LIVER FIBROSIS IN CHRONIC HEPATITIS B – A STUDY OF THE NATURAL HISTORY USING TRANSIENT ELASTOGRAPHY

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Dedicated to

Byron & Faith

DECLARATION OF ORIGINALITY

The work contained in this thesis is completely original and has not been submitted for any other degrees. The studies were performed in Prince of Wales Hospital, Hong Kong, under the supervision and guidance of Prof. Henry LY Chan, Professor, Department of Medicine and Therapeutics, The Chinese University of Hong Kong. I was responsible for the study design, patient recruitment, liver biopsy, transient elastography, morphometry, data entry and analysis, and manuscript preparation.

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PRECIS

Background

Chronic hepatitis B (CHB) is a global health problem, which currently affects about 400 million people. It is estimated that worldwide over 200,000 and 300,000 CHB patients die annually from complications of liver cirrhosis and hepatocellular carcinoma, respectively. Liver fibrosis is the byproduct of persistent necro-inflammation and the intermediate step to liver cirrhosis. Patients who have advanced liver fibrosis (bridging fibrosis) and liver cirrhosis have the highest risk of developing cirrhotic complications. Understanding the factors influencing the progression of liver fibrosis is of paramount importance on managing patients with CHB. Liver biopsy has been the only tool in the past to determine liver fibrosis but it is limited by its invasiveness.

Transient elastography is a new non-invasive method to assess the degree of liver stiffness, which reflects the severity of liver fibrosis. Based on the experience in chronic hepatitis C patients in Europe, liver stiffness measurement (LSM) has high sensitivity and specificity to detect advanced liver fibrosis. Before LSM can be widely used in clinical practice among Asian CHB patients, validation of its performance is warranted. Nonetheless, it is a potentially useful investigation to study the natural history of liver fibrosis, particularly among patients who have no clinical indication for liver biopsy.

Aims

1. To validate the accuracy of transient elastography to detect advanced liver fibrosis in CHB.
2. To study the optimal LSM cutoff values for different stages of liver fibrosis in CHB.
3. To study the clinical predictive factors of liver cirrhosis in CHB.
4. To study the effect of metabolic syndrome on liver cirrhosis in CHB.

Hypothesis

1. Transient elastography can accurately diagnose advanced liver fibrosis.
2. Different cutoff values of LSM can be defined for different stages of liver fibrosis.
3. The risk of liver cirrhosis increases with higher ALT and hepatitis B virus (HBV) DNA levels.
4. Metabolic syndrome is an independent risk factor of liver cirrhosis in CHB.

Investigation Methods

1. Serological assays

Hepatitis B surface antigen (HBsAg) was tested by commercially available enzyme-linked immunosorbant assay kits (Abbott GmBH Diagnostika, Wiesbaden-Delkenheim, Germany). Hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) were measured by enzyme-linked immunosorbant assay (Sanofi Diagnostics, Pasteur, France).

2. HBV DNA assay

Serum HBV DNA level was quantified by TaqMan real-time polymerase chain reaction using Eurohep standard to set the standard curve. The range of HBV DNA detection was from 10^2 to 10^9 copies/ml.

3. Histology

Liver fibrosis and necroinflammatory activity were evaluated semi-quantitatively according to the Metavir scoring system.

4. Morphometry

Morphometric analysis was used for the quantitative assessment of fibrosis in the liver biopsy using a computerized image analysis system and the Bioquant Nova Prime software (Bioquant Image Analysis Corporation, Nashville, TN, USA).

5. Transient elastography

LSM was performed using transient elastography according to the instructions and training provided by the manufacturer. The liver stiffness was expressed in kiloPascal (kPa). A higher kPa reflected a stiffer liver and more severe liver fibrosis.

Statistical Analysis

Statistical analysis was performed by Statistical Package for Social Science (SPSS version 11.5, Chicago, IL, USA). Qualitative and quantitative differences between subgroups were analyzed using Chi-square or Fisher's exact test for categorical parameters as appropriate and Mann–Whitney test for continuous parameters. Spearman's rank correlation coefficient was used to analyze the correlations between morphometric scores and the histologic (Metavir and Brunt) fibrosis scores. Pearson's correlation coefficient was used to test the correlations between morphometric scores and LSM. The overall accuracy of LSM in diagnosing histologic bridging fibrosis and cirrhosis was calculated using the receiver of characteristic (ROC) curve and its 95% confident interval (CI). All statistical tests were two-sided. Statistical significance was taken as $P<0.05$.

Research Models and Results

1. Validation of transient elastography

Consecutive patients with chronic liver diseases undergoing liver biopsy and transient elastography examinations were recruited. Morphometric analysis was performed to evaluate the distribution of liver fibrosis. One hundred thirty-three patients were studied (Study 1, Clin Gastroenterol Hepatol 2008;6:1027-1035). Morphometric analysis revealed a higher correlation between LSM and pericellular fibrosis (r=0.43) than periportal (r=0.21) or perivenular fibrosis (r=0.25). Area under ROC curves of LSM for bridging fibrosis was 0.87 (95% CI: 0.81–0.93) and for cirrhosis was 0.89 (95% CI 0.83–0.94). Higher LSM was associated with higher serum ALT level. Patients with the same fibrosis staging but higher ALT levels tend to have higher LSM. The area under ROC curve of LSM for cirrhosis was lower among patients who had ALT higher than upper limit of normal (ULN) (0.86) as compared to that of patients with normal ALT levels (0.93, P=0.03). In conclusion, transient elastography can accurately diagnose advanced fibrosis because of its good correlation with pericellular fibrosis. Transient elastography may over-estimate liver fibrosis when ALT is elevated.

The LSM cutoff values for different stages of liver fibrosis in CHB and the effect of ALT on LSM was investigated in Study 2 (J Viral Hepat 2009;16:36-44). Consecutive CHB patients undergoing liver biopsy and transient elastography examinations were prospectively recruited. Diagnostic

performance of LSM for different degrees of liver fibrosis was evaluated. One hundred sixty-one CHB patients with adequate liver biopsy sample sizes were studied. Area under ROC curves of LSM for no fibrosis (F0 *vs.* F1–4), bridging fibrosis (F0–2 *vs.* F3–4) and liver cirrhosis (F0–3 *vs.* F4) was 0.80 (95% CI 0.68–0.92), 0.87 (95% CI 0.82–0.93) and 0.93 (95% CI 0.89–0.97), respectively. Patients with the same fibrosis staging but higher ALT levels tend to have higher LSM, and the diagnostic performance for low stage fibrosis was most seriously affected when ALT was elevated. Different LSM cutoff values and algorithms were derived for normal and elevated ALT levels. Insignificant fibrosis was defined as LSM ≤6.0 kPa for normal ALT and ≤7.5 kPa for ALT >1-5x ULN; whereas advanced fibrosis as LSM >9.0 kPa for normal ALT and >12.0 kPa for ALT>1-5x ULN, respectively. Based on these algorithms, liver biopsy can be avoided in 62% and 58% of patients with normal and elevated ALT, respectively. In conclusions, transient elastography may perform differently in patients with different ALT levels, and different cutoff values are warranted for patients with normal and elevated ALT levels. Transient elastography is a reasonable noninvasive tool to substitute liver biopsy among the lowest and highest risk patients for the assessment of liver fibrosis.

2. Factors associated with advanced liver fibrosis in hepatitis B e antigen-positive chronic hepatitis B

Four hundred fifty-three treatment-naïve HBeAg-positive patients were prospectively recruited for LSM by transient elastography (Study 3, Clin Gastroenterol Hepatol 2009;7:227-233). The main aim of this study was to define the immune tolerance, which should be associated with insignificant liver fibrosis and did not require any anti-viral treatment. Insignificant fibrosis was defined as LSM ≤6.0 kPa for normal ALT and ≤7.5 kPa for ALT >1-5x ULN; whereas advanced fibrosis as LSM >9.0 kPa for normal ALT and >12.0 kPa for ALT>1-5x ULN, respectively based on the results of the previous study with histologic validation. Patients with ALT>5x ULN were excluded from analysis due to unreliable LSM. Among the 74 patients who also had liver biopsy, the cutoff values for advanced fibrosis had 95% specificity. Patient age and ALT level, but not HBV DNA level, were the independent factors associated with liver fibrosis severities by LSM. Based on ROC curve analysis, age above 35 had the highest specificity for advanced fibrosis. The risk of advanced fibrosis started to increase when ALT was >0.5x ULN. Among the 47 patients who were aged ≤35 years and ALT ≤0.5x ULN, 39 patients (83%) had LSM indicative of insignificant fibrosis and only one patient (2%) had advanced fibrosis. Among the 217 patients who were aged >35 years with ALT >0.5x ULN, 61 (28%) had LSM indicative of insignificant fibrosis and 80 (37%) had advanced fibrosis. In conclusion, the risk of advanced liver fibrosis increased in HBeAg-positive patients older than 35 with ALT >0.5x ULN. Patients who

were younger than 35 year old with ALT ≤0.5x ULN were probably in immune tolerance.

3. Factors associated with liver cirrhosis in hepatitis B e antigen-negative chronic hepatitis B

One thousand one hundred and seventy-four treatment-naïve HBeAg-negative patients were prospectively recruited for LSM by transient elastography (Study 4, Am J Gastroenterol 2008;103:3071–3081). The main aim of this study was to define factors associated with liver cirrhosis in these patients. Possible and probable cirrhosis was defined according to ALT-based LSM algorithms derived in Study 2. In the subgroup of 100 patients with liver biopsy, the LSM algorithm of possible cirrhosis had a sensitivity of 84% to diagnose liver cirrhosis, and the LSM algorithm of probable cirrhosis had a specificity of 87% to diagnose liver cirrhosis. Possible and probable cirrhosis were present in 26% and 12% of the patients, respectively. The risk of cirrhosis was significantly increased when ALT level was >0.5x ULN or serum HBV DNA >10,000 copies/ml. Among patients who have ALT ≤0.5xULN and HBV DNA ≤10,000 copies/ml, 9% (26/283) and 2% (7/283) had possible and probable cirrhosis respectively, which were significantly lower when compared to 31% (279/891, $P<0.001$) and 15% (134/891, $P<0.001$) of those who had higher ALT and HBV DNA levels. In conclusions, liver cirrhosis was common among HBeAg-negative CHB patients. The risk of liver cirrhosis increased with higher ALT and HBV DNA levels. Patients with ALT levels >0.5x ULN and/or serum

HBV DNA >10,000 copies/ml have higher risk of cirrhosis and need further assessment for antiviral therapy.

4. Metabolic syndrome and liver cirrhosis in chronic hepatitis B

One thousand four hundred and thirty-five CHB patients from primary care and hospital clinics were studied on the relationship of metabolic syndrome and liver cirrhosis (Study 5, Gut 2009;58:111–117). Possible and probable cirrhosis was again defined according to ALT-based LSM algorithms derived in Study 2. 134 (9%) patients had adequate liver biopsy and histologic liver cirrhosis was present in 32/134 (24%) patients. One hundred and eighty-eight (13%) patients had metabolic syndrome. Histologic liver cirrhosis was more common among patients who had metabolic syndrome (38%) versus those who did not (11%, $P<0.001$). The specificity of probable cirrhosis on LSM for histologic cirrhosis was 90%. Probable cirrhosis was present in 201 (14%) patients. Metabolic syndrome was more prevalent in patients with probable cirrhosis (24%) than those without cirrhosis (11%, $P<0.001$). After adjustment for anthropometric, biochemical and virologic factors, metabolic syndrome remained an independent factor associated with probable cirrhosis (odds ratio 1.7, 95% confidence interval [CI] 1.1–2.6). The odds ratios of probable cirrhosis were 1.3 (95% CI 0.9–2.1), 2.4 (95% CI 1.4–4.0), 3.9 (95% CI 2.1–6.7), 4.1 (95% CI 1.9–8.4) and 5.2 (95% CI 1.6–14.2) in patients with 1, 2, 3, 4 and 5 components of metabolic syndrome, respectively. In conclusion, metabolic syndrome was an independent risk

factor of liver cirrhosis in CHB.

Conclusions

Results from these studies suggest that transient elastography is a reliable non-invasive tool to substitute liver biopsy to determine the presence of advanced liver fibrosis and early liver cirrhosis. It can diagnose advanced fibrosis because of its good correlation with pericellular fibrosis. Patients with the same fibrosis staging but higher ALT levels tend to have higher LSM, and the diagnostic performance for low stage fibrosis was most seriously affected when ALT was elevated. An algorithm composing of different LSM cutoff values for patients with normal and elevated ALT levels should be used. HBeAg-positive patients who are aged below 35 years with ALT equal or lower than half of the ULN usually have insignificant liver fibrosis and rarely have advanced liver fibrosis. These patients are probably in the immune tolerance phase. Liver cirrhosis is common among HBeAg-negative CHB patients, particularly among patients with ALT levels >0.5x ULN and/or serum HBV DNA >10,000 copies/ml. These high risk patients need further assessment for antiviral therapy. Metabolic syndrome is an independent risk factor of liver cirrhosis in CHB.

Captions for Tables

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Part I

LITERATURE REVIEW

CHAPTER ONE

NATURAL HISTORY OF CHRONIC HEPATITIS B IN ASIA

1.1 Epidemiology of Chronic Hepatitis B in Asia

Hepatitis B infection is a global health problem, which currently affects about 400 million people worldwide. It is estimated that over 200,000 and 300,000 chronic hepatitis B (CHB) patients die annually worldwide from liver cirrhosis and hepatocellular carcinoma (HCC) respectively [Chan et al., 2006; Perz et al., 2006]. In Hong Kong, it is the commonest cause of liver cirrhosis and HCC, accounting for 4.9% of all deaths in 2005 [Department of Health, 2005].

