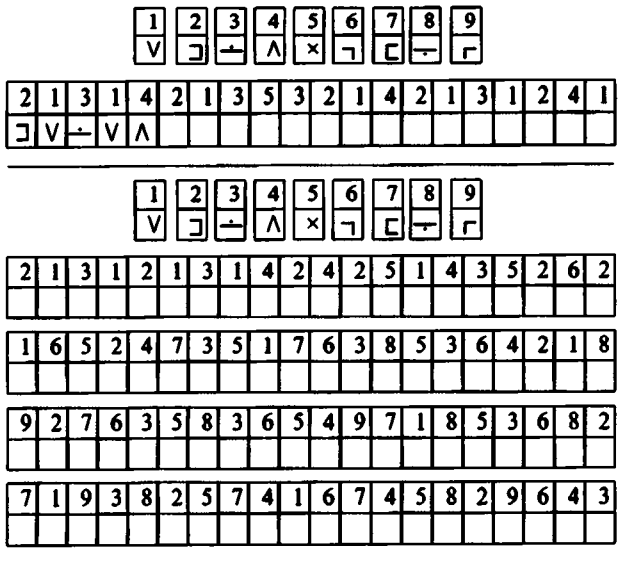


改良版 NCT-B：很多国内的研究应用的是改良的 NCT-B，即用中文数字“一～十二”取代字母“A ～ L”，使之适合我国国情。故经过改良的 NCT-B 将 1 至 13 和一至十二按 1 ～一、2 ～二对等顺序连接。在受试者明确理解后，开始计时并要求受试者尽快、正确地去做，主试者需要密切注意，一旦发现错误要立即指出纠正并从发生错误处继续做下去，记分是以 s 为单位的完成时间（包括纠正错误的时间）。

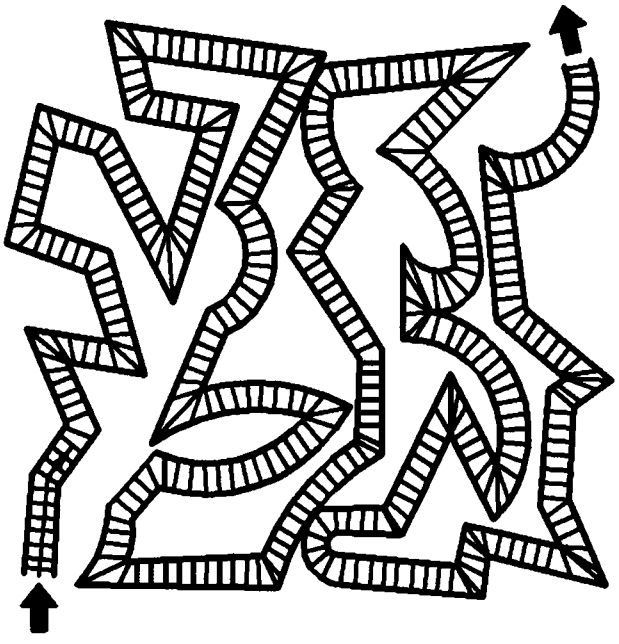
万方数据



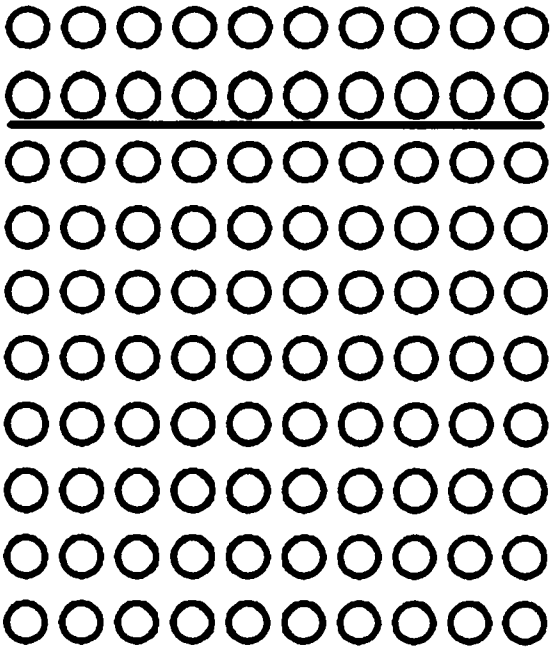
2. 数字符号试验（digit-symbol test，DST）：按照韦氏成人智力量表（WAIS-RC）进行，1到9数字规定了相应的九种简单符号，把符号填写在相应的数字下面，受试者在测定90 s内按顺序依次写出的与数字相应的符号数。先让受试者熟悉数字和符号并在样本上试做，待明确后，才开始计算在90 s内填充正确的个数，每一正确填充记1分，倒转符号记0.5分，错误为0分。主要测试知觉运动速度、视扫描、视觉运动综合能力。计算90 s内的总得分。异常值（x−2 s）：年龄＜35岁，得分＜40.5分；35～44岁，得分＜35分；45～54岁，得分＜28.5分；55～64岁，得分＜26分。



3. 轨迹描绘试验（line tracing test，LTT）：纸上有一连续的宽条纹，走行有直行、转弯和曲线，用铅笔延事先画好的线条在两条线中间由下往上画线，不要穿越过或接触宽条文的轮廓，描绘中不能移纸、也不能将笔离开纸面。尽可能减少错误，从开始描绘计算总共花费的时间，同时计算错误的积分（与界限连接但未越过为1，越过但未到纸边为2，越过纸边为3，按错误的类型及次数累计）。主要测量一般的知觉辨别能力。最后总得分＝所用时间 ×（1＋错误次数/100）。



4. 系列打点试验（serial dotting test，SDT）：受试者尽快在10行圆圈中打点，尽可能打在中心，先练习两行，然后开始，并计算所花费的时间。记分是以s为单位的完成时间（包括错误改正）。主要测量灵活性和知觉辨别能力。



附件3 手机版Stroop测试方法

1. 从Apple App商店下载APP。

2. 测试分2个部分：off阶段和on阶段。开始前均进行2次模拟训练。(1) off阶段测试：1个中性刺激符号(#)每次以红、绿或者蓝中任一种颜色出现，尽快做出反应。按屏幕下方对应的颜色按键；屏幕下方颜色按键也是随机排列分布。每轮要选择10次，所需时间及完成测试的表现会记录下来。一旦犯错，如按错颜色，本轮结束，自动停止，开始新的一轮；需要正确完成5轮，同时也记录下完成时所犯的错误次数。(2)on阶段测试:10个刺激中9个是不一致的，这部分需要正常按对单词字体的颜色，而单词字体与单词含义不一致;例如，红色可能以蓝色出现，正确的按键是蓝色，而非红色。正式开始后需正确完成5轮。

3. 结束后测试软件会自动记录所有数据：(1) 总off阶段时间：完成5轮所需的时间；(2) 正确完成off阶段部分5轮所需要的次数;(3)总on阶段时间:完成5轮所需的时间;(4) 正确完成on阶段部分5轮所需的次数；(5)总on阶段时间+总off阶段时间。

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ORIGINAL PAPER

Serum YKL-40 as a biomarker for liver fibrosis in chronic hepatitis B patients with normal and mildly elevated ALT

Linlin Yan[1] · Yongqiong Deng[1,2] · Jiyuan Zhou[1] · Hong Zhao[1] · Guiqiang Wang[1,3,4] · China HepB-Related Fibrosis Assessment Research Group

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Abstract

Purpose YKL-40 is a chitinase-like protein expressed in multiple tissues including liver and is reported as a fibrosis marker. This study aimed to determine whether YKL-40 could serve as a diagnostic marker for the assessment of liver fibrosis in chronic hepatitis B patients with normal and mildly elevated ALT.

Methods Six hundred and eighty-five patients with chronic hepatitis B infection were enrolled in this study from October 2013 to March 2016. All patients underwent liver biopsy and then staged based on Ishak histological system. Serum YKL-40 levels were measured by Human Magnetic Luminex Assays.

Results Among chronic hepatitis B patients with normal and mildly elevated ALT, almost more than 30% of patients have significant liver fibrosis. Serum YKL-40 levels increased significantly in parallel with the progression of fibrosis in patients with ALT less than two times the upper limit of normal range ($P < 0.0001$). Multivariate analysis revealed that serum YKL-40, hyaluronic acid, PLT, and AST were independently associated with significant fibrosis. We established a novel YKL-40-based fibrosis model for patients with ALT less than two times the upper limit of normal range (ULN). YKL-40 model was superior to APRI, FIB-4, Forns' index, and Hui model for diagnosis of significant fibrosis in patients with ALT < 2ULN, with AUROCs of 0.786 [95% confidence interval (CI) 0.726–0.846] in the training group, 0.831 (95%CI 0.752–0.910) in the validation group and 0.801 (95%CI 0.753–0.849) in the entire cohort.

Conclusion Serum YKL-40 is a feasible biomarker of liver fibrosis in chronic hepatitis B patients. YKL-40 model was superior to APRI, FIB-4, Forns' index and Hui model for diagnosis of significant fibrosis in patients with normal and mildly elevated ALT.

Keywords YKL-40 · Chronic hepatitis B · Liver fibrosis

Abbreviations

HBV	Hepatitis B virus
CHB	Chronic hepatitis B
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B e antigen
BMI	Body mass index
ALT	Alanine transaminase
AST	Aspartate transaminase
ALP	Alkaline phosphatase

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✉ Guiqiang Wang
John131212@126.com

Linlin Yan
ylinlin3@163.com

Yongqiong Deng
dengyongqiong1@126.com

Jiyuan Zhou
zhoujiyuan198703@163.com

Hong Zhao
minmin2001@126.com

[1] Department of Infectious Disease, Center for Liver Disease, Peking University First Hospital, No. 8, Xishiku Street, Xicheng District, Beijing 100034, China

[2] The Department of Dermatology, The Affiliated Hospital of Southwest Medical University, Luzhou 646000, Sichuan, China

[3] The Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Zhejiang University, Hangzhou, Zhejiang, China

[4] Peking University International Hospital, Beijing, China

GGT	Gamma-glutamyl transpeptidase
TBil	Total bilirubin
PT	Prothrombin time
PLT	Platelet counts
HA	Hyaluronic acid
LN	Laminin
PIIINP	N-terminal peptide of type III procollagen
YKL-40	Chitinase 3-like-1
sCD163	Soluble CD163
MMP	Matrix metalloproteinase
TIMP-1	Tissue inhibitor of metalloproteinase 1
ULN	Upper limit of normal
ROC	Receiver operating characteristic curve
AUROC	Area under the receiver operating characteristic curve
SD	Standard deviation
CI	Confidence interval

Introduction

Chronic hepatitis B (CHB) infection remains a major global health burden; approximately, 350–400 million individuals were infected [1]. The burden of chronic hepatitis B infection is serious in China, with an estimated 120 million people infected, and 0.3 million deaths annually [2]. In China, a proportion of CHB patients are asymptomatic accompanied by normal and mildly elevated alanine transaminase (ALT, ALT levels are less than two times the upper limit of normal). The guidelines of American Association for the Study of Liver Diseases (AASLD) and Asian Pacific Association for the Study of the Liver for the management of CHB recommend antiviral treatment when ALT levels are two times the upper limit of normal (ULN), and monitoring or performing liver biopsy (especially for patients > 40 years) to assess if significant histologic disease is present when ALT levels are less than two times the upper limit of normal (ULN) [3, 4]. However, CHB patients with normal and mildly elevated ALT levels may not have healthy livers. Several studies indicated that moderate inflammation and/or advanced fibrosis was present in 28–37% of CHB patients who had persistently normal ALT [5–7]. These studies suggested that CHB patients with normal ALT might have histologically significant disease, an indication for antiviral treatment. Use of ALT without resorting to liver biopsy may miss a certain proportion of patients with histologically significant disease who may benefit from antiviral therapy. Liver biopsy remains the gold standard for assessing liver fibrosis in CHB patients. However, liver biopsy has several limitations including invasiveness, risk of complications, sampling error, and cost [8], which limited its application in assessing and dynamic monitoring of liver fibrosis. Currently, multiple noninvasive methods based on laboratory tests have been developed as surrogates to assess liver fibrosis, such as aspartate aminotransferase–platelet index (APRI), fibrosis index based on the four factors (FIB-4), Forns' index [9], and Hui model [10]. Gao et al. [11] had reported a noninvasive model, consisting of aspartate transaminase (AST), HBsAg, platelet, and albumin, to predict significant liver histology change [necroinflammatory activity grade (G) ≥ 2 or fibrosis stage (S) ≥ 2] in HBeAg-positive CHB with ALT ≤ 2ULN. Gao's model had an area under the receiver operating characteristic curve of 0.868, which was significantly higher than APRI and FIB-4. However, there is no noninvasive method to predict significant fibrosis in CHB patients with ALT < 2ULN regardless of HBeAg status and HBV DNA levels.

YKL-40 (chitinase-3-like-1, or human cartilage glycoprotein-39) is a member of the mammalian chitinase family [12] and is secreted by a variety of cells, including neutrophils, macrophages, and vascular smooth muscle cells [13]. YKL-40 is thought to be involved in remodeling of the extracellular matrix and in inflammatory processes [14]. YKL-40, as the growth factor for fibroblasts and chemoattractant for endothelial cells, is also believed to modulate angiogenesis during tissue damage [15, 16]. Recently, YKL-40 mRNA expression was found in human liver [17], and serum YKL-40 levels were associated with liver fibrosis in patients with chronic liver disease [18]. Immunohistochemical studies have shown that YKL-40 is expressed in fibrotic areas of the liver [17, 19]. Based on these supporting evidences, serum YKL-40 has been evaluated as a noninvasive marker of fibrotic liver diseases, including alcoholic liver disease [20], non-alcoholic fatty liver disease [21] and chronic hepatitis C-induced liver fibrosis [22, 23]. Therefore, we recently proposed a hypothesis that serum YKL-40 may be a potential biomarker for differentiating significant fibrosis in chronic hepatitis B patients with normal and mildly elevated ALT.

In this study, we identified the proportion of significant fibrosis in CHB patients with normal and mildly elevated ALT. We measured the serum levels of YKL-40 and compared them with fibrosis stages to evaluate the feasibility of YKL-40 as a biomarker of liver fibrosis in patients with normal and mildly elevated ALT levels.

Patients and methods

Patients

A total of 685 patients with chronic HBV infection from 24 hospitals located in mainland China were enrolled in this study between October 2013 and March 2016. Of which, 460 patients have ALT levels less than two times the upper limit of normal range (ULN), and they were randomly divided into a training group (n = 307) and a validation

group ($n = 153$). They all underwent liver biopsies. Inclusion and exclusion criteria were described previously [24]. All patients provided written informed consent for research use of their clinical data and specimens. This study was approved by the Ethics Committee of Peking University First Hospital. The detailed protocol for the clinical trial was registered at clinicaltrials.gov (NCT01962155) and chictr.org (ChiCTR-DDT-13003724).

Histological staging

Ultrasonography-guided liver biopsies with a minimal length of 20 mm (at least 11 portal tracts) were routinely performed at each hospital according to a standardized protocol after receiving the patient's written informed consent. Pathological interpretations were conducted in the Department of Pathology at You An Hospital affiliated to the Capital Medical University. The histopathological examination rules were previously reported [24]. Fibrosis stages were assessed according to Ishak criteria [25]. Significant fibrosis was defined as F3.

Examination of serum markers

The biochemical and hematological parameters were routinely detected by standard methods in local hospitals. Serum HBV DNA (range 2.0×10^1–1.7×10^8 IU/ml) was measured by the COBAS AmpliPrep/COBAS TaqMan (Roche Diagnostics, Basel, Switzerland). Serum HBsAg (range of 20–52,000 IU/ml) was quantified using the Roche Elecsys HBsAg II assay (Roche Diagnostics, Penzberg, Germany). The serum levels of YKL-40 were determined using Human Magnetic Luminex® Assays (LXSAHM-08, R&D Systems, Inc, Minneapolis, MN, USA) according to the manufacturer's instructions. The serum concentrations of hyaluronic acid (range of 2–200 μg/L), laminin (5–900 μg/L), were measured using a chemiluminescence immunoassay kit (Yuande Bio-Medical Engineering Co., Ltd, Beijing, China).

Noninvasive fibrosis scores

Noninvasive assessment of fibrosis, APRI, and FIB4 was calculated according to the following formulae: APRI = [(AST/ULN)/platelet($\times 10^9$/L)] × 100; FIB4 = (age × AST)/[platelet($\times 10^9$/L) × $\text{ALT}^{1/2}$]. Forns' index [9] and Hui model [10] were obtained from reported research.

Statistical analysis

Quantitative variables were expressed as mean ± standard deviation (SD) and categorical variables were expressed as proportions. For normally and non-normally distributed variables, the differences between the groups were analyzed using Student *t* test and Mann–Whitney *U* test, respectively. For categorical variables, Chi-square test was used to compare the differences in proportions. Spearman's rank test was used to analyze the correlations between different variables and fibrosis stages. We performed multivariate backward logistic regression analysis to determine the independent variables of significant fibrosis. Receiver operating characteristic curve (ROC) was used to assess the performance of noninvasive models for staging significant fibrosis. The diagnostic performance of different variables was evaluated based on the area under the receiver operating characteristic curve (AUROC). SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses. $P < 0.05$ were considered statistically significant.

Results

Patient's characteristics

A total of 685 patients were enrolled in this study; seven patients were excluded because of unqualified liver tissue. The remaining 678 patients with chronic HBV infection were analyzed, of which 460 patients with ALT less than two times the upper limit of normal range (ULN). The baseline characteristics of the study patients are shown in Table 1. There were no significant distributional differences in fibrosis stages between the group of patients with ALT ≥ 2 × ULN and the group of patients with ALT < 2 × ULN ($P = 0.312$, Table 1). This result indicated the presence of significant or more severe fibrosis in patients with ALT < 2 × ULN, patients who do not meet the treatment criteria recommended by AASLD guideline.

ALT was not a perfect surrogate marker for liver histology

For patients with ALT < 2 × ULN, they were stratified (from G1 to G5) according to the status of HBeAg and the levels of HBV DNA, as shown in Table 1. In patients with normal ALT, differences in the proportion of significant fibrosis were statistically significant ($P = 0.015$, Fig. 1a). Overall, more than 30% of patients had significant fibrosis, besides G1 (immuno-tolerant phase) with 17.8% incidence of significant fibrosis. Similar results were obtained in patients with mildly elevated ALT ($P < 0.0001$, Fig. 1b). Regarding the incidence of significant fibrosis between patients with normal ALT and patients with mildly elevated ALT, there were no significant differences (data not shown). This result

Table 1 Patients' characteristics

	ALT ≥ 2 × ULN (n = 218)	ALT < 2 × ULN (n = 460)	P value
Age (median, ≥ 40 years %)	36, 77 (35.3%)	38, 205 (44.6%)	0.024
Gender (male %)	184 (84.4%)	345 (75.0%)	0.006
BMI (median, ≥ 24 kg/m^2 %)	23.3, 76 (34.9%)	23.0, 165 (35.9%)	0.864
HBsAg ($\log_{10}$IU/mL)	3.59 ± 0.77	3.56 ± 0.88	0.409
AST (U/L)	116.55 ± 109.99	35.50 ± 17.84	< 0.001
ALP (U/L)	91.64 ± 29.93	77.31 ± 26.07	< 0.001
GGT (U/L)	82.62 ± 69.51	41.50 ± 47.42	< 0.001
Albumin (g/L)	43.67 ± 5.85	44.48 ± 5.26	0.002
TBil (μmol/L)	18.30 ± 15.33	16.94 ± 22.78	0.017
PT (s)	12.94 ± 1.51	12.56 ± 1.49	0.001
PLT (× 10^9/L)	170.64 ± 52.76	172.33 ± 59.08	0.635
Hyaluronic acid (ug/L)	149.05 ± 102.82	115.26 ± 71.14	< 0.001
Laminin (ug/L)	179.54 ± 302.39	84.24 ± 177.79	< 0.001
PIIINP (ug/L)	5.65 ± 11.42	3.65 ± 5.04	< 0.001
Collagen IV (pg/mL)	1120.00 ± 628.19	896.98 ± 540.96	< 0.001
YKL-40 ($\log_{10}$ pg/mL)	4.46 ± 0.38	4.47 ± 0.38	0.718
sCD163($\log_{10}$ pg/mL)	6.20 ± 0.36	6.01 ± 0.33	< 0.001
MMP-1 ($\log_{10}$ pg/mL)	3.47 ± 0.31	3.48 ± 0.32	0.566
MMP-2 ($\log_{10}$ pg/mL)	5.28 ± 0.10	5.26 ± 0.10	0.058
MMP-3 ($\log_{10}$ pg/mL)	4.17 ± 0.25	4.17 ± 0.26	0.624
MMP-9 ($\log_{10}$ pg/mL)	4.85 ± 0.41	4.87 ± 0.45	0.770
TIMP-1 ($\log_{10}$ pg/mL)	5.08 ± 0.12	5.06 ± 0.13	0.018
HBeAg status/HBV DNA(IU/mL) (n %)			0.020
G1 e+, HBV DNA ≥ 2 × 10^7	86 (39.4%)	128(27.6)	
G2 e+, 20,000 ≤ HBV DNA < 2 × 10^7	54 (24.8)	116 (25.4)	
G3 e+, HBV DNA < 20,000	9 (4.1)	25 (5.7)	
G4 e−, HBV DNA ≥ 2000	59 (27.1)	152 (32.8)	
G5 e−, HBV DNA < 2000	10 (4.6)	39 (8.5)	
Fibrosis stages (n %)			0.312
*F*0–2	128 (58.7%)	291 (63.2%)	
*F*3	48 (22.0%)	85 (18.5%)	
*F*4	33 (15.1%)	68 (14.8%)	
*F*5–6	9 (4.1%)	16 (3.5%)	

Data presented as mean ± SD or no. (%)

BMI body mass index, *HBsAg* hepatitis B surface antigen, *AST* aspartate transaminase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transpeptidase, *TBil* total bilirubin, *PT* prothrombin time, *PLT* platelet counts, *PIIINP* N-terminal peptide of type III procollagen, *YKL-40* chitinase 3-like-1, *sCD163* soluble CD163, *MMP* matrix metalloproteinase, *TIMP-1* tissue inhibitor of metalloproteinase 1, *HBeAg* hepatitis B e antigen, *HBV* hepatitis B virus, *ULN* upper limit of normal

suggested that ALT levels and fibrosis are not always consistent in CHB patients.

Serum YKL-40 levels increased with the progression of fibrosis

Serum YKL-40 levels were measured to assess the feasibility of YKL-40 as a biomarker of fibrosis in CHB patients. Serum levels of YKL-40 throughout different fibrosis stages are shown in Fig. 2. In the total patients, serum YKL-40 levels increased in parallel with the progression of fibrosis, showing significant difference between fibrosis stages (F01 vs F2–F56, F2 vs F3–F56) (P < 0.0001, Fig. 2a). In patients with ALT < 2 × ULN, similar results were obtained as in the total patients (Fig. 2b). In addition, serum YKL-40 levels were positively correlated with hyaluronic acid, laminin, PIIINP, Collagen, and AST, while they were negatively correlated with platelet count (Supplementary Table 1).

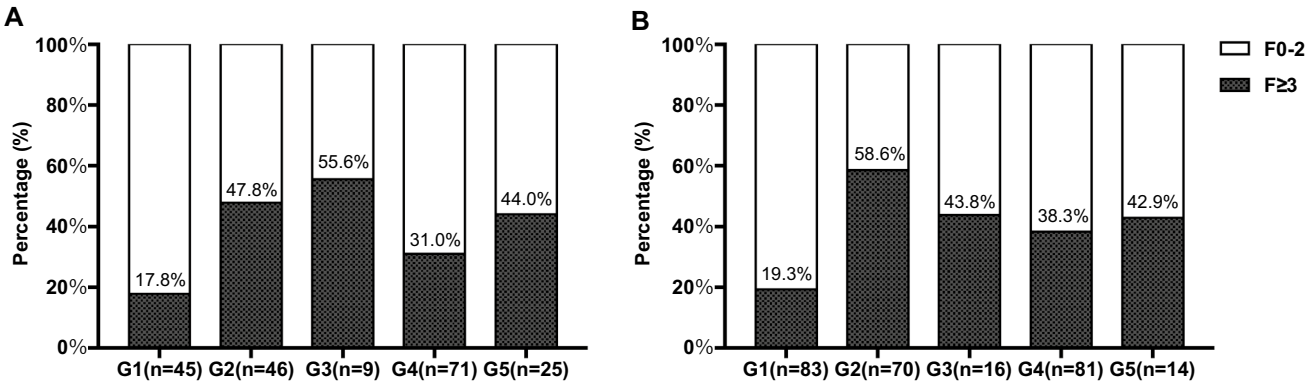

Fig. 1 Proportion of patients with significant fibrosis in the group of G1–G5 in chronic hepatitis B patients. Patients with **a** normal ALT and **b** mildly elevated ALT

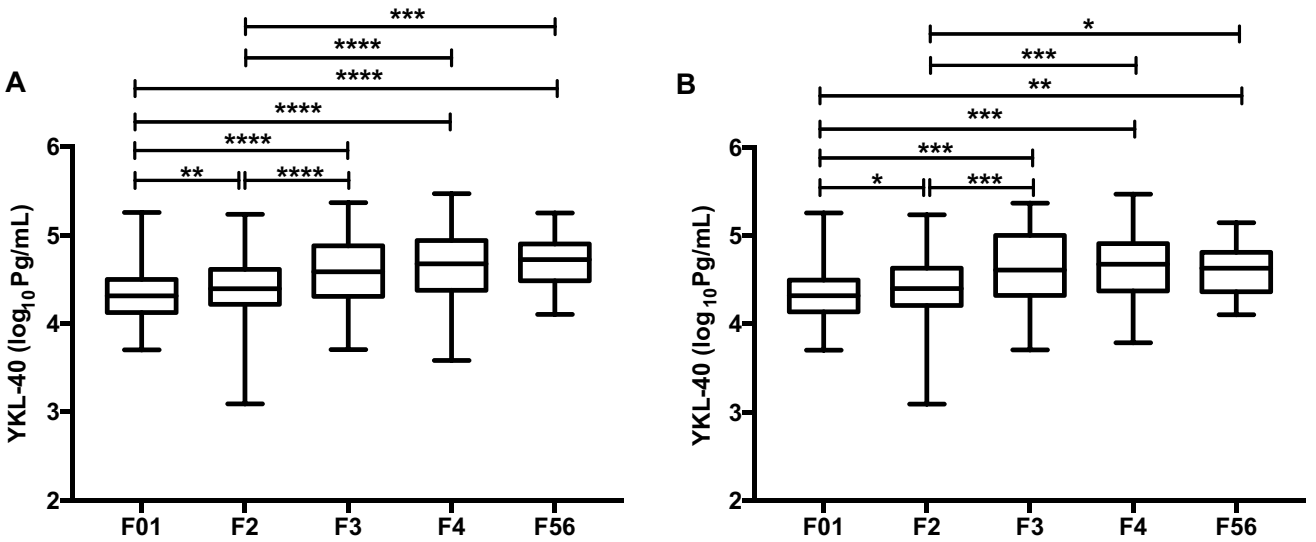

Fig. 2 Associations between serum YKL-40 levels and liver fibrosis. **a** YKL-40 in total patients, **b** YKL-40 in patients with ALT < 2 × ULN. $P < 0.0001$ for all fibrosis stags. ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

Development of YKL-40-based fibrosis model in patients with ALT < 2 × ULN

To determine the ability of YKL-40 to diagnose significant fibrosis, all CHB patients with ALT < 2 × ULN were divided into a training group and a validation group. There was no statistical difference between training group and validation group about any parameters (Supplementary Table 2). In the training group, univariate analysis found that serum YKL-40, hyaluronic acid, laminin, PIIINP, Collagen IV, sCD163, and MMP-2 were positively associated with significant fibrosis (Table 2). PLT was inversely associated with significant fibrosis (Table 2). Multivariate analysis revealed that YKL-40 [odd ratio (OR) 2.330, 95% confidence interval (CI) 1.019–5.330, $P = 0.045$], hyaluronic acid (HA), PLT, and AST were independent factors of significant fibrosis (Table 3). We performed backward logistic regression analysis and established a novel YKL-40 based model for CHB patients with ALT < 2 ULN:

YKL-40 model = 0.032 × AST − 0.012 × PLT + 0.012 × HA + 0.846 × log10 (YKL-40) − 4.752.

Diagnostic performance of YKL-40 model for significant fibrosis

YKL-40 model had an area of 0.786 (95%CI 0.726–0.846) under the ROC curve in predicting significant fibrosis in the training group, with 71.74% sensitivity, 72.85% specificity, 61.68% PPV, and 80.88% NPV at the cut-off point

Table 2 Univariate analysis of clinical parameters and biomarkers with significant fibrosis in the training group ($n=307$)

	*F*0–2 ($n=194$)	$F\geq 3$ ($n=113$)	*P* value
Age (≥ 40 years %)	37.54 ± 10.12	42.54 ± 10.92	< 0.0001
Gender (male %)	141 (72.68%)	83 (73.45%)	> 0.9999
BMI (≥ 24 kg/m^2 %)	22.88 ± 2.95	23.50 ± 2.74	0.096
HBsAg ($\log_{10}$IU/mL)	3.71 ± 0.88	3.28 ± 0.72	< 0.0001
ALT (U/L)	42.16 ± 17.61	44.55 ± 16.04	0.258
AST (U/L)	32.52 ± 15.72	42.36 ± 21.75	< 0.0001
ALP (U/L)	72.61 ± 19.21	85.90 ± 33.97	0.002
GGT (U/L)	35.60 ± 45.56	54.91 ± 47.21	< 0.0001
Albumin (g/L)	45.06 ± 4.53	43.58 ± 6.78	0.003
TBil (μmol/L)	15.45 ± 17.01	20.84 ± 38.75	0.002
PT (s)	12.32 ± 1.27	12.85 ± 1.43	0.002
PLT ($\times 10^9$/L)	187.06 ± 49.56	145.23 ± 53.21	< 0.0001
Hyaluronic acid (ug/L)	93.89 ± 41.27	151.04 ± 96.27	< 0.0001
Laminin (ug/L)	48.21 ± 91.38	126.52 ± 204.21	< 0.0001
PIIINP (ug/L)	3.12 ± 6.14	4.53 ± 4.56	< 0.0001
Collagen IV (pg/mL)	782.48 ± 387.26	1037.84 ± 584.14	< 0.0001
YKL-40 (log10 pg/mL)	4.39 ± 0.35	4.62 ± 0.40	< 0.0001
SCD163 (log10 pg/mL)	5.94 ± 0.32	6.12 ± 0.32	< 0.0001
MMP-1 (log10 pg/mL)	3.49 ± 0.32	3.46 ± 0.31	0.501
MMP-2 (log10 pg/mL)	5.24 ± 0.10	5.28 ± 0.10	0.001
MMP-3 (log10 pg/mL)	4.17 ± 0.27	4.18 ± 0.26	0.740
MMP-9 (log10 pg/mL)	4.86 ± 0.46	4.86 ± 0.40	0.752
TIMP-1 (log10 pg/mL)	5.05 ± 0.13	5.08 ± 0.13	0.134

BMI body mass index, *HBsAg* hepatitis B surface antigen, *ALT* alanine transaminase, *AST* aspartate transaminase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyl transpeptidase, *TBil* total bilirubin, *PT* prothrombin time, *PLT* platelet counts, *PIIINP* N-terminal peptide of type III procollagen, *YKL-40* chitinase 3-like-1, *sCD163* soluble CD163, *MMP* matrix metalloproteinase, *TIMP-1* tissue inhibitor of metalloproteinase 1

Table 3 Multivariate logistic regression analysis of independent predictors for significant fibrosis in the training group ($n=307$)

	Coefficient	OR	95%CI	*P* value
AST (U/L)	0.032	1.033	1.009–1.057	0.007
PLT ($\times 10^9$/L)	−0.012	0.988	0.982–0.995	< 0.0001
Hyaluronic acid (ug/L)	0.012	1.013	1.005–1.020	0.001
YKL-40 (log10 pg/mL)	0.846	2.330	1.019–5.330	0.045
Constant	−4.758	0.009	–	0.018

YKL-40 model = 0.032 × AST − 0.012 × PLT + 0.012 × HA + 0.846 × log10 (YKL-40) − 4.752

of −0.56. It was superior to that of APRI [0.736 (95%CI 0.670–0.803)], FIB-4 [0.735 (95%CI 0.669–0.801)], Forns' index [0.753 (95%CI 0.688–0.817)], and Hui model [0.734 (95%CI 0.667–0.801)] (Fig. 3a, Table 4). The area under the ROC curve of YKL-40 model in the validation group was 0.831 (95%CI 0.752–0.910), with 71.79% sensitivity, 85.33% specificity, 71.79% PPV, and 85.33% NPV at the cut-off point of −0.33, which was also higher than that of APRI, FIB-4, Forns' index, and Hui model (Fig. 3b, Table 4). In the entire cohort, YKL-40 model had an area of 0.801 (95%CI 0.753–0.849) under the ROC curve in predicting significant fibrosis (data not shown).