The prevalence of hepatitis B carriers varies from 0.1% to 20% in different areas [Alter et al., 1990]. In high prevalence areas such as Southeast Asia and sub-Saharan Africa, the carrier rate is 10% to 20%. A study performed in 1978-1979 involving 16,334 subjects in Hong Kong found that 43% and 10% of the local population had evidence of past infection and were in the carrier state, respectively [Yeoh et al., 1984]. In intermediate prevalence areas such as the Mediterranean countries, India, Japan and Singapore, the carrier rate ranges from 3% to 5%. The prevalence varies from 0.1% to 2% in low prevalence areas such as United States and Canada, Western Europe, Australia and New Zealand. The wide range in carrier rates in different parts of the world is largely related to differences in the age at infection, which is inversely related to the risk of chronicity (Table 1.1).

In high prevalence areas such as Hong Kong and China, perinatal route is the

major mode of transmission, which accounts for 40% to 50% of CHB [Stevens et al., 1975; Lok et al., 1987a]. The reason for the predominance of perinatal transmission among Asians can be partly explained by the difference in hepatitis B virus (HBV) genotypes. It was recently found that more than 50% of patients cleared hepatitis B e antigen (HBeAg) by age of 20 if they were infected with HBV of genotypes A, B, D, and F. On the other hand, 50% of patients infected with HBV of genotype C cleared HBeAg by the age of 48 [Livingston et al., 2007a]. As HBV genotype C is common among Asian CHB patients, significant proportions of CHB females remain HBeAg-positive with high viral load at childbearing age. This accounts for the high frequency of maternal-infant transmission in Asian countries [Livingston et al., 2007a]. In contrast, horizontal transmission, particularly in early childhood, accounts for most cases of CHB in intermediate prevalence areas. Horizontal transmission among children may result from close bodily contact leading to transfer of virus across minor skin or mucosal breaks. Unprotected sexual intercourse and intravenous drug use in adults are the major routes of spread in low prevalence areas.

The age at infection has significant effect on the rate of chronicity. The rate of chronicity is approximately 90% for perinatally acquired infection [Stevens et al., 1975], as compared to 20% to 50% for infections acquired in early childhood (age 1 to 5 years) [Beasley et al., 1982a; Coursaget et al., 1987], and less than 5% for adult-acquired infection [Tassopoulos et al., 1987].

Table 1.1 Epidemiology and modes of transmission of hepatitis B virus infection

Prevalence	High	Intermediate	Low
Carrier rate	≥8%	2-7%	≤1%
Geographic distribution	Southeast Asia China Pacific islands Sub- Saharan Africa Alaska (Eskimos)	Mediterranean basin Eastern Europe Central Asia Japan Latin and South America Middle East	United States Canada Western Europe Australia New Zealand
Predominant age at infection	Perinatal and early childhood	Early childhood	Adult
Predominant mode of infection	Maternal-infant Percutaneous	Percutaneous Sexual	Sexual Percutaneous

1.2 Natural History in Asian Patients

The natural course of CHB is determined by the interplay between virus replication and the host immune response. In the settings of recurrent episodes of hepatitis, the risk of fibrosis and various complications may be increased either directly or indirectly through immune-mediated injury. The outcome of CHB depends upon the severity of liver fibrosis at the time HBV replication is arrested.

Adult-acquired CHB generally consists of two phases: an early replicative phase with active liver disease and a late or low replicative phase with remission of liver disease (Figure 1.1) [Hoofnagle et al., 1981; Realdi et al., 1980]. In patients with perinatally acquired HBV infection, which is particularly common in Asian countries and areas including Hong Kong, there is an additional immune tolerance phase in which virus replication is not accompanied by active liver disease (Figure 1.2) [Lok, 1992]. The natural course of CHB in patients infected early in life can be divided into four phases: immune tolerance, immune clearance, low or non-replication, and reactivation phase [EASL 2009; Lok et al., 2007].

A history of acute hepatitis is elicited in only a small proportion of CHB patients. In low or intermediate prevalence areas, approximately 30% to 50% of patients with CHB have a past history of acute hepatitis; such a history is

lacking in the remaining patients in these areas and in the majority of patients in high prevalence areas including Hong Kong, in which CHB is predominantly perinatal infection.

1.2.1 Immune tolerance phase

In patients with perinatally acquired CHB, the initial immune tolerance phase is characterized by high levels of HBV replication. HBeAg and high levels of HBV DNA in serum are present, but these is no evidence of active liver disease as manifested by lack of symptoms, normal serum ALT level, normal liver or only minimal histological activity and scanty fibrosis on liver biopsy [Chang et al., 1988; Lok et al., 1988]. The lack of necroinflammation and liver fibrosis despite high levels of HBV replication is believed to be due to immune tolerance to the virus [Hsu et al., 1992]. The exact mechanisms by which this tolerance occurs are unknown. Animal models suggested that transplacental transfer of maternal HBeAg may induce specific unresponsiveness of T cells to HBeAg and hepatitis B core antigen (HBcAg), resulting in ineffective cytotoxic T cell lysis of infected hepatocytes [Milich et al., 1990]. Immune tolerance is believed to be the major reason for the poor response to interferon therapy in HBeAg-positive Asian patients who have normal serum ALT levels.

The immune tolerance phase usually lasts 10 to 30 years, during which there is a very low rate of spontaneous HBeAg clearance [Liaw et al., 1984a; Lok et al., 1987b]. On the other hand, this phase is generally short-lived or absent in

childhood or adult-acquired HBV infection. Studies involving HBV infected Chinese children have found HBeAg in as many as 90% below the age of 5, and up to 80% below the age of 20 [Lok et al., 1988; Liaw et al., 1984a]. The cumulative rate of spontaneous HBeAg clearance is estimated to be around 2% during the first three years and only 15% after 20 years of infection [Lok et al., 1987b; Chang et al., 1995]. Genotypes of HBV also play a role in the timing of HBeAg clearance. It was found that more than 50% of patients cleared HBeAg by age of 20 if infected with genotypes A, B, D, and F, while 50% of genotype C patients cleared HBeAg only at age of 48 [Livingston et al., 2007a]. The low rate of viral clearance in adolescence and early adulthood accounts for the high frequency of maternal-infant transmission in Asian countries, particularly because genotype C is common among Asian CHB patients and hence significant proportions of CHB patients remain HBeAg-positive at childbearing age [Livingston et al., 2007a].

1.2.2 Immune clearance phase

After a variable period of HBeAg positivity, depending on the age of acquiring the infection, immune tolerance to the virus is lost and the immune system mounts an attack on infected hepatocytes. This phase is characterized by fluctuating, but progressively decreasing HBV DNA levels, ALT elevation and necroinflammation in liver histology. Transition from immune tolerance to immune clearance phase usually occurs at the second and third decades in patients with perinatally acquired CHB. Patients who acquire CHB at late

childhood, adolescence or adulthood usually present in the immunoactive phase with HBeAg positive CHB with elevated serum ALT and moderate or severe necroinflammation with variable amounts of fibrosis on liver biopsy. During the immune clearance phase, spontaneous HBeAg clearance increases to an annual rate of 10% to 20% [Liaw et al., 1984a; Lok et al., 1987b]. A seroconversion rate of 70% during 10 years of follow-up was described in a population-based study of 1536 Alaskan natives who acquired HBV as adults [McMahon et al., 2001].

HBeAg seroconversion is frequently, though not exclusively, accompanied by biochemical exacerbations characterized with abrupt increases in serum ALT levels [Lok et al., 1987b; Liaw et al., 1983; Liaw et al., 1987a]. Exacerbations are believed to be due to a sudden increase in immune-mediated lysis of infected hepatocytes. They are often preceded by an increase in serum HBV DNA [Maruyama et al., 1993] and a shift of HBcAg from nuclear to cytoplasmic sites within hepatocytes [Hsu et al., 1987], suggesting that immune clearance may be triggered by an increase in viral load or change in the presentation of viral antigens. How these changes occur is not yet known.

Most exacerbations are asymptomatic and are discovered during routine follow-up. However, some are accompanied by symptoms of acute hepatitis and may lead to the incorrect diagnosis of acute hepatitis B in patients who are unknown to have CHB [Chu et al., 1989]. Exacerbations may be

associated with an elevation in the IgM anti-HBc titer, though to a level generally lower than that in acute hepatitis B, such that leading to a misdiagnosis of acute hepatitis B. There may also be an increase in the serum alpha-fetoprotein concentration, which may raise concerns about the diagnosis of HCC [Lok et al., 1988; Liaw et al., 1984b]. Exacerbations are more commonly observed in men than women [Lok et al., 1988]. The reason for this gender difference is not clear, but this higher frequency of exacerbations in men may partly explain why more male CHB patients have cirrhosis and HCC.

HBeAg seroconversion and clearance of HBV DNA from the serum is not always present after exacerbation of CHB, such phenomenon is called abortive immune clearance [Lok et al., 1988, Liaw et al., 1987b]. These patients may develop recurrent exacerbations with intermittent disappearance of serum HBV DNA with or without transient loss of HBeAg. Such repeated episodes of hepatitis may increase the risk of developing liver fibrosis, cirrhosis and its complications.

1.2.3 Low or nonreplication phase (inactive carrier state)

Patients in the low or nonreplicating phase (i.e. inactive carrier state) are HBeAg negative and anti-HBe positive. In some patients, virus replication has ceased although they remain hepatitis B surface antigen (HBsAg) positive. These patients have undetectable HBV DNA in serum, even when tested by

polymerase chain reaction (PCR) assays. The liver disease is in remission as evidenced by normal serum ALT levels and resolution of necroinflammation with a usually minimal amount of fibrosis in liver histology.

Some CHB patients in this low or nonreplicating phase become HBsAg negative. The annual rate of delayed HBsAg clearance has been estimated to be 0.5% to 2% in Western patients and much lower (0.1% to 0.8 %) in Asian countries [Alward et al., 1985; Liaw et al., 1991]. Longitudinal studies have demonstrated that HBsAg seroclearance usually confers excellent long-term prognosis, provided that HBsAg loss occurred at a younger age, in the absence of concurrent viral infections, and preceded the development of severe liver fibrosis or cirrhosis. As shown in the studies presented in Table 1.2, clinical outcomes of disease progression such as decompensation, HCC or death may occur in these patients, particularly those with cirrhosis or in patients with hepatitis C virus (HCV) or hepatitis delta virus (HDV) co-infection at the time of HBsAg clearance [Yuen et al., 2004; Ahn et al., 2005; Fattovich et al., 1998; Chen et al., 2002; Arase et al., 2005]. One of the largest series to address this issue focused on 218 such patients who had been followed for a median of 62 months [Chen et al., 2002]. Of 189 patients who were not cirrhotic at the time of HBsAg clearance, three developed cirrhosis, two developed HCC, and one died of HCC. However, these complications developed only in patients who had concurrent HCV or HDV infection. In another series of 55 patients who spontaneously cleared HBsAg,

complications developed in 33% (11 HCC, 6 cirrhosis, 1 subfulminant liver failure) during a mean follow-up of 23 months [Huo et al., 1998]. The ability of HBV to cause complications despite clearance of HBsAg probably results from its integration into the genome of patients, reflected by the persistence of HBV DNA when measured using sensitive PCR assays. However, this study [Huo et al., 1998] might overestimate the frequency of complications as it included 20 patients (36 %) who had co-infection with either hepatitis C or hepatitis D. Furthermore, some of the patients may have had undocumented cirrhosis or irreversible liver damage prior to HBsAg seroconversion.

A recent study reported the replicative and transcriptional levels, and the risk of HCC in a cohort of 298 Hong Kong patients who spontaneously cleared HBsAg [Yuen et al., 2008]. The median age of HBsAg seroclearance was 49.6 years and the median follow-up duration was 108 months. Intrahepatic total HBV DNA and cccDNA were detected in 100% and 79.3% of patients, while serum HBV DNA were detectable in 13.4%, 6.1%, and 3.7% of patients within 1 year and 5-10 and >10 years after HBsAg seroclearance, respectively. All patients had undetectable surface and precore/pregenomic RNA transcripts. Cumulative risk for HCC was higher in patients with HBsAg seroclearance at ages above 50 years compared with those with HBsAg seroclearance at ages below 50 years. The authors concluded that HBV persisted at low replicative and transcriptional levels after HBsAg seroclearance; HBsAg seroclearance at age below 50 years was associated with a lower risk for the development of

HCC [Yuen et al., 2008].

1.2.4 Reactivation phase

As viral supercoiled DNA persists in the liver, a number of inactive HBsAg carriers may eventually develop HBV reactivation with recrudescence of liver disease either spontaneously or triggered by active immunosuppression. Reactivation of viral replication may occur due to reactivation with the wild type virus with reversion back to the HBeAg positive state, or much more frequently with replication-competent HBV variants that prevent HBeAg expression. This phase is characterized by HBeAg negativity and anti-HBe positivity, detectable serum HBV DNA, ALT elevation and moderate or severe necroinflammation with variable amounts of fibrosis on liver biopsy. Immunosuppression, as occurs with cancer chemotherapy or after organ transplantation, can lead to reactivation of hepatitis in patients even after HBsAg clearance achieved.

Patients in the low replication phase have undetectable HBV DNA in serum when tested by hybridization assays. However, many of these patients, up to 28% in one series, remained HBV DNA positive when tested by PCR assays [Chung et al., 1995]. These patients may have low level HBV replication, but liver disease is usually inactive. A small proportion of these patients may be infected with a mixture of the wild-type virus and HBV variants with a deletion in the pre-S1 region, which are associated with a reduction in HBsAg

synthesis [Cabrerizo et al., 2000]. Reactivation of HBV replication with reappearance of HBeAg and HBV DNA (by hybridization assays in one study) in serum and recrudescence of liver disease may occur when these patients are immunosuppressed [Lok et al., 1991]. The reactivation can vary in severity from mild and asymptomatic to severe with possible fulminant hepatic failure [Davis et al., 1984].