Discussion

Serum ALT is commonly used to assess liver histology activity and to guide antiviral therapy in patients with liver disease. However, results of the present study showed that, ALT levels and fibrosis are not always consistent in CHB patients. We observed that a high proportion (> 30%) of CHB patients with normal and mildly elevated (1-2ULN) ALT have significant fibrosis regardless of the state of HBeAg and the levels of HBV DNA (Fig. 1, G2–G5). Even for patients in the immunO-tolerant phase (Fig. 1, G1), 17.8 and 19.3%, respectively, have significant fibrosis. Our present findings are consistent with the previous reports that patients with chronic HBV infection can display normal and mildly elevated ALT levels despite significant histological injury [6, 7, 26]. A meta-analysis [26] concluded that approximately one-fifth of CHB patients with ALT ≤ 40 IU/L may have significant hepatic fibrosis. Lai et al. [7] found that 37% of CHB patients with persistently normal ALT had significant fibrosis and inflammation. According to current guidelines, antiviral therapy should be initiated immediately for patients with significant fibrosis [3, 4]. Our results confirmed that ALT was not a perfect surrogate marker for liver histology, because ALT failed to identify many patients who might benefit from antiviral therapy. The "gray zone" patients were defined as those patients with normal and mildly elevated ALT. Because of the high proportion of significant liver disease in the "gray zone" patients, it is highly important to assess liver fibrosis. Liver biopsy, a gold standard for assessing liver fibrosis, is not suitable for regular applications due to the limitations of invasive, finite, complications, and cost [8]. Noninvasive models such as APRI and FIB-4 using biochemical laboratory index have been proposed to replace liver biopsy to assess liver fibrosis. Therefore, it is reasonable to evaluate "gray zone" patients based on such noninvasive methods, and then to decide whether initiating antiviral treatment or not.

Within the present study, we assessed the relationship between serum markers, including YKL-40, hyaluronic

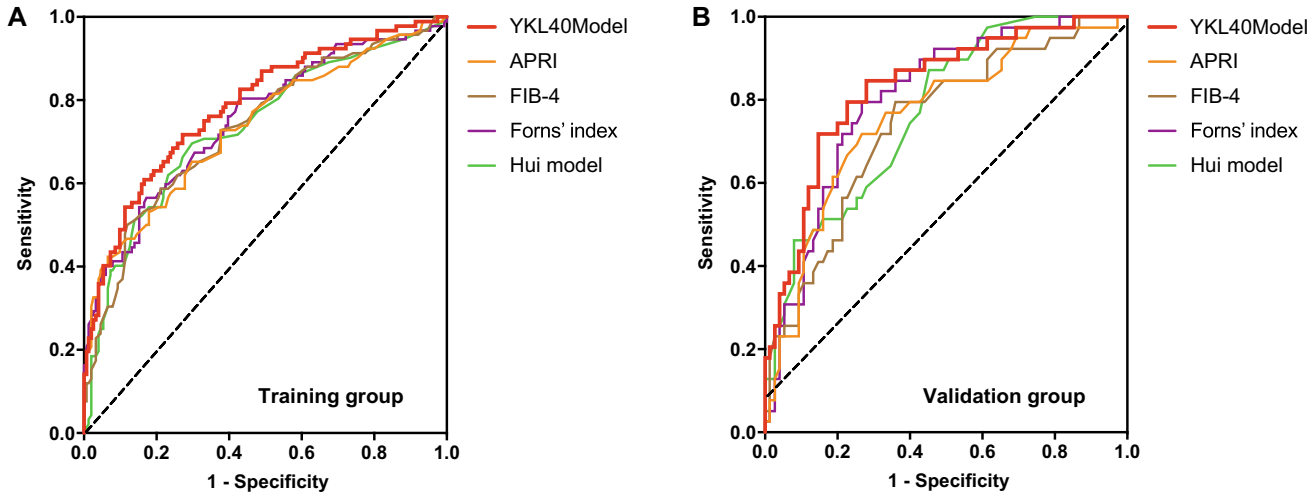

Fig. 3 Receiver operating characteristic curve (ROC) analysis showing the diagnostic performance of noninvasive models for significant fibrosis. Area under the ROC curves (AUROCs) of YKL-40 model, ARPI, FIB4, Forns' index and Hui model in the diagnosis of significant fibrosis in CHB patients with ALT < 2ULN. **a** Training group and **b** validation group

Table 4 Receiver operating characteristics curve (ROC) analysis of noninvasive models for the diagnosis of significant fibrosis in CHB patients with ALT < 2ULN

	AUROC (95%CI)	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Training group						
YKL-40 model	0.786 (0.726–0.846)	−0.56	71.74	72.85	61.68	80.88
APRI	0.736 (0.670–0.803)	0.76	42.39	93.38	79.60	72.68
FIB-4	0.735 (0.669–0.801)	1.33	50.00	88.08	71.88	74.30
Forns' index	0.753 (0.688–0.817)	7.75	56.52	82.78	66.66	75.76
Hui model	0.734 (0.667–0.801)	0.12	69.57	70.20	58.72	79.11
Validation group						
YKL-40 model	0.831 (0.752–0.910)	−0.33	71.79	85.33	71.79	85.33
APRI	0.762 (0.668–0.855)	0.50	71.79	73.33	58.33	83.33
FIB-4	0.743 (0.648–0.838)	1.14	79.49	64.00	53.45	85.72
Forns' index	0.804 (0.723–0.886)	7.12	79.49	73.33	60.78	87.30
Hui model	0.771 (0.684–0.857)	0.08	87.18	54.67	50.00	89.13

acid, laminin, PIIINP, Collagen IV, sCD163 and metalloproteinases, and liver fibrosis in patients with normal and mildly elevated ALT. Of note, our results indicated that serum YKl-40 levels significantly correlated with fibrosis stages as assessed by Ishak score. Serum levels of YKL-40 also increased in non-alcoholic fatty liver disease (NAFLD) and chronic hepatitis C-induced liver fibrosis [21, 22, 27, 28]. It has been reported that YKL-40 is a growth factor for fibroblasts and is expressed in active liver fibrotic areas [29, 30]. In addition, the progression of fibrosis rate per year linearly correlates with the serum levels of YKL-40 [23]. These observations further strengthen the possibility that YKL-40 is involved in hepatic fibrogenesis in patients with HBV infection and is a useful biomarker for hepatic fibrosis. It is critical to discriminate patients with significant fibrosis, a stage which represent an indication for antiviral therapy, from the "gray zone" patients. Our univariate analysis revealed that serum YKL-40, hyaluronic acid, laminin, PIIINP, Collagen IV, sCD163, and MMP-2 were associated with significant fibrosis. However, multivariate analysis showed that only YKL-40, hyaluronic acid and two laboratory parameters, PLT and AST, retained significance when combined with other clinical parameters. Series studies have demonstrated that combination of multiple serum markers could improve the sensitive, specific, and reproducible [31, 32]. Based on our findings, a four-variable model including two serum fibrosis markers ($\log_{10}$YKL-40, hyaluronic acid) and two routinely laboratory tests (PLT, AST) was derived via backward logistic regression analysis to detect significant fibrosis. Hyaluronic acid is synthesized by stellate cells and

is involved in fibrogenesis; it has been identified as one of the serum markers of liver fibrosis in non-alcoholic steatohepatitis (NASH) and chronic hepatitis C [31–34]. Regarding PLT, our finding is consistent with the previous studies that found decreased platelet counts are associated with more severe hepatic fibrosis [35, 36].

Identification of patients, who actually had significant hepatic fibrosis, diagnosed as "none treatment required" according to ALT levels, is very important. Significant fibrosis is an important endpoint of clinical antiviral therapy [37, 38]. The aim of this study was to develop an accurate noninvasive fibrosis model applied to "gray zone" CHB patients. Over the past 20 years, various noninvasive fibrosis models have emerged. The most widely used two scores, APRI and FIB-4, and Forns' index, are based on patients with hepatitis C infection [39]. APRI and FIB-4 have been validated and recommended for evaluation of liver fibrosis in CHB patients [39, 40]. Hui model is based on patients with HBV [10] while lacking of clinical validation (Supplementary Table 3). Furthermore, the diagnostic performances of the above models for fibrosis assessment in CHB patients with normal and mildly elevated ALT have not been validated in large cohorts. A recent analysis of APRI and FIB-4 in 231 HBV-infected patients with normal and mildly elevated ALT founded limited diagnostic value for significant fibrosis [41]. In this study, we developed a YKL-40 model in 460 CHB patients with normal and mildly elevated ALT, and then, we compared the performances of the five noninvasive models to diagnose significant fibrosis. For the identification of patients with significant fibrosis, the AUROCs for patients with ALT < 2ULN were 0.736 for APRI and 0.735 for FIB-4 in the training group, compared with 0.762 for APRI and 0.743 for FIB-4 in the validation group, showing similar performance as previous reported [41]. We found that YKL-40 model produced the best performances compared to existing scores, with AUROCs of 0.786 in the training group, 0.831 in the validation group and 0.801 in the entire cohort in predicting significant fibrosis for patients with ALT < 2ULN.

These findings indicated that combined measurement of serum YKL-40, hyaluronic acid, PLT and AST, via YKL-40 model can help identify "gray zone" CHB patients with significant fibrosis who should be treated immediately.

The limitation of this study is that the performance of YKL-40 model has not been validated by longitudinal data and future prospective studies should be performed. In addition, the mechanisms of YKL-40 in liver fibrogenesis of chronic HBV infection have not been clarified and this will require the basic research works.

In conclusion, the present study supports a fact that in China, significant liver fibrosis is present in a high proportion of CHB patients with normal and mildly elevated ALT levels regardless of HBeAg status and HBV DNA levels. In CHB patients with ALT < 2ULN, serum YKL-40 levels were independently associated with significant fibrosis and could be a feasible biomarker reflecting liver fibrosis. YKL-40 model was superior to existing scores in diagnosing significant fibrosis in CHB patients with normal and mildly elevated ALT. This finding offered a promising method to identify those "gray zone" patients who may benefit from antiviral therapy.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval This study was approved by the local ethics committee of Peking University First Hospital.

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· 综述 ·

壳多糖酶 3 样蛋白 1 在肝纤维化诊断中的研究进展

马莹莹 王麟 邬田港 林标扬 尤红

肝纤维化是肝细胞慢性损伤的一种修复反应，乙型肝炎是中国人群中引起肝纤维化和肝硬化的主要因素。但纤维组织在肝脏的过度沉积，会造成严重的并发症，包括门静脉高压症和肝功能衰竭。肝纤维化导致的肝硬化被定义为肝纤维化的晚期阶段[1]，肝硬化被证实为肝细胞癌（hepatocellular carcinoma，HCC）的高危因素，肝硬化患者累计 5 年患 HCC 的风险为 5%～30%[2-3]，明确肝纤维化分期对确定最佳抗病毒时间十分关键[4]。

肝脏活组织检查是诊断和分期肝纤维化的金标准[4]，但是由于其一致性差，或有严重的并发症[5-6]，加上患者的接受程度差，费用高等，应用受到极大限制。近年来，一些无创的技术包括影像学技术和血液标志物检测技术被积极研究和推崇。FibroScan 区分肝硬化或者轻微肝纤维化相对可靠，但对中间区域的肝纤维化不能明确分期，且受患者转氨酶水平、胆红素、腹水、肥胖和操作人员经验不同的影响而造成偏差[7]。FibroTest 需同时检测多个指标，综合判断得出结论，但其并不能将肝纤维化准确分期[8]，且和病理检测结果之间的差异达 28.7%[9]。基于上述分析，针对中国肝纤维化患者分期的血清标志物的需求越来越迫切。壳多糖酶 3 样蛋白 1（chitinase 3-like 1，CHI3L1）是壳多糖酶家族一员，能够参与炎性反应、细胞增殖和分化、保护细胞凋亡、促进血管生成及细胞外基质重构等病理过程[10]。本文就 CHI3L1 在肝纤维化诊断中的研究进展作一综述。

一、CHI3L1 概况

壳多糖酶 3 样蛋白 1（chitinase 3-like 1，CHI3L1）常被称为 YKL-40，其相对分子质量为 40×10^3。CHI3L1 从蛋白一级结构上分析，属于糖基水解酶家族 18。根据蛋白一级结构的相似性，糖基水解酶家族 18 包括了壳多糖酶（chitinases）和壳多糖酶样蛋白（chitinase-like proteins，CLP）。它们的保守序列（DXXDXDXE）是参与催化作用的，其中的谷氨酸（E）是催化的碱基。壳多糖酶（又称几丁质酶）是一种降解几丁质（β-1-4 连接的 N-乙酰-*D*-葡萄糖胺聚合物）的酶。在人类和小鼠中，有活性的几丁质酶是由两个基因编码，壳三糖酶（Chit1）和酸性哺乳动物几丁质酶（AMCase）组成。chitl 是第一个被克隆和纯化的有活性的哺乳动物几丁质酶。AMCase 则是后来被发现的，并由于其酸性的等电点而被命名。

CLP 和几丁质酶结构类似，但缺少几丁质酶降解几丁质的能力。这种催化能力的丧失是由于在进化保守的催化结构域关键残基谷氨酸和天冬氨酸的突变导致的。CHI3L1 虽然在体外实验中可以结合壳多糖，但到目前为止，还未证明人体中存在壳多糖。因此 CHI3L1 这个名字经常导致不符合其实际功能的推测和误解。

CHI3L1 的基因和蛋白序列在 1993 年被阐明（GenBank 编码 M80927），由 10 个外显子组成，位于人类染色体 1q32.1 上的一个 8 kb 的 DNA 上。其编码的蛋白有 383 氨基酸序列，相对分子质量为 40.476×10^3，等电点为 7.6[11]。在人类基因组中，它是唯一一个 CLP。而在小鼠中，则有 3 个 CLP，chi3l1、chi3l3 和 chi3l4。chi3l1 的人类同源基因是 CHI3L1，而 chi3l3 和 chi3l4 在人类不存在相应的同源基因。

二、CHI3L1 作为肝脏组织富集或特异性表达的蛋白的研究

关于 CHI3L1 在人不同组织中的表达，一直被错误地认为是均匀分布在不同的组织中低量表达，这可能是由于原先用来分析 CHI3L1 表达的 RNA 印迹（Northern blot）分析技术，或芯片杂交技术不能区分不同的 CHI3L1 家族。2008 年 Dezso 等[12]对人的 31 个不同组织进行微阵列分析，发现 CHI3L1 在肝脏组织中表达水平最高。但是由于微阵列的动态范围有限，CHI3L1 在肝组织中的高表达的程度并未引起重视。

为了更精确地定量 CHI3L1 的表达，Ohno 等[13]建立一个实时定量 PCR（qPCR）CHI3L1 mRNA 水平评价体系，然后通过对人体正常组织进行 RNA 表达分析，发现 CHI3L1 基因的表达模式呈现出明显的组织特异性。在肝脏组织中检测到最高的 CHI3L1 基因的 mRNA 水平，其次为肾、气管和肺。肾和肝中 YKL-40 的表达水平比 chitl 和 AMCase 表达水平高 100 倍以上[13]。

近年来二代测序技术的迅猛发展，使得更精准地定量基因表达水平成为可能（达到数值化定量，digital counts）。RNA 测序（RNA-Seq）相较于较老的技术，能够比原来的芯片技术在一个大得多的动态范围检测基因表达水平，并且定量更为精确。从世界著名的测序公司 Illumina 所提供的人体表达图谱 Illumina Human Body Map 2.0（http://genomicdbdemo.bxgenomics.com/）获得的数据显示，在肝脏中 CHI3L1 表达水平为 552 每 100 万个测序片段映射到外显子的每 1 000 碱基上的测序片段数（FPKM），而在其他如心脏、脑、乳腺等 15 个组织的数据显示其表达水平较低[14]。CHI3L1 在肝脏中的表达水平比肾脏高 15.3 倍，比心脏高 276 倍。这些数据表明，CHI3L1 是肝特异性或高度肝富集的基因，同时它的绝对表达水平也比较高，高达 552 FPKM。这个表达水平甚至比前列腺特异抗原[（PSA），正式基因命名是激肽释放酶 3（KLK3）]更高，在 Illumina Human Body

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作者单位：310058 杭州，浙江大学浙江加州国际纳米研究院系统生物学平台（马莹莹、邬田港）；首都医科大学附属友谊医院肝病中心（王麟、尤红）；美国华盛顿大学医学院泌尿系（林标扬）

通信作者：林标扬，Email：biaoylin@gmail.com

万方数据

Map 2.0 数据库中，前列腺特异性基因和前列腺癌的标志物 PSA 的表达水平为 349 FPKM[14]。

最近，日本理化研究所(RIKEN)牵头推动的国际合作项目 FANTOM5 数据库，建立了一个国际权威的数据库，这个数据库也显示 CHI3L1 是肝脏特异表达的基因[15]。CHI3L1 在肝脏中的表达水平为 3 187 每 100 万测序片段中的标签数(TPM)，其次是尾状核(497.6TPM)和附睾(333.4 TPM)，在其他如脑、肺、肾脏、乳房、子宫、卵巢等 32 个组织中表达水平更低，其中在精囊中不表达(http://www.proteinatlas.org/ENS00000133048-CHI3L1/tissue)。

总之，CHI3L1 作为一个高表达且其表达富集在肝脏，已经被多个数据库和方法学验证，这是对 CHI3L1 表达水平在许多组织非特异表达的误解的更正，为其能作为肝脏疾病的良好标志物提供了依据[16]。

三、CHI3L1 作为乙型和丙型肝炎相关肝纤维化和肝硬化的标志物的研究

Yan 等[17]研究结果显示，无论是转氨酶正常，还是少量升高的 CHB 患者，不同肝纤维化分期之间的血清 CHI3L1 表达水平差异有统计学意义。进一步研究分析，发现 CHI3L1 不仅可以区分转氨酶正常组 CHB 人群的 F0～F2 和 F≥3[受试者工作特征曲线曲线下面积(AUC)为 0.80]，F0～F3 和 F≥4 (AUC 为 0.81)肝纤维化，且对转氨酶少量增高人群，其区分效果基本不变[F0～F2 和 F≥3 (AUC为0.82)，F0～F3 和 F≥4 (AUC 为 0.79)]。研究还与常见的分期模型进行了比较，发现 CHI3L1 的区分 F0～F2 和 F≥3，或者 F0～F3 和 F≥4 能力(根据 AUC 判断)，无论是针对转氨酶正常的还是少量增高的人群，都比天冬氨酶转氨酶-血小板指数(APRI)、FIB-4、Forn 指数和 Hui 模型强。

Huang 等[14]比较分析了 98 例不同肝纤维化分期(S)肝活组织检查标本的 CHI3L1 的表达水平，发现血清 CHI3L1 表达水平随着肝纤维化程度的增加而增加。在 S0 和 S1 患者的 CHI3L1 蛋白水平差异无统计学意义。在 S0～S1 患者组合中，CHI3L11 蛋白表达水平的中位值为 46.51 μg/L，平均值为 64.79 μg/L。在 S2 患者中，CHI3L1 蛋白表达水平的中位值为 69.48 μg/L，平均值为 130.04 μg/L。在 S3～S4 的患者中，CHI3L1 蛋白表达水平的中位值增加到 188.88 μg/L，平均值增加到277.46 μg/L。对 3 组不同肝纤维化阶段的患者所做的盒形图中显示，CHI3L1 蛋白水平在 S0～S1 患者组和 S2 患者组中差异有统计学意义。同时发现 S2 和 S3～S4 患者的 CHI3L1 蛋白水平差异亦有统计学意义。因此，血清 CHI3L1 蛋白水平能够区分与 HBV 相关的中国肝纤维化患者的不同肝纤维化阶段。当选用 CHI3L1 水平>73.4 μg/L 的标准诊断晚期纤维化时，实质性和晚期肝纤维化的 CHI3L1 水平差异敏感度为0.941，特异度为 0.877 。

Huang 等[14]在 168 位正常人和 85 例 S3、S4 肝纤维化患者的验证试验中发现，以 73.4 μg/L 为临界值时，验证集合的敏感度为 0.918，特异度为 0.871。当以 78.48 μg/L 为临界值时，CHI3L1 诊断晚期肝纤维化的 AUC 为 0.96，敏感度和特异度分别为 0.918 和 0.917。在 36 例显著肝纤维化患者和 50 位正常人血清中比较 CHI3L1 和透明质酸(HA)、原骨胶原(PCⅢ)、层黏连蛋白(LN)和四型胶原(CIV)发现，诊断晚期纤维化 CHI3L1 的 AUC 值为 0.99，是 5 个标志物中诊断肝纤维化分期的最佳血清学标志物。

四、CHI3L1 作为酒精性肝硬化和日本血吸虫肝纤维化标志物的研究

Johansen 等[18]比较了 51 例酒精性肝硬化和正常人的血清 CHI3L1 的表达，发现 CHI3L1 表达水平增加了约 4.5 倍。Zheng 等[19]比较了 CHI3L1 和 HA 对日本血吸虫导致的肝纤维化中的血清表达情况，发现 CHI3L1 在正常人群、轻微肝纤维化患者和严重肝纤维化患者中的表达水平分别为(49.0±10.4)、(92.3±18.5)和(172.1±35.9) g/L。认为 CHI3L1 比 HA 能够更好地区分日本血吸虫引起的肝纤维化的不同程度。

五、CHI3L1 作为非酒精性脂肪性肝病(NAFLD)导致的肝纤维化和肝硬化标志物的研究

NAFLD 是慢性非病毒性肝病的常见原因，发病率较高(25%～45%)。Kumagai 等[20]对 111 例 NAFLD 患者和 23 例肝癌患者的血清 CHI3L1 水平进行了定量分析，发现随着肝纤维化的进展，NAFLD 患者的血清 CHI3L1 水平升高。多变量分析表明，CHI3L1 是与显著肝纤维化(F3～F4)密切相关的独立因素。研究还发现，CHI3L1 和Ⅳ型胶原蛋白 7s、HA、血清多花紫藤凝集素阳性 Mac-2 结合蛋白(WFA^+-M2BP)、FIB-4 指数呈正相关，但与 ALT 和血小板计数呈负相关，与脂肪变性指数、炎性反应和肝细胞气球样变性不相关。

六、CHI3L1 作为抗病毒治疗疗效跟踪和预后的研究

Nøjgaard 等[21]比较了 CHI3L1 在丙型肝炎患者 α 干扰素和利巴韦林治疗前后的变化，在 30 例治疗有效患者中，第 18 周的血浆 CHI3L1 的水平与治疗前比较显著降低；而在 19 例治疗无效患者中，CHI3L1 不发生变化。Nunes 等[22]研究发现在慢性丙型肝炎患者中，对干扰素治疗有反应者 CHI3L1 水平降低。Wang 等[23]比较了 CHB 患者抗病毒治疗 78 周后和治疗前的 CHI3L1 水平，发现 CHI3L1 水平显著下降。因此，CHI3L1 的检测有望替代肝脏活组织检查，来连续地跟踪抗病毒治疗和抗纤维化治疗的疗效。

Pungpapong 等[24]研究发现，血清中 CHI3L1 的水平可以作为丙型肝炎患者肝移植后是否会快速纤维化进展(rapid fibrosis progression，RFP)的标志物。肝移植后 6 个月内血清 CHI3L1≥200 μg/L 预测 RFP 的准确率高达 96%。Fontana 等[25]在慢性丙型肝炎患者使用聚乙二醇干扰素联合利巴韦林长期治疗对肝硬化的远期疗效临床试验中发现，CHI3L1 持续升高预示肝纤维化的持续进展，预后不佳。

CHI3L1 在肝脏中高度特异表达，且能够在肝脏库普弗细胞中被诱导表达从而激活肝脏星状细胞，可能直接参与肝纤维化的形成和维持。CHI3L1 肝纤维化检测可以用来协助诊断病毒性肝炎、酒精性肝硬化和脂肪肝等导致的肝纤维化和肝硬化，同时能够比较精准区分不同时期的肝纤维化，对患者进行抗病毒治疗或抗纤维化治疗的指导有一定的意义。另外，CHI3L1 检测具有无创和简便的特征，可以用来连续多次检测从而跟踪抗病毒治疗和抗纤维化治疗的疗效，减少或替代肝脏穿刺，极大地减少患者的痛苦和风险，降低医疗费用。

万方数据

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Original Article

Changes in serum chitinase 3-like 1 levels correlate with changes in liver fibrosis measured by two established quantitative methods in chronic hepatitis B patients following antiviral therapy

Lin Wang, Tianhui Liu, Jialing Zhou, Hong You and Jidong Jia

Liver Research Center, Beijing Friendship Hospital, Capital Medical University, National Clinical Research Center for Digestive Disease, Beijing, China

Aim: Non-invasive assessment of changes in liver fibrosis is still an unmet medical need in the era of antiviral therapy. Therefore, we explore whether chitinase 3-like 1 (CHI3L1), a serum marker of liver fibrosis, can be used as a non-invasive surrogate marker of fibrosis change during treatment.

Methods: We correlated serum CHI3L1 levels with liver tissue collagen proportionate area (CPA) in a cohort of 131 patients with chronic hepatitis B (CHB) receiving entecavir-based antiviral therapy for 78 weeks. In addition, we compared this marker with the liver stiffness measurement (LSM). Multivariate regression analyses were undertaken to determine the clinical factors associated with the CHI3L1 levels.

Results: Before treatment, correlation analysis showed that there were positive correlations between CHI3L1 levels and the CPA ($r = 0.351$, $P < 0.001$), and between CHI3L1 and LSM ($r = 0.412$, $P < 0.001$). After 78 weeks treatment, serum CHI3L1 levels decreased compared with that at baseline (87.8 vs. 69.6 ng/mL, $P < 0.001$), and CHI3L1 levels were also correlated with CPA ($r = 0.293$, $P = 0.001$) and LSM ($r = 0.443$, $P < 0.001$). Furthermore, there were positive correlations between the changes in CHI3L1 and CPA ($r = 0.366$, $P < 0.001$), and changes in CHI3L1 and LSM ($r = 0.438$, $P < 0.001$). Multivariate regression analyses indicated that CPA values were related with pre- ($\beta = 5.450$, $P = 0.019$) and post-treatment CHI3L1 levels ($\beta = 7.460$, $P = 0.023$).

Conclusions: Chitinase 3-like 1 is not only a useful noninvasive marker for the assessment of liver fibrosis in CHB patients before treatment, but also a potential useful marker for monitoring the change in liver fibrosis during therapy.

Key words: antiviral therapy, chitinase 3-like 1, liver fibrosis, noninvasive, quantitative measurement of liver fibrosis

INTRODUCTION

IMPROVEMENT OF LIVER fibrosis is an important goal for chronic hepatitis B (CHB) patients receiving antiviral therapy, therefore, dynamic assessment of liver fibrosis changes is important.[1,2] Although liver biopsy is still the gold standard to assess liver fibrosis, its invasive nature prevents it from wide use. There is an unmet clinical need to develop a non-invasive and quantitative measurement for liver fibrosis change during treatment.

Chitinase 3-like 1 (CHI3L1) is a member of the chitinase-like protein family.[3] Previous studies have shown that CHI3L1 levels are significantly correlated with stages of fibrosis in CHB patients.[4] However, whether changes in CHI3L1 levels can be used to monitor fibrosis changes in CHB patients during antiviral therapy has not been assessed.

In this study, we correlated the serum CHI3L1 levels with the collagen proportionate area (CPA) and liver stiffness measurement (LSM) in CHB patients who received antiviral therapy. We wanted to evaluate the change in CHI3L1 levels pre- and post-therapy as a practical and non-invasive method to monitor fibrosis changes.

Correspondence: Dr Jidong Jia, Liver Research Center, Beijing Friendship Hospital, Capital Medical University, 95 Yong-an Road, Xi-Cheng District, Beijing 100050 China,. Email: jia_jd@ccmu.edu.cn

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METHODS

Study cohort

CLINICAL DATA AND serum samples were from patients of the "Regression Study" (ClinicalTrials.gov: NCT01938781 and NCT01938820). A total of 131 CHB patients were included in the final analysis. Inclusion criteria were: men or women aged 18 to 65 years; hepatitis B surface antigen positivity for at least 6 months and treatment-naive before screening; serum hepatitis B virus (HBV)-DNA load >20 000 IU/mL in hepatitis B envelope antigen (HBeAg)-positive patients or 2000 IU/mL in HBeAg-negative patients at baseline (prior to antiviral therapy). Exclusion criteria included: co-infection with hepatitis C or HIV; presence of chronic liver disease caused by other etiologies; decompensated cirrhosis; α-fetoprotein >100 ng/mL; creatinine >1.5 times the upper limit of normal; malignant tumors; severe disease of heart, lung, kidney, brain, blood system, or other organs; and pregnant or lactating women.

All patients received entecavir (ETV)-based treatment after initial evaluation of liver fibrosis by biopsy, and a second liver biopsy was carried out after 78 weeks of treatment.

Collection of clinical data and LSM

Demographic data were collected at baseline (0 week), and clinical laboratory tests were carried out at baseline and at 26-week intervals including blood cell counts, HBV-DNA level, serological markers of HBV, liver biochemical parameters, α-fetoprotein, prothrombin time, liver ultrasonography, and LSM.

Transient elastography (FibroScan; Echosens, Paris, France) was undertaken on the right lobe of the liver through the right intercostal according to previously described methods.[5] The obtained value (LSM) was reported as the median of 10 successful measurements. The results were expressed in kPa. The values with at least 10 valid measurements, with success rates ≥60% and with the interquartile range over the median ratio less than 30%, were considered reliable, and were included in the analysis.

Histological assessment

Liver tissues were formalin fixed and paraffin embedded. The specimens were then stained with hematoxylin–eosin, reticulin, and Masson trichrome and independently evaluated by two experienced hepatopathologists. Necroinflammation was assessed by the modified histology activity index grading system (scale, 0–18) and fibrosis was staged with the Ishak fibrosis scores (scale, 0–6).