Some patients continue to have moderate levels of HBV replication and active liver disease (elevated serum ALT and chronic inflammation on liver biopsies) but remain HBeAg negative [Bonino et al., 1986; Lok et al., 1984]. These patients with HBeAg-negative CHB may have residual wild type virus or HBV variants that cannot produce HBeAg due to precore or core promoter variants [Carman et al., 1989; Lok et al., 1994; Okamoto et al., 1994; Brunetto et al., 1991]. Patients with HBeAg-negative CHB are older and have more severe liver fibrosis. They also tend to have fluctuations in HBV DNA and ALT levels. In one study, 67% of 283 patients who had sustained biochemical remission after spontaneous HBeAg seroconversion were followed for a median of 8.6 (range 1 to 18) years [Hsu et al., 2002]. The risk of cirrhosis and HCC were negligible in those with sustained remission while the risk was significantly higher in those with ALT elevation after HBeAg seroconversion. Based on the knowledge of the natural history of CHB as discussed above, patients can be classified according to their serologic status as shown in Table 1.3.

Table 1.2 Occurrence of complications after HBsAg seroclearance

Clinical status at HBsAg loss	Publication	Area	No. patients Δ	Mean age at entry (years)	% of male patients	Mean follow-up (years)	No. HCC	No. decomp	No. death/ OLT
Chronic hepatitis	Chen et al., 2002	Taiwan	189 (43)	43	79	5.1	2 (2)	0	1 (1)
	Ahn et al., 2005	Korea	32*	50	73	1.6	1	n/a	n/a
	Arase et al., 2005	Japan	164*	51	80	5	0	0	0
Chronic hepatitis	Yuen et al., 2004	China	92*	43	71	4.2	5#	0	n/a
or cirrhosis	Yuen et al., 2008		298*	43	71	9	7	0	n/a
Cirrhosis	Fattovich et al., 1998	Europe	32 (n/a)	45	90	4.6	1 (1)	2	2 (1)
	Chen et al., 2002	Taiwan	29 (12)	54	79	5.1	1 (1)	4 (2)	1 (1)
	Ahn et al., 2005	Korea	17*	50	73	1.6	4	n/a	n/a
	Arase et al., 2005	Japan	67*	51	80	6.1	2	0	0

Δ Numbers in blankets refer to the numbers of patients with hepatitis C virus or hepatitis D virus co-infection.

*None of the patients had hepatitis C virus or hepatitis D virus co-infection.

#Four of the five patients had cirrhosis.

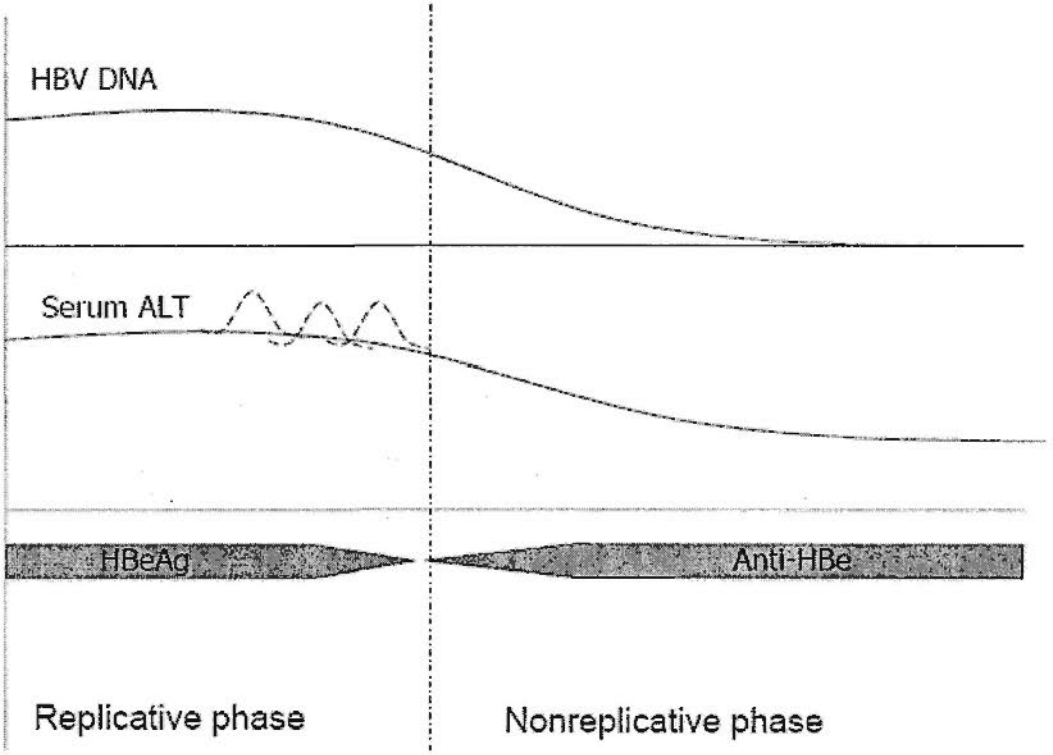
HCC = hepatocellular carcinoma; decomp = decompensation; OLT = orthotopic liver transplantation; n/a = not available.

Table 1.3 Serological profiles of chronic hepatitis B virus infection

Phase	Serum ALT	HBeAg	Anti-HBe	HBV DNA	
					Copies/ml
					IU/ml
Immune tolerance	Normal or minimally elevated	Positive	Negative	Very high levels	10^8–10^{11} 20 million–20 billion
HBeAg-positive chronic hepatitis	Persistently elevated	Positive	Negative	High levels	10^6–10^{10} 200,000–2 billion
HBeAg-negative chronic hepatitis	Elevated and often fluctuating	Negative	Positive	Moderate levels, often fluctuating	10^4–10^8 2000–20 million
Inactive carrier	Normal	Negative	Positive	Low or no detectable levels	$<10^4$ <2000

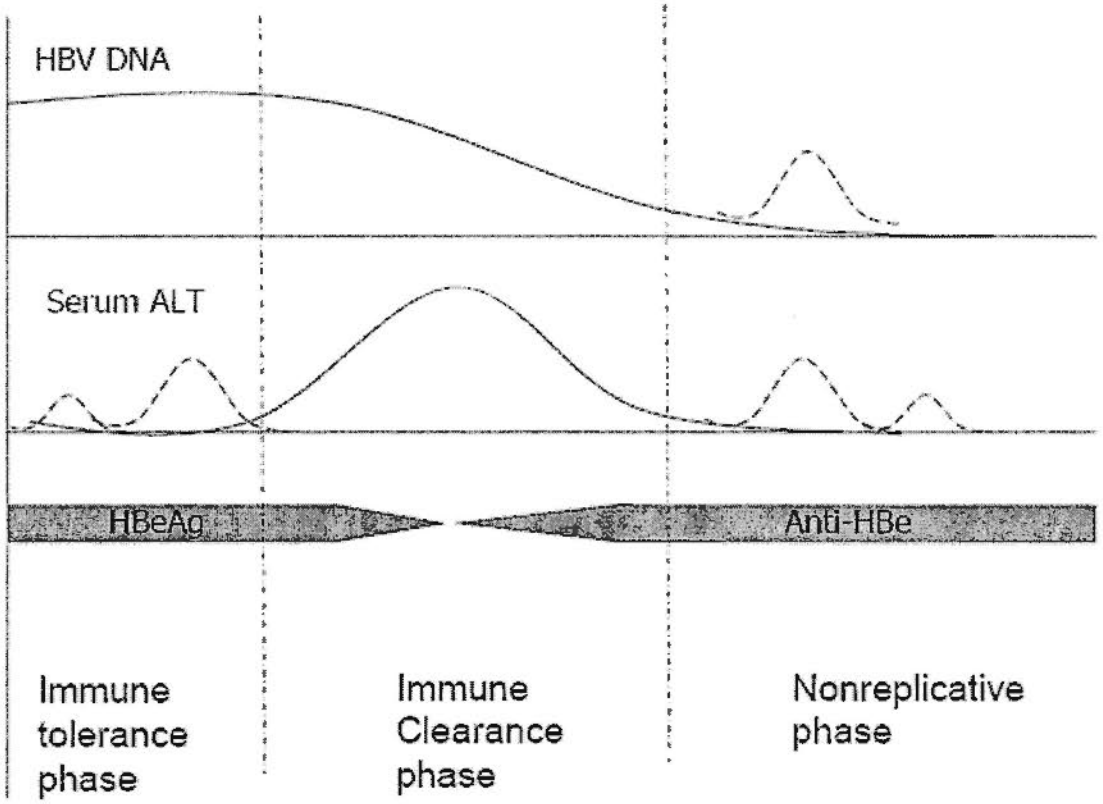
ALT = alanine aminotransferase

Figure 1.1 The natural history of adult-acquired chronic hepatitis B



The natural history of adult-acquired CHB infection generally consists of two phases: an early replicative phase with active liver disease, as manifested by an elevated serum ALT and circulating HBV DNA and HBeAg; and a late nonreplicative phase with remission of liver disease that is characterized by the disappearance of HBV DNA and HBeAg, normalization of serum ALT, and the appearance of anti-HBe antibodies.

Figure 1.2 The natural history of perinatally acquired chronic hepatitis B



The natural history of perinatally acquired CHB generally consists of three phases: an immune tolerance phase, an immune clearance phase, and a late nonreplicative phase. The immune tolerance phase, which lasts 10 to 30 years, is characterized by high levels of HBV replication, as manifested by the presence of HBeAg and high levels of HBV DNA in serum, but no evidence of active liver disease as manifested by normal serum ALT levels. During the immune clearance phase, HBeAg seroconversion is frequently, but not always, accompanied by biochemical exacerbations (abrupt increases in serum ALT). The nonreplicative phase is usually characterized by the absence of HBV DNA and normalization of serum ALT.

1.3 HBeAg-Positive Chronic Hepatitis B

The natural history of HBeAg-positive CHB is very heterogeneous. Age at acquisition is a major factor in determining the natural history of chronic infection. The vigor of the host immune response to the virus, viral factors (genotype, core promoter mutations, and duration of viral replication) and exogenous factors (alcohol, immune suppression) influence the severity of disease [Feld et al., 2006]. Although HBeAg positivity is known to be associated with more active hepatic inflammation and higher risk of HCC in the long run [Yang et al., 2002], most Asian patients acquire the infection at infancy and do not have significant liver disease during the immune tolerant phase [Chan, 2002a]. During this phase, there is little immune response against the infected hepatocytes, resulting in high viral replication (represented by high HBV DNA level), presence of HBeAg, normal serum alanine ALT levels and minimal changes on liver biopsy [Chang et al., 1988; Lok et al., 1988].

Not every HBeAg-positive CHB patient with normal ALT level is in the immune tolerant phase. Some patients may have abortive immune clearance resulting in advanced liver fibrosis and even liver cirrhosis [Feld et al., 2006]. While patients in the immune tolerant phase do not require anti-viral therapy, treatment in patients with advanced liver fibrosis can lower the risk of HCC and various cirrhotic complications [Liaw et al., 2004a]. Differentiating the two

conditions by indiscriminate liver biopsies are unlikely to gain general acceptance. The American Association for the Study of Liver Diseases recommends liver biopsy in patients with borderline or mildly elevated ALT or aged older than 40 [Lok et al., 2007]. The latest Asian-Pacific guideline recommends liver biopsy prior to therapy in patients with HBV DNA levels >100,000 copies/ml and raised ALT level, or those with high normal ALT and age older than 40 [Liaw et al., 2008].

Although higher HBV DNA is associated with a higher risk of liver cirrhosis and HCC regardless of the ALT levels in a longitudinal follow-up study in Taiwan, only 15% of these patients had positive HBeAg [Iloeje et al., 2006; Chen et al., 2006b]. The true relationship between HBV DNA, ALT and advanced liver fibrosis among HBeAg- positive patients, particularly the young patients in immune tolerance phase, remains to be defined.

1.4 HBeAg-Negative Chronic Hepatitis B

HBeAg seroconversion has been considered as an indicator of immune clearance, which is generally believed to have reduced risk of hepatic decompensation and improved survival [Fattovich et al., 1991; Villeneuve et al., 1994]. However, 10% to 30% of patients continue to have elevated ALT and high HBV DNA levels after HBeAg seroconversion [Chan et al., 2000a; Davis et al., 1984; Liaw et al., 1987]. These HBeAg-negative CHB patients may have residual wild type virus or HBV variants that cannot produce HBeAg due to precore or core promoter variants [Carman et al., 1989; Lok et al., 1994; Okamoto et al., 1994; Brunetto et al., 1991]. Patients with HBeAg-negative CHB are older and have more severe liver fibrosis. They also tend to have fluctuations in HBV DNA and ALT levels. In one study, 67% of 283 patients who had sustained biochemical remission after spontaneous HBeAg seroconversion were followed for a median of 8.6 (range 1 to 18) years [Hsu et al., 2002]. The risk of cirrhosis and HCC were negligible in those with sustained remission while the risk was significantly higher in those with ALT elevation after HBeAg seroconversion.

HBeAg-negative CHB patients have increased risk of developing complications of liver cirrhosis [Lok et al., 1987b; McMahon et al., 2001; Hsu et al., 2002]. In a Taiwanese longitudinal follow-up study, in which 85% of the study population had negative HBeAg and 94% of patients had normal ALT levels,

high HBV DNA has been found to associate with a higher risk of liver cirrhosis regardless of the ALT levels [Iloeje et al., 2006].

Among patients with severe fibrosis or early cirrhosis, suppression of HBV DNA by anti-viral agent could reduce the risk of liver decompensation and HCC [Liaw et al., 2004a]. Differentiation between HBeAg-negative chronic hepatitis and the inactive carrier-state is sometimes difficult as HBeAg-negative patients may have fluctuating HBV DNA and ALT levels [Sung et al., 2002; Chan et al., 2003; Chu et al., 2002a; Hadziyannis et al., 2001]. Liver biopsy is sometimes needed to confirm the disease activity and staging of liver fibrosis in HBeAg-negative patients.

Serum ALT level has been one of the major criteria during consideration of treatment for HBeAg-negative CHB. According to the latest Asian-Pacific and American guidelines, HBeAg-negative patients is recommended for treatment when ALT levels higher than 2 times upper limit of normal (x ULN) and HBV DNA levels higher than 100,000 copies/ml (or 20,000 IU/ml) [Lok et al., 2007; Liaw et al., 2008]. A more aggressive recommendation was made by a panel of American hepatologists [Keeffe et al., 2006]. In this guideline, they recommended that HBeAg-negative patients with elevated ALT should be treated; while liver biopsy should be considered among patients with normal ALT as far as the HBV DNA is higher than 10,000 copies/ml (or 2,000 IU/ml). In all the practice recommendations, severe liver fibrosis and early liver

cirrhosis with active viremia are indications of treatment regardless of serum ALT levels in HBeAg-negative patients [Lok et al., 2007; Liaw et al., 2008; EASL 2009].

1.5 Clinical Factors Associated with Severe Liver Disease

1.5.1 Host-related factors

1.5.1.1 Age

Clinical parameters affecting severity of liver fibrosis and disease progression can be divided into host-related, viral-related or other factors (Table 1.4). Host factors having impact on the progression of liver fibrosis include advanced age, male gender and host genetic factors. Several studies found that Asian patients aged 40 years or above are at higher risk of cirrhosis and HCC than are younger individuals [Yu et al., 1997; Iloeje et al., 2006; Liaw et al., 1988; Huo et al., 2000; Chen et al., 2006b; Park et al., 2007]. Western studies also demonstrated that the incidence of cirrhosis and HCC increased significantly with increasing age [Brunetto et al., 2002; Moreno-Otero et al., 1991; Fattovich et al., 1991; Fattovich et al., 2004]. Older age appears to be an important determinant of disease progression probably because it implies a longer duration of HBV infection and liver disease.

1.5.1.2 Gender

Male gender has been identified as an independent risk factor of cirrhosis [Iloeje et al., 2006; Huo et al., 2000]. The pathological mechanism by which sex affects fibrosis progression remains undefined. The antifibrogenic effect of estrogen may be one of the explanations, possibly through the inhibition of stellate cells [Shimizu et al., 2003]. Overall, the risk of HCC is three to six

times higher in male than in female patients [Chen et al., 2006b; Park et al., 2007].

1.5.1.3 Host genetic factors

There is increasing interest in the study of genetic markers that can reliably identify patients with chronic viral hepatitis developing progressive fibrosis. For that purpose, susceptibility or protective polymorphisms have been sought. Experimental data from animal models and clinical data from patients with HBV and HCV chronic infection suggest that inflammation associated cytokines including pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and transforming growth factor beta (TGF-β) and anti-inflammatory cytokines such as interleukin 10 (IL-10) are involved in the development and progression of liver fibrosis. IL-10 promoter allelic frequencies of T and A at positions −819 and −592 as well as the frequencies of ATA haplotypes at positions −1082/−819/−592 are significantly higher in asymptomatic carriers relevant to a genetically low capacity of IL-10 production than in patients with progressive chronic liver disease who carry the GCC haplotype identified as a high IL-10 producer phenotype [Zhang et al., 2006, Cheong et al., 2006].

Angiotensin II, the main peptide of the renin-angiotensin system, is involved in liver fibrosis through activation of hepatic stellate cells [Bataller et al., 2003; Hirose et al., 2007] and polymorphisms in the promoter region of the

angiotensinogen gene have been shown to be associated with cirrhosis in CHB patients [Xiao et al., 2006]. Single nucleotide polymorphisms at various foci of the TGF-β gene with a higher TGF-β production and a lower risk of HBV-related HCC have been described [Kim et al., 2003; Migita et al., 2005], thus supporting the concept of the tumour suppressor activity of TGF at the early stage of tumourigenesis [Lutz et al., 2002].

1.5.2 Viral-related factors

1.5.2.1 Viral load

Several lines of evidence support the association between sustained high levels of HBV replication with accompanying hepatitis and risk of severe liver fibrosis, cirrhosis and its complications. Ongoing HBV replication, defined by serum HBV DNA detectable by hybridization assays (higher than 10^5–10^6 copies/ml) or presence of HBeAg, may accelerate the progression of chronic hepatitis to cirrhosis [Hsu et al., 2002; Brunetto et al., 2002; Iloeje et al., 2006; Liaw et al., 1988; Fattovich et al., 1991]. Delayed HBeAg seroconversion (over 40 years of age) and HBeAg seroreversion after spontaneous HBeAg seroconversion, indicating a prolonged period of viral replication and necroinflammation, were associated with increased risk of cirrhosis [Hsu et al., 2002; Chu et al., 2004].

In Asia, a population-based study on 11,893 Taiwanese men found that the risk of HCC increased 10-fold among men positive for HBsAg alone and 60-

fold for those who were positive for both HBsAg and HBeAg at diagnosis compared to those negative for both markers [Yang et al., 2002]. The increased risk of HCC in individuals seropositive for HBeAg remained significant regardless of serum level of ALT and status of liver cirrhosis [You et al., 2004]. Another population-based cohort study of more than 3500 untreated Taiwanese HBsAg carriers, among whom 85% were HBeAg negative and 94% with normal ALT, found that the risk of cirrhosis and HCC increased significantly with increasing baseline serum HBV DNA levels detected by sensitive PCR assays [Iloeje et al., 2006; Chen et al., 2006b]. The adjusted relative risks of cirrhosis were 2.5, 5.6 and 6.5 when baseline HBV DNA levels were equal to or greater than 10^4, 10^5 and 10^6 copies/ml, respectively [Iloeje et al., 2006]. The risk of HCC increased significantly at the level of 10^4 copies/ml and was highest for patients with the highest baseline HBV-DNA level (>10^6 copies/ml) with hazard risk values of 2.3 and 6.1, respectively [Chen et al., 06]. HBV DNA level remained an independent predictor of cirrhosis or HCC after adjustment for risk factors of age, sex, smoking, alcohol and HBeAg status and serum ALT levels at enrolment [Iloeje et al., 2006, Chen et al., 2006b]. The authors suggested that HBV DNA level of 10^4 copies/ml or more are the strongest predictor of future cirrhosis or HCC risk, regardless of HBeAg status and serum ALT levels at baseline [Iloeje et al., 2006; Chen et al., 2006b].

In Europe, a 25-year longitudinal study of Italian CHB patients whom were

HBeAg positive at diagnosis showed that the risk for liver-related death was increased 33-fold in patients who remained HBeAg positive and 38-fold in those with HBeAg negative chronic hepatitis or HBeAg reversion relative to inactive carriers [Fattovich et al., 2008]. Other studies showed that patients with compensated cirrhosis and HBeAg positivity and/or serum HBV DNA detectable by hybridization assays are at increased risk of decompensation and liver-related death [Fattovich et al., 2002].

Overall, these studies support the concept that the higher the level of HBV replication, the greater the risk of severe liver fibrosis, cirrhosis and its complications including HCC, decompensation and liver related mortality. However, the above findings from population-based cohort studies in Taiwan showing a direct association between serum HBV DNA levels above 10^4 copies/ml and cirrhosis or HCC risk should be considered with caution because only baseline measures of HBV-DNA level were tested, which are poorly related with the levels of HBV replication and disease activity during the whole follow-up of an individual [Iloeje et al., 2006; Chen et al., 2006b].

Persistent reduction of HBV replication during follow-up predicts a favourable outcome. Long-term follow-up studies of adult inactive HBsAg carriers have shown that these patients rarely progress to cirrhosis or HCC [Hsu et al., 2002; Manno et al., 2004]. Cirrhotic patients who clear HBeAg with sustained reduction of HBV DNA, ALT normalization, and eventually, HBsAg loss have a

very low risk of developing HCC and decompensation and increased survival compared to cirrhotics with persistently high levels of HBV replication [Fattovich et al., 2003]. In addition it has been reported that survival can be prolonged in HBsAg positive decompensated cirrhotics who subsequently lose HBsAg [Fattovich et al., 2003].

1.5.2.2 HBV genotypes

HBV is currently classified into 8 genotypes, A to H, based on a more than 8% difference in the entire nucleotide sequence. HBV genotypes can be further segregated into subgenotypes based on a more than 4% but less than 8% difference, with distinct ethnic or geographic origin [Kao et al., 2006]. Genotypes B and C are most common in Asia, genotype A in Northwest Europe, North America, India and Africa and genotype D in Southern Europe, the Middle East and India. Increasing evidence supports the view that different HBV genotypes have a role in determining the clinical outcome of liver disease. Various cross sectional studies found that patients with genotype C have more severe liver fibrosis than those with genotype B [Kao et al., 2006; Kramvis et al., 2005]. In a longitudinal study of 202 HBeAg-positive CHB patients from Taiwan, genotype C was predictive of cirrhosis development [Chu et al., 2005]. A lower rate of spontaneous HBeAg seroconversion, and a longer duration of high levels of HBV replication associated with more severe hepatitis activities may contribute to a higher risk of progression to cirrhosis among genotype C patients compared to those

with genotype B infection [Chu et al., 2002b]. In cohort studies of 426 CHB patients from Hong Kong [Chan et al., 2004] and of 4841 HBsAg positive men from Taiwan [Yu et al., 2005], genotype C was associated with a 3- and 5-fold increased HCC risk, respectively, compared with other HBV genotypes. Subgenotype Ce, together with high HBV DNA level, increased the risk of HCC in CHB patients in Hong Kong [Chan et al., 2008]. The main discrepant finding comes from a study in Taiwan reporting that genotype B is associated with an increased risk of HCC in young adult patients mostly non-cirrhotic [Ni et al., 2004]. Similarly, in Taiwan the majority of children with HCC and CHB have genotype B [Kao et al., 2000].

A cross-sectional study from India [Thakur et al., 2002] and a longitudinal report from Spain [Sanchez-Tapias et al., 2002] found that genotype D was associated with more severe liver fibrosis compared to genotype A. A study involving Vietnamese patients has reported that genotype D correlated positively with the presence of liver cirrhosis and genotype C and D were significantly associated with HCC [Toan et al., 2006]. A case-control study in sub-Saharan Africans showed a 4.5-fold increased risk for HCC in CHB patients infected with genotype A compared with those infected with other genotypes, and this increased risk was entirely attributable to subgenotype A1 [Kew et al., 2005]. Another study found a significant association between genotype F and the development of HCC among native Alaskans suffering from CHB [Livingston et al., 2007b]. Overall these data suggest that genotype

D infection is associated with more active liver disease and advanced liver fibrosis compared with genotype A infection, whereas the association with HCC is controversial.

1.5.2.3 HBV mutations

The HBV virus is prone to mutations due to its asymmetric replication via reverse transcription of an RNA intermediate [Buti et al., 2005]. The pre-S HBV deletion, the precore and the basal core-promoter (BCP) mutations occur in various stages of the CHB infection and have been demonstrated to be associated with progression of the disease leading to liver cirrhosis and HCC [Chen et al., 2006a; Lin et al., 2005]. Combination of mutations rather than a single mutation have been associated with the development of liver disease. Further mapping of the pre-S deletion sequences found that all the deletion regions encompassed T- and B-cell epitopes [Chen et al., 2006a]. The most common naturally occurring HBV mutations are the precore G1896A mutation and the dual BCP A1762T/G1764A mutation. Cross-sectional studies have reported that core promoter mutations are associated with more severe liver fibrosis and necroinflammation [Orito et al., 2001; Kao et al., 2003; Yuen et al., 2005a]. Genotype C tends to have a higher proportion of BCP T1762/A1764 mutation than does genotype B [Orito et al., 2001; Kao et al., 2003] and this fact may partly explain the association between genotype C and liver fibrosis [Yuen et al., 2005a]. Studies have indicated that BCP T1762/A1764 mutation is associated with an increased risk of developing HCC

independent of HBV genotype [Kao et al., 2003; Chen et al., 2006; Liu JID 06] and serum viral load [Liu et al., 2006a; Liu et al., 2006b]. BCP T1762/A1764 mutation might contribute to HCC risk because it is associated with changes in the overlapping X gene [Zheng et al., 2004].

A case-control study found a higher prevalence of pre-S deletion, BCP T1762/A1764 mutation and precore G1896A mutation in patients with progressive liver disease, including patients with HCC, as compared to age-matched chronic HBV carriers with persistently normal ALT [Chen et al., 2006]. The same study also found that combination of mutations rather than single mutation was associated with progressive liver fibrosis and HCC, especially in combination with pre-S deletion [Chen et al., 2006]. HBeAg-negative CHB patients that have a higher frequency of mutations at core promoter nucleotides 1753 and 1773 and precore nucleotides 1846, 1896, and 1899 than HBeAg-positive Taiwanese patients also show a more rapid progression of the disease [Chen et al., 2005].

1.5.2.4 Concurrent infection

Concurrent infection with HCV is present in 10% to 15% of patients with CHB, particularly in area where both HBV and HCV are endemic [Gaeta et al., 2003]. Most patients who have dual HCV and HBV infections have detectable serum HCV RNA but undetectable or low HBV DNA levels, indicating that HCV is the predominant cause of liver disease in these patients. Liver disease is usually

more severe than in patients infected by HBV alone [Raimondo et al., 2006]. Patients with HBV and HCV co-infection may also have a higher rate of HCC compared to patients infected by either virus alone, particularly those who are anti-HCV and HBeAg positive [Huang et al., 2005; Benvegnu et al., 1994; Yu et al., 1991b]. HCV co-infection also increased the risk of cirrhosis [Liaw et al., 2004b].

Although HDV can replicate autonomously, the simultaneous presence of HBV is required for complete virion assembly and secretion. As a result, individuals with hepatitis D are always dually infected with HDV and HBV. An increase in the risk of cirrhosis due to HDV co-infection was observed, with relative risks of 2.6 and 2.8 [Fattovich et al., 1987; Tamura et al., 1993].