Assessment of CPA

The CPA was measured on unstained liver sections by a second-harmonic generation (SHG)/two-photon-excited fluorescence (TPEF) technology-based microscope (Genesis200; HistoIndex, Singapore). The specimen was scanned to generate multiple adjacent images under a 20× objective and the images stitched together to form a whole slide picture (Fig. S1). The SHG microscope was used to visualize collagen and TPEF was used to identify cell structures. After normalizing the SHG signals by the area percentage of TPEF signals, the CPA was computed.[6,7]

Serum CHI3L1 measurement

The concentrations of serum CHI3L1 were measured using CHI3L1 ELISA kits (Hangzhou Proprium Biotech, Hangzhou, China) according to the manufacturer's instructions.[4] A standard curve was generated using the four-parameter logistic regression model. The correlation coefficients of CHI3L1 ELISA kits were >0.9900. The detection limit was 0.035 ng/mL. The measurement for our study passed the quality control with the values of the low and high concentration quality control samples at 0.14–0.15 ng/mL and 0.57–0.66 ng/mL, respectively, within the ranges for the low concen-tration (0.12–0.18 ng/mL) and high concentration (0.48–0.72 ng/mL) values of quality control. The intra-assay coefficients of the variations of the low and high concentration samples were 4.5% and 4.1%, respectively.

Statistical analysis

Normally distributed continuous variables were expressed as means with standard deviations and compared by Student's *t*-test. Non-normally distributed variables were reported as medians with interquartile ranges and compared by the Wilcoxon matched-pairs test and the Kruskal–Wallis test. Categorical variables were compared using the χ^2-test and Fisher's exact test. Correlations between parameters were undertaken using Spearman's rank test. The performance of biomarkers to identify liver fibrosis was assessed by receiver operating characteristic (ROC) curve analysis. Linear regression analyses were used to determine the associated factors with pre- and post-treatment CHI3L1 levels. A *P*-value <0.05 was considered statistically significant. Statistical analyses were undertaken using SPSS version 20.0 (IBM, Chicago, IL, USA).

Ethics

Our study was registered with ClinicalTrials.gov (registration nos. NCT01938781 and NCT01938820).

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The study protocol was approved by the Ethics Committee of Beijing Friendship Hospital (Beijing, China). The study was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki (2008 revision). Written informed consent was provided by all patients.

RESULTS

Baseline characteristics and virologic and histological changes after 78 weeks of treatment

A TOTAL OF 131 patients were included in the final analysis (Fig. 1). Most of the patients were men (78%), the mean age was 39 years, and 71% of patients were HBeAg-positive. The median level of baseline serum HBV-DNA was 6.7 log IU/mL. After 78 weeks of treatment, 71% patients (93/131) achieved virologic and biochemical responses, the HBV-DNA was undetectable (virologic response) in 85% of patients, and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels declined to the normal range (biochemical response) in 83% of patients. Histological assessment also showed improvement in necroinflammation and fibrosis (Table 1). The median inflammation score decreased from 7 to 4 ($P < 0.001$) and the proportion of patients with pronounced necroinflammation (≥10) decreased from 18.3% (24/131) at baseline to 2.3% (3/131) at week 78 (Fig. 2a). Fibrosis scores decreased in 40% of patients (53/131), and the proportion of patients with significant fibrosis and more (Ishak ≥2) decreased from 95.4% (125/131) to 86.2% (113/131) (Fig. 2b). The serum CHI3L1 level also declined significantly compared with that at baseline (median, 87.8 vs. 69.6 ng/mL, $P < 0.001$) (Fig. 2c). There was no significant difference in CPA, CHI3L1, or LSM between patients with biochemical and virologic response and non-responders (Table S1).

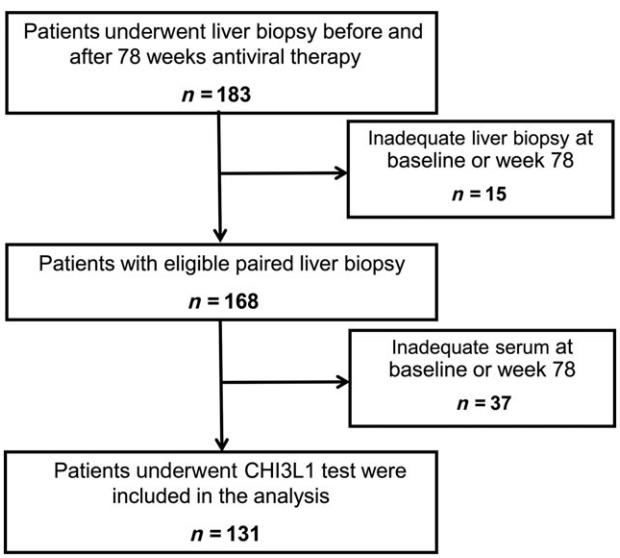

Figure 1 Flowchart for the study of changes in liver fibrosis and serum chitinase 3-like 1 levels following antiviral therapy in a cohort of 131 patients with chronic hepatitis B.

Baseline CHI3L1 levels correlated with CPA and LSM

At baseline, the median levels of CHI3L1 were high, which increased with fibrosis stage and were highest in cirrhosis patients (Fig. S2). The correlation analysis found positive correlations between serum CHI3L1 levels and CPA ($r = 0.351$, $P < 0.001$) (Fig. 3a), and between CHI3L1 levels and LSM ($r = 0.412$, $P < 0.001$) (Fig. 3b). The ROC curve analysis revealed that the performance of CHI3L1 in identifying significant liver fibrosis (Ishak≥F2) was superior to APRI and FIB-4 with a cut-off value of 60.9 ng/mL (area under the curve = 0.86) (Fig. S3).

Post-treatment CHI3L1 levels also were correlated with CPA and LSM

After 78 weeks treatment, CPA decreased from baseline 3.39% to post-treatment 2.42% ($P < 0.001$) (Fig. 4a), and the LSM from 10.3kpa to 6.3 kPa ($P < 0.001$) (Fig. 4b). There were positive correlations between serum CHI3L1 levels and CPA ($r = 0.293$, $P = 0.001$) (Fig. 4c), and between serum CHI3L1 levels and LSM ($r = 0.443$, $P < 0.001$) (Fig. 4d).

Changes in CHI3L1 levels after treatment positively correlated with changes in CPA and LSM

We found there were positive correlations between the changes in CHI3L1 levels and the changes in CPA and LSM pre- and post-treatment. The correlation coefficient was 0.366 between CHI3L1 and CPA, and 0.438 between CHI3L1 and LSM. Both correlations had statistical significance ($P < 0.001$) (Fig. 5).

Factors associated with CHI3L1 levels using multivariate regression analysis

The multivariate linear regression analysis showed that the factors associated with pretreatment CHI3L1 levels were fibrosis severity assessed by CPA measurement and inflammatory score. There were positive correlations between CPA, inflammatory score, and CHI3L1 levels ($\beta = 5.450$, $P = 0.019$; $\beta = 2.864$, $P = 0.047$) (Table 2). In the multivariate analysis, the factors contributing to post-treatment CHI3L1 levels were treatment drugs and CPA values. The

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Table 1 Characteristics of the study cohort of chronic hepatitis B patients at baseline and after 78 weeks of antiviral therapy

Parameter	Baseline ($n = 131$)	Week 78 ($n = 131$)	*P*-value
Age, years	39 ± 10	–	–
Male gender, *n* (%)	102 (78)	–	–
HBeAg positive, *n* (%)	93 (71)	–	–
HBV-DNA, log IU/ml	6.7 (2.1)	0 (0)	<0.001
ALT, U/L	83 (106)	25 (18)	<0.001
AST, U/L	53 (57)	24 (11)	<0.001
Albumin, g/L	42.1 ± 4.9	44.8 ± 3.5	<0.001
Bilirubin, mg/dL	0.8 (0.6)	0.73 (0.39)	<0.001
Prothrombin time, s	12.7 ± 1.5	11.6 ± 1.6	<0.001
Platelets, $\times 10^9$/L	170 ± 55	160 ± 53	0.045
CH3L1, ng/mL	87.8 (70.9)	69.6 (42.2)	<0.001
LSM, kPa	10.3 (7.4)	6.3 (2.7)	<0.001
Histology			
Necroinflammation, 0–3 / 4–6 / 7–9 /≥10, *n*	14/50/43/24	51/77/3/0	<0.001
Fibrosis, 0–2 / 3–4 / 5–6, *n*	37/76/18	54/63/14	<0.001

–, not applicable; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHI3L1, chitinase 3-like 1; HBeAg, hepatitis B envelope antigen; HBV, hepatitis B virus; LSM, liver stiffness measurement.

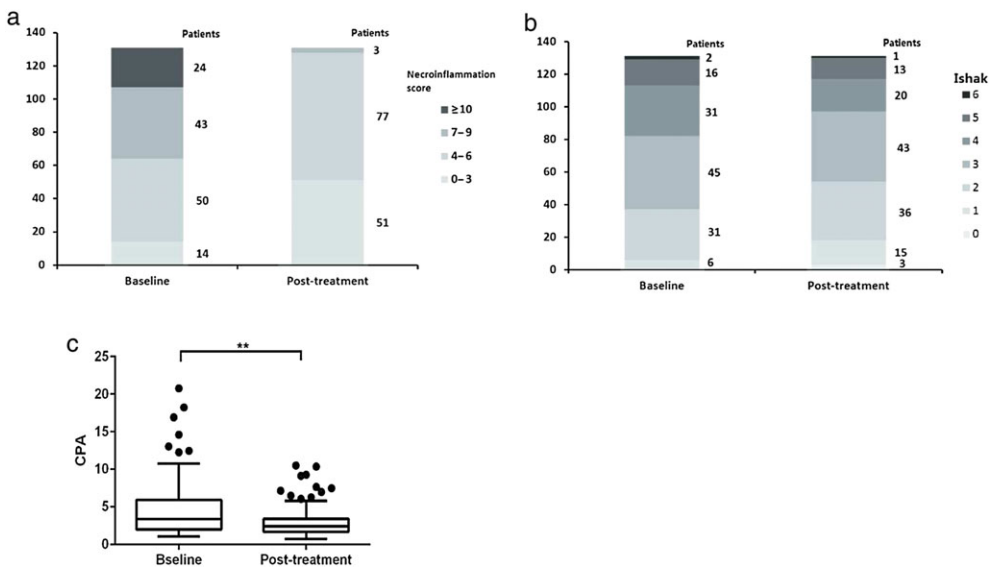

Figure 2 Histological change and serum chitinase 3-like 1 (CHI3L1) change in patients with chronic hepatitis B after 78 weeks of antiviral therapy. The proportion of patients with pronounced necroinflammation (≥10) and significant fibrosis (Ishak score ≥2) decreased on post-treatment (a,b), and the CHI3L1 levels also decreased significantly ($P < 0.001$) (c). $^{**}P < 0.001$. [Color figure can be viewed at wileyonlinelibrary.com]

CPA values had positive correlation with post-treatment CHI3L1 levels (β = 7.460, $P = 0.023$). Some patients received ETV and pegylated interferon (peg-IFN) combination treatment ($n = 19$). The CHI3L1 levels in patients who received the combination treatment were higher than in those treated with ETV alone ($n = 112$) (74.2 vs. 61.5 ng/mL).

DISCUSSION

IN THIS STUDY, we found that serum CHI3L1 levels not only had positive correlations with CPA and LSM before treatment, they also had correlations at 78-week post-treatment. More importantly, the changes in CHI3L1 levels after treatment also were correlated with the changes in

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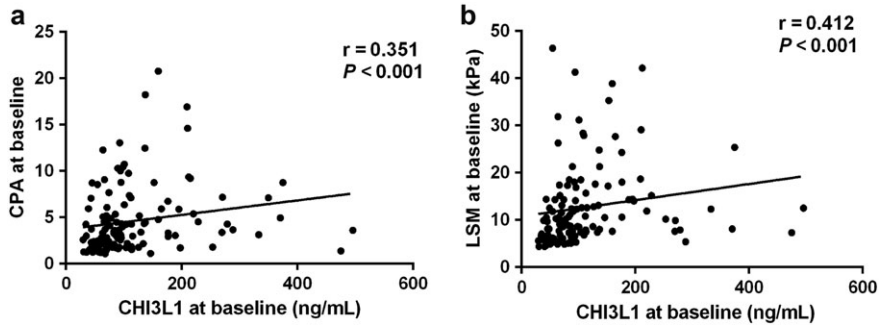

Figure 3 Correlations between serum chitinase 3-like 1 (CHI3L1) levels and liver tissue collagen proportionate area (CPA) and liver stiffness measurement (LSM) at baseline in patients with chronic hepatitis B. There were positive correlations between serum CHI3L1 levels and CPA (r = 0.351, $P < 0.001$) (a), and between CHI3L1 and LSM (r = 0.412, $P < 0.001$) (b).

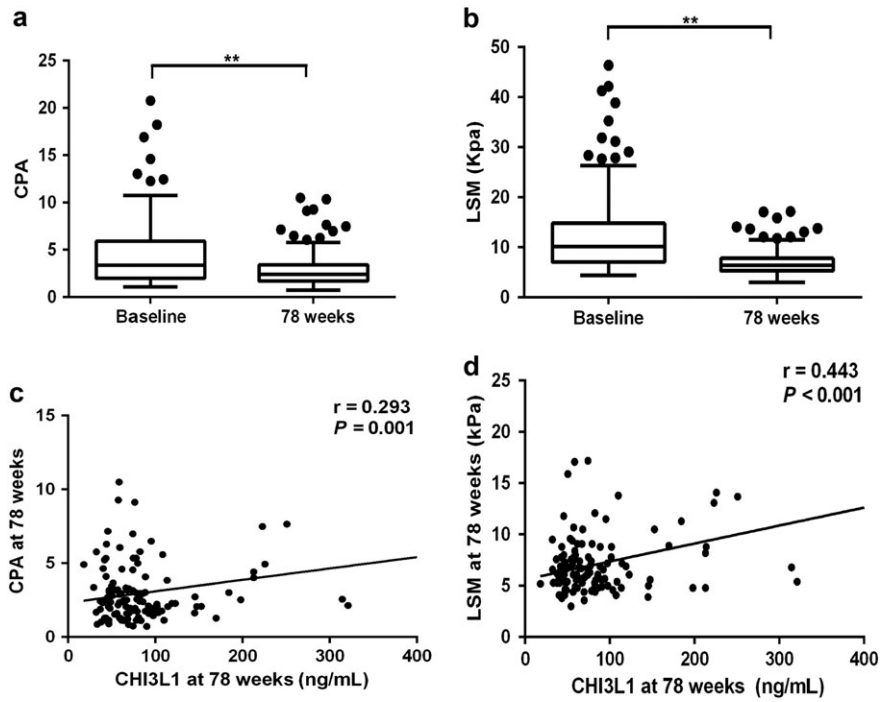

Figure 4 Correlations between chitinase 3-like 1 (CHI3L1) levels and liver tissue collagen proportionate area (CPA) and liver stiffness measurement (LSM) in patients with chronic hepatitis B after 78 weeks of antiviral therapy. (a,b) Changes in CPA (a) and liver stiffness measurement (LSM) (b) before and after treatment. There were significant decreases in CPA values and LSM after treatment compared to that at baseline ($P < 0.001$). (c,d) CHI3L1 levels had positive correlations with CPA (r = 0.293, $P = 0.001$) (c) and LSM (r = 0.443, $P < 0.001$) (d) at 78 weeks.

CPA and LSM. Multivariate linear regression analyses further indicated that there were positive correlations between CPA value and CHI3L1 level pre- and post-treatment. Our results suggested that serum CHI3L1 would be used as a potential surrogate marker for monitoring fibrosis change during treatment.

Also known as YKL-40, CHI3L1 belongs to the chitinase family but lacks chitinolytic activity, which highly enriches in the liver.[8,9] It was reported to act as a growth factor for fibroblasts and to be involved in matrix remodeling.[10,11] Serum CHI3L1 levels were reported to be associated with the severity of liver fibrosis caused by non-alcoholic fatty liver disease, schistosomiasis, hepatitis C virus, and HBV.[12–15] Interestingly, we found that CHI3L1 levels declined in CHB patients with liver fibrosis regression after antiviral therapy, whereas it had been reported that

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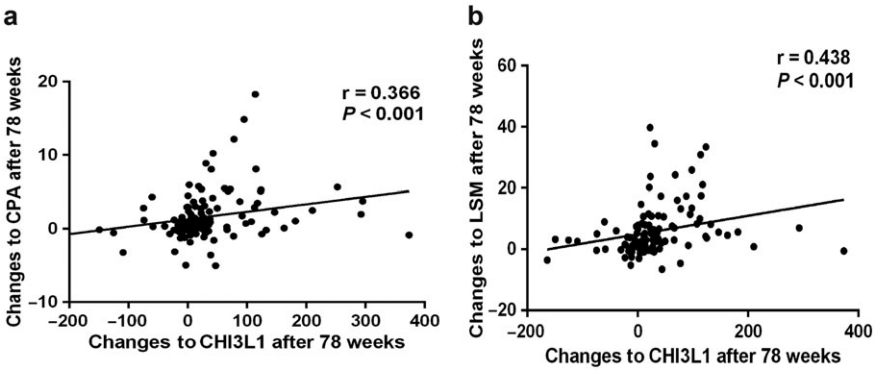

Figure 5 Correlations between changes of chitinase 3-like 1 (CHI3L1) and changes in the liver tissue collagen proportionate area (CPA) and liver stiffness measurement (LSM) in patients with chronic hepatitis B pre- and post-treatment with entecavir. The correlation analysis found positive correlations between changes in CHI3L1 levels and changes in CPA (r = 0.366, $P < 0.001$) (a) and LSM (r = 0.438, $P < 0.001$) (b) after 78 weeks of antiviral therapy.

Table 2 Linear regression analysis for factors associated with chitinase 3-like 1 level in patients with chronic hepatitis B infection

	Univariate analysis			Multivariate analysis		
	β	SE	*P*-value	β	SE	*P*-value
Baseline						
Age, per year	0.992	0.695	0.156			
Gender, female	−1.580	18.043	0.930			
HBV-DNA, per log IU/L	−1.913	5.154	0.711			
ALT, per U/L	0.314	0.239	0.072			
AST, per U/L	0.766	0.668	0.093			
Albumin, per g/L	−3.400	1.537	0.029	−2.148	1.784	0.231
Bilirubin, per μmol/L	0.306	0.533	0.568			
Inflammatory score	7.747	2.462	0.002	2.864	3.031	0.047
CPA, %	7.171	2.031	0.001	5.450	2.291	0.019
Week 78						
Biochemical response	−6.751	17.600	0.702			
Virologic response	−3.298	17.608	0.852			
Treatment, ETV : ETV + peg-IFN	−7.091	1.796	0.006	−6.939	1.896	0.011
Albumin, per g/L	−0.193	0.126	0.129			
Bilirubin, per μmol/L	0.787	1.163	0.500			
Inflammatory score	11.836	5.337	0.028	7.904	5.579	0.159
CPA, %	11.138	3.288	0.008	7.460	3.427	0.023

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPA, collagen proportionate area; ETV, entecavir; HBV, hepatitis B virus; peg-IFN, pegylated interferon; SE, standard error.

CHI3L1 levels were significantly increased in chronic hepatitis C patients with risk of disease progression.[16,17]

To obtain further histological information, we applied the automatic CPA measurement as the gold standard, instead of the conventional histological scoring algorithm, in evaluating fibrosis changes.[18–22] This new technology enables CPA measurement to be carried out on unstained liver sections automatically using a SHG/TPEF technology-based microscope. It could maximize the reproducibility by avoiding staining procedures and operator variances.[6,7]

Collagen proportionate area, the quantitative fibrosis measurement, was more sensitive than the semiquantitative Ishak scoring system in evaluating fibrosis change after treatment. Our recent study found that fibrosis reversal had taken place, as assessed by the new CPA classification, in many patients who were assessed as having post-treatment non-regressive fibrosis measured by Ishak

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score.[23] According to the new assessment, we found that the levels of CPA and CH3L1 were lower in patients with regressive liver fibrosis than that in progressive fibrosis patients (Table S2), and the difference in CPA had statistical significance.

We also compared serum CHI3L1 levels with LSM, which is widely used as a non-invasive method for assessing liver fibrosis.[24–26] Previous studies have shown that the LSM declined significantly in CHB patients after antiviral treatments. However, whether dynamic changes of LSM could be used to monitor the changes in liver fibrosis remained controversial.[27–29] We found that changes in CHI3L1 levels were correlated with the changes in LSM, suggesting both of them could be used for monitoring dynamic changes of liver fibrosis in CHB patients during antiviral therapy.

There were some confounding factors that influenced the CHI3L1 in evaluating liver fibrosis. Liver necroinflammation was an important influencing factor on the CHI3L1, especially at baseline. However, ALT and AST were not related with CHI3L1 in this study; the reason might be that many patients received traditional Chinese medicines before antiviral treatment that could decrease aminotransferase. The treatment was an important confounding factor influencing the post-treatment CHI3L1 levels. Although the CHI3L1 levels declined in both the ETV and peg-IFN combination treatment group (from 82.7 to 74.2 ng/mL) and the ETV monotherapy group (from 97.8 to 61.5 ng/mL), the reduction from pre- to post-treatment was greater in the monotherapy group than that in the combination treatment group (26.5 vs. 8.0 ng/mL).

There were several limitations in the present study. The number of patients was limited. However, the sample size of our study was large enough to undertake the statistical analyses, and the power was sufficient. Additionally, changes in histological measurements might lag behind the biochemical changes, thus introducing bias for the comparisons.

In conclusion, we found that changes in serum CHI3L1 levels were correlated with changes in quantitative assessments of liver fibrosis (CPA and LSM) among CHB patients who received antiviral therapy. It indicated that CHI3L1 change could be a potentially useful, non-invasive method to monitor fibrosis changes.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found online in the supporting information tab for this article.

Figure S1 Liver tissue collagen proportionate area (CPA) measurement using second-harmonic generation (SHG)/two-photon-excited fluorescence (TPEF) microscopy. Collagen and hepatocyte morphology were detected by SHG and TPEF signals, denoted in green and red, respectively. CPA was the ratio (percentage) of area of fibrillar collagen to its corresponding liver tissue.
Figure S2 Chitinase 3-like 1 (CHI3L1) levels in patients with different liver fibrosis stages (1–6, Ishak scores) at baseline. CHI3L1 levels increased with fibrosis stages and there were significant differences among patients with mild fibrosis (1), significant fibrosis (2/3), severe fibrosis (4), and cirrhosis (5/6). $^{*}P < 0.05$; $^{**}P < 0.01$.
Figure S3 Areas under the receiver operating characteristic curves of chitinase 3-like 1 (CHI3L1), aspartate aminotransferase to platelet ratio index (APRI), Fibrosis-4 (FIB-4) index, and platelets were: 0.86, 0.78, 0.51, and 0.34, respectively, for diagnosing pre-treatment Ishak score ≥F2; 0.65, 0.59, 0.62, and 0.33, respectively, for Ishak ≥F3; 0.70, 072, 0.74, and 0.21, respectively, for Ishak ≥F4; and 0.67, 0.69, 0.68, and 0.23, respectively, for Ishak ≥F5. CHI3L1 showed significantly better performance for diagnosis of Ishak ≥F2 than other markers. Cut-offs for diagnosing ≥F2, ≥F3, ≥F4, and ≥F5 were 60.9 ng/mL (sensitivity, 82%; specificity, 83%), 73.8 ng/mL (sensitivity, 53%; specificity, 70%), 91.9 ng/mL (sensitivity, 69%; specificity, 67%), and 106.9 ng/mL (sensitivity, 61%, specificity, 70%), respectively.
Table S1 Characteristics of patients with chronic hepatitis B classified as biochemical and virologic responders or non-responders at pre- and post-treatment with entecavir
Table S2 Characteristics of markers according to the new fibrosis activity classification of "P-I-R" (Progressive - Indeterminate - Regressive) pre- and post-treatment with antivirals

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慢性乙型肝炎患者抗病毒治疗后血清中壳多糖酶 3 样蛋白 1 水平变化与两种公认的定量方法测定的肝纤维化变化相关

目的

肝纤维化变化的无创性评估在抗病毒治疗时代仍然是一个尚未满足的医学需求。因此，我们探讨了治疗过程中肝纤维化的血清标志物-壳多糖酶 3 样蛋白 1（CHI3L1），可以作为一种无创的标志物来反映治疗过程中纤维化的变化。

方法

研究队列中共有 131 例慢性乙型肝炎患者接受了 78 周的恩替卡韦抗病毒治疗，我们对血清 CHI3L1 水平与肝组织胶原比例面积（CPA）进行了相关性分析。此外，我们还比较了该标志物与肝脏硬度测量（LSM）间的相关性。为了确定临床因素与 CHI3L1 水平的相关性，我们还进行了多元回归分析。

结果

相关分析表明治疗前 CHI3L1 水平与 CPA（r＝0.351，*P*＜0.001）和 LSM（r=0.412，*P*＜0.001）均呈正相关，治疗 78 周后，血清 CHI3L1 水平与基线（治疗前）相比（87.8 vs 69.6 ng/ml，*P*＜0.001）下降，并且也与治疗 78 周后 CPA（r＝0.293，*P*＝0.001）和 LSM（r＝0.443，*P*＜0.001）相关。此外，CHI3L1 变化与 CPA（r = 0.366，*P*＜0.001）和 LSM（r = 0.438，*P*＜0.001）变化呈正相关。多元回归分析表明，CPA 值与治疗前（β= 5.450，*P* = 0.019）和治疗后（β= 7.460，P = 0.023）的 CHI3L1 水平相关。

结论

对于慢性乙型肝炎患者，CHI3L1 不仅是治疗前用于评估肝纤维化的无创标志物，更是治疗过程中用于监测肝纤维化变化的潜在标志物。

关键词

抗病毒治疗，壳多糖酶 3 样蛋白 1，肝纤维化，无创，肝纤维化定量测定

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CHI3L1 Is a Liver-Enriched, Noninvasive Biomarker That Can Be Used to Stage and Diagnose Substantial Hepatic Fibrosis

Haijun Huang,[1#] Tiangang Wu,[2#] Jian Mao,[2] Yongxing Fang,[2] Jiajie Zhang,[1] Lihua Wu,[3] Shu Zheng,[4] Biaoyang Lin,[2,4,5] and Hongying Pan,[1]

Abstract

Liver fibrosis is a major disease that is primarily caused by hepatitis virus infections, toxins, and alcohol abuse. Diagnosing and staging liver fibrosis are critical in guiding the treatment of chronic liver diseases, according to several international and Chinese guidelines. Liver biopsy is the gold standard for diagnosing and staging liver fibrosis, but it is invasive and suffers from several limitations. Consequently, much research has focused on the search for a noninvasive serum biomarker of fibrosis. In this study, we determined that Chitinase 3-like 1 (CHI3L1) is an abundantly expressed liver gene whose expression is highly enriched in the liver. We then compared serum levels of CHI3L1 among patients with various stages of liver fibrosis, as determined by liver biopsies, and found that the CHI3L1 levels were able to differentiate early stages of liver fibrosis (S0–S2) from late stages of liver fibrosis (S3–S4). We further showed that CHI3L1 is a good marker of substantial fibrosis, with areas under the ROC curves (AUCs) of 0.94 for substantial (S2, S3, S4) fibrosis and 0.96 for advanced (S3, S4) fibrosis. Finally, we showed that CHI3L1 is superior to hyaluronic acid (HA), type III procollagen (PCIII), laminin (LN), and type IV collagen (CIV), which are also serum biomarkers of liver fibrosis, in identifying advanced liver fibrosis in patients with HBV-related liver fibrosis in China.

Introduction

Liver fibrosis is a wound-healing response of liver cells to chronic injuries caused by viral infections, toxins, alcohol abuse, and other causes. Liver fibrosis is accompanied by a constant process of destruction and repair of the hepatic parenchyma that is caused by inflammation, and it often results in serious complications, including portal hypertension and liver failure. It can also give rise to hepatocellular carcinoma (HCC). Liver fibrosis can lead to cirrhosis, which is defined as the end stage of liver fibrosis (Pellicoro et al., 2014).

In China, hepatitis B is the major cause of injuries leading to liver fibrosis and cirrhosis (Liao et al., 2013; Xu et al., 2003). Cirrhosis is a important factor in the development of HCC because the cumulative 5-year risk of developing HCC in patients with cirrhosis ranges from 5% to 30%, depending on several factors, including the presence and stage of underlying liver disease, ethnicity, age, gender, and the duration of exposure to primary hepatotropic viruses. Therefore, staging liver fibrosis before cirrhosis develops could allow early-stage liver fibrosis to be detected soon enough for potentially curative treatments to be administered.

According to several international and Chinese guidelines for the treatment of chronic liver diseases, including hepatitis B virus (HBV) infection, accurate determination of fibrosis stages is critical for optimizing the timing of antiviral treatment (Asia-Pacific Consensus on Hepatitis B and C, 2000; National Institutes of Health, 2002). Elevated alanine transaminase (ALT) levels equal to or greater than the upper limit of normal (ULN) have been used as a major factor in deciding to initiate antiviral therapy (Chao et al., 2014). Approximately

[1]Department of Infectious Diseases, Zhejiang Provincial People's Hospital, Hangzhou, Zhejiang, China.
[2]System Biology Division, Zhejiang-California International Nanosystem Institute (ZCNI), Zhejiang University, Hangzhou, Zhejiang, China.
[3]The Research Center for Clinical Pharmacy, State Key Laboratory for Diagnosis and Treatment of Infectious Disease, The First Affiliated Hospital of Zhejiang University, Hangzhou, Zhejiang, China.
[4]The Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, China National Ministry of Education), The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China.
[5]Department of Urology, University of Washington, Seattle, Washington.
[#]These authors contributed equally to this work.

one-fifth of patients with ALT levels less than the ULN have substantial liver fibrosis; these patients would be missed using ALT alone as the determining factor for initiating treatment (Chao et al., 2014). Therefore, additional staging markers are needed.

Liver biopsy is the gold standard for detecting and staging liver fibrosis (Papatheodoridis and Manolakopoulos, 2009). However, liver biopsy is a complicated procedure that includes sampling and staining tissue and having the resulting slides read by a pathologist. Liver biopsies may also cause complications, including post-procedure pain or bleeding, sampling error (as only 1/50,000th of the liver is sampled), and inter- and intra-pathologist variability (Afdhal, 2004). Over the past decades, many noninvasive techniques have been developed with the aim of either replacing liver biopsies or conducting pre-screening for liver biopsies.

These techniques rely on either of two distinct but complementary approaches: a non-biomarker-based approach, which relies on the measurement of liver stiffness using elastography-based technologies, such as the widely proposed FibroScan method; or a serum marker-based approach, which relies on the quantification of biomarkers of fibrosis in serum.

The FibroScan method, which uses transient elastography, reliably detects cirrhosis in most HBV and HCV patients; however, it cannot be used in approximately 20% of HBV and HCV patients, particularly those with ascites and obesity, and its performance varies with operator experience (Degos et al., 2010). For serum biomarkers, the most common test platforms are the FibroTest (Biopredictive, Houilles, France) and the ActiTest (Biopredictive), both of which use a combination of levels of alpha-2-macroglobulin, alpha-2 globulin (or haptoglobin), gamma globulin, apolipoprotein A1, gamma-glutamyl transpeptidase (GGT), or total bilirubin, and age and sex information to generate their results. Together, these tests are marketed as the HCV-FibroSure Test (LabCorp, Burlington, NC); this test is the most widely used test for the assessment of fibrosis.