Studies conducted before the introduction of the highly active anti-retroviral therapy (HAART) have shown that human immunodeficiency virus (HIV)-related immune deficiency modifies the natural history of CHB with higher levels of HBV replication and a lower rate of spontaneous HBeAg seroconversion, leading to a more rapid liver fibrosis progression towards cirrhosis [Di Martino et al., 2002; Puoti et al., 2006]. A higher rate of cirrhosis decompensation and liver-related death, but not of HCC, was reported in HIV and HBV co-infected individuals with cirrhosis before the introduction of HAART [Di Martino et al., 2002; Puoti et al., 2006; Thio et al., 2002].

1.5.3 Other factors

1.5.3.1 Alcohol

Heavy alcohol intake was found to increase the risk of progression to cirrhosis 6-fold relative to alcohol abstinence among CHB patients [Ikeda et al., 1998]. A population based cohort study from Korea found that in the subgroup of CHB patients the HCC risk started to increase significantly with an alcohol intake of 50 grams per day or more with a relative risk of 1.2 (95% confident interval 1.0–1.5) for 50 to 99 grams per day and of 1.5 (95% confident interval 1.2–2.0) for more than 100 grams per day [Jee et al., 2004].

1.5.3.2 Metabolic factors

The interaction between metabolic factors, including obesity and diabetes, hepatic steatosis and liver fibrosis will be discussed in details in Chapter 2.

1.5.3.3 Environmental factors

Environmental hepatotoxins including tobacco smoke and aflatoxins may increase the risk of HCC. Few and discordant data are available on the association of cigarette smoking with HCC in CHB patients [Chen et al., 2006; Yang et al., 2002]. It has been reported that exposure to even modest levels of aflatoxin, a mycotoxin which contaminates food in humid conditions, triples HCC risk in CHB patients [Ming et al., 2002]. However, data concerning the effect of these environmental factors on liver fibrosis or cirrhosis are lacking.

Table 1.4 Factors associated with increased risk of progression to cirrhosis

Host related factors	**Virus related factors**	**Other factors**
Older age (>40 years)	High HBV DNA levels during follow-up	Heavy alcohol consumption
Male gender	HBV genotype (C worse than B; D worse than A)	Steatosis
Genetic diversity	HBV variant (core promoter; pre-S)	Diabetes
	HCV co-infection	Obesity
	HDV co-infection	
	HIV co-infection	

1.6 Risks of Complications and Liver Fibrosis

Liver fibrosis stage was found as an independent predictive factor of liver cirrhosis in a group of 90 Chinese CHB patients [Wu et al., 2007]. Relationship of different stages of liver fibrosis and long-term outcomes in CHB patients has been recently studied in a cohort of 188 Korean CHB patients [Park et al., 2007]. During a mean follow-up of 119.8 months, cirrhosis developed in 62 patients (33%), decompensation in 20 patients (11%), and HCC in 21 patients (11%). The cumulative probability of developing cirrhosis increased significantly with progression in liver fibrosis stage, and was directly proportional to the stage. The cumulative probability of developing cirrhosis for patients with Metavir stage F0 or F1 fibrosis at baseline was 0%, 11% and 11% at 5 years, 10 years and 15 years respectively. The probabilities were increased significantly with baseline fibrosis at stage F2 (12%, 33% and 47% at 5 years, 10 years and 15 years respectively) and stage F3 (22%, 47% and 65% at 5 years, 10 years and 15 years respectively) [Park et al., 2007]. In another cohort of 2215 patients with chronic viral hepatitis (among them 645 patients were suffering from CHB), the relative risk of developing cirrhosis and HCC in patients with F3 fibrosis was 6.3 (95% confidence interval or CI, 3.9–10.1) and 4.4 (95% CI, 2.4–7.8) compared with those with F1 and F2 fibrosis [Ikeda et al., 1998]. In summary, the severity of fibrosis stage at presentation correlates with the risk of cirrhosis, which was 4-fold higher for stage F3 as compared to stage F1 or F2 [Park et al., 2007; Ikeda et al., 1998].

The morbidity of CHB patients suffering from compensated cirrhosis was studied in a cohort of 349 European patients (86% men; mean age, 44 years; mean follow-up 73 months). HCC developed in 32 (9%) of the 349 patients and decompensation was observed in 88 (28%) of 317 tumor-free patients. The probability of HCC appearance was 6% and the probability of decompensation was 23% at 5 years after decompensation. However the rates of CHB-related complications may be over-estimated in this study as 20% of the patients were anti-HDV-positive [Fattovich et al., 1995]. In a cohort of 76 Taiwanese CHB patients with recent development of cirrhosis and a mean follow-up period of 34.4 months, hepatic decompensation, esophageal variceal bleeding and HCC developed relatively late in the course of the disease with a calculated annual incidence of 2.3%, 2.3% and 2.8%, respectively [Liaw et al., 1989]. In summary, the estimated five-year rates of progression have been reported to be: 12% to 20% from chronic hepatitis to cirrhosis; 20% to 23% from compensated cirrhosis to hepatic decompensation; and 6% to 15% from compensated cirrhosis to HCC [Fattovich et al., 1991; Fattovich et al., 1995; Liaw et al., 1988; Liaw et al., 1989].

CHAPTER TWO

METABOLIC SYNDROME AND HEAPTITIS

2.1 Metabolic Syndrome

Metabolic syndrome, comprising of type 2 diabetes, hypertension, central obesity and dyslipidemia, is increasingly prevalent worldwide [Eckel et al., 2005]. This increase is associated with the global epidemic of obesity and diabetes [Zimmet et al., 2001]. Increased caloric intake, increased consumption of refined carbohydrates, and physical inactivity has led to an explosion in the incidence of abdominal obesity and an emerging epidemic of insulin resistance. Abdominal obesity has tripled in the United States during the past four decades [Okosun et al., 2004]. More than one quarter of the population in the United States has the metabolic syndrome [Ford et al., 2002], and the incidence is increasing [Ford et al., 2004].

Metabolic syndrome can be diagnosed with International Diabetes Federation (IDF) criteria [IDF 2007], which are modified from National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III), the most commonly used diagnostic criteria for metabolic syndrome [Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001]. IDF criteria are different from NCEP-ATP III as it includes ethnic specific definition of central obesity. Metabolic syndrome is defined with the presence of central obesity (waist circumference ≥90 cm for men and ≥80 cm for women in Asia) plus two or more of the following four factors: 1) raised concentration of triglycerides: >1.7 mmol/l or specific treatment for this lipid

abnormality; 2) reduced concentration of HDL cholesterol: <1.03 mmol/l in men and <1.29 mmol/l in women or specific treatment for this lipid abnormality; 3) raised blood pressure: systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg or treatment of previously diagnosed hypertension; and 4) raised fasting plasma glucose concentration ≥5.6 mmol/l or previously diagnosed type 2 diabetes.

The most accepted and unifying hypothesis to describe the pathophysiology of metabolic syndrome is insulin resistance (Figure 2.1) [Eckel et al., 2005]. Free fatty acids (FFAs) are released in abundance from an expanded adipose tissue mass. In the liver, FFAs produce an increased production of glucose, triglycerides and secretion of very low density lipoproteins (VLDL) [Lewis et al., 1995]. Associated lipid/lipoprotein abnormalities include reductions in high density lipoprotein (HDL) cholesterol and an increased density of low density lipoproteins (LDL) [Brinton et al., 1991]. FFAs also reduce insulin sensitivity in muscle by inhibiting insulin-mediated glucose uptake. Associated defects include a reduction in glucose partitioning to glycogen and increased lipid accumulation in triglyceride (TG) [Lewis et al., 1995]. Increases in circulating glucose and to some extent FFAs increase pancreatic insulin secretion resulting in hyperinsulinemia [Seufert et al., 2004]. Hyperinsulinaemia may result in enhanced sodium reabsorption and increased sympathetic nervous system activity and contribute to hypertension [Anderson et al., 1991].

Superimposed and contributory to the insulin resistance produced by excessive FFAs is the paracrine and endocrine effect of the proinflammatory state. Produced by a variety of cells in adipose tissue including adipocytes and monocyte-derived macrophages, the enhanced secretion of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) among others results in more insulin resistance and lipolysis of adipose tissue triglyceride stores to circulating FFA [Fernandez-Real et al., 2003]. IL-6 and other cytokines also are increased in the circulation and may enhance hepatic glucose production, the production of VLDL by the liver and insulin resistance in muscle. Cytokines and FFAs also increase the production of fibrinogen and plasminogen activator inhibitor-1 (PAI-1) by the liver that complements the overproduction of PAI-1 by adipose tissue [Aubert et al., 2003], which results in a pro-thrombotic state. Reductions in the production of the anti-inflammatory and insulin sensitizing cytokine adiponectin are also associated with the metabolic syndrome and may contribute to the pathophysiology of the syndrome [Nawrocki et al., 2004].

Metabolic syndrome, a multiplex comprises atherogenic dyslipidemia, glucose intolerance, hypertension, proinflammatory state, and prothrombotic state [Grundy, 2006] (Table 2.1), is a predictor of type 2 diabetes meliitus and future cardiovascular events. The incidence of diabetes meliitus is increased at least 7-fold in the presence of metabolic syndrome [Wilson et al., 2005]. A recent meta-analysis of nearly 175,000 patients showed that metabolic

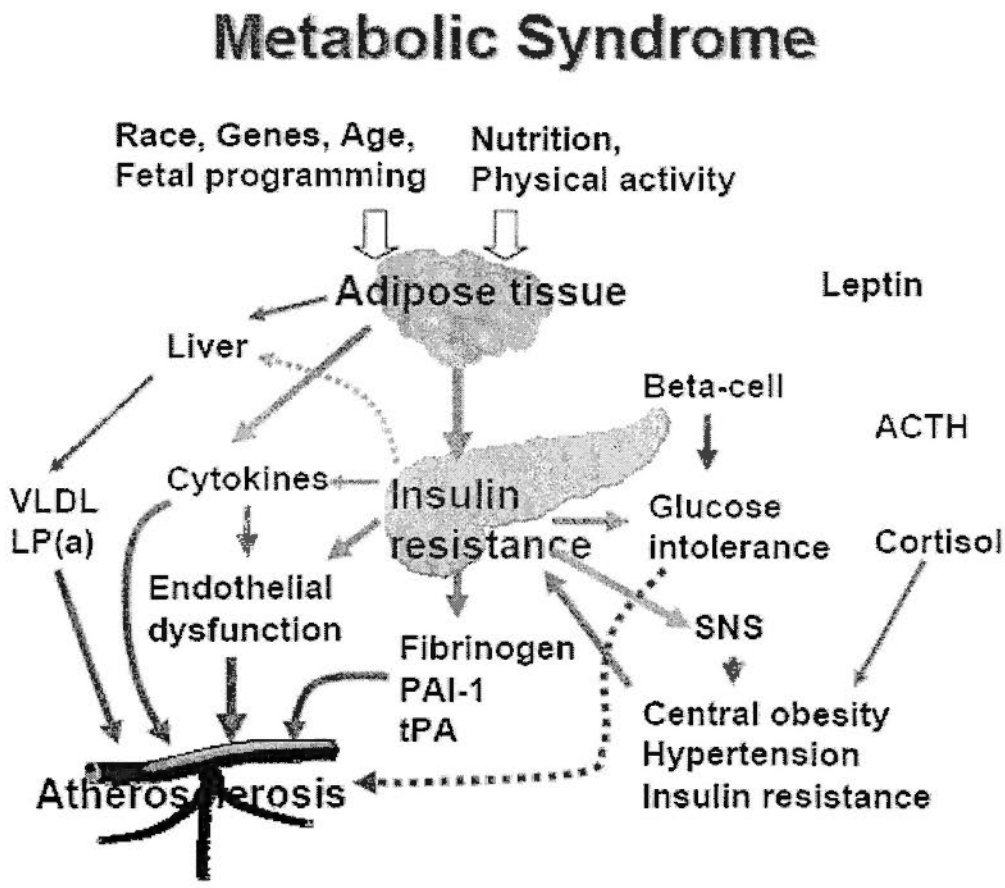
syndrome confers a relative risk of 1.54 for cardiovascular events and death after adjustment for traditional risk factors [Gami et al., 2007]. Apart from its significant effect on cardiovascular events, its importance in chronic liver diseases has been increasing recognized. Adipokines including leptin, adiponectin and resistin, which are secreted by the metabolically active visceral adipose tissue and involved in metabolic syndrome, have gained attention in the pathogenesis of fibrogenesis in various chronic liver diseases [Tsochatzis et al., 2008a; Tsochatzis et al., 2006; Schäffler et al., 2005; Wong et al., 2006].

Table 2.1 Metabolic risk conditions that constitute metabolic syndrome

Metabolic risk condition	Clinical markers
Atherogenic dyslipidemia	↑ Triglycérides, VLDL-C, non-HDL-C ↓ HDL-C, LDL-C particle size
Glucose intolerance	↑ Fasting glucose, hemoglobin A1c Impaired glucose tolerance
Hypertension	Elevated blood pressure
Proinflammatory state	↑ WBC, hs-CRP, IL-6
Prothrombotic state	↑ Fibrinogen, vWF, PAI-1

HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; IL-6 = interleukin 6; PAI-1 = plasminogen activator inhibitor 1; VLDL-C= very lowdensity lipoprotein cholesterol; vWF = von Willebrand factor; WBC = white blood cell count.

Figure 2.1 Pathophysiology of metabolic syndrome



(Modified from Eckel et al., 2005)

2.2 Metabolic Syndrome and Non-alcoholic Fatty Liver Disease

Insulin resistance is accompanied by many other alterations that are not included in the diagnostic criteria for metabolic syndrome. Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) is known to be associated with insulin resistance. As the characteristics of metabolic syndrome are also risk factors for hepatic steatosis, it has been hypothesized that NAFLD is the hepatic manifestation of the metabolic syndrome [Marchesini et al., 2001].

Hepatic steatosis and NAFLD are at focus of increasing interest in clinical hepatology [Farrell et al., 2006]. In fact, the growing epidemic of obesity has rendered NAFLD as the leading cause of referral to hepatology clinics in most western countries [Farrell et al., 2006]. NASH is becoming an important health problem that is present in 2% to 3% of individuals in the USA and other western countries [Neuschwander-Tetri et al., 2003]. As the incidence of overweight, obesity and metabolic syndrome increases, NAFLD could become one of the more frequent causes of end stage liver disease and HCC.

In recent years, it has become evident that hepatic steatosis is not an innocent bystander. A small but significant proportion of NAFLD patients may develop NASH, such that these patients have a subsequent increased risk of

cirrhosis and even HCC [Matteoni et al., 1999]. NASH is strongly associated with obesity and presence of insulin resistance and type 2 diabetes mellitus [Lonardo et al., 2005; Machado et al., 2006], while abdominal obesity is a risk factor for NAFLD even in patients with normal body mass index (BMI) [Farrell et al., 2006]. Most patients with NASH or liver cirrhosis have features of metabolic syndrome (Table 2.2). The strongest clinic predictive factors of NAFLD severity are hyperglycemia, type 2 diabetes mellitus, hypertension, obesity, and age [Angulo et al., 1999; Dixon et al., 2001; Harrison et al., 2003; Ratziu et al., 2002]. As these are the features of metabolic syndrome, hepatic steatosis is now considered an early indicator of insulin resistance [Farrell et al., 2006].

Table 2.2 Clinical predictors for the severity of fibrosis in non-alcoholic steatohepatitis

Parameters	Angulo et al., 1999	Harrison et al., 2003	Ratziu et al., 2002	Dixon et al., 2001
Body mass index	√	√	√	--
Age	√	--	√	--
AST:ALT	√	√	--	--
ALT	--	--	√	√
Diabetes mellitus	√	--	--	--
HbA1c, hyperglycemia	--	√	--	--
Insulin resistance index	--	--	--	√
Triglycerides	--	--	√	--
Hypertension	--	--	--	√

AST = aspartate aminotransferase; ALT = alanine aminotransferase; HbA1c= hemoglobin A1c.

2.3 Metabolic Syndrome and Viral Hepatitis

Evidence concerning the relationship of metabolic syndrome and viral hepatitis mainly comes from studies in patients suffering from chronic hepatitis C. Hepatic steatosis is present in up to 30% to 70% of chronic hepatitis C patients [Rubbia-Brandt et al., 2004; Tsochatzis et al., 2007]. Even after exclusion of the usual causes of steatosis including obesity, diabetes, alcohol, and drugs, the prevalence of steatosis is still around 30% to 40% [Rubbia-Brandt et al., 2000; Kumar et al., 2000]. It has been suggested that HCV genotype 3 is directly responsible for hepatocyte steatosis [Rubbia-Brandt et al., 2000; Kumar et al., 2000; Mihm et al., 1997]. Steatosis could be the morphologic expression of a cytopathic effect of HCV genotype 3 [Rubbia-Brandt et al., 2000; Kumar et al., 2000]. HCV genotype 3 core protein could inhibit very low-density lipoprotein (VLDL) liver secretion and induce liver steatosis together with hypobeta-lipoproteinemia [Serfaty et al., 2001]. One strong argument for a direct effect of HCV virus is the disappearance of steatosis with the clearance of the virus. In previous studies, the disappearance of steatosis has been observed in a small number of patients infected by HCV genotype 3 and successfully treated with interferon or with interferon and ribavirin combo-therapy [Rubbia-Brandt et al., 2000; Kumar et al., 2000]. Additionally, hypobeta-lipoproteinemia was corrected in 7 HCV sustained responders but without significant change in liver steatosis [Serfaty et al., 2001].

On the other hand, patients with chronic hepatitis C and hepatic steatosis have more rapid progression of liver fibrosis and poorer response to peginterferon and ribavirin treatment, independent of hepatitis C genotypes [Poynard et al., 2003]. In patients with HCV genotypes 1 and 4, insulin resistance is associated with high viral load and significant fibrosis independent of hepatic steatosis [Moucari et al., 2008]. It has also been shown that obesity, insulin resistance, and the metabolic syndrome are strong predictors of increased ALT levels in patients suffering from viral hepatitis [Ioannou et al., 2005]. Metabolic syndrome is independently associated with more severe liver fibrosis but not with the severity of steatosis in patients suffering from chronic viral hepatitis [Tsochatzis et al., 2008b].

A recent retrospective study involving 153 Chinese CHB patients showed that hepatic steatosis is more frequently present in CHB patients than in the general population [Peng et al., 2008]. The authors hypothesized that steatosis in CHB patients may be due to metabolic factors and the ability of HBV to indirectly facilitate the development of steatosis. In contrary to the previous studies, steatosis in CHB patients was not found to be associated with the severity of fibrosis in this retrospective study [Peng et al., 2008]. Another retrospective study involving a large cohort of 1915 CHB patients showed that hepatic steatosis was present in 14% of the patients [Shi et al., 2008]. Steatosis was found independently associated with metabolic factors including body mass index, serum triglyceride, apolipoprotein B, uric acid,

fasting blood glucose, but not liver fibrosis. Authors concluded that hepatic steatosis was associated with metabolic factors and did not affect the severity of liver disease. The real relationship between metabolic syndrome and CHB remains to be defined.

2.4 Metabolic Syndrome and Hepatocellular Carcinoma

In Hong Kong, HCC together with liver cirrhosis account for 4.9% of all deaths in Hong Kong in 2005 [Department of Health, Hong Kong 2005]. The incidence of HCC has increased significantly over the past two decades in the USA [El-Serag et al., 2003a]. The cause of the increase in HCC is incompletely understood. Approximately half of this increase is attributed to HCV, while a minimal or no increase has been related to HBV or alcoholic liver disease [El-Serag et al., 2000]. In general, approximately 15% to 50% of HCC cases remain idiopathic, suggesting that other risk factors are responsible for this increase in HCC.

Diabetes has been suggested as a potential risk factor for HCC. It is a known risk factor for NAFLD and NASH [Angulo et al., 1999; Dixon et al., 2001], which can lead to cirrhosis and subsequently HCC. On the other hand, end stage liver disease itself can cause glucose intolerance and overt diabetes [Petrides et al., 1994]. Moreover, some causes of chronic liver disease, such as HCV and haemochromatosis, have been associated with an increased risk of diabetes. Several studies have examined the association between HCC and diabetes. Earlier studies reported no association between diabetes and HCC while more recent studies have identified diabetes as a risk factor for HCC [El-Serag et al., 2003b; Wideroff et al., 1997; Adami et al., 1991; Adami et al., 1996; Fugino et al., 2001; Yu et al., 1991a; Hassan et al., 2002]. For example,

a cohort study conducted in patients identified at hospitals of Veterans Affairs found a greater than two-fold increase in the relative risk of HCC among male veterans with diabetes in the absence of HCV, HBV, and alcoholic cirrhosis [El-Serag et al., 2003]. However, the generalisability of these results to the overall US population may be limited; all except for one small study were based on referral samples [Yu et al., 1991a]. Moreover, selection bias may have occurred in which patients with diabetes were more likely to be tested and diagnosed with HCC, and therefore more likely to be enrolled in these studies.

A population-based case-control study using the linked records of patients with HCC identified from the Surveillance, Epidemiology, and End-Results (SEER) registries to Medicare claims has been performed to minimize the issue of referral bias [Warren et al., 2002; Davila et al., 2005]. This study included 2061 HCC patients and 6183 non-cancer controls. The proportion of HCC patients with diabetes (43%) was significantly greater than non-cancer controls (19%). Diabetes was found to be associated with a three-fold increase in the risk of HCC in multiple logistic regression analyses. In a subset of patients without major risk factors of HCC (HCV, HBV, alcoholic liver disease, and haemochromatosis), the adjusted odds ratio for diabetes declined but remained significant (adjusted odds ratio 2.9, 95% confidence interval 2.5–3.3). Hence the authors concluded that diabetes is independently associated with a 2- to 3-fold increase in the risk of HCC, regardless of the presence of other major HCC risk factors [Davila et al., 2005]. The risk factors for

metabolic syndrome tended to lower the survival rates in patients suffering from HBV or HCV infections [Takamatsu et al., 2008].

CHAPTER THREE

IMPORTANCE OF LIVER FIBROSIS ASSESSMENT

3.1 Importance of Assessing and Monitoring of Liver Fibrosis

Liver fibrosis is a key and prognostically relevant phase that is directly related to the severity of the process itself in CHB. The sequelae of CHB may vary from an inactive carrier state to the development of cirrhosis, hepatic decompensation, HCC, and death, which are mostly related to the severity of liver fibrosis. Hence assessment of the presence and severity of liver fibrosis together with inflammation is critical to determine prognosis, potential risks for complications and therapeutic strategies. With drugs that potentially reverse liver fibrosis, methods of assessing fibrosis is essential to monitor disease progression, clinical outcomes, and response to treatment are warranted.

3.1.1 Severity of liver fibrosis and survival

It is undisputable that most of the mortalities in CHB patients occur in those with cirrhosis and that the evolution to cirrhosis is characterized by a progressive increase in liver fibrosis. To assess the severity of liver fibrosis is therefore of tremendous importance. Most of the evidence of survival rates came from studies concerning the severity of cirrhosis [Fattovich et al., 1995; Liaw et al., 1989; de Jongh et al., 1992; Realdi et al., 1994]. Among Chinese CHB patients irrespective of the presence of cirrhosis, the lifetime risk of a liver-related death has been estimated at 40% to 50 % for men and 15 % for

women [Beasley et al., 1982b]. The estimated 5-year survival rate was 80% in a cohort of 76 Taiwanese CHB patients suffering from compensated liver cirrhosis, 7 patients (9%) of whom died of liver failure or variceal bleeding, which usually happened more than 3 years after entry [Liaw et al., 1989].

In a cohort of 349 European CHB patients, the probability of survival was 35% at 5 years after the first episode of decompensation [Fattovich et al., 1995]. Another European multicenter longitudinal study involving 366 Caucasian CHB patients with compensated cirrhosis and a mean follow-up period of 72 months, the cumulative probability of survival was 84% and 68% at 5 and 10 years, respectively [Realdi et al., 1994]. Death occurred in 84 (23%) patients, mainly due to liver failure (45 cases) or HCC (23 cases). Age, albumin, platelet count, splenomegaly, serum bilirubin and HBeAg positivity at time of diagnosis was found to be independently correlated with survival. In this cohort, 20% of patients were positive for anti-HDV. However, this study did not identify any difference in survival in HDV-infected or uninfected patients. In a group of 98 Dutch CHB patients with mean follow-up of 4.3 years, the overall one-year, three-year and five-year survival rate was 92%, 79%, and 71%, respectively. The five-year survival rates of patients with compensated and decompensated cirrhosis was 14% and 84%, respectively. Age, serum bilirubin, and presence of ascites ascites were independently related to survival [de Jongh et al., 1992]. In summary, the five-year survival rate of CHB patients with compensated cirrhosis was approximately 85%. The

one-year and five-year survival rates of patients with decompensated cirrhosis were 55% to 70% and 14% to 35% respectively.

3.1.2 Severity of liver fibrosis and risks of complications

The prognosis has known to be poor in CHB patients from endemic areas [Fattovich et al., 1991; Fattovich et al., 1995; Liaw et al., 1988; Liaw et al., 1989]. Liver fibrosis stage was found as an independent predictive factor of liver cirrhosis in a group of 90 Chinese CHB patients [Wu et al., 2007]. Relationship of different stages of liver fibrosis and long-term outcomes in CHB patients has been recently studied in a cohort of 188 Korean CHB patients [Park et al., 2007]. They have ultrasonography and clinical assessment performed regularly to detect the development of cirrhosis and complications. During a mean follow-up of 119.8 months, cirrhosis developed in 62 patients (33%), decompensation in 20 patients (11%), and HCC in 21 patients (11%). The cumulative probability of developing cirrhosis increased significantly with progression in liver fibrosis stage, and was directly proportional to the stage. The cumulative probability of developing cirrhosis for patients with Metavir stage F0 or F1 fibrosis at baseline was 0%, 11% and 11% at 5 years, 10 years and 15 years respectively. The probabilities were increased significantly with baseline fibrosis at stage F2 (12%, 33% and 47% at 5 years, 10 years and 15 years respectively) and stage F3 (22%, 47% and 65% at 5 years, 10 years and 15 years respectively) [Park et al., 2007].

In another cohort of 2215 patients with chronic viral hepatitis (among them 645 patients were suffering from CHB), the relative risk of developing cirrhosis and HCC in patients with F3 fibrosis was 6.3 (95% CI, 3.9–10.1) and 4.4 (95% CI, 2.4–7.8) compared with those with F1 and F2 fibrosis [Ikeda et al., 1998]. Overall the severity of fibrosis stage at presentation correlates with the risk of cirrhosis, which was 4-fold higher for stage F3 as compared to stage F1 or F2 [Park et al., 2007; Ikeda et al., 1998].

The morbidity of CHB patients suffering from compensated cirrhosis was studied in a cohort of 349 European patients (86% men; mean age, 44 years; mean follow-up 73 months). HCC developed in 32 (9%) of the 349 patients and decompensation was observed in 88 (28%) of 317 tumor-free patients. The probability of HCC appearance was 6% and the probability of decompensation was 23% at 5 years after decompensation. However the rates of CHB-related complications may be over-estimated in this study as 20% of the patients were anti-HDV-positive [Fattovich et al., 1995]. In a cohort of 76 Taiwanese CHB patients with recent development of cirrhosis and a mean follow-up period of 34.4 months, hepatic decompensation, esophageal variceal bleeding and HCC developed relatively late in the course of the disease with a calculated annual incidence of 2.3%, 2.3% and 2.8%, respectively [Liaw et al., 1989]. In summary, the estimated five-year rates of progression have been reported to be: 12% to 20% from chronic hepatitis to cirrhosis; 20% to 23 % from compensated cirrhosis to hepatic

decompensation; and 6% to 15% from compensated cirrhosis to HCC [Fattovich et al., 1991; Fattovich et al., 1995; Liaw et al., 1988; Liaw et al., 1989].