However, this test does not stage liver fibrosis well. Rossi et al. (2003) investigated FibroTest scores of 125 patients with hepatitis C and found that 57 of these patients had FibroTest scores either less than 0.1 (indicating no fibrosis) or greater than 0.6 (indicating substantial fibrosis). They found that 6 (18%) of 33 patients who had FibroTest scores less than 0.1 and were therefore deemed unlikely to have fibrosis in fact had substantial fibrosis. Conversely, five (21%) of the 24 patients with scores greater than 0.6 who were thus predicted to be likely to have substantial fibrosis instead had mild fibrosis. The investigators found large discrepancies between the test results and the biopsy results in approximately 19% of the patients. The discordance between the FibroTest and liver biopsy results was similarly reported to be 28.7% (154 of 537 patients) by Poynard et al. (2012). Therefore, serum markers that can be used to stage fibrosis with greater accuracy are needed.

Chitinase 3-like 1 (CHI3L1, also known as YKL-40) is a member of the chitinase family but lacks chitinase activity; it encodes a glycoprotein that is a member of the 18-glycosyl hydrolase family (Libreros et al., 2013). The function of this glycoprotein is unclear, but it has been hypothesized that CHI3L1 plays a role in both inflammation and tissue remodeling (Libreros et al., 2013). Immunohistochemical analysis demonstrated positive staining for CHI3L1 antigens in areas with fibrosis, particularly areas with active fibrogenesis. Several studies have established that CHI3L1 is a biomarker for alcoholic cirrhosis (Johansen et al., 1997) and HCV-induced liver fibrosis (Johansen et al., 2000; Tran et al., 2000; Nojgaard et al., 2003). However, to our knowledge, the performance of CHI3L1 in staging or diagnosing HBV-related liver fibrosis has not been systematically analyzed. Our laboratory seeks to identify novel biomarkers of liver fibrosis in the Chinese population. Therefore, we sought to determine whether CHI3L1 is a good biomarker for staging or diagnosing liver fibrosis in HBV-related chronic liver disease in the Chinese population.

Materials and Methods

Patients

Ninety-eight consecutive treatment-naive chronic hepatitis B (CHB) patients who had undergone percutaneous liver biopsies were prospectively enrolled in this study in the Department of Infectious Diseases of the Zhejiang Provincial People's Hospital from June 2012 through December 2013. The inclusion criteria for the study were age greater than 20 years, positive HBsAg for more than 6 months, HBV DNA levels $\geq 10^3$ copies/mL, and ALT levels ≤ 2 ULN (ULN = 50 U/L); ALT and HBV DNA levels were monitored monthly for 6 months prior to enrollment to ensure that ALT levels ≤ 2 ULN and HBV DNA levels $\geq 10^3$ copies/mL were maintained. Exclusion criteria for the study included co-infection with human immunodeficiency virus (HIV) or hepatitis C virus (HCV), compensated or decompensated liver cirrhosis, alcoholic liver diseases, non-alcoholic fatty liver disease (NAFLD), autoimmune liver diseases, chronic liver diseases due to other causes, renal insufficiency, inadequate biopsy samples, and incomplete clinical data. In addition, 146 serum samples from stage S3 and S4 hepatic fibrosis patients were collected at three other major hospitals in Hangzhou, China, including the First Affiliated Hospital of College of Medicine, the Second Affiliated Hospital of College of Medicine of Zhejiang University, and Sir Run Run Shaw Hospital. Informed consent was obtained from each patient, and the study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the institutional review board of each hospital.

Enzyme-linked immunosorbent assay (ELISA)

CHI3L1 ELISA kits (Hangzhou Proprium Biotech Co. Ltd, Hangzhou, Zhejiang, China) were used to quantify the serum CHI3L1 levels.

Liver biopsies and the staging of fibrosis

The staging of fibrosis was confirmed by liver biopsies. Percutaneous liver biopsies were conducted using an 18G biopsy needle guided by ultrasound. The specimens were then fixed, paraffin-embedded, and stained with hematoxylin and eosin (HE). For the diagnosis of fibrosis, 1.5–2.5 cm of liver tissue containing at least six portal tracts was used in analyses. Liver fibrosis stages (S0–S4) were determined using Scheuer's classification system by a single pathologist who was blinded to the patients' clinical data.

Statistical analysis

All statistical analyses were performed using MedCalc software (Version 13.0.0.0). Differences between groups were tested using the Mann-Whitney *U*-test (for continuous variables and for nonparametric analyses for independent samples). Comparative ROC analyses were conducted using a nonparametric approach previously described by Delong et al. (1988).

Results

CHI3L1 is an abundantly expressed liver gene whose expression is highly enriched in the liver

Under normal physiological conditions, CHI3L1 expression is low or absent in many tissues (Johansen, 2006). For example, CHI3L1 expression is absent in normal human monocytes but is strongly induced during the late stages of human macrophage differentiation (Krause et al., 1996). However, a systematic analysis of CHI3L1 expression in multiple tissues was not conducted before the arrival of high-throughput technologies. In 2008, Dezso et al. performed a microarray analysis of 32 human tissues and found that the highest levels of expression of CHI3L1 were observed in the liver, out of all of the 32 tissues that were tested (data not shown). However, because the dynamic range of microarrays is limited, we did not initially appreciate that CHI3L1 is, in fact, highly expressed in liver tissue.

RNA sequencing (RNA-seq), which is capable of detecting expression levels over a much greater dynamic range than is possible using older technologies, such as cDNA arrays, allows CHI3L1 expression levels to be determined over a large dynamic range in many normal human tissues. The data from the Illumina Human Body Map 2.0 (http://genomicdbdemo.bxgenomics.com/) show that CHI3L1 is expressed at a level of 552 FPKM (fragments per kb of exon per million fragments mapped) in the liver, whereas it is expressed at very low levels (median 15, with a maximum of 36 in the kidney) in all of the other 15 tissues for which data are available, including all of the major organs: the heart, brain, breast, colon, kidney, lung, muscle, lymph node, and thyroid, and leukocytes (Fig. 1).

The level of expression of CHI3L1 in the liver is 15.3-fold (compared with that in the kidney) to 276-fold higher (compared with that in the heart) than its level of expression in other tissues. These data suggest that CHI3L1 is a liver-specific or a highly liver-enriched gene and that it is also abundantly expressed. At 552 FPKM, the level of expression of CHI3L1 is even higher than that of PSA (KLK3), at 349 FPKM in the Illumina Human Body Map 2.0 database, and PSA is a prostate-specific gene and a marker of prostate cancer. The establishment of CHI3L1 as an abundantly expressed gene whose expression is enriched in the liver is important because this corrects the misconception that CHI3L1 is expressed at similar levels in many tissues and thus should alleviate concerns that it might not be a good marker of liver disease (Johansen, 2006).

CHI3L1 is able to differentiate early stages of liver fibrosis (S0-S2) from late stages of liver fibrosis (S3-S4)

To investigate whether CHI3L1 was able to differentiate early stages of liver fibrosis from late stages of liver fibrosis, we compared serum levels of CHI3L1 and stages of liver fibrosis determined from liver biopsies from 39 patients with stage S0 liver fibrosis, 36 patients with stage S1 liver fibrosis, 16 patients with stage S2 liver fibrosis, and 153 patients with stage S3 or S4 liver fibrosis. Representative images of staining of liver biopsy tissue for different stages of fibrosis are shown in Figure 2. All of the raw data used in this analysis are shown in Supplementary Table S1 (supplementary material is available online at www.liebertpub.com/omi). We calculated the

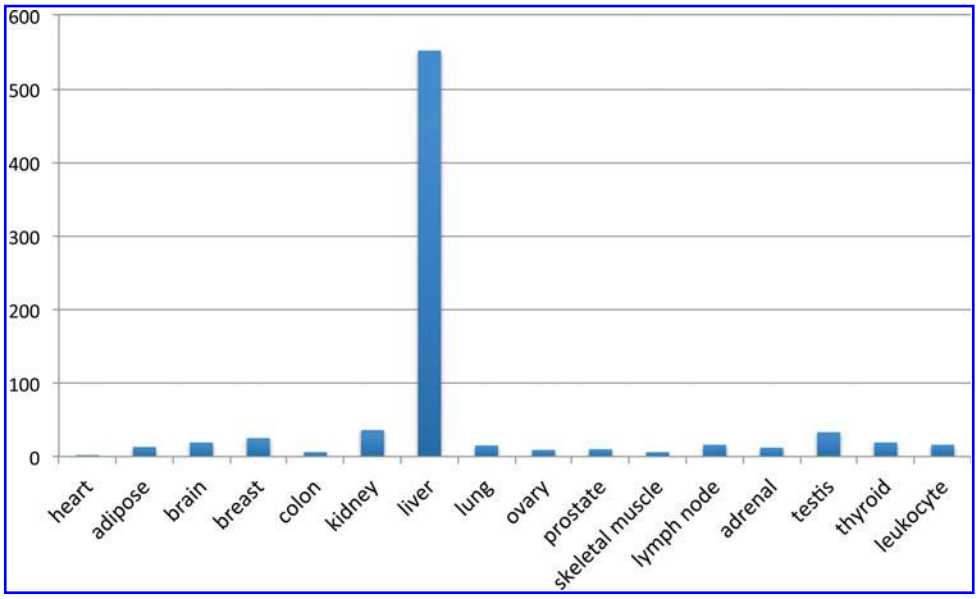


FIG. 1. CHI3L1 expression levels among 16 normal human tissues, as determined using the Illumina Human Body Map 2.0 (http://genomicdbdemo.bxgenomics.com/). Y-axis: FPKM (fragments per kb of exon per million fragments mapped) values. Each column represents a different tissue.

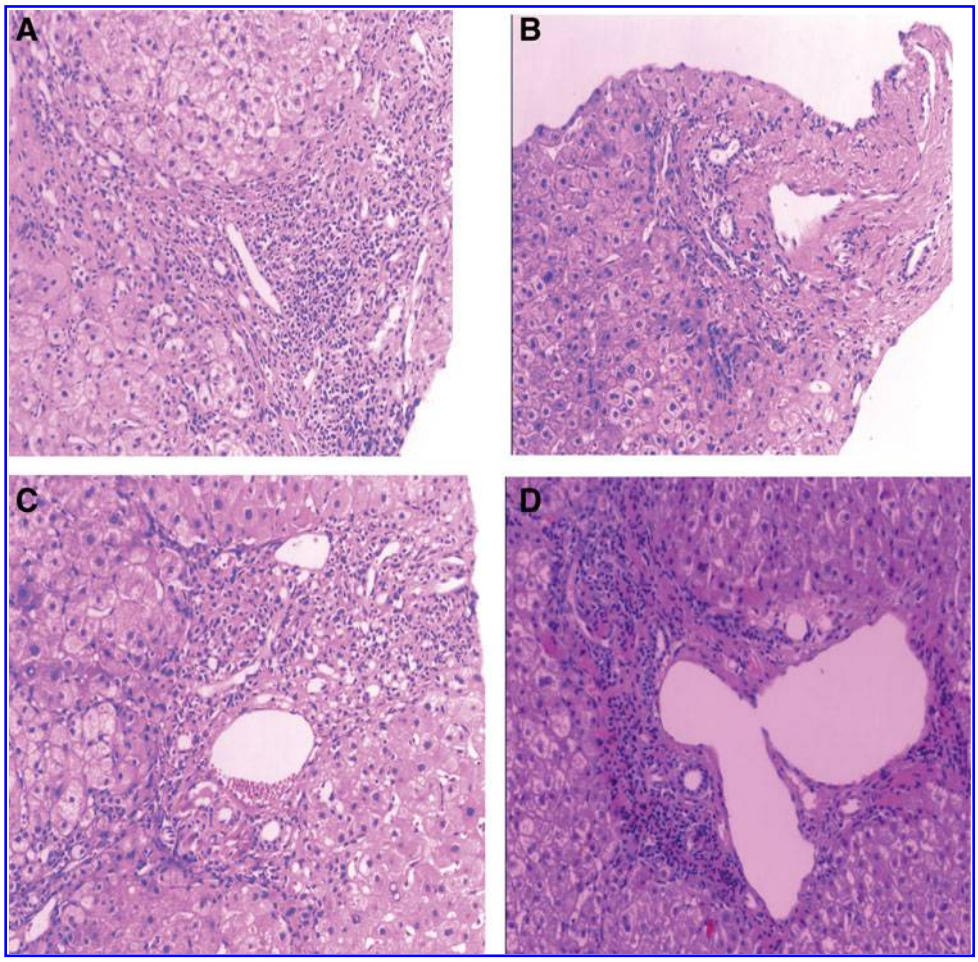


FIG. 2. Pathology staining of liver biopsies at different stages of fibrosis. Representative images of liver fibrosis at stages S0, S1, S2, and S4, *clockwise from the top left*.

median levels of CHI3L1 in serum samples from patients with different pathological stages of fibrosis (Table 1). We found little difference in the expression level of CHI3L1 between patients with no fibrosis (S0) and those with the earliest stage of fibrosis (S1); therefore, we grouped patients with early-stage fibrosis (S0–S1) together. The median expression level of CHI3L1 was 46.51 ng/mL, and the mean expression level of CHI3L1 was 64.79 ng/mL in the S0–S1 group of patients.

In patients with stage S2 fibrosis, the median and mean CHI3L1 levels increased to 69.48 ng/mL and 130.04 ng/mL, respectively. In patients with stage S3–S4, the median and mean CHI3L1 levels further increased to 188.88 ng/mL and 277.46 ng/mL, respectively. A box-and-whisker plot for the three groups of patients with different stages of liver fibrosis is shown in Figure 3A. We found that the difference in CHI3L1 levels between the group of patients with stage S0-S1 fibrosis and the group of patients with stage S2 fibrosis patients is highly statistically significant ($p = 0.0015$, Mann-Whitney *U*-test, two-tailed). We also found a highly statistically significant difference ($p = 0.0002$, the Mann-Whitney *U*-test, two-tailed) between the group of patients with stage S2 liver fibrosis and those with stage S3–S4 liver fibrosis. Thus, we found that serum CHI3L1 levels could differentiate between early-stage (S0–S1), middle-stage (S2), and late-stage (S3–4) liver fibrosis in patients with HBV-related liver fibrosis in China.

Table 1. Median Levels of CHI3L1 Expression in Patients with Different Stages of Liver Fibrosis

Stage	*N*	*Median*	*95% CI*
S0	39	46.150	38.692–55.790
S1	36	47.050	35.963–55.396
S2	16	69.475	57.165–125.007
S3–S4	153	188.800	169.408–228.196

CHI3L1 is a diagnostic marker of substantial or advanced liver fibrosis

Determining whether substantial fibrosis, defined as fibrosis at stages greater than or equal to S2 (i.e., stage S2, S3, or S4 fibrosis), in chronic HBV patients is critical for guiding the prognosis and treatment of patients with hepatitis B (Asia-Pacific Consensus on Hepatitis B and C, 2000; National Institutes of Health, 2002). Encouraged by our finding that CHI3L1 is a good marker for staging fibrosis, we sought to determine whether CHI3L1 is a good marker for identifying substantial fibrosis. ROC curve analysis produced areas

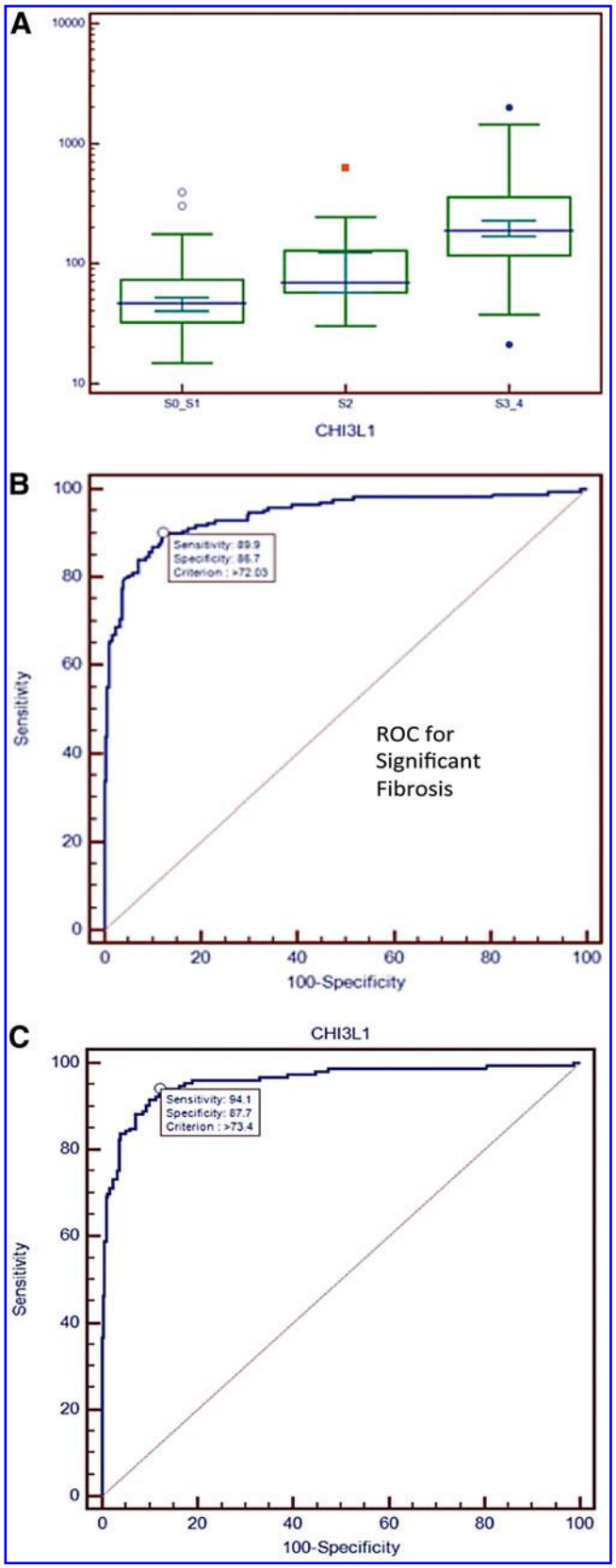


FIG. 3. Analysis of CHI3L1 as a staging and diagnostic marker for liver fibrosis. **(A)** Box-and-whisker plots of CHI3L1 in different groups of patients with various stages of fibrosis. **(B)** ROC curve analysis for substantial fibrosis (S2, S3, S4). **(C)** ROC curve analysis for advanced (S3, S4) fibrosis.

under ROC curves (AUCs) of 0.94 and 0.96 for substantial (S2, S3, S4) fibrosis and advanced (S3, S4) fibrosis, respectively (Fig. 3B–C). CHI3L1 levels differentiated between substantial and advanced fibrosis with a sensitivity of 94.1% and a specificity of 87.7% when a criterion of CHI3L1 level > 73.4 ng/mL was used to diagnose advanced fibrosis. We next recruited patients from Sir Run Run Shaw Hospital (Hangzhou) as a validation set for testing predictions made using serum CHI3L1 levels. We recruited 168 normal individuals and 85 advanced (S3, S4) fibrosis patients (Supplementary Table S2). The area under the ROC curve (AUC) for advanced fibrosis for the validation set is 0.96. The sensitivity and specificity were 91.8% and 91.7%, respectively, when a cutoff value of 78.48 ng/mL was used. When using a cutoff value of 73.4 ng/mL as determined previously, the sensitivity was 91.76% for the validation set, and the specificity was 87.06%.

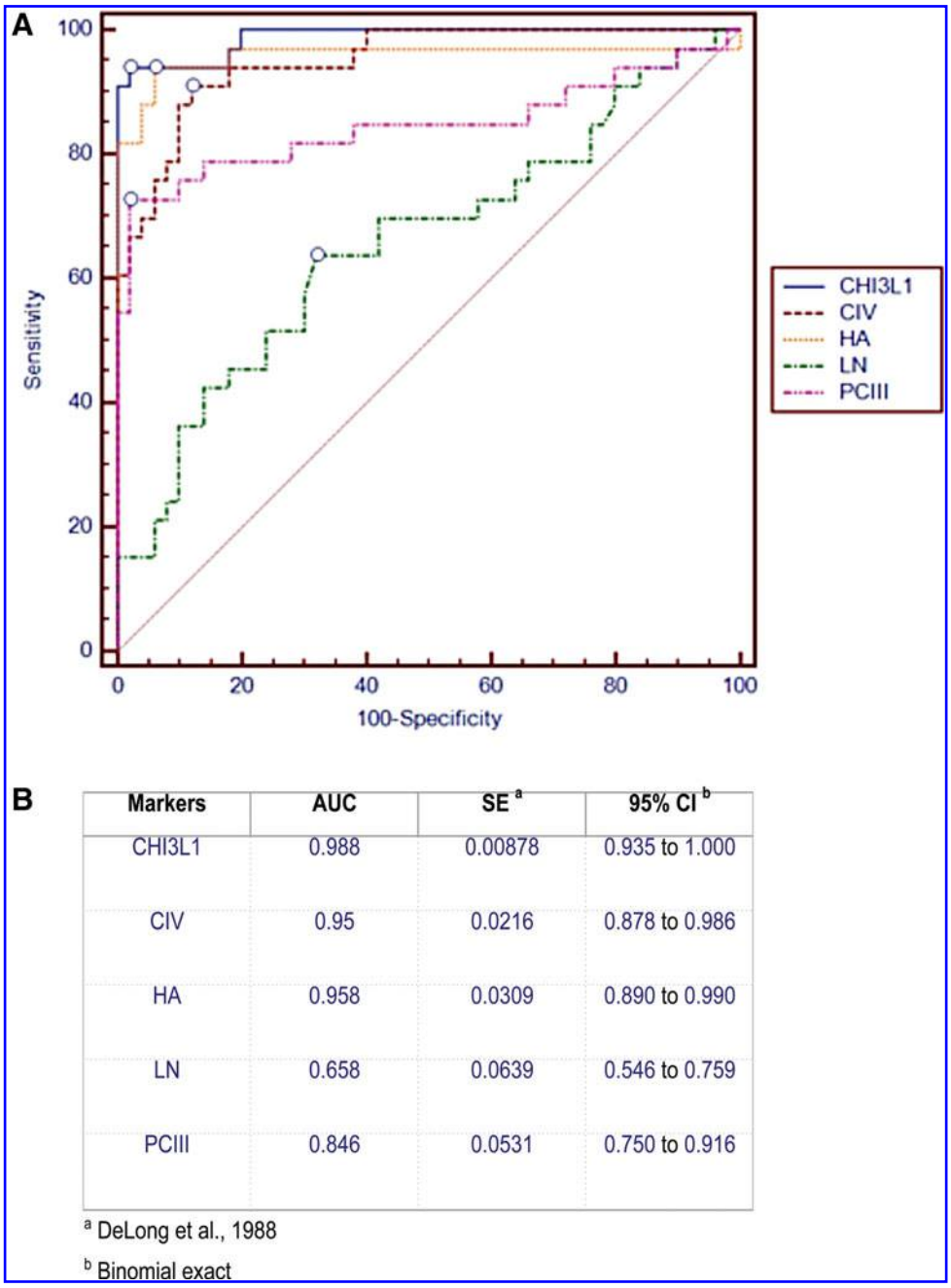
Comparison of CHI3L1 and several commonly used serum markers for diagnosing advanced liver fibrosis

Traditionally, the serum markers hyaluronic acid (HA), type III procollagen (PCIII), laminin (LN), and type IV collagen (CIV) have been used to diagnose liver fibrosis or cirrhosis (Rossi et al., 2007). We compared the performance of CHI3L1 to the performance of these serum fibrosis markers for the detection of advanced liver fibrosis. We measured the levels of CHI3L1 side-by-side with the levels of these older four markers—HA, PCIII, LN, and CIV—in 36 patients with advanced-stage liver fibrosis and 50 healthy individuals. All data are presented in Supplementary Table S3. We conduced a comparative ROC analysis for these 5 markers individually for diagnosing advanced liver fibrosis (Fig. 4A). CHI3L1 performed the best among the five markers, with an AUC of 0.99 (Fig. 4B).

Discussion

The correct staging of liver fibrosis is critical for guiding the treatment of chronic hepatitis. The gold standard for staging liver fibrosis, the liver biopsy, is an invasive procedure and has many limitations (Motola et al., 2014). First, only approximately 1/50,000 the volume of the liver is sampled in a liver biopsy; therefore, a biopsy is unable to reflect fibrotic changes occurring throughout the entire liver and hence does not detect cirrhosis in 10%–30% of patients (Motola et al., 2014). Additional disadvantages include disagreements between pathologists and a risk of complications that range from mild abdominal pain to severe hemorrhage and injury to the biliary system (Motola et al., 2014). Therefore, many investigators are pursuing the development of noninvasive procedures or tests for staging liver fibrosis or diagnosing substantial liver fibrosis.

In this study, we showed that CHI3L1 is a marker that is able to differentiate early-stage fibrosis from late-stage fibrosis (Fig. 3A) in HBV-related liver fibrosis patients in China. Such determinations are critical for guiding the clinical treatment of chronic HBV carriers (Asia-Pacific Consensus on Hepatitis B and C, 2000; National Institutes of Health, 2002). Adams et al. (2005) sought to create an algorithm that accurately and reliably predicts liver fibrosis stages among hepatitis C patients based on the levels of several serum markers and developed a model (HepaScore) based on bilirubin levels, gamma-glutamyl transferase levels,



B

Markers	AUC	SE [a]	95% CI [b]
CHI3L1	0.988	0.00878	0.935 to 1.000
CIV	0.95	0.0216	0.878 to 0.986
HA	0.958	0.0309	0.890 to 0.990
LN	0.658	0.0639	0.546 to 0.759
PCIII	0.846	0.0531	0.750 to 0.916

[a] DeLong et al., 1988

[b] Binomial exact

FIG. 4. Comparison of CHI3L1 with four other serum markers for the detection of liver fibrosis. **(A)** Comparative ROC analysis of CHI3L1 and four other serum markers, hyaluronic acid (HA), type III procollagen (PCIII), laminin (LN), and type IV collagen (CIV), for the detection of advanced liver fibrosis. **(B)** AUC (area under the curve) values for the five serum markers.

hyaluronic acid (HA) levels, alpha-2-macroglobulin levels, age, and gender that produced areas under the ROC curves (AUCs) of 0.85, 0.96, and 0.94 for substantial (S2, S3, S4) fibrosis, advanced (S3, S4) fibrosis, and cirrhosis (S4), respectively.

We further showed that CHI3L1 is capable of identifying substantial liver fibrosis ($\geq$S2) or advanced liver fibrosis (>S3; Fig. 3B, C). We showed that CHI3L1 identifies advanced liver fibrosis in patients with HBV-related liver fibrosis in China better than hyaluronic acid (HA), type III procollagen (PCIII), laminin (LN), and type IV collagen (CIV), all of which are other serum markers of liver fibrosis (Fig. 4A, B). Our observations in Chinese patients with HBV-related liver fibrosis are similar to previous observations in HCV-related liver fibrosis. Rath et al. (2011) tested the abilities of many biomarkers, including CHI3L1 (YKL-40), hyaluronic acid (HA), laminin, C-terminal procollagen I peptide, MMP-9, TIMP-1, TIMP-2, and a complex of MMP-9 and TIMP-1, to detect HCV-related liver fibrosis and found that CHI3L1 performed the best among the biomarkers tested.

Conclusions

We have shown that CHI3L1 is a liver-enriched gene that may aid in the staging of liver fibrosis and in the diagnosis of advanced liver fibrosis in chronic HBV patients in China.

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Address correspondence to:
Professor Shu Zheng
The Cancer Institute
The Second Affiliated Hospital
Zhejiang University School of Medicine
Hangzhou, Zhejiang
China

E-mail: zhengshu@zju.edu.cn

Address correspondence to:
Professor Biaoyang Lin
Zhejiang University
Hangzhou
China
and
Department of Urology
University of Washington
Seattle, WA 98195

E-mail: bylin@u.washington.edu

Address correspondence to:
Professor Hongying Pan
Department of Infectious Diseases
Zhejiang Provincial People's Hospital
Hangzhou, Zhejiang
China

E-mail: hypanhz@139.com

CHI3L1 是一种肝脏富集表达的、无创的肝纤维化诊断和分期标志物

摘要

肝纤维化是一种常见的疾病，可由感染肝炎病毒、毒素和酗酒引发。根据国内外的多种指导原则，肝纤维化的分期和诊断在慢性肝病的治疗中至关重要。肝脏的活检是诊断和分期肝纤维化的金准则，但是容易伤害机体并且受到多种限制。因此，很多研究都致力于发现一种无创的纤维化血清生物标志物。我们的研究发现壳多糖酶 3 样蛋白 1（CHI3L1）在肝脏中高度和富集性表达。接着我们比较了不同阶段的肝纤维化分期（根据活检的金标准）病人血清中的 CHI3L1 表达水平，发现肝纤维化早期（S0-S1）和晚期（S3-S4）的 CHI3L1 含量是不同的。研究进一步发现 CHI3L1 是实质性纤维化 (substantial fibrosis) 的一个很好的标志物，诊断实质性纤维化（S2、S3、S4）和晚期纤维化（S3、S4）的 ROC 曲线(AUCs)值分别为 0.94 和 0.96。 结论：在诊断 HBV 相关的中国晚期肝纤维化病人上，壳多糖酶 3 样蛋白 1，优于其他的肝纤维化标志物如透明质酸（HA）、原骨胶原（PCIII）、层粘连蛋白（LN）和 4 型胶原蛋白。

关键字：CHI3L1、肝纤维化、生物标志物

for FM (0.254 and 0.585) and ELF (8.64 and 10.0). In the lower interval below the 90% sensitivity threshold, negative predictive value was 88.0% for FM and 86.2% for ELF. In the higher interval above the 90% specificity threshold, positive predictive value was 76.6% for FM and 75.2% for ELF. 39.3% of patients were included in the grey zone between the two thresholds with FM, versus 45.3% with ELF (p=0.065). **Conclusion:** The diagnostic accuracy of FibroMeter^{V2G} and ELF are not significantly different in NAFLD. These two blood fibrosis tests perform significantly better than simple blood tests such as FIB4 and NAFLD fibrosis score.

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NIS4 for the Detection of Active Nash (NAS≥4) and Significant Fibrosis (F≥2) in 714 Patients at Risk of Nash: Diagnostic Metrics Are Not Affected By Age, Sex, Presence of Type 2 Diabetes or Obesity.

Remy Hanf[1], Stephen A. Harrison[2], Pierre Bedossa[3], Quentin M. Anstee[4], Vlad Ratziu[5], Sven M. Francque[6], Zouher Majd[1], Emilie Praca[1], Fouad Ben Sudrick[1], John Brozek[1], Bart Staels[7], Sophie Megnien[8], Dean Hum[1], Suneil Hosmane[1], Pierre Chaumat[1] and Arun J. Sanyal[9], (1)Genfit, (2)Pinnacle Clinical Research Center, San Antonio, TX, USA, (3)Pathology, Hôpital Beaujon, Clichy, France, (4)Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, UK, (5)Hôpital Pitié-Salpêtrière and Sorbonne Université, (6) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerp, Belgium, (7)Inserm U1011, (8) Genfit SA, (9)Virginia Commonwealth University

Background: After training of NIS4 (a non-invasive score combining circulating levels of miR-34a, Alpha2-macroglobulin, YKL-40 and HbA1c) for the identification of patients with active NASH and significant fibrosis in the GOLDEN cohort, we have validated NIS4 performances in an independent population of patients prospectively screened for inclusion into RESOLVE-IT. The aim was to validate diagnostic performances of NIS4 in subpopulations of patients sorted according to gender, age and concomitant diseases like type-2 diabetes or obesity. Diagnostic metrics were compared at three cut-offs defining low, low-medium, medium high and high risk ranges of having the condition. **Methods:** The data set comprised 714 patients (239 from GOLDEN + first 475 screened for inclusion in RESOLVE-IT) with a NIS4 value and liver biopsy scores (Central reading according to NASH-CRN scoring system). AUROC and diagnostic metrics (sensitivity/SN, specificity/SP, positive predictive value/PPV and negative predictive value/NPV) were calculated at optimal, low (90% SN) and high (90% SP) cut-offs. Patients were grouped according to age (<55 year vs ≥55 years), sex (male vs female), BMI (<30 kg/m^2 vs ≥30 kg/m^2) or type 2 diabetes status (yes vs no). AUROCs and diagnostic metrics at the three cut-offs (low, optimal and high) and in the different subgroups were then compared. AUROCs are expressed as mean and 95% CI and statistical significance between AUC's was assessed using the DeLong test. **Results:** The prevalence of active NASH (NAS≥4) and significant fibrosis (F≥2)in the cohort was 51%. NIS4 AUROC in this cohort was 0.83; [0.795-0.858]. At the optimal cut-off of 0.5, SN = 74%, SP = 75%, PPV = 76% and NPV= 73%. At a low cut-off of 0.3 (SN=90%), SP =51 % and NPV=83%. At a high cut-off of 0.7 (SP = 90%), SN=52%, PPV=84%. In this cohort, 49% were >55 year old, 48% were male, 68% had a BMI>30 and 38% had type 2 diabetes. As shown in table 1, AUROCs were not statistically different and diagnostic metrics were comparable in all subgroups analyzed. **Conclusion:** In a large cohort of patients prospectively screened because of accumulating rik factors for NASH, NIS4 had good diagnostic performance for the identification of patients with active NASH (NAS≥4) and significant fibrosis (F≥2), irrespective of patient sex, age, obesity or type 2 diabetes status. Defining 3 cut-offs could allow stratification of patients according to their risk of having the condition and guide medical intervention.

Table 1

	Subgroup	Prevalence NAS≥4 and F≥2	AUC [95% CI]	NIS4 Cut-off	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Obesity	≤30 N=224	45%	0.79 [0.733;0.849]	**0.3**	**86**	**50**	**58**	**81**
				0.5	70	74	69	75
				0.7	***49***	***91***	***82***	***68***
	>30 N=490	54%	0.84 [0.802;0.871] p=0.204	**0.3**	**92**	**51**	**68**	**85**
				0.5	76	76	78	73
				0.7	***52***	***90***	***86***	***62***
Diabetes	No N=439	44%	0.83 [0.790;0.868]	**0.3**	**87**	**58**	**62**	**85**
				0.5	67	80	73	75
				0.7	***44***	***93***	***84***	***68***
	yes N=275	61%	0.80 [0.749;0.851] p=0.382	**0.3**	**95**	**32**	**69**	**79**
				0.5	83	64	79	71
				0.7	***59***	***84***	***86***	***56***
age	≤55 N=361	42%	0.80 [0.758;0.848]	**0.3**	**85**	**56**	**59**	**84**
				0.5	66	78	69	76
				0.7	***41***	***92***	***79***	***68***
	>55 N=353	59%	0.83 [0.791;0.871] p=0.358	**0.3**	**94**	**42**	**71**	**83**
				0.5	81	71	81	71
				0.7	***59***	***88***	***88***	***59***
gender	female N=369	50%	0.84 [0.802;0.881]	**0.3**	**93**	**48**	**64**	**86**
				0.5	78	75	76	77
				0.7	***53***	***93***	***89***	***66***
	male N=345	51%	0.81 [0.767;0.855] p=0.345	**0.3**	**88**	**53**	**67**	**81**
				0.5	71	75	75	71
				0.7	***49***	***87***	***80***	***62***

Disclosures:

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Bart Staels – Genfit SA: Consulting

Dean Hum – Genfit: Employment

Arun J. Sanyal – Sanyal Bio: Employment; Exhalenz: Stock Shareholder; Conatus: Consulting; Akarna: Stock Shareholder; Genfit: Stock Shareholder; Gilead: Consulting; Elsevier: Consulting; Echosens: Consulting; Malinckrodt: Consulting; Immuron: Consulting; Intercept: Consulting; Pfizer: Consulting; Salix: Consulting; Uptodate: Consulting; Boehringer Ingelhie

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Denotes AASLD Foundation Abstract Award Recipient

NIS4 用于检测具有活动性 NASH(NAS≥4)和显著纤维化(F≥2)的 NASH 患者：诊断指标不受年龄、性别、2 型糖尿病或肥胖的影响

Dr. Arun J. Sanyal1, Dr. Remy Hanf2, Dr. Stephen A. Harrison3, Dr. Pierre Bedossa4, Prof. Quentin M. Anstee5, Prof. Vlad Ratziu6, Prof. Sven M. Francque7, Dr. Zouher Majd2, Mr. Fouad Ben Sudrick2, Mrs. Emilie Praca2, Mr. John Brozek2, Prof. Bart Staels8, Dr. Sophie Megnien9, Dr. Dean Hum2, Mr. Suneil Hosmane2 and Mr. Pierre Chaumat2.

(1)Virginia Commonwealth University, Virginia, USA;

(2)Genfit;

(3)Pinnacle Clinical Research Center, San Antonio, TX, USA;

(4)Pathology, Hôpital Beaujon, Clichy, France;

(5)Institute of Cellular Medicine, Newcastle University,Newcastle-upon-Tyne, UK; (6)Hôpital Pitié-Salpêtrière and Sorbonne Université;

(7)Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerp, Belgium;

(8)Inserm U1011, (9)Genfit SA

背景：经过 NIS4（一种非侵入性评分，结合循环水平的 miR-34a、alpha2 -巨球蛋白、YKL-40 和 HbA1c）在 GOLDEN 队列中鉴别出有活动性 NASH 和显著纤维化的患者后，我们在一组独立的患者中验证了 NIS4 的表现，并对其进行了前瞻性筛选以纳入解决方案。目的是验证 NIS4 在以性别、年龄和并发疾病（如 2 型糖尿病或肥胖症）进行分类的亚组患者中的诊断性能。诊断指标定义为低、低-中、中高-高三个风险范围的 cut-off 值，并进行比较。

方法：该队列包括 714 名患者(239 名患者来自 GOLDEN + 前 475 名患者经过筛选纳入解决方案)，并且全部有 NIS4 值和肝脏活检评分结果(根据 NASH-CRN 评分系统的中心读数)。AUROC 和诊断指标（敏感性/SN，特异性/SP，阳性预测值/PPV，阴性预测值/NPV）在最佳、低（90% SN）和高（90% SP）cut-off 值下计算。患者按年龄（<55 岁 vs ≥55 岁）、性别（男 vs 女）、BMI（<30 kg/m^2 vs ≥30 kg/m^2）或 2 型糖尿病状态（有 vs 无）分组，然后比较三个 cut-off 值下（低、最优和高）和不同亚组的 AUROCs 和诊断指标，AUROCs 以均值和 95% CI 来表示，并且通过 DeLong 检验来评估 AUC 之间的统计学意义。

结果：队列中活动性 NASH（NAS≥4）和显著纤维化（F≥2）的患病率为 51%，队列中 NIS4 的 AUROC 为 0.83[0.795-0.858]。当最优 cut-off 值为 0.5 时，SN = 74%，SP = 75%，PPV = 76%，NPV= 73%。当低 cut-off 值为 0.3 时（SN=90%,）SP =51 % 和 NPV=83% 当高 cut-off 值为 0.7 时（SP = 90%），SN=52%，PPV=84%。该队列中，49%的患者年龄>55 岁，48%的患者为男性，68%的患者 BMI>30，38%的患者有 2 型糖尿病。如表 1 所示，AUROCs 无统计学差异，诊断指标在所有分析的亚组中具有可比性。

结论：由于 NASH Rik 因子的积累，队列中的患者进行了前瞻性筛选。NIS4 对活动性 NASH（NAS≥4）和显著纤维化（F≥2）患者具有良好的诊断价值，与患者性别、年龄、肥胖或 2 型糖尿病状态无关。定义的 3 个 cut-off 值可以允许患者根据自己的病情分级，并指导医疗干预。

表 1

	Subgroup	Prevalence NAS≥4 and F≥2	AUC [95% CI]	NIS4 Cut-off	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Obesity	≤30 N=224	45%	0.79 [0.733;0.849]	**0.3**	**86**	**50**	**58**	**81**
				0.5	70	74	69	75
				0.7	***49***	***91***	***82***	***68***
	>30 N=490	54%	0.84 [0.802;0.871] p=0.204	**0.3**	**92**	**51**	**68**	**85**
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Diabetes	No N=439	44%	0.83 [0.790;0.868]	**0.3**	**87**	**58**	**62**	**85**
				0.5	67	80	73	75
				0.7	***44***	***93***	***84***	***68***
	yes N=275	61%	0.80 [0.749;0.851] p=0.382	**0.3**	**95**	**32**	**69**	**79**
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	male N=345	51%	0.81 [0.767;0.855] p=0.345	**0.3**	**88**	**53**	**67**	**81**
				0.5	71	75	75	71
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and Masson's Trichrome for content of collagen. Medium was collected for potential markers of tissue damage.
Results: The combination of fructose and fatty acids produced an increase in smooth muscle actin and collagen. Nutrient stimuli alone produced NASH-type liver pathology including steatosis, inflammation, ballooning, and fibrosis. Further damage could also be produced by adding a pulse challenge with LPS. The histological damage could be reduced by adding MSDC-0602 either in parallel with or up to one week after the challenge. Analysis of media demonstrated that the nutrient damage response included the release to the medium of mitochondrial DNA.
Conclusion: These data show that the human in vitro 3D bioprinted liver model can be adapted for demonstrating NASH-type liver pathology and the pharmacology of the novel MPC modulator can be modeled in this system. Three exposures of MSDC-0602K are currently being evaluated in a large Phase 2b clinical trial in subjects with biopsy-confirmed NASH. Samples collected for biomarkers in this trial will be evaluated for changes in parameters that are being identified by this human organoid system including evidence of protection of mitochondria.

LBP-019

13C-methacetin breath test is a highly accurate non-invasive point of care test for detecting CSPH in patients with NASH

J. Bosch[1], C. Bureau[2], N. Chalasani[3], E. Lawitz[4], M. Abdelmalek[5], M. Rinella[6], S. Harrison[7], A. Berzigotti[8], A. Sanyal[9], M. Noureddin[10], D. Rockey[11], T.I. Ber[12], Y. Ilan[13], G. Garcia-Tsao[14]. *[1]Hospital Clinic, Spain; [2]Hopital Purpan, France; [3]Indiana University School of Medicine, United States; [4]The Texas Liver Institute, United States; [5]Duke University, United States; [6]Northwestern, United States; [7]San Antonio Military Medical Center, United States; [8]Inselspital Bern, Switzerland; [9]Virginia Commonwealth University, United States; [10]Cedars Sinai Medical Center, United States; [11]Medical University of South Carolina, United States; [12]Exalenz Bioscience, Israel; [13]Hadassah, Israel; [14]Veteran Affairs Hospital, West Haven, United States*
Email: jbosch@clinic.cat

Background and Aims: Hepatic Venous Pressure Gradient (HVPG), a measurement of portal pressure, correlates with chronic liver disease severity. Clinically significant portal hypertension (CSPH), defined as HVPG ≥ 10 mmHg is associated with an increased risk of decompensation in patients with compensated cirrhosis. Esophageal varices (EV) indicate the presence of CSPH and are also predictive of decompensation. The ^{13}C-Methacetin Breath Test (MBT) using the Exalenz BreathID® System, is a non-invasive, real-time molecular correlation spectroscopy assay that quantitates hepatic cytochrome p450 1A2 metabolism of ingested non-radioactive ^{13}C-labeled methacetin by measuring the abundance of $^{13}CO_2$ in expired breath. The MBT measures a relevant liver metabolic function that has been shown to reflect the degree of overall liver impairment. Here we aimed to determine the accuracy of the MBT in the detection of CSPH.
Method: MBT was performed on 257 patients with NASH- compensated cirrhosis (i.e. no prior variceal hemorrhage, ascites or encephalopathy), pooled from two prospective studies, all of whom had HVPG measured and upper endoscopy performed in a period near the MBT.
Results: Of the 257 NASH-cirrhosis patients, 158 were female (61.5%), median age was 58.7 years, median BMI was 34.6 g/m^2, and median HVPG was 10.6 mmHg (range 1.5–27.5 mmHg). Of the total, 122 (47.5%) had CSPH and/or EV; 61 had CSPH and EV, 47 had CSPH but no EV and 14 had EV without CSPH. MBT values, adjusted by percentage dose recovered (PDR) and noise, allowed to establish a cut-off to accurately rule–in the presence of CSPH/EV. Only 15/257 (5.8%) of patients were wrongly classified as having CSPH. The MBT-based rule-in model had a sensitivity of 76.3%, specificity of 84.5%, PPV was 89.1% (82.6%-93.7%) and NPV was 68.3% with a CI 95% of 0.837–0.925 (p < 0.0001) and AUROC of 0.881.

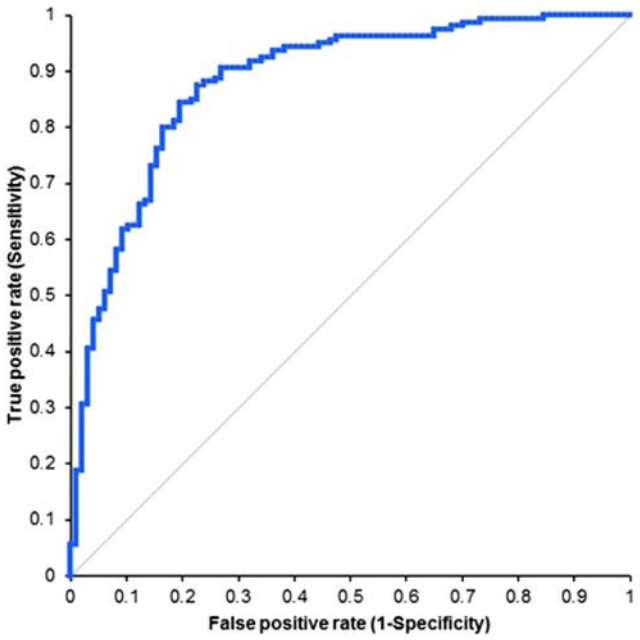


Figure 1: Shows a ROC curve for the detection of CSPH.

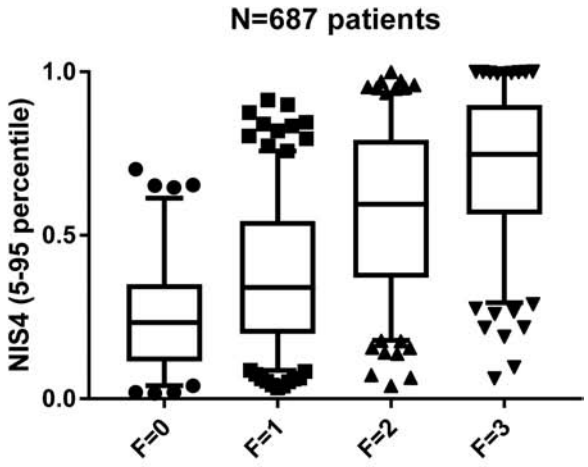
Conclusion: The MBT is highly accurate at detecting CSPH in patients with NASH- compensated cirrhosis. MBT provides a valid point-of-care tool for identifying patients at increased risk for hepatic decompensation in a non-invasive and non-operator dependent fashion.

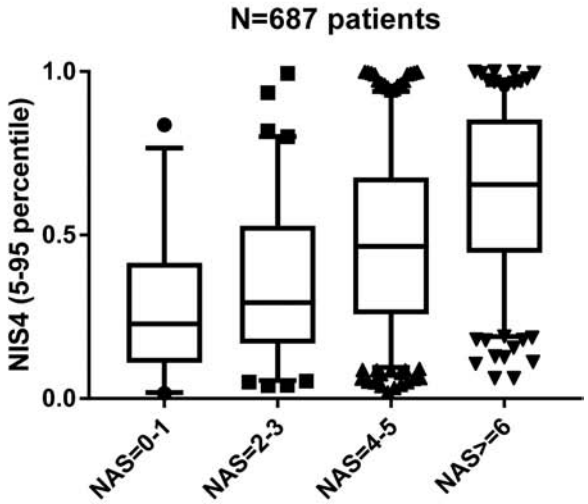
LBP-020

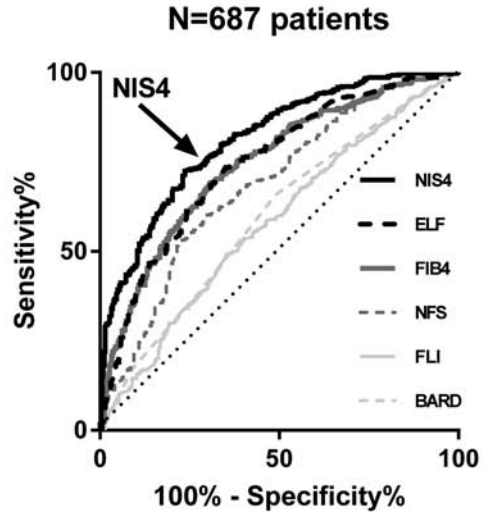
Validation of NIS4 algorithm for detection of NASH at risk of cirrhosis in 467 NAFLD patients prospectively screened for inclusion in the RESOLVE-IT trial

R. Hanf[1], C. Pierre[2], M. Zouher[2], G. Cordonnier[1], J. Brozek[1], E. Praca[1], F.B. Sudrick[1], P. Bedossa[3], Q. Anstee[4], S. Francque[5], S. Harrison[6], V. Ratziu[7], S. Megnien[2], A. Roudot[2], D. Hum[8], A. Sanyal[9]. *[1]GENFIT sa, Loos, France; [2]GENFIT sa, Paris, France; [3]Department of Pathology, Hopital Beaujon, Paris, France; [4]Institute of cellular Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle Upon Tyne, United Kingdom; [5]Antwerp University Hospital, Belgium; [6]Pinnacle Clinical Research, San Antion, United States; [7]Hopital Pitié Salpétrière, Paris, France; [8]GENFIT sa, Loos, France; [9]Virginia Commonwealth University, Richmond, United States*
Email: remy.hanf@genfit.com

Background and Aims: Using the GOLDEN trial as a training cohort, we have previously reported diagnostic performances of a non-invasive score (NIS4) using four circulating biomarkers (miR-34a, Alpha2-macroglobulin/A2M, YKL-40 and HbA1C) for calculation of the risk (0–1) of NASH progression to cirrhosis. The aim of this study was clinical validation of NIS4 in a large independent population of patients prospectively screened for inclusion in RESOLVE-IT trial.
Method: NASH patients At-Risk-of-Cirrhosis (ARC) were defined by NAS ≥ 4 and F ≥ 2 and patients Not-At-Risk-of-Cirrhosis (NARC) by NAS < 4 and/or F < 2. The training cohort (GOLDEN or G) comprised 220 patients (ARC/NARC = 95/125). The validation cohort (RESOLVE or R) comprised 467 patients (ARC/NARC = 255/212). Diagnostic performances (ARC vs NARC) in G and R were compared (AUROC, sensitivity, specificity). Merged cohort (M) with 687 patients was used for optimization of coefficients, assessment of relations with NAS and Fibrosis score (F), and comparison with existing scores.







Results: A higher rate of ARC was obtained in R vs G (55% vs 43%), but distributions of patients according to NAS or fibrosis score in ARC and NARC groups were comparable in the 2 cohorts. In R as in G, circulating levels were higher in ARC vs NARC ($p < 0.0001$) for miR-34a (2.82 ± 0.26 vs 2.54 ± 0.3 log10 copies/µL), A2M (2.61 ± 0.87 vs 2.04 ± 0.78 g/l), YKL40 (119 ± 162 vs 56 ± 41 ng/ml) and HbA1c (6.36 ± 0.97 vs 5.96 ± 0.89%). NIS4 was significantly ($p < 0.0001$) higher in ARC vs NARC patients (0.659 ± 0.015 vs 0.345 ± 0.016). NIS4 showed similar diagnostic performances in G and R cohorts for detection of ARC: AUROC = 0.81 (0.73–0.86) in G and AUROC = 0.81 (0.77–0.85) in R. Comparison of NIS4 in R vs G at optimal cutoff for G, sensitivity (68% vs 74%), specificity (77% vs 82%) total accuracy (72% vs 79%), PPV (78% vs 76%) and NPV (66% vs 81%) were only slightly to moderately affected. In M (n = 687) after optimization, AUROC reached 0.82 (0.78–0.85). At optimal cutoff for M, sensitivity and specificity were 76% and 76% respectively. In M, NIS4 gradually increased with NAS and fibrosis and was more potent than existing scores for detection ARC vs NARC (see figures).

Conclusion: This study validates NIS4 clinical performances for detection of ARC in a large population of patients prospectively screened for suspicion of progressive NASH at 133 hepatology centers in 25 countries. In this context, NIS4 outperforms existing scores, supporting its use in medical practice.

LBP-021

The percentage of patients with HCV infection in need of a liver transplant is rapidly declining while their survival after transplantation is improving: A study based on European liver transplant registry

G. Perricone[1], C. Mazzarelli[1], R. Viganò[1], C. Duvoux[2], P. Cortesi[3], R. Facchetti[3], M. Vangeli[1], V. Karam[4], R. Adam[4], M. Strazzabosco[5], L.S. Belli[6]. *[1]ASST GOM Niguarda, Hepatology and Gastroenterology Unit, Milan, Italy; [2]Henri Mondor Hospital-APHP, Liver Transplant Unit, Creteil, France; [3]University of Milan-Bicocca, Research Centre on Public Health, Monza, Italy; [4]Hôpital Paul Brousse, Centre Hépatobiliaire, Villejuif, France; [5]Yale University, Section of Digestive Diseases, New Haven, United States; [6]ASST GOM Niguarda, Hepatology and Gastroenterology Unit, Milan*
Email: giovanni.perricone@ospedaleniguarda.it

Abstract LBP-021 is under embargo until Friday 13 April 2018, 07:00. This abstract has been selected to be highlighted during official EASL Press Office activities or in official EASL Press Office materials that will be made publicly available on the congress website at 07:00 (CET) on the day of their presentation at the congress.
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Progression of Fibrosis in Hepatitis C With and Without Schistosomiasis: Correlation with Serum Markers of Fibrosis

Sanaa M. Kamal,[1,2] Bradley Turner,[1] Qi He,[1] Jens Rasenack,[3] Leonardo Bianchi,[3] Ahmed Al Tawil,[4] Ahmed Nooman,[2] Mahmoud Massoud,[2] Margaret James Koziel,[1] and Nezam H. Afdhal[1]

Serial liver biopsies are the gold standard by which the progression of fibrosis is evaluated. This longitudinal cohort study assessed the different rates in the progression of fibrosis using serial liver biopsies and serum fibrosis markers YKL-40 and PIIINP and the cytokines, transforming growth factor beta (TGF-β) and tumor necrosis factor alpha (TNF$-\alpha$). A 10-year cohort study was performed in patients with hepatitis C virus (HCV) alone or HCV and schistosomiasis. Patients were enrolled at the time of acute HCV infection and prospectively evaluated with two liver biopsies (at entry and end of follow-up), and true rates in the progression of fibrosis were calculated per year. Serum YKL-40, N-terminal propeptide of collagen III (PIIINP), TGF-β, and TNF-α were measured, as well as the expression of TGF-β, TNF-α, and YKL-40 mRNA in liver tissue. A significant increase in the progression rates of fibrosis occurred in the coinfected group (0.61 ± 0.13) compared with the HCV monoinfection group (0.1 ± 0.06; $P < .001$)). The progression of fibrosis rate/year had a direct linear correlation for YKL-40 ($r = 0.892$, $P < .001$) and for PIIINP ($r = 0.577$, $P < .01$). YKL-40 showed a linear correlation with TGF-β ($r = 0.897$, $P < .001$). Hepatic mRNA levels of YKL-40 and TGF-β correlated with the serum levels, confirming a hepatic source for the elevated serum levels. In conclusion, serial cytokine and fibrosis markers can accurately determine the rate at which fibrosis is progressing, identifying both those with rapid fibrosis and those with stable disease. *Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).* (HEPATOLOGY 2006;43:771-779.)

Hepatitis C virus (HCV) infection is characterized by silent onset in most infected individuals, a high rate of viral persistence, and the potential for development of chronic liver disease, ranging from chronic hepatitis to cirrhosis and hepatocellular carcinoma.[1,2] However, the progression of fibrosis in chronic hepatitis C is highly variable, and the natural history of the disease usually extends over several decades.[3,4] In epidemiological studies of chronic HCV infection, age, duration of infection, alcohol consumption, male sex, and coinfection with HIV, hepatitis B virus, or schistosomiasis have been related to histological severity.[5-10] Key cytokines secreted in response to cell injury such as tumor necrosis factor-alpha (TNF-α) and transforming growth factor beta-1 (TGF-β1) have been implicated in the development of liver inflammation and fibrosis.[11-13] TNF-α has been shown to modulate hepatic stellate cell activation as well as synthesis of some extracellular matrix proteins and proteins involved in matrix degradation.[14]

Serial liver biopsies are the current gold standard to evaluate the progression of fibrosis.[15] A number of serological and urinary compounds such as procollagens, tissue inhibitors of metalloproteinases (TIMP), type IV S collagen, hyaluronic acid, and laminin and mediators of extracellular matrix production such as TGF-β have been

Abbreviations: HCV, hepatitis C virus; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; TIMP, tissue inhibitors of metalloproteinases; ALT, alanine aminotransferase; PCR, polymerase chain reaction; PIIINP, aminoterminal propeptide of type III procollagen; AST, aspartate aminotransferase; ECM, extracellular matrix.

From the [1]Liver Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; [2]Division of Gastroenterology and Hepatology, Department of Internal Medicine, University of Ain Shams, Cairo, Egypt; [3]Department of Pathology, University of Basel, Switzerland; and the [4]Department of Pathology, University of Ain Shams, Cairo, Egypt.

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Address reprint requests to: Nezam H. Afdhal M.D., Liver Center, Beth Israel Deaconess Medical Center, 110 Francis street, Boston, MA 02215. E-mail: nafdhal@caregroup.harvard.edu; fax: 617-632-1066.

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evaluated as noninvasive markers of liver fibrosis.[16-26] Most of these studies have focused on using these markers in cross-sectional studies to diagnose the stage of liver fibrosis.

We recently proposed that YKL-40, also known as human cartilage glycoprotein 39 or CHONDREX is an excellent marker for staging fibrosis in the liver and differentiating cirrhosis from chronic hepatitis with stage 1 and 2 fibrosis in patients with HCV.[27-30]

Schistosomiasis is a chronic helminthic disease infecting more than 200 million people worldwide.[31] Concomitant schistosomiasis and HCV infection is common in many developing countries[32,33] and exhibits a unique clinical, virological, and histological pattern manifested by virus persistence with high HCV RNA titers, higher necroinflammatory and fibrosis scores in liver biopsies, and poor response to interferon therapy.[33-35] Patients with hepatitis C and *Schistosoma mansoni* coinfection show markedly accelerated hepatic fibrosis.[9,10]

Therefore, in this study, we used serum fibrosis markers and profibrogenic and pro-inflammatory cytokines to predict differences in the rate of progression of fibrosis in a rapidly progressive cohort versus a traditional HCV slowly progressive cohort. Our studies indicate that serum YKL-40 and TGF-β can accurately predict the progression of fibrosis over an 8- to 10-year period in patients with progressive HCV and Schistosomiasis coinfection and are also effective in identifying stable patients without progression of fibrosis.

Patients and Methods

Study Population. Patients were enrolled into this longitudinal cohort study from patients with acute HCV who failed to clear viremia within 6 months of initial infection. The diagnosis of acute HCV infection was based on the following criteria: elevated values of serum alanine aminotransferase (ALT) to more than 10 times above the upper limit of normal; seroconversion from negative to anti-HCV–positive antibody status assessed by second-generation enzyme-linked immunosorbent assay (Abbott Laboratories, Abbott Park; IL); positive polymerase chain reaction (PCR) for HCV RNA (Amplicor, Roche Diagnostics, Branchburg, NJ); with or without a history of sudden onset of malaise, jaundice, fever, and other symptoms related to liver disease in a previously healthy individual. Overall, 87 patients were enrolled and divided into two groups; HCV monoinfection (n = 39) and HCV coinfection with *Schistosoma mansoni* (n = 48). Shistosomiasis was diagnosed by history, detection of *S. mansoni* ova in stools (modified Kato test) or rectal biopsy; and seropositivity to schistosomal antibodies (indirect hemagglutination: Femouz laboratories, Cedex, France). No patient had clinically active schistosomiasis.). An initial experimental study cohort comprised 42 patients (M:F 26:16; mean age, 29.0 $\pm$ 8.3 years), and a second group of 45 patients were used as a validation cohort for the YKL-40 biomarker.

Patients were followed prospectively for 96 $\pm$ 4.6 months (range, 97-125 months). Patients were examined semi-annually until the end of study. All patients participating in the study presented oral and written informed consent. In the extremely rare case in which literacy was an issue, patients had the consent form read and carefully explained to them in the presence of a family member, both had to consent and the form was stamped, and both patient and family member made their mark. The study was approved by the Office for Human Protections Research Board of An Shams University (P-002104), and the protocol and all procedures of the study were conducted in conformity with the ethical guidelines of the Declaration of Helsinki and the human experimentation guidelines of the U.S. Department of Health and Human Services.

Laboratory Tests of Liver Disease and Virological Markers. Serum ALT, albumin and bilirubin concentrations, and prothrombin time were determined at entry and semi-annually until the end of follow-up. Serum HCV RNA was estimated by PCR, using a commercial kit (Amplicor HCV; Roche Diagnostics, Branchburg, NJ), and genotyping was performed using a second-generation reverse hybridization, line-probe assay (Inno-LiPA HCV II; Innogenetics, Zwijndrecht, Belgium). The entire cohort had ultrasonography and endoscopy, and the results are given for the end of the study procedures.

Histological Assessment. All patients were subjected to a baseline liver biopsy within 8 to 10 months after the onset of symptoms. Another liver biopsy was performed at the end of follow-up (mean of 96 $\pm$ 4.6 months after onset of symptoms). The study commenced in 1992, and interferon-based therapy became available in Egypt in 1999, but with limited access because of lack of national insurance and cost. The 2nd liver biopsy was performed in some patients before commencing interferon therapy and in the remainder to determine disease progression. A second biopsy after a minimum of 4 to 5 years is standard of care at many U.S. centers, including BIDMC, to evaluate disease progression and is clinically justified. Two passes were performed at each biopsy time point, one for histology and one for intrahepatic RNA studies. Liver biopsies were stained with hematoxylin-eosin and a connective tissue stain (chromotrope aniline blue). Liver biopsies were read by two pathologists in a blinded fashion, adopting the grading and scoring system proposed by

Ishak et al.[36] Moreover, biopsies were assessed for morphological features of schistosomiasis and graded as follows: 0: no evidence for schistosomiasis, 1: poor evidence, 2: suggestive of schistosomiasis, 3: strong evidence for schistosomiasis.

The progression rate of fibrosis per year was estimated as the difference between fibrosis scores of the baseline and follow-up biopsies divided by the interval between the two biopsies.