3.2 The Role of Liver Fibrosis on Decision of Treatment for Chronic Hepatitis B

In the treatment guidelines issued by different authorities of liver diseases, severity of liver fibrosis, together with ALT and HBV DNA level, has a central and important role on the decision-making process of CHB. The latest Asian-Pacific consensus statement by the Asian-Pacific Association for the Study of the Liver (APASL) pointed out that the short-term goal of treatment in terms of HBeAg seroconversion and/or HBV DNA suppression, ALT normalization, and prevention of hepatic decompensation is aimed to reduce liver necroinflammation and fibrosis during and after therapy. This intermediate goal leads to the ultimate long-term goal of preventing hepatic decompensation, progression to cirrhosis and/or HCC, and prolong survival [Liaw et al., 2008]. This APASL statement pointed out that severity of liver fibrosis would alter management particularly in patients with persistently normal ALT, as treatment is usually not indicated to patients with persistently normal ALT levels unless they have evidence of advanced fibrosis or cirrhosis [Han et al., 2008]. Liver biopsy is recommended before therapy to assess the fibrosis state, necroinflammatory grade, and exclude other possible causes of raised ALT levels as a guide to the indication for antiviral treatment. A liver biopsy should be considered in patients older than 40 [Chu et al., 2007], especially those with high normal ALT levels as these patients are more likely to have significant liver fibrosis [Lai et al., 2007].

Instead of liver biopsy for all patients before antiviral treatment, the Americian Association for the Study of Liver Diseases (AASLD) recommended to selectively perform sssessment of liver fibrosis and necroinflammation for patients who remain HBeAg positive with HBV DNA levels >20,000 IU/ml after 3 to 6 month of elevated ALT levels between 1 to 2x ULN, or who remain HBeAg positive with HBV DNA levels >20,000 IU/ml and are older than 40 [Lok et al., 2007]. Treatment would be indicated in the presence of significant fibrosis, and/or moderate or severe inflammation for these patients [Lok et al., 2007]. Treatment can be initiated without liver biopsy for patients whose serum HBV DNA levels above 20,000 IU/ml and ALT levels >2x ULN and patients with icteric ALT flares. The AASLD also mentioned that the severity of liver fibrosis poses implication on the treatment strategy if drug resistance developed. It was recommended that majority of patients with confirmed lamivudine-resistance should receive rescue therapy with antiviral agents that are effective against lamivudine-resistant HBV mutants. A minority of patients may consider stopping treatment, particularly if they have normal ALT, or if the biopsy showed mild inflammation and no or minimal fibrosis prior to initiation of treatment [Liaw et al., 2004c; Wong et al., 2004].

The latest European Association for the Study of the Liver (EASL) clinical practice guidelines recommended liver biopsy in patients with either HBV DNA levels >2000 IU/ml (a level one log lower than that of AASLD guideline) or increased ALT (or both) [EASL 2009]. On the other hand, the EASL guidelines

did not recommend liver biopsy in patients with clinical evidence of cirrhosis or in those in whom treatment is indicated irrespective of the grade of activity or the stage of fibrosis.

3.3 Liver Fibrosis and Therapeutic Response

As severity of liver fibrosis has been well established as an indicator of prognosis, improvement of liver fibrosis has been an important end-point of treatment response in various large-scaled clinical trials of anti-viral treatments of CHB.

3.3.1 Nucleos(t)ide analogues

Lamivudine is the first generation nucleoside analogue which was found to reduce progression of liver fibrosis when compared to placebo [Lai et al., 1998; Dienstag et al., 1999]. Adefovir dipivoxil was found to be associated with significantly improvement and less worsening of liver fibrosis in HBeAg-positive [Marcellin et al., 2003] and HBeAg-negative CHB patients [Hadziyannis et al., 2003]. Entecavir was found to result in similar improved liver fibrosis when compared to lamivudine [Chang TT et al., 2006; Lai et al., 2006]. Emtricitabine was also found to produce a significant decrease in fibrosis compared to placebo [Lim et al., 2006]. So far there has been no evidence concerning which of the nucleos(t)ide analogues is superior to the other on improving liver fibrosis.

3.3.2 Inteferon-based therapy

Interferon-alpha (IFN-α) was found to reduce liver fibrosis progression compared to placebo [Papatheodoridis et al., 2005]. Extended treatment of

IFN-α2b for 24 months retarded progression of liver fibrosis in HBeAg-negative CHB patients who sustainedly responded [Lampertico et al., 2003]. Virologic response (HBeAg seroconversion and HBV DNA <10,000 copies/ml at week 78) occured significantly more often in patients with advanced fibrosis when treated pegylated-interferon alpha-2b (pIFN-α2b) alone or in combination with lamivudine 100 mg daily [Buster et al., 2007].

Improvement of fibrosis score occurred more often in patients treated with combination therapy of IFN-α2b and lamivudine than those treated with lamivudine monotherapy [Barbaro G et al., 2001]. HBeAg-positive CHB patients received combination therapy of pIFN-α2b for 32 weeks plus lamivudine for 52 weeks tended to have less progression and more reduction of liver fibrosis when compared to lamivudine monotherapy for 52 weeks [Chan et al., 2005]. Similar trend was observed in another study comparing pIFN-α2b combined with lamivudine and pIFN-α2b monotherapy [Janssen et al., 2005] and in a study comparing pIFN-α2a combined with lamivudine and pIFN-α2a or lamivudine monotherarpy [Marcellin et al., 2004].

In summary, antiviral treatments, either nucleos(t)ide analogues, interferon treatment or combination therapy, improved liver fibrosis and/or reduce progression of liver fibrosis when compared to placebo. On the other hand, none of these treatments was proved superior to the others in this aspect.

CHAPTER FOUR

LIVER BIOPSY – THE CURRENT GOLD STANDARD FOR LIVER FIBROSIS ASSESSMENT

4.1 Technique of Percutaneous Liver Biopsy

Percutaneous liver biopsy varies with regard to the technique (blind, percussion–palpation approach or image-guided approach) [Cadranel et al., 2000; Gilmore et al., 1995]. The percussion–palpation (or blind) approach is commonly used by experience hepatologists. Caudal percussion is helpful in selecting the site for the biopsy over the hemithorax between the anterior and midaxillary lines, until an intercostal space is reached where dullness is maximal at the end of expiration. The intercostal space below this point is used.

The liver biopsy can be performed under imaging control or guidance using different imaging modalites, including ultrasonography (USG), computer tomography or magnetic resonance imaging. USG is the most common imaging modality used because it is readily and widely available, simple, the least costly and does not expose the patient to radiation. When USG is used in obtaining the liver biopsy, it is done either immediately before (site marking) or throughout the entire procedure (real time). Pre-biopsy USG helps to detect focal hepatic tumours (benign or malignant), cysts, ascites, intrahepatic biliary dilatation or hepatic anatomical variation. For focal hepatic lesions, it is an accepted standard of practice that image guidance is used in order to guide and direct the liver biopsy [Buscarini et al., 1990; Sbolli et al., 1990].

4.2 Contraindications and Risks of Liver Biopsy

4.2.1 Contraindications of liver biopsy

The contraindications to a percutaneous liver biopsy are listed in Table 4.1. Liver biopsy is a safe procedure when performed by experienced operators. A lower complication rate for physicians who performed more than 50 biopsies a year was reported [Froehlich et al., 1993]. Prior ultrasonographic localization of the biopsy site may decrease the rate of complications for physicians who perform liver biopsies infrequently. Liver biopsy via the blind or percussion–palpation approach should be performed by experienced gastroenterologists, hepatologists, or transplantation surgeons and not by general internists [Garcia-Tsao et al., 1993].

4.2.2 Risks and complications of liver biopsy

Although the liver has a rich vascular supply, complications associated with percutaneous liver biopsy are rare. Pain and hypotension are the predominant complications for which patients are hospitalized [Garcia-Tsao et al., 1993; Janes et al., 1993]. Minor complications after percutaneous liver biopsy include transient, localized discomfort at the biopsy site; pain requiring analgesia; and mild, transient hypotension (due to a vaso-vagal reaction). Approximately one fourth of patients have pain in the right upper quadrant or right shoulder after liver biopsy. The pain is usually dull, mild, and brief [Castera et al., 1999]. Ongoing, severe pain in the abdomen should alert the

physician to the possibility of a more serious complication, such as penumothorax, hemothorax, hemoperitoneum or peritonitis after puncturing the gallbladder or bowel.

Clinically significant intraperitoneal hemorrhage is the rare but most serious bleeding complication of percutaneous liver biopsy; it usually becomes apparent within the first two to three hours after the procedure [Piccinino et al., 1986; Van Thiel et al., 1993]. Risk factors for hemorrhage after liver biopsy are older age, more than three passes with the needle during biopsy, and the presence of cirrhosis or liver cancer [Piccinino et al., 1986; Janes et al., 1993]. Other rare complications of percutaneous liver biopsy include biliary ascites, bile pleuritis, bile peritonitis, pneumothorax, hemothorax, subcutaneous emphysema, pneumoperitoneum, pneumoscrotum, subphrenic abscess, carcinoid crisis, anaphylaxis after biopsy of an echinococcal cyst, pancreatitis due to hemobilia, and breakage of the biopsy needle [Piccinino et al., 1986; Van Thiel et al., 1993; Ruben et al., 1987].

The mortality rate among patients after percutaneous liver biopsy is approximately 1 in 10,000 to 1 in 12,000 [McGill et al., 1990; Van Thiel et al., 1993]. Mortality is highest among patients who undergo biopsies of malignant lesions. Cirrhosis is another risk factor for fatal bleeding after liver biopsy.

Table 4.1 Contraindications to percutaneous liver biopsy

Absolute contraindications	**Relative contraindications**
Uncooperative patient	Morbid obesity
History of unexplained bleeding	Ascites
Bleeding tendency	Infection in the right pleural
Prothrombin time ≥3 to 5 seconds above control	cavity or below the right
Platelet count <50,000/mm^3	hemidiaphragm
Prolonged bleeding time (≥10 minutes)	
Use of a nonsteroidal antiinflammatory drug	
within previous 7 to 10 days	
Blood for transfusion unavailable	Hemophilia
Suspected hemangioma or other vascular tumor	
Inability to identify an appropriate site for biopsy	
by percussion or ultrasonography	
Suspected echinococcal cysts in the liver	

4.3 Histologic Scoring Systems for Chronic Viral Hepatitis

The histologic classification system for chronic hepatitis was first proposed in 1968, which recognized two major histologic patterns: chronic persistent hepatitis, and chronic aggressive hepatitis [De Groote et al., 1968]. These patterns represented mild and more aggressive histologic categories of liver disease of any cause. The histologic characteristics of chronic persistent hepatitis included chronic inflammatory infiltration mostly involving the portal areas, with preserved lobular architecture and little or no fibrosis. Piecemeal necrosis or interface hepatitis was absent or slight. The chronic inflammatory infiltrate in chronic aggressive hepatitis involved the portal tracts and extended into the parenchyma with prominent piecemeal necrosis (interface hepatitis) and formation of intralobular septa. The term chronic aggressive hepatitis eventually became synonymous with chronic active hepatitis, which was originally used to describe clinical features of chronic hepatitis. Subsequently, several other specific histologic patterns were recognized, and terms added to the common lexicon. Examples include chronic lobular hepatitis, chronic portal hepatitis, and chronic periportal hepatitis [Popper et al., 1971].

Since these terms were first introduced, the recognition of specific forms of liver diseases and their clinical course permitted a better understanding of histologic correlates. As a result, the nomenclature of chronic hepatitis has continued to change. However, despite these advances, the assessment of

liver histology is usually based upon a percutaneous biopsy, which samples only a small portion of the liver and has several limitations. First, the quality of liver biopsy specimens can vary. Specimens shorter than 2 cm in length may be difficult to interpret. Second, liver disease does not always affect the liver in a homogeneous pattern, leading to the possibility of sampling variability [Abdi et al., 1979; Regev et al., 2002]. Small specimens may underestimate the degree of inflammatory activity and fibrosis in patients with viral hepatitis [Colloredo et al., 2003; Bedossa et al., 2003]. Third, the interpretation of liver biopsy specimens is subject to both inter- and intra-observer variability or error [Soloway et al., 1971]. Fourth, histologic changes obtained at a single point in time may not reflect overall disease activity, as which may fluctuate.

Several histologic classification systems have been proposed to minimize these uncertainties and provide a uniform standard that can be used to compare histologic findings in clinical trials. Although some of these classifications are qualitative, quantitative systems are most frequently used in clinical trials since they are amenable to statistical analysis [Bonis et al., 1997]. The most common quantitative system that has been used in the assessment of chronic viral hepatitis is the Knodell score [Knodell et al., 1981]. In addition, another semiquantitative system, the Metavir score, has been increasingly used for chronic hepatitis C [Bedossa et al., 1996; The French METAVIR Cooperative Study Group, 1994].

4.3.1 Knodell score

The Knodell score, also known as the histologic activity index (HAI), is composed of the summation of four individual scores representing periportal and/or bridging necrosis, intralobular degeneration and focal necrosis, portal inflammation, and fibrosis; the score ranges from 0 to 22 (Table 4.2). Several modifications of the HAI have also appeared, which were designed, in part, to address histologic features specific to the disease under study [Honkoop et al., 1997; Haque et al., 1996; Kaplan et al., 1997]. One modification referred to as the Ishak score, has six stages of fibrosis, permitting more detailed evaluation of changes in fibrosis compared with the standard Knodell fibrosis score, which has only three stages [Ishak et al., 1996]. The Knodell score is frequently used in drug trials in chronic hepatitis, particularly chronic hepatitis C. The score serves two purposes: to assure that baseline histologic features in treatment groups are equally matched, and to assess histologic changes after therapy. A decrease in the Knodell score is considered to represent histologic improvement. No histologic feature represented in the Knodell score can predict the response to interferon [Ikawa et al., 2001].