Serum TGF-β, TNF-α, YKL-40, Aminoterminal Propeptide of Type III Procollagen Measurement: Fasting serum TGF-β, TNF-α, YKL-40, and aminoterminal propeptide of type III procollagen (PIIINP) levels were quantitated at baseline and annually until the end of the study (96 $\pm$ 4.6 months) in the experimental study group. Serum TGF-β (BioSource -International Inc, Nivelles, Belgium), serum TNF-α (Boehringer Mannheim, Germany), and YKL-40 (Metra, Biosystems, Mountain View, CA) were measured by commercially available ELISA assay according to the manufacturer's instructions. PIIINP was measured by radioimmunoassay (Orion Diagnostica, Espoo, Finland) following the manufacturer's instructions.

RNA Studies. Intra-hepatic TGF-β and TNF-α transcript expression was assessed in baseline and follow-up biopsies using standard techniques. YKL-40 mRNA gene expression was measured using TaqMan quantitative PCR. (See supplemental data at the HEPATOLOGY website: http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).

YKL-40 Validation Cohort. The validation group consisted of 45 patients, 19 with HCV alone and 26 with HCV plus schistosomiasis coinfection. The validation cohort was used only to validate the YKL-40 serum marker. This cohort was derived from patients enrolled in a study of immune responses and progression of fibrosis in HCV and schistosomiasis and has been previously published.[10] The validation cohort again included patients with acute HCV who developed chronic hepatitis, and their clinical characteristics are given in Table 4. Patients from this cohort who had adequate serum stored for YKL analysis were included. Patients in this cohort were followed a mean of 114 $\pm$ 12 months, once again with a baseline liver biopsy 6 months after the onset of acute HCV and at the end of the follow-up. Serum tests for YKL-40 were performed on serum stored at the baseline biopsy, 5 years of follow-up, and at year 10, the end of the follow-up period when the second biopsy was performed. The validation cohort did not have any studies performed on liver tissue and was only used to confirm the serial changes in YKL-40 over time. No difference was found between the validation cohort and the initial experimental cohort with respect to clinical characteristics such as genotype (95% genotype 4), viral load, and ALT/aspartate aminotransferase (AST) at baseline. Analysis of fibrosis markers in this cohort was limited to only YKL-40 due to a limited supply of available serum.

Table 1. Demographic and Baseline Characteristics of Patients With Hepatitis C Virus (HCV) Monoinfection, and HCV/*S. mansoni* Coinfection in Experimental Group

Parameter	Group A HCV Monoinfection	Group B HCV & *S. mansoni* Co-infection
Number	20	22
M/F	12/8	13/9
Age(y):mean $\pm$ S.D	30.6 $\pm$ 5.1	29.2 $\pm$ 6.7
Risk factors		
i. Occupational exposure	10	15
ii. Blood transfusion	3	4
iii. Dental procedures	2	2
iv. Intravenous drug use	4	1
V. Surgery	1	0
Disease duration (mo)	7.4 $\pm$ 4.1	8.5 $\pm$ 3.9
ALT (U/mL) mean $\pm$ S.D	123.5 $\pm$ 31.1	108.2 $\pm$ 28.5
AST (U/mL) mean $\pm$ S.D	98.5 $\pm$ 27.3	113.5 $\pm$ 30.8
Albumin (g/dL) mean $\pm$ S.D	4.2 $\pm$ 0.3	4 $\pm$ 0.4
Platelets (per microliter) mean $\pm$ SD	198,000 $\pm$ 50,000	170,000 $\pm$ 38,000
RNA (cop $\times$105/mL) mean $\pm$ SD	16.5 $\pm$ 4.8*	38.8 $\pm$ 8.7*

NOTE. **Group A:** 20 patients with chronic hepatitis C, **Group B: 22** patients co-infected with HCV and *S. mansoni.*

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.
*$P < .01$ between groups A and B.

Statistical Analysis. Results were expressed as mean $\pm$ SD and analyzed using paired and unpaired Student *t* test, chi squared, nonparametric Mann-Whitney *U* test, Wilcoxon rank sum test, or Fisher's exact test where appropriate. Correlation between different parameters was performed using Pearson or Spearman's rank test. *P* values of .05 or less were regarded as significant. All statistical procedures were performed using an SSPS for windows version 10 package (SPSS Inc., Chicago, IL).

Results

Baseline Clinical Characteristics of Patients. The clinical, virological, and histological profile of the experimental cohort patients is shown in Table 1. No statistically significant differences were found between the monoinfected and coinfected patients for age, sex, peak ALT at entry, or source of infection and HCV genotype (4a). HCV patients coinfected with *S. mansoni* had significantly higher HCV RNA titers ($P < .001$).

Histological Hepatic Inflammation. The clinical baseline biopsy features of the Schistosomiasis group are shown in Table 2. The total necroinflammatory scores

Table 2. Histological Evidence of Schistosomiasis at Baseline in Patients With HCV/*S. mansoni* Coinfection

Parameter	HCV & *S. mansoni* Co-infection (n = 22)
S. mansoni ova	17/22 (77.2%)
Eosinophils	16/22 (78%)
Granuloma	12/22 (54.4%)
Pigment	15/22 (68.2%)
Fibrosis of pipestem type	1/22 (4.5%)
Grading for schistosomiasis:	
• Grade 0	0
• Grade 1	0
• Grade 2	5 (22.7%)
• Grade 3	17 (77.2%)

Table 3. End of Follow-up Characteristics of Patients With Hepatitis C Virus (HCV) Monoinfection, and HCV/*S. mansoni* Coinfection in Experimental Group

Parameter	Group A HCV Monoinfection	Group B HCV & *S. mansoni* Co-infection
ALT (U/mL) mean ± SD	84.5 ± 24.5	93.1 ± 31.7
AST (U/mL) mean ± SD	77.9 ± 31.5	91.9 ± 40.2
Albumin (g/dL) mean ± SD	3.9 ± 0.7	2.8 ± 1.3*
Platelets (per microliter) mean ± SD	187,000 ± 54,000	121,000 ± 27,000
RNA (cop × 105/mL) mean ± SD	10.6 ± 2.3†	19.2 ± 2.1†
Splenomegaly: n (%)	1 (5)‡	20 (91)‡
Esophageal varices n (%)	1 (5)‡	21 (95)‡

*$P < .01$.
†$P < .05$.
‡$P < .001$.

were significantly higher at liver biopsy 1 (baseline biopsy) in coinfected patients ($P < .05$). Coinfected patients had significantly higher degrees of interface hepatitis (1.5 ± 0.7 vs. 0.6 ± 0.5; $P = .027$) and periportal necrosis (1.9 ± 0.9 vs. 1.1 ± 0.2; $P = .0016$). No significant difference was seen in necroinflammatory scores between monoinfected and coinfected patients in liver biopsy 2 (follow-up biopsy) (Fig. 1A).

In both monoinfected and coinfected patients, neither ALT levels nor viral load correlated with the necroinflammatory scores in baseline or follow-up biopsies (Wilcoxon's signed rank test $P = .5$, $P = .7$ respectively; data not shown).

Clinical Follow-up. The clinical and virological data of the experimental cohort patients is shown in Table 3. At baseline, only HCV RNA levels were significantly higher in coinfected patients. At the end of treatment, however, statistically significant differences were found between the mono-infected and coinfected patients for serum albumin levels, platelet counts, and HCV RNA titers. Unlike the biomarkers, reduction in platelets and albumin were only seen late in follow-up once cirrhosis had developed. There was no difference in ALT or AST at baseline, throughout the follow-up and at the end of the study. At the end of follow-up, almost all HCV patients coinfected with *S. mansoni* had splenomegaly and esophageal varices (see Table 3).

Histological Progression of Fibrosis. Initially at baseline biopsy, both monoinfected and coinfected patients had no fibrosis (stage: 0). Only one patient in the coinfected group had mild pipestem fibrosis. In the coinfected group, 2 of 22 (9.1%) progressed to stage 1 fibrosis, 2 of 22 (9.1%) progressed to stage 2 fibrosis, 4 of 22

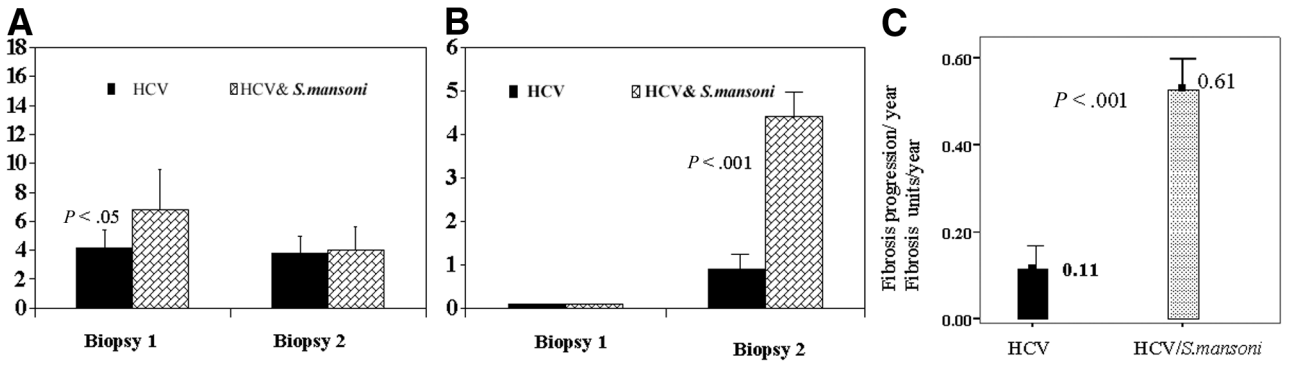

Fig. 1. (A) Comparison of the necroinflammatory scores at baseline biopsies (biopsy 1, performed 6-8 months after acute hepatitis) and follow-up biopsies (biopsy 2, performed at end of follow-up) in 20 monoinfected patients (black bars) and 22 coinfected patients (white bars). Bars represent means. There was significant difference in necroinflammatory scores between monoinfected and coinfected patients in baseline biopsies ($P < .05$) but not in follow-up biopsies. (B) Fibrosis scores at baseline biopsies and follow-up biopsies in 20 monoinfected patients (black bars) and 22 coinfected patients (white bars). Bars represent means. At liver biopsy 1, both monoinfected and coinfected patients had no fibrosis (stage: 0). Coinfected patients had significantly greater increase in fibrosis scores detected in biopsy 2 compared with monoinfected individuals (4.3 ± 0.9 vs. 0.8 ± 0.5, respectively; $P < .001$). (C) Fibrosis progression rates (fibrosis units per year) in monoinfected patients (black) versus coinfected patients (shaded).The rate of liver fibrosis progression was significantly higher in coinfected patients than in monoinfected patients (0.61 ± 0.13 in the coinfected group vs. 0.1 ± 0.06 in the monoinfected group; $P = .001$).

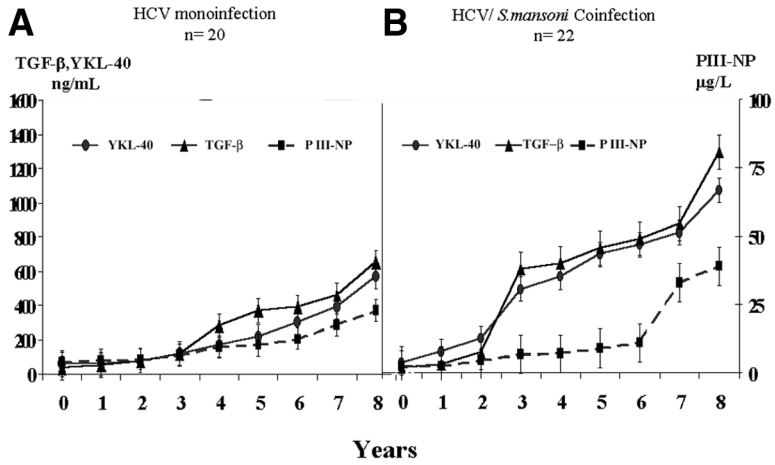

Fig. 2. Scattergrams showing the mean serum levels of each of the fibrosis markers (YKL-40, PIII-NP, and TGF-β) at different points in the monoinfected group and the coinfected group.

(18.2%) progressed to stage 3 fibrosis, 8 of 22 (36.4%) to stage 4, and 6 of 22 (27.3%) to stage 5 fibrosis.

In the HCV alone group, 2 of 20 (10%) progressed to stage 1 fibrosis, 1 of 20 (5%) progressed to stage 2 fibrosis, and 17 of 20 (85%) remained the same with stage 0 fibrosis. Overall, coinfected patients showed a striking increase in fibrosis scores detected in biopsy 2 compared with monoinfected individuals (4.4 ± 0.9 vs. 0.8 ± 0.5, respectively; $P < .001$) (Fig. 1B). The rate of progression of liver fibrosis (fibrosis units per year) was significantly accelerated in coinfected patients in comparison with monoinfected patients (0.61 ± 0.13 in the coinfected group versus 0.1 ± 0.06 in the monoinfected group; $P < .001$) (Fig. 1C). The increased fibrosis in the coinfected cohort was statistically significant by chi-square analysis ($P < .001$).

Fibrosis Markers. The mean baseline and follow-up values of YKL-40 and PIII-NP in both groups are shown in Fig. 2.

At baseline, no significant difference was seen in serum YKL-40, PIII-NP, and TGF-β in monoinfected and coinfected patients. The rate of increase during the first 2 years was comparable in the two groups. Coinfected patients showed a sharp increase in serum YKL-40 levels and serum TGF-β levels starting the 3rd to the 4th year of follow-up (Fig. 2B). The highest YKL-40 levels were de-

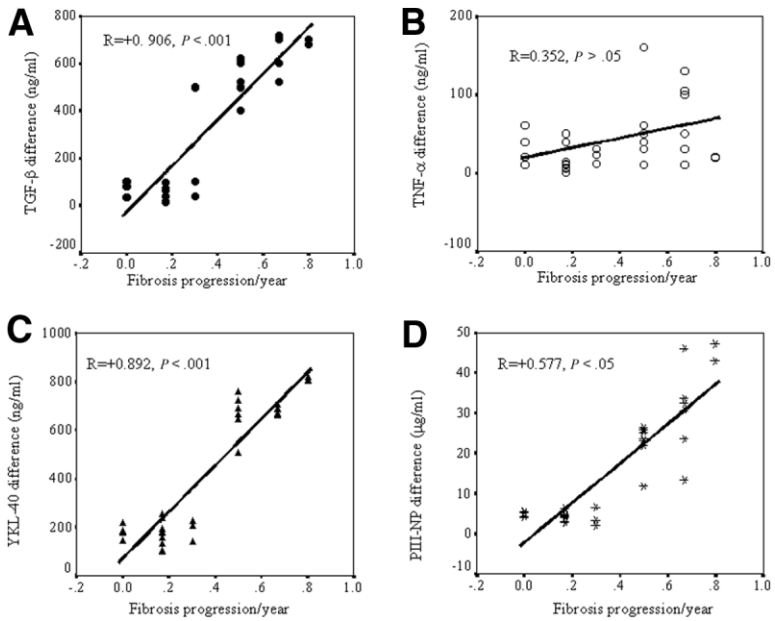

Fig. 3. Scattergrams showing the relationship between rate of fibrosis progression and TGF-β, TNF-α and the fibrosis markers (YKL-40, PIIINP). TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; PIIINP, aminoterminal propeptide of type III procollagen.

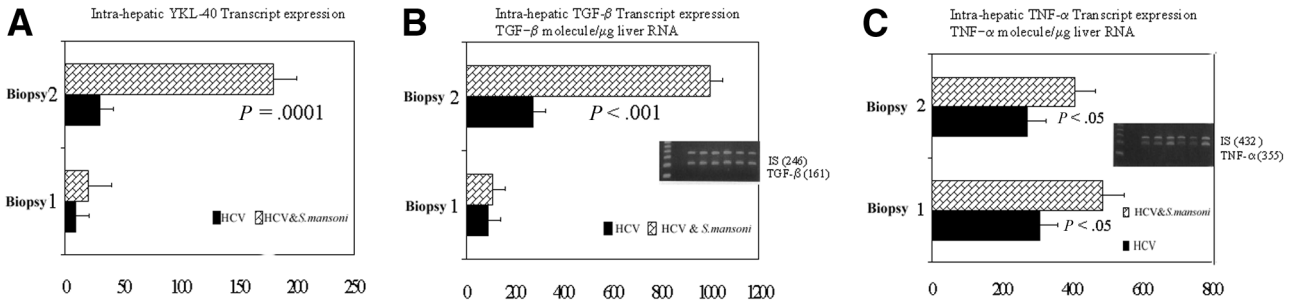

Fig. 4. Expression of transcripts specific for YKL-40 (A), TGF-β (B), and TNF-α (C) within the liver tissue from 20 HCV monoinfected patients and 22 HCV/*S. mansoni*–coinfected patients. RNA preparations from baseline liver biopsies and follow-up biopsies were analyzed for YKL-40 expression by TaqMan (TM) quantitative real-time RT-PCR. and for TGF-β and TNF-α by a competitive RT-PCR technique. TGF-β and TNF-α cDNA has been coamplified with an appropriate concentration of respective internal standard (SI). TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; RT-PCR, reverse transcription polymerase chain reaction.

tected in coinfected patients, who showed marked worsening of fibrosis (n = 17 with fibrosis progression rate > 0.3 fibrosis units/year) (data not shown). YKL-40 paralleled serum TGF-β levels at all times with a highly statistically significant relationship between YKL-40 and TGF-β ($r = +0.897$, $P < .001$).

The peak increase in PIII-NP levels in coinfected patients was detected at later times (years 7 and 8). A weaker correlation was detected between PIII-NP and TGF-β ($r = +0.403$, $P < .05$). Early on, serum TNF-α levels were higher in coinfected patients compared with levels in monoinfected patients; however, the levels were fluctuating over time and did not correlate with either YKL-40 or PIII-NP (data not shown).

Serum TGF-β levels increased in parallel with severity of liver damage and progression of fibrosis, which was markedly accelerated in coinfected patients. The association between serum TGF-β and rates of progression of fibrosis is shown in Fig. 3A. Patients who had fibrosis scores (>3) at the end of follow-up (17 coinfected patients) showed higher mean and median serum TGF-β levels starting year 3 ($R = +0.903$, $P < .001$).

We found no significant relationship between overall degree of fibrosis or progression rates of fibrosis and TNF-α (Fig. 3B). Serum TNF-α did, however, correlate at all points with the necroinflammatory score ($R = +0.4$, $P < .05$; data not shown).

To determine whether changes in serum fibrosis markers would parallel the changes in progression of fibrosis, we correlated serum YKL-40 and serum PIIINP change rate (difference between baseline and follow-up values) to the fibrosis progression rate (fibrosis unit/year) (Fig. 3C-D). A stronger direct linear correlation was observed between YKL-40 levels ($r = +0.892$, $P < .001$) and fibrosis progression rate when compared with PIIINP ($r = 0.577$, $P < .05$), suggesting that YKL-40 may be more efficient than PIII-NP in early detection of fibrosis and in monitoring progression of fibrosis.

Hepatic mRNA Expression. We then analyzed TGF-β TNF-α, and YKL-40 messenger RNA (mRNA) expression in liver tissue of baseline and follow-up biopsy specimen from the two groups of patients. Data have been normalized for β-actin transcript expression. The levels of both TGF-β and YKL-40 m RNA expression in the follow-up biopsies were 6-fold higher than the levels in baseline biopsies only for the coinfected patients (Fig. 4A and B) and were highest in those with the more advanced fibrosis stage. There was no significant correlation between mRNA levels of either YKL-40 or TGF-β and histological inflammatory index. These increases in hepatic message paralleled the changes seen in serum expression of both YKL-40 and TGF-β, confirming a hepatic source

Table 4. Clinical Characteristics, Fibrosis Progression and YKL-40 Levels in the Validation Cohort

Parameter	HCV Monoinfection	HCV & *S. mansoni* Co-infection
Number	19	26
M/F	11/8	17/9
Age (yrs):mean ± S.D	36.6 ± 8.1	34.2 ± 7.6
ALT/AST (U/mL)	74/88	68/75
Fibrosis score		
Baseline	0	0
Year 10	1.52 ± 1.3	5.0 ± 0.6
Fibrosis progression rate (U/yr)	0.16	0.56
YKL (ng/mL)		
Baseline	53 ± 35	80 ± 45
Year 5	110 ± 64	278 ± 92*
Year 10	172 ± 76	503 ± 106*
Change in YKL from baseline (ng/mL)		
Year 5	59 + 39	190 + 83*
Year 10	117 + 56	423 + 101*

*$P < .0001$ between groups, two-tailed t test.

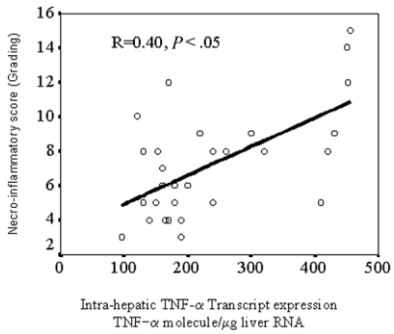

Fig. 5. Correlation between necroinflammatory score and intrahepatic TNF-α expression. The two parameters show a mildly significant positive correlation ($R = +0.40$, $P < .05$). TNF-α, tumor necrosis factor alpha.

of origin for these markers. In monoinfected patients, who did not show progression of fibrosis, there was no major change in hepatic expression of mRNA for either YKL-40 or TGF-β at the second biopsy (Fig. 4A-B).

TNF-α mRNA expression was not different between baseline and follow-up liver biopsy in either coinfected or HCV-alone patients. However, levels of TNF-α mRNA were significantly higher in coinfected patients than in HCV alone at both baseline and follow-up (Fig. 4C) and appeared to correlate best with degree of inflammation and necroinflammatory scores ($R = 0.40$, $P < .05$, Fig. 5) but not with fibrosis scores or the rate of progression of fibrosis.

Validation Cohort. Because YKL-40 is a relatively new marker for HCV-related fibrosis, we examined YKL-40 serum levels in a further cohort of 45 patients with matched liver biopsies. The baseline clinical and demographic data for the validation cohort is given in Table 4. The rate of disease progression in the validation cohort was identical to that seen in the experimental cohort. A very significant correlation with YKL levels and disease progression was seen in the HCV/Schistosomiasis coinfected group and with no disease progression in the mono-infected group (Table 4). All patients in the HCV and schistosomiasis group had significant increases in YKL compared with the HCV alone group, as shown in Fig. 6. Using an increase in YKL-40 of 100 ng/mL from baseline at year 5 and 200 ng/mL at year 10 to indicate disease progression was both highly specific and sensitive. In the entire combined cohort, only two patients with mild disease progression (<2 points increase over 10 years on Ishak) had increases in YKL-40 at years 5 or 10 as listed above (96% sensitivity). Similarly, only two patients with progressive disease failed to increase their YKL levels, giving a specificity of 96%.

Discussion

This unique cohort study clearly confirms previous reports showing the more rapid rate of progression of liver fibrosis in patients who have both schistosomiasis and HCV compared to HCV alone.[9,10,33] The rate of progression of fibrosis at 0.61 units per year has most coinfected patients developing cirrhosis within 10 years of exposure to HCV and was seen in both the experimental and validation cohorts. This rate of progression of fibrosis would be comparable to patients with HIV and HCV or HCV patients with significant alcohol consumption (>50 g/d). The fibrosis rate of 0.1 units per year seen in the HCV alone patient more closely resembles that proposed by Poynard et al.[37] for most studies of hepatic fibrosis in uncomplicated HCV, with cirrhosis occurring between 20 and 40 years. The study is clearly limited by the relatively small number of patients and the low rate of disease progression in the HCV monoinfection group. Combining both experimental and validation cohorts, the rate of progression in HCV monoinfected was only 0.15 U/year. This slow rate of progression can be best explained by the relatively few cofactors for disease progression, as patients had no alcohol consumption, no HIV or hepatitis B virus coinfection and are infected at a young age (mean age at infection, 30 years).

Examining the hepatic and serum levels of TGF-β and TNF-α gives us some insight into the potential mechanism for the more rapid fibrosis in patients with schistosomiasis. Initially, there is no difference in liver fibrosis; however, histological liver inflammation and TNF-α levels are higher in the coinfected group, suggesting these patients are primed by the schistosomal infection to a more aggressive level of inflammation. Within 2 years, we begin to see increases in serum pro-fibrogenic TGF-β levels and YKL-40 in the coinfected group, suggesting that the fibrotic process is progressing with changes in the extracellular matrix (ECM). These continue throughout the next 6 years of follow-up and, assuming some linearity to the progression of fibrosis, they strongly parallel the changes seen on the repeat liver biopsy. The serum levels

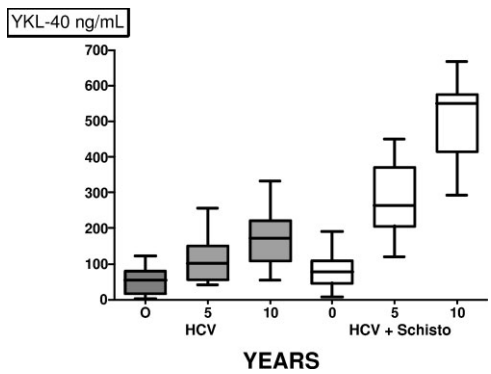

Fig. 6. Boxplot of YKL levels in both groups in the validation cohort at baseline, year 5 and year 10.

closely parallel the hepatic mRNA increases for both YKL-40 and TGF-β and suggest that the serum levels are a reflection of ECM modeling in the liver.

Perhaps the most important finding in this study is the very close clinical correlation of the panel of markers for fibrosis and cytokines with disease progression. Using YKL-40, PIINP, and TGF-β, we would have clearly been able to identify differences in progression rates of fibrosis between the two cohorts in the experimental study group. In the validation group, YKL-40, as a single serum marker for fibrosis, was able to differentiate progression rates between slow and rapid fibrosing patients. In our prior studies in a U.S. population, a YKL-40 level of >350ng/mL indicated stage 3 or above fibrosis,[27] and this was seen in all patients in the coinfected group with rapidly progressive disease to stage 4 or 5. The lack of a marker for a change of fibrosis in the HCV alone group also shows how useful serial markers can be to determine lack of disease progression. Interestingly, in patients with disease progression, changes also occur in standard clinical markers such as platelets and albumin but not in ALT or AST. However, because the study started approximately 12 years ago, we were unable to longitudinally evaluate other clinical markers such as platelet count or APRI, which may have performed equally well as the markers of fibrosis we measured.

In addition, patients with schistosomiasis developed evidence of portal hypertension with splenomegaly and esophageal varices. However, this was independent of liver fibrosis and reflects the underlying pre-hepatic portal hypertension associated with schistosomiasis. The associated hypersplenism is also a factor in the development of thrombocytopenia. However, these clinical changes tend to occur later in disease progression, whereas the markers of fibrosis start to rise when there is an estimated transition to Ishak stage 3 with bridging fibrosis.

Most studies of markers of fibrosis have been cross-sectional and focused on the ability of markers to diagnose cirrhosis. Some studies have also shown that successful therapy of HCV can be associated with a reduction in serum markers of fibrosis such as PIIINP,[38,39] but there are no long-term studies on the role of markers in predicting resolution or stabilization of fibrosis. This study represents a truly unique cohort of patients followed sequentially for almost 10 years and thus is an excellent model for fibrosis studies such as this one.

When examining liver ECM turnover, patients with more rapidly progressive diseases such as alcoholic hepatitis have the highest levels of ECM markers.[22,40] This has been shown for markers such as type IV collagen and hyaluronic acid, which correlate best with the degree of alcoholic hepatitis and perivenular fibrosis.[23,24] Rather than reflect the total collagen level, they accurately correlate with the degree of new collagen production and turnover and will also fall with abstinence from alcohol. In a similar fashion, the levels of YKL-40 were higher than that seen in some patients (>110 ng/mL) in the coinfected group, and these levels are reflecting the very active ECM with rapidly progressive liver diseases. In prior studies, YKL-40 has been shown to be an excellent marker in active alcoholic liver disease.[28,41] Because we are using the markers for monitoring disease, the absolute levels are not as important as the rate of increase over time, and certainly YKL-40 and TGF-β in individuals show excellent sensitivity to disease progression. In fact, in the validation cohort, the sensitivity and specificity of YKL-40 for predicting disease progression was greater than 95% and represents one of the first cohort studies to really use longitudinal markers of serum fibrosis.

This variation of markers of fibrosis in individuals with disease progression could have a strong potential clinical role in patients with HCV. Many HCV patients have slowly progressive disease and at initial diagnosis have only minor histological changes of fibrosis and inflammation and are not candidates or refuse interferon-based treatment. The standard of care has been to follow these patients and repeat liver biopsies in 4 to 5 years. However, as demonstrated by the HCV-alone group, who had a disease progression rate of 0.1 ± 0.06 fibrosis units per year, these markers of fibrosis can be used longitudinally to determine patients with slow rates of disease progression who do not need biopsy or therapy. Larger clinical cohorts need to verify these results in patients who are not treated for HCV but are followed clinically for disease progression before these biomarkers can be truly integrated into clinical practice.

Although this study demonstrates an important use for markers of fibrosis and their ability in serial analysis over time to predict progression of liver disease, an alternative important area for investigation is the role in predicting disease regression. Several ongoing large studies with both alpha and gamma interferons are looking at fibrosis as end points of therapy, serial measurements of markers of fibrosis can predict regression of fibrosis. These studies will potentially determine whether a clinically useful panel of markers could be used to replace or guide the use of liver biopsy.

In summary, this study shows the rapid rate of progression of fibrosis in patients with HCV and schistosomiasis compared to HCV alone. Progression of fibrosis may be mediated by an initially increased inflammatory response caused by elevated TNF-α and subsequent activation of hepatic TGF-β. The utilization of serum markers of fibrosis shows great potential in disease monitoring, and

larger studies will be required to confirm the findings of this initial cohort study.

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伴有或不伴有血吸虫病的丙型肝炎纤维化进展：与纤维化血清标志物相关

摘要：

肝活检是评估纤维化进展的金标准。本研究采用肝活检和血清肝纤维化标志物 YKL-40、PIIINP、细胞活素类、转化生长因子-β（TGF-β）和肿瘤坏死因子-α（TF）对纤维化进行了评估。对单独的丙型肝炎病毒（HCV）或 HCV 伴有血吸虫病患者进行了 10 年队列研究。患者在急性丙型肝炎病毒感染时进行了登记，通过进行两次肝活检（随访和随访结束）进行了前瞻性评估，并且每年都进行纤维化进展的真实率的计算。血清中 YKL-40，N-末端前肽 III 型胶原（PIIINP）、TGF-β、TNF-α 的进行了测定，以及在肝组织中对 TGF-β、TNF-α、和 YKL-40 的 mRNA 进行表达。肝纤维化显著增加发生在感染组其进展率为 0.61±0.13，相比 HCV 感染组的进展率为 0.1±0.06（P＜0.001）。纤维化每年的进展率与 YKL-40 呈直接线性相关性（r= 0.892，P＜0.001），与 PIIINP 的相关性为（r= 0.577，P<0.01）。YKL-40 与 TGF-β 呈线性相关性（r= 0.897，P<0.001）。肝脏中 YKL-40 和 TGF-β 的 mRNA 水平与血清水平相关，确认为升高血清水平的肝源。综上所述，细胞活素和纤维化标志物可以精确地判断纤维化进展的速度，可鉴别患者是处于快速纤维化进展期还是和病情稳定期。本文的补充材料见 HEPATOLOGY 网站

（***http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html***）。

(HEPATOLOGY 2006;43:771-779.)

双抗体夹心法测定血清YKL-40在肝硬化中的诊断价值研究

饶春美，高致远，徐玉兵，房萌，蒋倩倩，洪松，高春芳
（上海东方肝胆外科医院 实验诊断科，上海 200438）

摘要：探讨肝硬化(liver cirrhosis，LC)患者外周血中血清YKL-40(壳多糖酶3样蛋白1)蛋白水平及其在LC中的诊断意义。采用ELISA方法共检测112例LC患者以及114例健康者血清中YKL-40蛋白水平，并进一步分析其在LC中的诊断价值及其与LC患者肝功能和现有肝纤维化指标的相关性。LC组血清YKL-40蛋白水平高于正常对照组($P<0.001$)；将LC组和对照组作比较，ROC曲线分析血清YKL-40蛋白对LC的诊断效能，曲线下面积(area under the curve，AUC)为0.934(95%置信区间为：0.904～0.964)，YKL-40在cutoff值为92.25 ng/mL时，敏感度为81.3%，特异度为90.4%；通过相关性分析发现血清YKL-40蛋白水平与肝功能Child-Pugh分级和FIB-4指数正相关。YKL-40对LC具有良好的诊断效力，能辅助诊断LC并有助于判断LC的严重程度。

关键词：肝硬化；YKL-40；Child-Pugh；FIB-4指数
中图分类号：R392.12 **文献标志码**：A **文章编号**：1001-2478(2017)04-0313-04

肝硬化是肝脏炎症、慢性损害导致的肝纤维化长期进展所致的弥漫性肝损害，早期没有明显的临床表现，晚期出现肝功能损害、门脉高压等症状，甚至上消化道出血、肝性脑病等严重并发症，危及患者生命。

YKL-40(壳多糖酶3样蛋白1，人软骨糖蛋白39)是分子质量为40 kDa、N末端序列为酪氨酸-赖氨酸-亮氨酸结构的包含383个氨基酸序列的糖蛋白[1]，大量研究表明YKL-40参与炎症、细胞迁移、组织重塑、纤维化和肿瘤发生过程[2-5]。酒精性肝硬化、肝炎后肝硬化、非硬化性肝纤维化患者中血清YKL-40水平均显著高于正常人群、脂肪肝患者和无纤维化肝炎患者，中重度纤维化患者血清YKL-40水平与肝纤维化程度相关[6]。我们采用双抗体夹心法，分析了YKL-40在健康者和肝硬化患者中的表达差异并进一步探讨了YKL-40在肝硬化中的诊断价值及其与肝硬化患者肝功能和现有肝纤维化指标的相关性。

1 材料和方法

1.1 对象 2013年1月至2015年12月于上海东方肝胆外科医院就诊LC患者112例，男女之比为3.15∶1，平均年龄为(50.70±12.24)岁；以2013年以来在我院进行体检的114例健康者作为健康对照组，男女之比为2.08∶1，平均年龄为(48.49±8.34)岁。血清样本经3 000 r/min离心10 min后分离并于－80℃保存。本研究所有患者均已签署知情同意书并经上海东方肝胆外科医院伦理委员会批准。

1.2 仪器和试剂 YKL-40检测试剂盒(ELISA法，杭州普望生物技术有限公司)；MIK3酶标仪(上海热点仪器有限公司)；Elx50洗板机(美国Biotek公司)。

1.3 实验方法 双抗体夹心酶联免疫吸附试验(ELISA)：①取出试剂盒室温平衡15 min以上，复融标准品、质控品；②标准品、质控品和稀释后待检样本加入抗人CHI3L1抗体包被微孔板37 ℃孵育1 h，形成抗原抗体复合物；③洗板机洗板5次；④加入生物素标记的抗人CHI3L1抗体37 ℃孵育1 h，形成抗体-抗原-生物素标记抗体复合物；⑤洗板机洗板5次；⑥加入HRP标记的亲和素37 ℃孵育30 min，形成抗体-抗原-生物素标记抗体-酶标亲和素复合物；⑦洗板机洗板5次；⑧加入3，3，5，5′-四甲基联苯胺底物系统，显色15 min，显色反应结束后加终止液，酶标仪检测450 nm吸光度值。

1.4 统计学处理 实验结果采用SPSS Statistics

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作者简介：饶春美，硕士生，从事临床检验工作
通信作者：高春芳(E-mail：gaocf1115@163.com)

22 和 GraphPad Prism 6 统计软件进行分析，以 $\bar{x}\pm s$ 表示正态分布和接近正态分布的计量资料，median(range)表示非正态分布数据。2 组比较采用秩和检验或 t 检验，多组比较采用方差分析，相关性分析采用 Pearson 双变量相关分析。检验水准 $\alpha=0.05$。

2　结果

2.1　外周血 YKL-40 蛋白水平在 LC 组和正常对照组中的差异　外周血中 LC 组 YKL-40 蛋白水平 195.8(103.3～330.4) ng/mL，显著高于正常对照组 46.8(30.7～66.4) ng/mL($P<0.001$，图 1)。

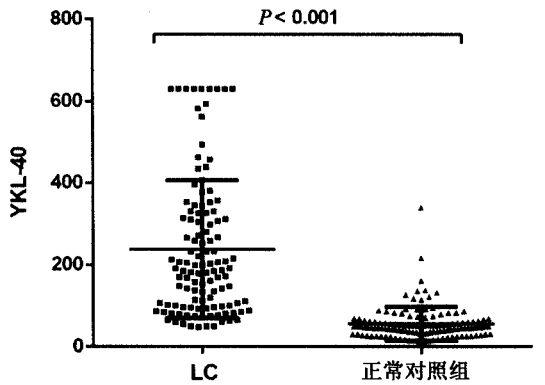

图 1　LC 组、对照组外周血中 YKL-40 蛋白表达差异

2.2　ROC 曲线分析血清 YKL-40 蛋白水平对 LC 的诊断效力　将 112 例 LC 患者同 114 例正常对照者作比较，ROC 曲线分析血清 YKL-40 蛋白水平对 LC 的诊断效能，曲线下面积(area under the curve，AUC)为 0.934(95%CI 为 0.904～0.965，图 2)，YKL-40 在 cutoff 值为 92.25 ng/mL 时，敏感度为 81.3%，特异度为 90.4%。

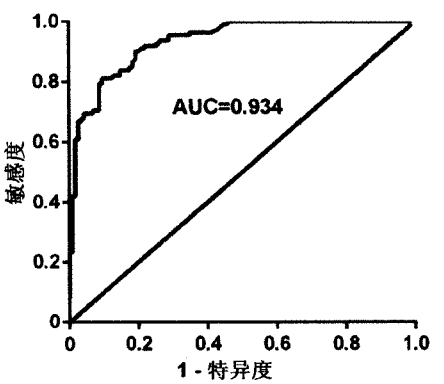

图 2　YKL-40 对 LC 的诊断效力

2.3　LC 组 YKL-40 水平与肝纤维化 FIB-4 指数正相关　LC 组 YKL-40 蛋白水平与 FIB-4 指数[FIB-4＝年龄×AST（IU/L）/PLT（1×10^9/L）×$ALT^{1/2}$(IU/L)]存在正相关($n=111$，其中 1 例患者未检测 PLT，无法计算 FIB-4 指数)，Pearson 相关系数为 0.237($P=0.013$)。

2.4　LC 组 YKL-40 和 Child-Pugh 分级正相关　112 例 LC 组患者，按 Child-Pugh 分级将 Child-Pugh A 级患者(48 例)归为肝功能良好组，Child-Pugh B 级患者(44 例)和 Child-Pugh C 级患者(20 例)归为肝功能不良组(64 例)；比较发现肝功能不良组 YKL-40 蛋白水平 210.5(135.5～385.38) ng/mL，高于肝功能良好组的 168.75(82.25～270.5) ng/mL($P=0.009$，图 3)。

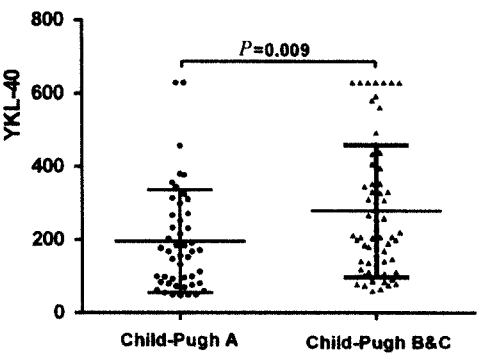

图 3　YKL-40 在 Child-Pugh 分级中的表达差异

3　讨论

YKL-40 基因位于人类染色体 1q32.1，包含 7 948 个碱基对和 10 个外显子[7]，是几丁质酶蛋白家族的一员，隶属于 18 糖基水解酶家族，高度保守，具有几丁质酶样结构但缺乏几丁质酶活性。YKL-40 在人类组织中广泛表达，但也表现出明显的组织特异性，表达最丰富的是肝脏，其次为肾、气管和肺[8]。

Kjaergaard 等[9]在一项对 86 258 例个体的随机研究中发现 YKL-40 水平升高是酒精性肝硬化的血清标志物。YKL-40 通过参与炎症反应和促进细胞外基质重塑而参与到肝脏纤维化发生中，与肝纤维化严重程度相关[10]。肝纤维化是形成与消退动态平衡的过程，早期肝纤维化可以消退逆转，血清 YKL-40 水平可以区分轻度与中重度肝纤维化[11]，早期诊断肝纤维化并加以干预，有助于预防肝硬化的发生。本研究表明，肝硬化组的血清 YKL-40 蛋白水平明显高于正常组，差异具有统计学意义($P<0.05$)，揭示患者发生 LC 时 YKL-40 表达水平显著增加。

将 LC 组与健康者作比较，以血清 YKL-40 水平作 ROC 曲线分析，AUC 为 0.934。YKL-40 在 cutoff 值为 92.25 ng/mL 时，敏感度为 81.3%，特异度为 90.4%，提示血清 YKL-40 水平在区分正常肝和硬化肝上具有很好的诊断效能。YKL-40 诊断严重肝纤维化（F2-F4）ROC AUC（F2-4：0.792，0.914，0.936）与瞬时弹性成像相近，其诊断准确度优于 HA、层黏连蛋白、MMP-9 等血清标志物[12]，并且可提高 TE 诊断早期肝纤维化的准确度。

LC 是肝脏受病毒、酒精、慢性炎症等长期损害，肝纤维化长期进展导致的弥漫性肝损害病变。Child-Pugh 分级是临床上评估患者肝脏功能的常用指标，根据凝血酶原时间、总胆红素、血清白蛋白、腹水和肝性脑病综合评价患者肝功能水平。但是，迄今为止文献中未见 YKL-40 与临床指标 Child-Pugh 分级和 FIB-4 指数的关联分析。因此，在本研究中我们首次尝试 YKL-40 水平与这 2 个临床指标关系的分析。本研究中 112 例 LC 患者中，属 Child-Pugh A 级患者 48 例，属 Child-Pugh B 级 44 例，属 Child-Pugh C 级 20 例。肝功能不良组（Child-Pugh B 级和 Child-Pugh C 级）YKL-40 蛋白水平高于肝功能良好组（Child-Pugh A 级），说明 YKL-40 蛋白作为一个定量指标能直接有效地评估 LC 患者的肝功能水平，随着患者肝功能的下降 YKL-40 蛋白表达增加。

FIB-4 指数[FIB-4＝年龄×AST(IU/L)/PLT(1×10^9/L)×$ALT^{1/2}$(IU/L)]是另一个利用临床常用指标评估慢性肝病患者肝纤维化的无创性方法[13]，可以较准确地估计慢性乙型肝炎感染者有无显著纤维化[14]。本研究结果显示，YKL-40 蛋白水平与 FIB-4 指数之间存在正相关，进一步说明 YKL-40 在评估患者肝脏纤维化水平方面具有重要意义。因此，联合 YKL-40 蛋白水平、FIB-4 指数、Child-Pugh 分级可更好地评估患者的肝纤维化程度，使患者免于肝穿刺活检。

血清 YKL-40 水平可早期诊断肝纤维化，对 LC 具有较高的诊断效能，同时也可用于 LC 患者肝功能的评估。YKL-40 参与炎症反应和促进细胞外基质重塑，在促进肝纤维化上的作用已经得到大量文献的证实。有文献报道，YKL-40 可用于评估 IFN 治疗肝纤维化的效果[15]，在肝纤维化诊疗上具有重要的临床价值。但是，YKL-40 在评估、预测儿童严重肝纤维化上却没有明显的价值[16-17]，需要进一步的研究验证。

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Diagnostic value of double-antibody sandwich ELISA for serum YKL-40 in patients with liver cirrhosis

RAO Chun-mei, GAO Zhi-yuan, XU Yu-bing, FANG Meng, JIANG Qian-qian, HONG Song, GAO Chun-fang(*Department of Laboratory Medicine, Shanghai Eastern Hepatobiliary Sugery Hospital, Shanghai* 200438, *China*)

Abstract: We aimed to compare the concentrations of YKL-40 in the sera from healthy people and patients with liver cirrhosis (LC) and asses its diagnosis values for LC. The study consisted of 112 patients with LC and 114 healthy individuals. The concentration of YKL-40 was determined by a commercial Enzyme-linked Immunosorbent Assay(ELISA) kit. The YKL-40 level in the serum of LC group was significantly higher than that of healthy control group ($P<0.001$). The ROC curve was established by using the YKL-40 concentrations in serum. Compared with the healthy control group, the area under the curve of LC group was 0.934(95% confidence interval: 0.904-0.964). When the cutoff value of YKL-40 was set at 92.25 ng/mL, the sensitivity was 81.3%, and the specificity was 90.4%. In addition, we found that the level of YKL-40 correlated with Child-Pugh stage and the FIB-4 index in LC group. In conclusion, YKL-40 is highly expressed in patients with liver cirrhosis which might be a helpful marker in the diagnosis of LC and in judgement of severity of LC.

Key words: liver cirrhosis(LC); YKL-40; Child-Pugh; FIB-4 index

血清壳多糖酶3样蛋白1检测在不同肝脏疾病中的应用价值*

石玉如[1],岳 莉[1],赵长城[1],姚余有[2],戚应杰[1]
(1. 安徽省立医院感染病院,合肥 230022;2. 安徽医科大学,合肥 230032)

摘 要:目的 探讨血清壳多糖酶3样蛋白1(CHI3L1)在慢性乙型肝炎(CHB)、肝硬化和肝癌患者中的应用价值。**方法** 收集安徽省立医院感染病院2016年1月~2017年2月经临床确诊的96例患者血清标本,其中CHB组28例,肝硬化组44例,肝癌组24例,另收集健康体检标本15例,采用酶联免疫吸附实验(ELISA)定量检测血清壳多糖酶3样蛋白1(CHI3L1)的浓度;利用双抗体夹心免疫层析法检测高尔基蛋白(GP73);采用化学发光法检测甲胎蛋白(AFP)。**结果** CHB组、肝硬化组、肝癌组、健康体检组之间血清CHI3L1差异具有统计学意义($\chi^2=70.249$,$P<0.001$),其中肝硬化组、肝癌组明显高于健康对照组($P<0.001$);各组之间血清GP73差异具有统计学意义($\chi^2=44.963$,$P<0.001$),其中CHB组、肝硬化组、肝癌组显著高于健康对照组($P<0.05$);各组之间血清AFP差异具有统计学意义($\chi^2=57.606$,$P<0.001$),其中肝癌组高于肝硬化组、CHB组、健康组($P<0.001$)。将CHB组与肝硬化组中的GP73,CHI3L1,AFP做ROC曲线,CHI3L1的AUC为0.953(95%可信区间:0.902~1.000),敏感度和特异度分别为88.6%和92.9%,高于GP73和AFP。将肝硬化组与肝癌组中的AFP,CHI3L1,GP73做ROC曲线,AFP的AUC为0.930(95%可信区间:0.871~0.989),敏感度和特异度分别为75.0%和97.7%,高于CHI3L1与GP73。对AFP,GP73,CHI3L1在CHB,肝硬化和肝硬化中的相关性进行分析,AFP与GP73呈正相关($r_s=0.491$,$P<0.001$),AFP与CHI3L1呈正相关($r_s=0.452$,$P<0.001$),GP73与CHI3L1呈正相关($r_s=0.554$,$P<0.001$)。**结论** 血清CHI3L1在肝硬化中具有很好的诊断效能,优于GP73与AFP,而AFP在肝癌中的诊断价值要高于CHI3L1与GP73。

关键词:壳多糖酶3样蛋白1;肝脏疾病;应用价值
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Clinical Application Value of Chitinase-3-like Protein 1 in Patients with Different Liver Diseases

SHI Yu-ru[1], YUE Li[1], ZHAO Chang-cheng[1], YAO Yu-you[2], QI Ying-jie[1]
(1. *Department of Infectious Diseases Hospital of Anhui Provincial Hospital, Hefei 230022, China*; 2. *Anhui Medical University, Hefei 230032, China*)

Abstract: Objctive To explore the clinical value of Chitinase-3-like protein 1(CHI3L1) in patients with chronic hepatitis B (CHB), liver cirrhosis and liver cancer. **Methods** 96 clinically diagnosed patients in Department of Infectious Diseases Hospital of Anhui Provincial Hospital from Jan 2016 to Feb 2017 (28CHB, 44livercirrhosis, 24liver cancer, 15healthy controls) were analyzed. The serum level of CHI3L1 was measured by enzyme-linked immunosorbentassay (ELISA). The Golgi protein (GP73) was tested by double-antibody sandwich immunochromatographic assay. The Alpha-fetoprotein (AFP) was tested by means of chemical luminescence. **Results** There were significant differences between the groups of chronic hepatitis B (CHB), liver cirrhosis, liver cancer and healthy controls on the CHI3L1 level ($\chi^2=70.249$, $P<0.001$). The CHI3L1 level of the liver cirrhosis group and the liver cancer group increased significantly compared with that of the healthy controls ($P<0.001$). The GP73 levels of these groups were significantly different ($\chi^2=44.963$, $P<0.001$). The GP73 levels of the CHB group, the liver cirrhosis group and the liver cancer group all increased significantly compared with that of the healthy controls ($P<0.05$). The AFP levels of these groups were significantly different ($\chi^2=57.606$, $P<0.001$). The AFP level of the liver cancer group increased significantly compared with that of the CHB group, the liver cirrhosis group and the healthy controls ($P<0.001$). Based on the receiver operating characteristic (ROC) curve of CHI3L1, GP73 and AFP in the CHB group and the liver cirrhosisgroup, the Area Under roc Curve (AUC) of CHI3L1 was 0.953 (95%CI: 0.902~1.000), the sensitivity was 88.6%, and the specificity was 92.9%, which was higher than GP73 and AFP. Based on the ROC curve of CHI3L1, GP73 and AFP in the liver cirrhosis group and the liver cancer group, the AUC of AFP was 0.930 (95% CI: 0.871~0.989), the sensitivity was 75.0%, and the specificity was 97.7%, which was higher than CHI3L1 and GP73. The correlation between the CHI3L1, GP73 and AFP in CHB, liver cirrhosis and liver cancer groups were analyzed. There was a posi-

* 作者简介:石玉如(1984-),女,研究生,主管检验技师,研究方向:临床感染性疾病的免疫与分子诊断,E-mail:290797374@qq.com。
通讯作者:戚应杰,男,副主任技师,研究方向:临床感染性疾病的实验室诊断与研究,E-mail:7yingjie@sina.com。

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tive correlation between AFP and GP73 ($r_s=0.491$, $P<0.001$), a positive correlation between AFP and CHI3L1 ($r_s=0.452$, $P<0.001$), a positive correlation between GP73 and CHI3L1 ($r_s=0.554$, $P<0.001$). **Conclusion** CHI3L1 is good at diagnosis of liver cirrhosism, better than GP73 and AFP. And AFP could be more beneficial in patients with liver cancer, better than CHI3L1 and GP73.

Keyword: CHI3L1; liver diseases; clinical application value

据《2017年全球肝炎报告》显示，全球有3.25亿人感染慢性乙肝病毒或丙肝病毒。如果病毒未得到有效控制，则会引发慢性肝炎、肝硬化甚至肝癌。因此，对肝脏损伤严重程度的监控显得尤为重要。肝组织活检虽是“金标准”，但有创伤，不适合广泛开展。寻找反映肝损伤严重程度-尤其是肝脏纤维化程度的无创检测技术，一直是国内外学者努力的方向。以往采用高尔基体蛋白-73(golgiprotein73, GP73)、甲胎蛋白(alpha fetoprotein, AFP)等指标监测肝纤维化和肝癌，其灵敏度和特异度都不够理想。近些年来随着诊断技术的发展，血清壳多糖酶3样蛋白1(CHI3L1)在肝脏疾病中的鉴别越来越引起研究人员的重视。研究表明，多种细胞可以表达分泌CHI3L1[1]，CHI3L1能够参与急慢性炎症及细胞外基质重构等病理过程[2]，在临床上有广泛的应用前景，但有关CHI3L1在CHB、肝硬化、肝癌等疾病的不同阶段水平变化的研究鲜有报道。因此本文对乙肝病毒感染的患者进行血清CHI3L1检测，同时与GP73，AFP指标进行比较，从而探讨CHI3L1在CHB、肝硬化以及肝癌患者中的应用价值。

1 材料与方法

1.1 *研究对象* 收集2016年1月～2017年2月期间在我院住院的HBV感染者血清标本96例，其中CHB组28例，男性20例，女性8例，平均年龄43.7±11.2岁；肝硬化组44例，男性40例，女性4例，平均年龄50.1±12.4岁；肝癌组24例，男性20例，女性4例，平均年龄57.5±11.3岁；健康对照组15例，男性10例，女性5例，平均年龄53.6±10.6岁，所有患者无其他系统性疾病。CHB的诊断参照中华医学会肝病学会联合修订的《慢性乙型肝炎防治指南(2015年版)》[3]。

1.2 *仪器与试剂* 血清CHI3L1定量检测试剂为杭州普望生物技术公司生产的ELISA试剂盒，酶标仪采用Wellscan K-3型；血清GP73检测采用北京热景生物技术公司试剂，仪器为北京热景发光仪UPT-3A型；血清AFP采用化学发光法检测，仪器与试剂均由罗氏公司提供。

1.3 *方法* 采集所有CHB、肝硬化、肝癌、健康体检对照组清晨空腹静脉血，3 000r/min离心10 min，分离血清后进行检测。采用ELISA法定量检测CHI3L1，利用双抗体夹心免疫层析法检测GP73，运用化学发光法定量检测AFP，完全按照仪器和试剂厂家说明书进行。每次检测均使用试剂厂家提供的标准品和质控品，且结果均在控。

1.4 *统计学分析* 采用SPSS23.0软件进行处理，计量资料以四分位间距进行表示，多组间的比较采用Kruskal-Wallis H检验，多组样本间的两两比较采用Nemenyi检验，$P<0.05$为差异有统计学意义；采用Spearman秩相关分析各试验指标的相关性，$P<0.05$为差异有统计学意义；采用ROC曲线进行不同指标的效能分析。

2 结果

2.1 *CHB组、肝硬化组、肝癌组和健康体检组CHI3L1，GP73，AFP水平比较* 见表1。分别对CHB组、肝硬化组、肝癌组、健康体检组的CH3L1，GP73，AFP的结果进行Kruskal-Wallis H检验并进行两两比较，结果显示各组间CH3L1差异有统计学意义($\chi^2=70.249$, $P<0.001$)，其中健康体检组与肝癌组和肝硬化组的CH3L1水平差异有统计学意义($P<0.001$)，但与CHB组差异无统计学意义($P>0.05$)；各组间GP73差异有统计学意义($\chi^2=44.963$, $P<0.001$)，其中健康组高于CHB组、肝硬化组和肝癌组，差异有统计学意义($P<0.05$)；各组间AFP差异有统计学意义($\chi^2=57.606$, $P<0.001$)，其中肝癌组显著高于CHB组、肝硬化组和健康体检组，差异有统计学意义($P<0.001$)。

表1 慢性乙肝组、肝硬化组、肝癌组、健康体检组CHI3L1，GP73，AFP浓度比较

组 别	CHI3L1(pg/ml)			GP73(ng/ml)			AFP(ng/ml)		
	M	P25	P75	M	P25	P75	M	P25	P75
CHB组	148.38	122.64	199.55	72.53	38.26	123.80	2.58	1.45	20.02
肝硬化组	655.38	343.46	982.78	146.89	73.81	298.98	5.12	2.96	27.74
肝癌组	792.10	247.61	1 082.44	107.96	52.21	172.92	931.65	96.9	1 210
健康组	65.3	49.9	87.9	56.76	42.09	64.87	1.1	0.8	2.2
χ^2		70.249			44.963			57.606	
P		<0.001			<0.001			<0.001	

万方数据

2.2 GP73,CHI3L1和AFP在CHB组、肝硬化组中的敏感度和特异度分析 为了评估CHI3L1在CHB和肝硬化组中的应用价值,将CHB组和肝硬化组中的GP73,CHI3L1以及AFP浓度做ROC曲线,GP73浓度的ROC曲线显示:GP73的AUC为0.710(95%可信区间:0.588～0.833),敏感度和特异度分别为50.0%和89.3%;CHI3L1的AUC为0.953(95%可信区间:0.902～1.000),敏感度和特异度分别为88.6%和92.9%;AFP的AUC为0.623(95%可信区间:0.487～0.760),敏感度和特异度分别为81.8%和50%。CHI3L1在CHB、肝硬化组中的特异度与敏感度均优于GP73和AFP。见图1。

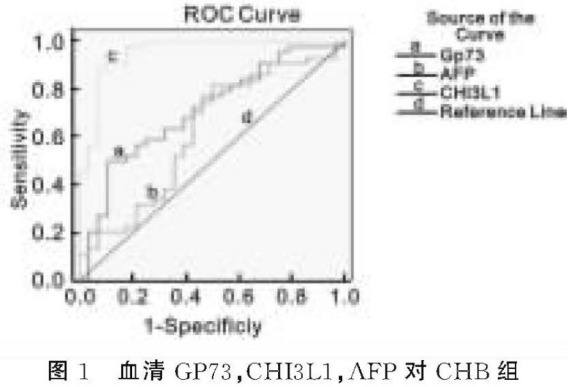

图1 血清GP73,CHI3L1,AFP对CHB组和肝硬化组鉴别效能的ROC曲线分析

2.3 AFP,GP73和CHI3L1在肝癌组和肝硬化组中的敏感度和特异度分析 为了评估血清CHI3L1在肝癌组和肝硬化组中的应用价值,将肝癌组和肝硬化组中的AFP,GP73和CHI3L1浓度做ROC曲线,AFP的AUC为0.930(95%可信区间:0.852～1.000),敏感度和特异度分别为75.0%和97.7%;GP73的AUC为0.377(95%可信区间:0.212～0.542),敏感度和特异度分别为33.3%和40.9%;CHI3L1的AUC为0.5(95%可信区间:0.291～0.709),敏感度和特异度分别为75%和2.3%。AFP在评价肝癌组和肝硬化组中的特异度与灵敏度要高于GP73与CHI3L1,见图2。

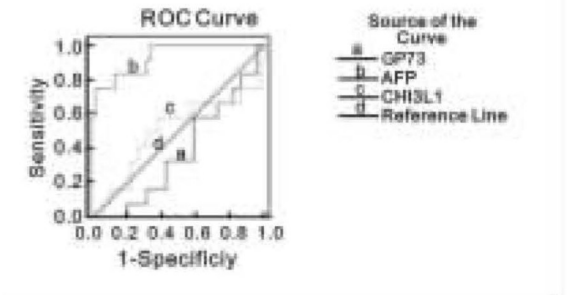

图2 血清GP73,CHI3L1,AFP对肝癌组和肝硬化组鉴别效能的ROC曲线分析

2.4 不同肝脏疾病组血清AFP,GP73和CHI3L1之间的相关性分析 采用Spearman秩相关分析CHI3L1,AFP和GP73之间的直线相关关系,结果显示,AFP与GP73呈正相关($r_s=0.491$,$P<0.001$),AFP与CHI3L1呈正相关($r_s=0.452$,$P<0.001$),GP73与CHI3L1呈正相关($r_s=0.554$,$P<0.001$)。

3 讨论 肝纤维化是由多种不同病因对肝脏造成的慢性损伤。如果得不到控制,任其发展就会使肝脏出现假小叶和结节[4],进而会发展成为肝硬化和肝癌,对患者的生活质量和预后造成严重的影响。因此,寻求新的可靠的血清学诊断指标,对于肝纤维化的诊断及动态监测具有重要的临床意义。

GP73目前经常用于肝纤维化的监控指标,AFP则是最常用的肝癌辅助诊断指标。但是,它们在监控肝病-尤其是早期肝硬化、肝癌时,灵敏度还不高。血清CHI3L1是一种含383个氨基酸,分子量42.6KD的单体糖基化蛋白,编码CHI3L1的基因位于小鼠和人类的1号染色体上[5]。既往研究表明,CHI3L1蛋白水平与乳腺癌、胃癌、肺癌、肝癌等多种肿瘤相关[6],谢而付等[7]对CHI3L1的性能进行评价,提出CHI3L1可以辅助肝癌的诊断,而Huang等[8]研究发现,在肝硬化发展的不同阶段,CHI3L1血清学指标会随肝纤维化严重程度逐渐上升。但是对CHI3L1与GP73,AFP在CHB、肝硬化和肝癌中的应用比较相关报道甚少。

为了评估CHI3L1,GP73和AFP在不同肝病中临床价值,本研究分别检测CHB组、肝硬化组、肝癌组的CHI3L1,GP73和AFP的浓度并进行比较。结果显示,肝癌组和肝硬化组的CHI3L1水平要高于健康对照组,并随着肝脏疾病的加重,血清CHI3L1的检测数值会不断升高,提示CHI3L1可以作为病情监测指标,辅助临床诊断与治疗。CHI3L1的表达途径可能存在两种调节方式,一种是慢性肝脏组织的重构和肝纤维化,一种是由急性肝脏细胞的损伤。但结果还显示,CHI3L1在健康组与CHB组差异无统计学意义,这与张巧娣等[9]人的研究结果一致,具体原因还有待进一步研究。对GP73分析显示,CHB组、肝硬化组、肝癌组要高于健康组,差异有统计学意义,尤其在肝硬化组,GP73的表达尤为显著,这与黄书明等[10]人的研究结果一致,说明GP73能够辅助鉴别健康人和肝损伤病人,可作为判定肝纤维化程度的一个很好指标。此外,AFP在肝癌组显著高于CHB组、肝硬化组和健康体检组,且差异有统计学意义($P<0.001$),进一步说明AFP在肝癌诊断上的重要性,它一直被认为是肝癌的首选肿瘤标志物[11,12]。

为了更好地了解CHI3L1,GP73和AFP在CHB、肝硬化和肝癌中的诊断效能是否存在差别,分别将CHB组与肝硬化组、肝硬化与肝癌组中CHI3L1,GP73和AFP进行AUC比较。结果显示,在鉴别CHB和肝硬化时,CHI3L1的敏感度和特异度要高于AFP和GP73,提示CHI3L1是一个很好的肝脏纤维化血清学指标来区分CHB和肝硬化。在肝硬化和肝癌的ROC曲线中,AFP的特异度和敏感度要优于CHI3L1和GP73,提示AFP在肝癌的血清学诊断上有很重要的意义。这有可能是因为AFP是由肝细胞分泌的,而CHI3L1是由巨噬细胞、中性粒细胞、软骨细胞、平滑肌细胞产生,导致灵敏度和特异度都不及AFP。

为了探讨CHI3L1,GP73和AFP在不同肝脏疾病中的相关性,本研究做了相关性分析,结果显示CHI3L1与GP73,AFP呈显著正相关,进一步说明血清CHI3L1的水平变化与肝纤维化、肝癌的程度密切相关,可将其作为肝硬化疾病进展的独立监测指标。

综上所述,血清CHI3L1在肝脏纤维化指标的诊断中,其灵敏度与特异度均优于GP73和AFP,可以作为肝脏纤维化的一种无创观察指标,并且在肝细胞癌的诊断中也有一定的辅助价值。

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万方数据

Comparison of chitinase-3-like protein 1, aspartate aminotransferase-to-platelet ratio index, and fibrosis-4 index with shear-wave elastography

Saadiya Mushtaq[a], Eijaz Ghani[a], Khalid Azam[b] and Tabinda Hussain[a]

Background In the past, there has been an exponential increase in the potential biomarkers that can be used for staging of liver fibrosis. In light of intraobserver and intralobular variations, criticism has been directed at liver biopsy, and its efficacy has been challenged. Shear-wave elastography (SWE) has become a routine method for pre-assessment of liver fibrosis. Serum markers such as chitinase-3-like protein 1 (CHI3L1) also known as YKL-40, aspartate aminotransferase-to-platelet ratio index, and fibrosis-4 (Fib-4) index have been researched as potential alternates to detect liver fibrosis.

Study A total of 150 enrolled patients with chronic hepatitis underwent serum analysis to estimate CHI3L1 or YKL-40 level, aspartate aminotransferase-to-platelet ratio index, and Fib-4 index. These patients also underwent SWE.

Results The distribution of fibrosis grade according to SWE was F0: 46 patients, F1: 31 patients, F2: 16 patients, F3: four patients, and F4: 53 patients. Receiver operating characteristic curve analysis for F0–F1 versus F2–F3, F0–F1 versus F4, and F2–F3 versus F4 gave area under curve values of 0.56 ($P > 0.05$), 0.76 ($P < 0.01$), and 0.75, respectively ($P < 0.01$) for aspartate aminotransferase-to-platelet ratio index; of 0.65 ($P < 0.05$), 0.78 ($P < 0.01$), and 0.7, respectively ($P < 0.05$) for Fib-4 index; and 0.98, 0.99, and 0.95, respectively ($P < 0.01$ for all) for CHI3L1.

Conclusion CHI3L1 could be used as a preliminary tool to assess mild/absent fibrosis from significant fibrosis and cirrhosis. Eur J Gastroenterol Hepatol 00:000–000

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Introduction

Fibrosis is one of the best predictors of management and outcome in chronic liver diseases. Liver fibrosis is a common clinical manifestation in response to liver injury caused by viral infections of hepatitis B virus (HBV) and hepatitis C virus (HCV). Fibrosis can also occur as a result of alcoholic steatohepatitis or nonalcoholic steatohepatitis.

Fibrosis in the liver is characterized by increased accumulation of extracellular matrix proteins (ECM) as a result of persistent liver injury. During fibrogenesis, hepatocytes are replaced by ECM proteins including collagen, fibronectin, and others. The deposition of fibrotic tissue also varies with the site of liver injury [1].

For a long time, liver biopsy has been considered the 'gold standard' for determining the presence and subsequently the stage of fibrosis. A crucial drawback of setting biopsy as the gold standard is the inconsistency between observer judgments [2,3]. In addition to this, sampling size variabilities and intra-lobular variation in fibrosis grade can also affect the diagnosis [4]. Moreover, there are chances of sampling error when just 1/50 000 of the total liver is taken under consideration [5]. A comparison of various surrogate methods to assess the stage of fibrosis with liver biopsy has suggested that biopsy may be the 'best' available standard but certainly not the gold standard, and there is need to research and standardize the use of nonbiopsy related markers [6–11].

There are various scoring systems for estimating the stage of fibrosis based on liver biopsies which have been replicated for use in noninvasive methods as well. METAVIR scoring system is a routinely used method that categorizes fibrosis between F0 and F4 stages [12], where F0 signifies no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. One such noninvasive technique is shear-wave elastrography (SWE), which uses radiations from focused ultrasound beams to estimate liver stiffness [13]. SWE has shown good agreement with liver biopsy, with area under curve (AUC) ranging between 0.84 and 0.87 in one study [14] and as high as 0.97 for assessment of different stages of fibrosis assigned by liver biopsy in another study [15].

Fibrosis is a reversible disease, and timely diagnosis can prevent progression to cirrhosis or liver failure. The benefit of noninvasive markers for diagnosing fibrosis has been established in various studies, and their use in differentiating between cirrhosis and mild fibrosis has also been proven. In this study, we aimed to understand how well different serum makers such as chitinase-3-like protein 1 (CHI3L1), aspartate-to-platelet ratio index (APRI), and fibrosis-4 index (Fib-4) performed against SWE as a reference method.

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[a]Department of Virology, Armed Forces Institute of Pathology and [b]Department of Otorhinolaryngology, Combined Military Hospital, Rawalpindi, Pakistan

Correspondence to Saadiya Mushtaq, MBBS, FCPS, Department of Virology, Armed Forces Institute of Pathology, Rawalpindi 54810, Pakistan

Tel: +92 333 536 3239; fax: +92 042 9950 5553;
e-mails: saadiyakhalid2008@gmail.com, sajukhagu@hotmail.com

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Patients and methods

Study population

Patients with chronic HCV and HBV were enrolled in the study. The sample included 128 (85.4%) HCV-positive cases and 21 (14%) were HBV positive, whereas the infection status of one patient could not be confirmed. The study was approved by Internal Review Board of the institution, and all patients gave informed consent for participating in this study.

Patient data such as age, sex, and previous history of disease were collected. A blood sample was drawn from each patient, which was then analyzed using standard diagnostic protocols to determine prothrombin time, alanine aminotransferase (ALT), aspartate aminotransferase (AST) (U/l), platelet count ($\times 10^9$/l), albumin (g/l), bilirubin (μmol/l), and CHI3L1 protein (ng/ml) levels.

Shear-wave elastography

After collection of blood samples, each patient underwent SWE on APLIO 500 system (Devon Medical Equipment Ltd, South West, UK). The respective values of liver stiffness were recorded in kilopascals, and METAVIR fibrosis grade corresponding to the SWE result was assigned, where F0 corresponded to no fibrosis, F1 to portal fibrosis without bridges/septa, F2 to portal fibrosis with rare bridges/septa, F3 to numerous bridges/septa without cirrhosis, and F4 stage signified cirrhosis. The reference ranges predefined by the radiologist for fibrosis grade interpretation were used, where less than 8 kPa = F0, 8–9 kPa = F1, 9–15 kPa = F2, 16–26 kPa = F3, and more than 26 kPa = F4.

Chitinase-3-like protein 1 protein immunoassay

CHI3L1 ELISA Kit manufactured by Proprium Biotech Co. (Hangzhou, China) was used to assess the levels of CHI3L1 (YKL-40) in the serum. Standard manufacturer's protocol was followed to perform ELISA, and the respective concentrations of CHI3L1 protein in the serum were recorded in ng/ml. Serum CHI3L1 protein levels of 11 healthy controls were also assessed, where a mean value of 42 ± 16 was observed and the levels ranged between 32 and 75 ng/ml. According to the manufacturer's instructions, CHI3L1 levels less than 79 ng/ml signified no significant fibrosis, between 79 and 177 ng/ml meant significant fibrosis, and more than 177 ng/ml implied cirrhosis.

Aspartate-to-platelet ratio index and fibrosis-4 index

APRI was calculated using the methodology by Lin and colleagues [5,16], whereas Fib-4 index was calculated using the model by Sterling *et al.* [17]. The relation used to compute each value was as follows:

$$\text{APRI} = \frac{\text{AST}/40}{\text{Platelets}} \times 100 \rightarrow \text{Fib4} = \frac{\text{Age} \times \text{AST}}{\text{Platelets} \times \sqrt{\text{ALT}}}.$$

Statistical analysis

All statistical tests were carried out in SPSS version 24 (IBM Corp., Armonk, New York, USA) and MedCalc version 17.4 (MedCalc, Ostend, Belgium). After descriptive analysis, one-way analysis of variance was used for parametric data and Kruskal–Wallis test was used for nonparametric data. Correlation of AST, ALT, platelet count, APRI, Fib-4, and CHI3L1 protein levels was calculated using Spearman's method. Inter-rater agreement κ was computed using Cohen's method. Receiver operating characteristic (ROC) curve analysis was used to compare diagnostic accuracy of APRI, Fib-4, and CHI3L1 protein against fibrosis grades assigned by SWE.

Results

Study population

The mean age of our study population was 48.6 ± 12.6 years. There were 95 (63.3%) male and 55 (36.7%) female patients. Although the HBV-positive group was small (21 HBV vs. 128 HCV), when CHI3L1 protein levels were compared against viral infection status, there was no significant difference in the mean values of ALT, AST, platelet count, and CHI3L1 protein levels between HBV-infected and HCV-infected patients. The mean value of each parameter is given in Table 1.

Aspartate aminotransferase, albumin, chitinase-3-like protein 1 protein levels, and platelet counts vary with fibrosis grade

According to SWE results, 46 patients had no fibrosis (F0), 31 had mild fibrosis (F1), 16 were graded as F2, four patients had F3 fibrosis, and 53 patients had cirrhosis (F4).

When variation between fibrosis grade versus ALT and bilirubin values was tested, their levels did not differ significantly between different stages of fibrosis. On the contrary, when AST, albumin, and CHI3L1 protein levels were compared against fibrosis grade, P value of less than 0.001 was obtained, whereas platelet count varied significantly, with P value of less than 0.05. These results implied significant variation of AST, albumin, CHI3L1 protein levels, and platelets with respect to fibrosis stage.

Chitinase-3-like protein 1 protein levels correlate well with fibrosis grade

For each parameter, Spearman's correlation with the METAVIR grade was computed. The correlation coefficient ρ between fibrosis grade and CHI3L1 protein level was 0.9, with a P value less than 0.01. Fib-4 and APRI returned a ρ value of 0.5 and 0.45, with P value less than 0.05. The distribution of APRI, Fib-4, and CHI3L1 protein levels with fibrosis grades is depicted in Figs 1–3.

Table 1. Basic history and biochemical features of study population

Parameters	Mean	Valid (*N*)
Age	48.6 ± 12.6	150
ALT	50.6 ± 43.9	148
Albumin	39.1 ± 6.3	148
AST	39.1 ± 37	146
CHI3L1	211 ± 235	150
Platelet count	192.3 ± 87.5	148
Bilirubin	30.8 ± 69.5	147
Prothrombin time	15.5 ± 3.8	149

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHI3L1, chitinase-3-like protein 1.

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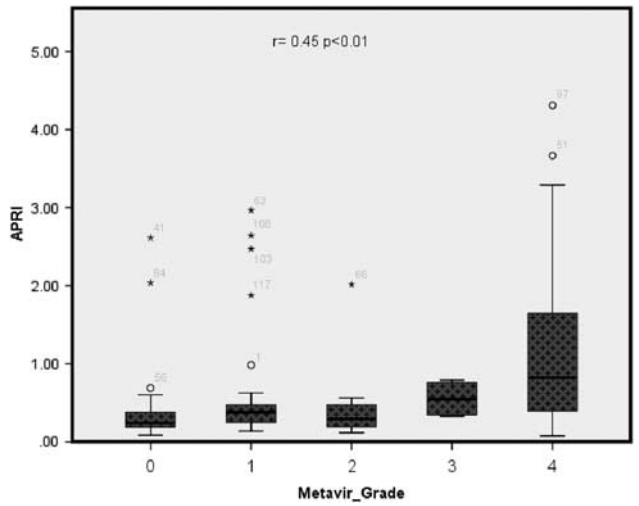

Fig. 1. Boxplot of aspartate-to-platelet ratio index (APRI) against fibrosis grade. Spearman's correlation ρ is 0.45, with *P* value less than 0.05. The boxes carries 50% of the data; horizontal bars indicate median value, and T-bars indicate minimum and maximum ranges. *Extreme outliers, °near outliers.

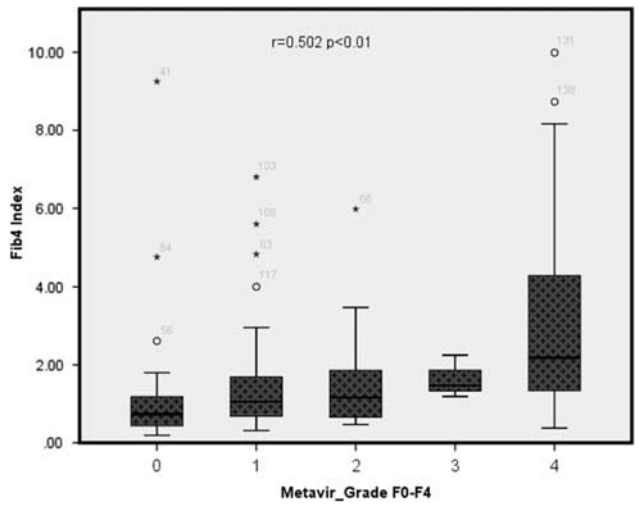

Fig. 2. Boxplot of fibrosis-4 (Fib-4) index against fibrosis grade. Spearman's coefficient ρ is 0.502, with *P* value less than 0.01. The boxes carries 50% of the data; horizontal bars indicate median value, and T-bars indicate minimum and maximum ranges. *Extreme outliers, °near outliers.

Diagnostic accuracy of chitinase-3-like protein 1 protein was greater than fibrosis-4 and aspartate-to-platelet ratio index

For the purposes of correlating the values of APRI, Fib-4, and CHI3L1 protein assay against SWE, the results of SWE were grouped into three categories. The first being absent to mild fibrosis (F0, F1) versus significant fibrosis (F2, F3). The second group was absent to mild fibrosis (F0, F1) versus cirrhosis (F4), and in the third category, SWE results were categorized as significant fibrosis (F2, F3) versus cirrhosis (F4). These values were then compared against CHI3L1 protein levels, APRI, and Fib-4 to see how well each test could differentiate between the two diagnostic states of each group. The results of all ROC curves are summarized in Table 2.

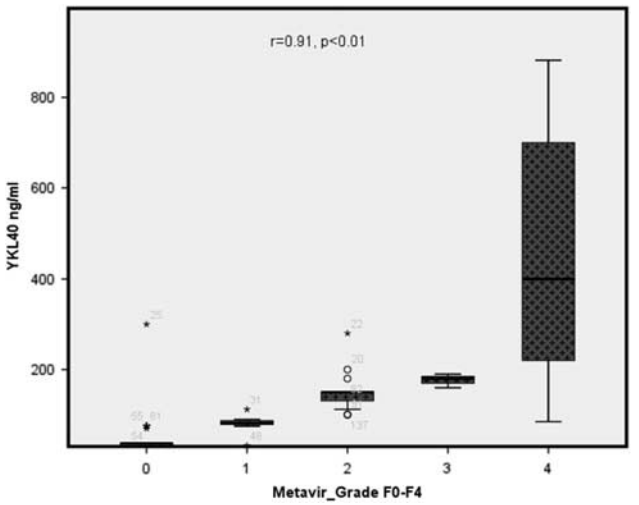

Fig. 3. Boxplot of chitinase-3-like protein 1 (CHI3L1) (YKL-40) against fibrosis grade. Spearman's coefficient ρ is 0.91, with *P* value less than 0.05. The boxes carries 50% of the data; horizontal bars indicate median value, and T-bars indicate minimum and maximum ranges. *Extreme outliers, °near outliers.

(1) ROC curve for differentiating between F0–F1 and F2–F3: In this group, the prevalence of positive disease state (significant fibrosis) was ~20%. CHI3L1 protein had the largest value of AUC that signified greater specificity and sensitivity in differentiating between absent to mild fibrosis from significant fibrosis (excludes cirrhosis).
(2) ROC curve for differentiating between F0–F1 and F4: The sensitivity and specificity for the detection of mild to absent fibrosis from cirrhosis are given in the Table 1. In this group, the prevalence of positive disease state (cirrhosis) was ~40%. The AUC of CHI3L1 protein against diseased and nondiseased state was 0.99, whereas APRI and Fib-4 had area under the curve of 0.76 and 0.78, respectively.
(3) Receiver operating characteristic curve for differentiating between F2–F3 and F4: In this category, the prevalence of positive disease state, that is, cirrhosis, was ~76%. The sensitivity and specificity for diagnosing significant fibrosis and cirrhosis are given in the Table 1. The AUC of CHI3L1 protein against diseased and nondiseased state was 0.95, whereas APRI and Fib-4 has area under the curve of 0.75 and 0.7, respectively.

Chitinase-3-like protein 1 protein levels and shear-wave elastography fibrosis grades agree with each other

According to the manufacturer's protocol, the CHI3L1 levels cannot differentiate between F0 versus F1 and F2 versus F3. Therefore, we predicted new cutoff ranges for differentiating between different fibrosis grades. Based on optimal cutoff obtained from ROC curve analysis, CHI3L1 protein levels below or equal to 75 were assigned F0 stage, whereas levels ranging from 76 to 90 were taken as F1 stage. As there were only a few samples that corresponded to the F3 grade, CHI3L1 protein levels ranging from 91 to 190 signified either F2 or F3 stage. CHI3L1 protein serum levels of at least 191 were assigned F4 grade.

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Table 2. Diagnostic accuracy of aspartate-to-platelet ratio index, fibrosis-4, and chitinase-3-like protein 1 in differentiating between F0–F1 versus F2–F3 versus F4 stage of fibrosis

Metavir stage	Marker	AUC	Optimal cutoff	Sensitivity	Specificity	PPV (%)	NPV (%)
F0–F1 vs. F2–F3	APRI	0.56#	> 0.258	75	47.95	27.7	87.82
	Fib-4	0.65*	> 0.786	73.68	53.4	29	88.66
	CHI3L1	0.98**	> 90	100	97.4	90.89	100
F0–F1 vs. F4	APRI	0.76**	> 0.486	70.6	82.19	73.26	80
	Fib-4	0.78**	> 1.26	80.39	71.2	65.98	83.9
	CHI3L1	0.99**	> 112	98.1	98.7	98.11	98.69
F2–F3 vs. F4	APRI	0.75**	> 0.56	66.0	88.2	94.37	46.37
	Fib-4	0.7*	> 1.257	81.1	56.2	85.9	47.3
	CHI3L1	0.95**	> 180	94.5	88.2	96.28	83.2

Optimal cutoff corresponds to the sensitivity and specificity values calculated with Youden index.
APRI, aspartate-to-platelet ratio index; AUC, area under characteristic curve; CHI3L1, chitinase-3-like protein 1; Fib-4, fibrosis-4; NPV, negative predictive value; PPV, positive predictive value.
#$P > 0.05$, insignificant outcome.
*$P < 0.05$, significant outcome.
**$P < 0.01$, significant outcome.

Cohen's κ can compute agreement/reliability between two observations that have continuous values; therefore, the stage of fibrosis from SWE estimation was compared against fibrosis stage calculated according to the above cutoffs for CHI3L1 levels. The inter-rater agreement between the two observations was $\kappa = 0.88$ ($P < 0.01$), if F2 and F3 stage of SWE estimated fibrosis was pooled as one group, whereas $\kappa = 0.72$ ($P < 0.01$), when F2 and F3 were treated as different groups. κ Values above 0.8 signify excellent agreement between the fibrosis stages determined by CHI3L1 protein levels against the fibrosis stages estimated by SWE, whereas between 0.6 and 0.8 are interpreted as good agreement.

Discussion

SWE is considered the best alternate to biopsy by most clinicians. In a recently published meta-analysis, SWE agreed very well with liver biopsy results. The pooled sensitivity and specificity of SWE ranged between 85–90 and 81–88%, respectively, for differentiating between different stages of fibrosis [18]. Therefore, we chose SWE as reference method and assessed the performance of different markers of fibrosis such as CHI3L1 protein or YKL-40, APRI, and Fib-4 index against it. Our results showed that serum CHI3L1 protein levels correlated most closely with the assessment made through SWE. CHI3L1 protein increased progressively with advancing stage of fibrosis, and there were very few outliers in each stage as shown in Fig. 3.

With respect to diagnostic accuracy, CHI3L1 had a mean AUC of 0.97 for differentiating between different stages of fibrosis. The highest sensitivity was 100% for differentiating between mild/absent fibrosis and significant fibrosis, and highest specificity of 98.7% for differentiating between mild/absent fibrosis and cirrhotic liver. Moreover, there was very good inter-rater agreement between CHI3L1 and SWE (Fig. 4).

Fib-4 index was the second to best at differentiating between fibrosis grades with a mean AUC of 0.7. The index had the highest sensitivity and specificity values of 80.4 and 70.2, respectively, for F0, F1 versus F4 ROC curve (Fig. 5).

The performance of APRI against SWE was not as good as CHI3L1 or Fib-4 in our study. However, the index has been previously performed, with AUC ranging between 0.77 and 0.83 for different stages of fibrosis [16] and achieved AUC between 0.8 and 0.9 in another study [5]. In an analysis of several serum markers in a group of 1252 HCV-infected patients, simple platelet count was observed as the most predictive for evaluation of fibrosis. Both APRI and Fib-4 use platelet count in determining the index value [19]. The use of these two indices in predicting liver-related mortality was also confirmed in a group of HCV and HIV-infected individuals [9].

There have been several studies that have compared the performance of CHI3L1 levels against fibrosis graded liver biopsies. In a recently published analysis that compared CHI3L1 levels with fibrosis in NAFLD, AUC of 0.76 for diagnosing advanced fibrosis was achieved [20]. A previous study compared digital quantification of fibrosis and several serum markers. Their analysis found lower AUC of CHI3L1 levels and higher rate of false positives compared with hyaluronic acid. However, Mehta *et al.* ([11]) concluded that CHI3L1 was effective at differentiating advanced fibrosis and cirrhosis from earlier stages of fibrosis.

The cellular function of CHI3L1 protein has been implicated as a growth factor [21], and its role in remodeling of ECM has also been suggested [22]. Recent research has suggested that this glycoprotein can promote angiogenesis by activating the mitogen-activated protein kinase/extracellular signal regulated kinase pathway, which is essential for cell proliferation [23,24].

Some researchers have also observed CHI3L1 role as a prognostic factor in determining disease outcome. Elevated serum levels of CHI3L1 protein have been reported as highly predictive of survival rate and disease progression in patients with chronic HCV [25,26]. The first study to correlate CHI3L1 levels with increasing grade of fibrosis was conducted by Nojgaard *et al.* [26] where they found that in case of normal liver histology, CHI3L1 had a mean value of 102 ng/ml. There is wide variation between the threshold values of CHI3L1 protein or YKL-40, which has been recommended in different studies [27]. In our study, we proposed that CHI3L1 protein levels less than 75 ng/ml, between 76 and 90 ng/ml, between 91 and 190 ng/ml, and more than 190 ng/ml were able to predict F0, F1, F2–F3, and F4 stage, respectively. Although markers such as Fib-4 and APRI have a standard range for interpretation of fibrosis stage, CHI3L1 protein serum levels are yet to be standardized to get cutoff values for each stage of fibrosis.

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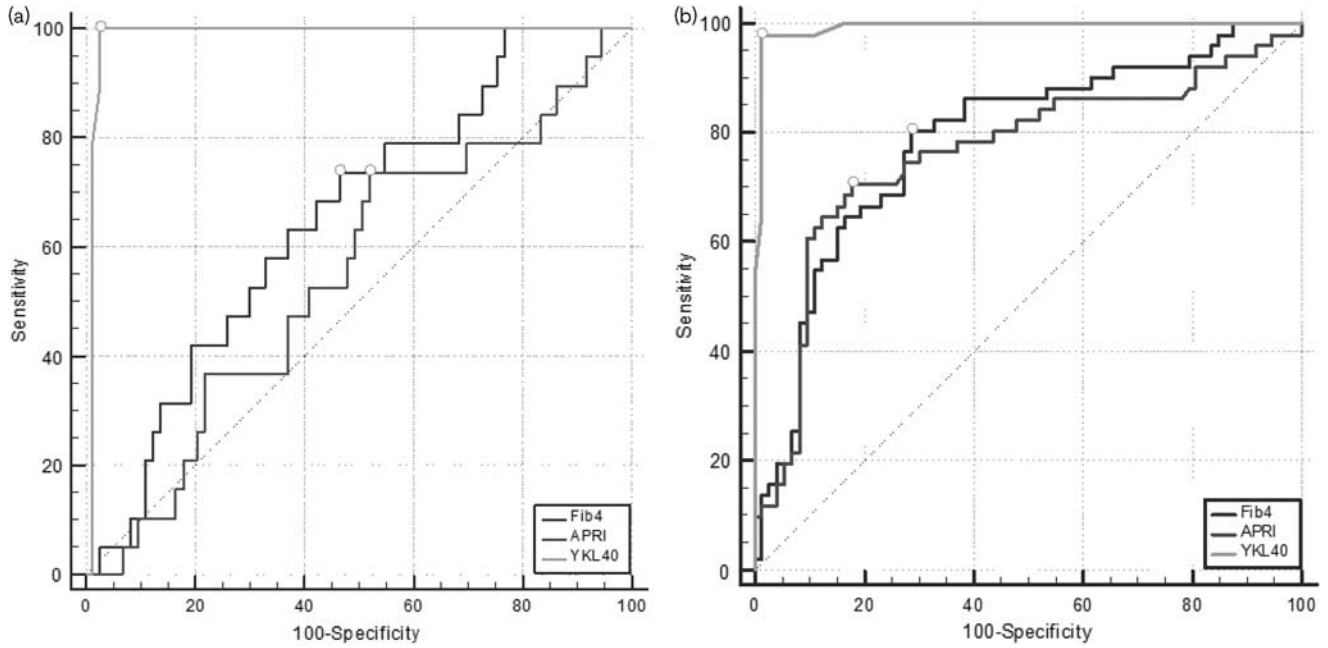

Fig. 4. (a) Receiver operator characteristic (ROC) curves outlining relationship between sensitivity (true positives) and 100-specificity (false positives) for three markers: aspartate-to-platelet ratio index (APRI), fibrosis-4 (Fib-4), and chitinase-3-like protein 1 (CHI3L1) (YKL-40), and shear-wave elastography. The area under curve indicates how well each marker can differentiate between mild/absent (F0–F1) fibrosis versus significant fibrosis (F2–F3). (b) ROC curves outlining relationship between sensitivity (true positives) and 100-specificity (false positives) for three markers: APRI, Fib-4, and CHI3L1 (YKL-40), and shear-wave elastography. The area under curve indicates how well each marker can differentiate between mild/absent (F0–F1) fibrosis versus significant fibrosis (F4).

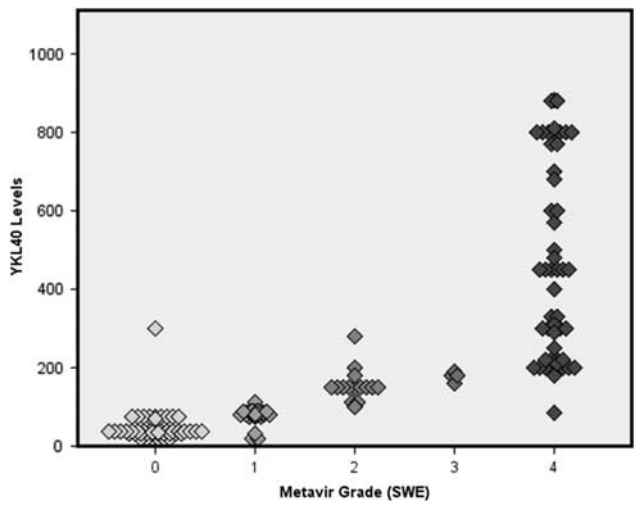

Fig. 5. Scatter-plot of chitinase-3-like protein 1 (YKL-40) for all samples against fibrosis grade. SWE, shear-wave elastography.

The benefit of using noninvasive serum markers to assess the stage of fibrosis is partly in ruling out advanced stages of fibrosis. Serum markers such as Fib-4 and CHI3L1 protein can identify liver fibrosis irrespective of the limitations associated with SWE, such as its inability to perform well in obese patients. Over-weight individuals have greater propensity to develop liver diseases [28,29]; therefore, a technique that makes clinical judgment harder in these cases compromises its efficiency.

The cellular source of CHI3L1 protein is thought to be hepatic stellate cells; more recent evidence suggests that this protein could have inducible expression in case of inflammatory signals. Macrophages, airway epithelial cells, and carcinoma tissue have all been observed to secrete CHI3L1 protein under various conditions [20,30,31]. There has also been some discussion on the association of CHI3L1 promoter polymorphism (G131→C) with the severity of fibrosis and other inflammatory diseases. However, the results have been inconclusive as there has been no consistent observation regarding this polymorphism's correlation with fibrosis severity and CHI3L1 serum levels [32–36].

The limitation of the current study was that we were unable to compare noninvasive biomarkers with the 'gold-standard' liver biopsy. However, each method that has been used for grading of fibrosis has its pitfalls, including the liver biopsy. In the analysis by Regev *et al*. [2], it was found that cirrhosis was misdiagnosed in 15% of the cases. Moreover, more than 30% of the patients had a different diagnosis for the left and right lobe of the liver. The study could have been improved by following up CHI3L1 protein levels and Fib-4 levels with progression and remission of disease in individual cases. A future study will be planned to cover these limitations as well.

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Conflicts of interest

There are no conflicts of interest.

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比较 CHI3L1、APRI、FIB-4 与剪切波弹性成像

Saadiya Mushtaq[a], Eijaz Ghania, Khalid Azam[b] and Tabinda Hussain

[a]Department of Virology, Armed Forces Institute of Pathology and [b]Department of Otorhinolaryngology, Combined Military Hospital, Rawalpindi, Pakistan

摘要：

背景：过去，用于肝纤维化分期的潜在生物标志物呈指数增长。根据观察者自身偏差和小叶内的变化，对肝活检提出了批评，其有效性也受到了挑战。剪切波弹性成像（SWE）已成为肝纤维化预评估的常规方法。血清标志物如壳多糖酶-3-样蛋白 1（CHI3L1）也被称为 YKL-40、天冬氨酸氨基转移酶-血小板比率指数（APRI）和纤维化-4（Fib-4）指数已被研究认为是检测肝纤维化的潜在替代物。

研究：我们对 150 例慢性肝炎患者进行了血清学分析，评估患者血清中 CHI3L1（YKL-40）水平、天冬氨酸氨基转移酶-血小板比值指数（APRI）和 Fib-4 指数，同时这些患者也进行了剪切波弹性成像（SWE）检测。

结果：根据 SWE 检测进行了纤维化分级：其中 F0：46 例、F1：31 例、F2：16 例、F3：4 例、F4：53 例。我们对 F0-F1 与 F2-F3、F0-F1 与 F4、F2-F3 与 F4 的受试者工作特性曲线进行了分析，APRI 的曲线下面积分别为 0.56 ($P > 0.05$)、0.76 ($P < 0.01$)、0.75 ($P < 0.01$)；Fib-4 指数的曲线下面积分别为 0.65 ($P < 0.05$)、0.78 ($P < 0.01$)、0.7 ($P < 0.05$)；CHI3L1 的曲线下面积分别为 0.98($P < 0.01$)、0.99($P < 0.01$)、0.95 ($P < 0.01$)。

结论：CHI3L1 可以作为评估轻度/无纤维化与明显纤维化和肝硬化的初步工具。