4.3.2 Metavir score

The Metavir score was developed in an attempt to address some of the problems with the Knodell score [Bedossa et al., 1996; The French METAVIR Cooperative Study Group, 1994]. In contrast to the Knodell score, which was designed as a generic scoring system for chronic hepatitis, the Metavir score

was specifically designed and validated for patients with hepatitis C. The Metavir score is a semiquantitative classifications system consisting of an activity and a fibrosis score. The fibrosis score is assessed on a five point scale: F0 represents no fibrosis; F1 represents portal fibrosis without septa; F2 represents few septa; F3 represents numerous septa without cirrhosis; and F4 represents cirrhosis. Compared to the Knodell fibrosis score (which has only four levels), the Metavir score permits recognition of subtler variation in the degree of fibrosis. The activity score was graded according to the intensity of necroinflammatory lesions: A0 represents no activity; A1 represents mild activity; A2 represents moderate activity; and A3 represents severe activity. The inter- and intra-observer reliability of the activity and fibrosis score of the Metavir system are similar to the Knodell score. In one study, the kappa coefficients of the Metavir activity score and the HAI, and the Metavir fibrosis score and the Knodell fibrosis score were found to be similar (approximately 0.5 and 0.8, respectively) [The French METAVIR Cooperative Study Group, 1994]. A subsequent study found that the interobserver agreement of the Metavir score depends highly upon the experience of the hepatopathologist [Rousselet et al., 2005]. Agreement was influenced more heavily by the experience of the interpreter compared with features of the specimen itself such as its length.

Another study evaluating the fibrosis and activity scores in specimens of various lengths suggested that the length of the biopsy was also important

[Schiano et al., 2005]. The kappa coefficients for fibrosis were 0.75, 0.85, and 0.92 comparing specimens of 5, 10, and 15 mm respectively (considering the fibrosis score of a 20 mm specimen as the reference standard). The corresponding figures for the activity scores were 0.73, 0.81, and 0.77, respectively. The authors concluded that a specimen length of at least 10 mm usually reflects the fibrosis and activity scores reliably. Thus, the main advantage of the Metavir score for chronic hepatitis C is its relative simplicity, its focus on necroinflammatory lesions, and its increased sensitivity in the fibrosis score due to the addition of one extra fibrosis level. However, many of the limitations of the Knodell score discussed above also apply to the Metavir score. In particular, the fibrosis stages of the Metavir score have not been well-correlated to the natural history of chronic hepatitis C. Thus, it is unclear whether individuals progress from early to late stages at a constant, linear rate, although this hypothesis has been proposed [Poynard et al., 1997].

Table 4.2 Knodell score

Score	I. Periportal ± bridging necrosis	II. Intralobular degeneration and focal necrosis	III. Portal inflammation	IV. Fibrosis
0	None	None	No portal inflammation	No fibrosis
1	Mild piecemeal necrosis	Mild (acidophilic bodies, ballooning degeneration and/or scattered foci of hepatocellular necrosis in < 1/3 of lobules or nodules	Mild (sprinkling of inflammatory cells in <1/3 of portal tracts)	Fibrous portal expansion
3	Moderate piecemeal necrosis (involves less than 50 % of the circumference of most portal tracts)	Moderate (involvement of 1/3 to 2/3 of lobules or nodules)	Moderate (increased inflammatory cells in 1/3 to 2/3 of portal tracts)	Bridging fiborisis (portal-portal or portal- central linkage)
4	Marked piecemeal necrosis (involves more than 50 % of the circumference of most portal tracts)	Marked (involvement of >2/3 of lobules or nodules)	Marked (dense packing of inflammatory cells in >2/3 of portal tracts)	Cirrhosis
5	Moderate piecemeal necrosis plus bridging necrosis			
6	Marked piecemeal necrosis plus bridging necrosis			
10	Multilobular necrosis			

4.4 Limitation of Liver Biopsy as the Gold Standard for Liver Fibrosis Assessment

4.4.1 Sampling error

The diagnostic value of liver biopsy is limited by the sampling variability. The average size of biopsy is 15 mm in length, which represents 1/50,000 the size of the entire liver. The number of portal triads present in the specimen is important; most hepatopathologists are satisfied with a biopsy specimen containing at least six to eight portal triads, especially in cases of chronic liver disease in which the extent of injury may vary among portal triads. An adequate specimen is usually provided by all the needles currently used for liver biopsy. Specimens obtained with standard thin-bore or spring-loaded needles measure between 1.4 and 1.8 mm in diameter, and those obtained with Menghini or Tru-cut needles measure up to 2 mm in diameter [Klatskin et al., 1993; Garcia-Tsao et al., 1993].

One study in patients with cirrhosis from different causes showed that three samples taken by the same route in the same patients were only concordant in 50% of the cases. A study in 124 chronic hepatitis C patients assessed the discordance between two liver biopsies done under laparoscopy, one from the right lobe and the other from the left lobe. Only non-fragmented biopsies larger than 15 mm and with at least five portal spaces were used, figures which eliminate half of the liver biopsies done in daily practice. Despite these

precautions, the percentage of discordance of at least one stage was 33% for fibrosis and 24% for the necroinflammatory activity score. Liver biopsy in 18 patients showed cirrhosis (i.e. F4) in one lobe and F3 fibrosis on the other. A discordance of two stages or two grades was seen in three (2.4%) and two (1.6%) patients, respectively. Very high coefficients of variation (55%) and high discordance rates (35%) for fibrosis staging (computer analysis) was observed in biopsies 15mm in length [Bedossa et al., 2003]. The variability improved in biopsies 25mm in length but was still very high with a 45% coefficient of variation and 25% discordance rate [Bedossa et al., 2003].

4.4.2 Intra- and inter-observer variability

There is significant variability in the histologic assessment of two readings of the same biopsy by the same pathologist, and between two pathologists, even among those who are highly specialized [Bedossa et al., 2003]. This variability is low for the diagnosis of cirrhosis (kappa coefficient of concordance higher than 0.80), moderate for earlier fibrosis stages (kappa between 0.70 and 0.80), but high for the activity grades (kappa between 0.40 and 0.50) [Bedossa et al., 2003].

4.4.3 Discontinuous semi-quantitative assessment

In contrast to the continuous nature of biochemical markers, histologic scoring systems are discontinuous and hence semi-quantitative. This limitation is significant in clinical practice since many infected patients have

intermediate stages of fibrosis. It is estimated that more than 50% of the patients with fibrosis are between the portal fibrosis stage (F1) and the moderate fibrosis stage with some septa (F2) [Bedossa et al., 2003].

4.4.4 Adverse effects of liver biopsy

Studies of biopsy complications show that pain is reported by one-third of the patients; a severe complication (which is life-threatening or prolongs hospitalization) occurs in 3 out of 1000 cases and death is reported in 3 out of 10,000 cases. Biopsy requires hospitalization as day case of 2 to 4 hours nowadays, with a waiting period for the appointment and results vary from days to weeks [Friedman, 2004; Piccinino et al., 1986].

4.4.5 Cost of liver biopsy

Liver biopsy is usually performed in the patient with a one-day hospitalization at a cost of at least $3,330 per day in the public hospital in Hong Kong, and a much higher cost for prolonged hospitalization and even intensive care if complications arise. In one cost-analysis study, the cost of an uncomplicated biopsy was estimated to be US$1032 and a biopsy with complications, US$2745. Further, not all patients would easily accept liver biopsy. Liver biopsy is not a risk-free procedure and can be experienced by patients as an aggressive procedure, therefore, becoming a potential obstacle to the effective management of chronic liver diseases.

CHAPTER FIVE

NON-INVASIVE ASSESSMENTS OF LIVER FIBROSIS

5.1 Imaging

5.1.1 Ultrasonography

Cross sectional imaging including ultrasonography (USG) can provide detailed images of the liver and surrounding structures. The presence of certain findings (such as splenomegaly, an enlarged caudate lobe or large varices) can establish the diagnosis of portal hypertension with high specificity in patients with known liver disease. Several modifications to standard USG have been proposed to increase accuracy for diagnosis of cirrhosis and portal hypertension. Sensitivity, specificity and accuracy for identifying cirrhosis were 84%, 100% and 94 %, respectively, in one study that used the ratio of the transverse caudate lobe width with the transverse right lobe width [Harbin et al., 1980]. In other reports, up to 11 ultrasonographic and doppler measurements reported accuracy of cirrhosis detection of between 82% to 88% [Aube et al., 1999; Gaiani et al., 1997]. However, the clinical utility of these approaches is limited by variations in anatomy and inconsistent interobserver agreement.

5.1.2 Hepatic vein transit time using contrast ultrasonagraphy

The introduction of contrast agents such as gas microbubbles has opened new diagnostic prospects. The study of transit times in a hepatic vein showed significantly different arrival and peak enhancement times in cirrhotic patients compared with non-cirrhotic subjects (healthy or with chronic liver disease).

In addition, a modest though significant further reduction in arrival times reflected greater disease severity in patients with Child's C cirrhosis compared with Child's A cirrhosis. Another interesting finding with considerable scope for application was the observation of a cutoff value in arrival times, with cirrhotic patients consistently exhibiting values below 17 seconds and all the other non-cirrhotic patients consistently showing higher values. Automatic sampling of time-intensity numerical data with a dedicated software allowed reproducible curve and result analysis. Because of its non-invasiveness, lack of adverse effects and cost-effectiveness, ultrasonographic imaging with contrast enhancement may become a more popular diagnostic tool [Giuseppetti et al., 2004].

5.1.3 Magnetic resonance imaging and elastography

Combined superparamagnetic iron oxide (SPIO) and gadolinium-enhanced T2-weighted magnetic resonance imaging (MRI) offers a unique opportunity to noninvasively visualize cirrhosis. SPIOs cause regenerating nodules to lose signal and appear as dark nodules; gadolinium causes fibrosis to gain signal and appears as bright reticulations. Using non-invasive scoring systems for cirrhosis on SPIO and gadolinium-enhanced MRI, authors have shown the areas under the receiver of characteristic curves (AUROCs) for the regenerating nodule score for fibrosis ≥F3 was 0.95 (95% CI: 0.91–0.99). At the optimal decision threshold, sensitivity was 89% and specificity was 97% for detecting fibrosis ≥F3. The AUROCs for the MR fibrosis score was 0.95

(95% CI: 0.91–1.00) with optimized sensitivity and specificity of 90% and 90%. The AUROCs of a combined regenerating nodule and fibrosis score was 0.98 (95% CI: 0.95–1.00) with optimized sensitivity and specificity of 90% and 97%. Internal liver architecture can be visualized and scored on MRI after combined SPIO and gadolinium administration. This capability shows promise for non-invasive detection of liver fibrosis [Aguirre et al., 2004].

Magnetic resonance elastography (MRE), a technique for quantitatively assessing the mechanical properties of soft tissues, has been recently shown to be a safe, noninvasive technique with excellent diagnostic accuracy for assessing liver fibrosis in 35 normal volunteers and 50 patients with chronic liver disease [Yin et al., 2007]. Receiver operating curve analysis showed that, with a shear stiffness cutoff value of 2.93 kilopascals (kPa), the predicted sensitivity and specificity for detecting all grades of liver fibrosis are 98% and 99%, respectively. MRE can discriminate between patients with moderate and severe fibrosis (≥F2) and those with mild fibrosis (sensitivity, 86%; specificity, 85%). Hepatic stiffness does not appear to be influenced by the degree of steatosis. In a more recent prospective study, the success rate and diagnostic accuracy of MRE, the technical success rate of magnetic resonance elastography was high (133/141, 94%), and the AUROCs of MRE were also large (0.994 for ≥F2; 0.985 for ≥F3; 0.998 for F4) [Huwart et al., 2008].

5.2 Serum Tests

5.2.1 Combined clinical-laboratory criteria

Sixty-three clinical, biochemical (prothrombin index, gamma-glutamyl transpeptidase and apolipoprotein A1 levels [PGA score]; and hyaluronate, alpha2-macroglobulin, N-terminal peptide of type III procollagen, laminin, and TGF-β1 levels), doppler ultrasonographic, and endoscopic variables were recorded in 243 patients who were divided into four groups: whole, compensated, alcohol-compensated, and viral-compensated liver disease. In three groups, hyaluronate and prothrombin index were the best predictive factors (accuracy ≥85%). Accuracy for the diagnosis of cirrhosis varied from 90% to 95% with global discriminant analysis and from 91% to 94% with stepwise analysis according to the group. In the compensated group, hyaluronate concentration of ≥60 microgram/l had a sensitivity of 97% and a specificity of 73%. Diagnostic accuracy was 87% globally for extensive fibrosis. Prothrombin index and hyaluronate were two independent variables predictive of the area of fibrosis (r^2=0.66). Hence cirrhosis can be correctly diagnosed in 91% to 94% of patients with chronic liver disease. Serum hyaluronate concentration is the most sensitive variable for screening with the use of a few noninvasive criteria [Oberti et al., 1997].

In a retrospective study on 235 treatment-naïve viremic CHB patients, body mass index, platelet count, serum albumin, and total bilirubin levels were

identified as independent predictors of bridging fibrosis or cirrhosis (Ishak stage 3 to 6). The AUROC of the best model was 0.80 (95% CI: 0.73-0.88) for the training cohort (150 patients), 0.77 (95% CI: 0.64-0.89) for the validation cohort (85 patients), and 0.79 (95% CI: 0.73-0.85) for the entire cohort. Using the low cutoff probability of 0.15, significant fibrosis could be excluded in 83 patients of the total patient population (negative predictive value 0.92) [Hui et al., 2005].

5.2.2 AST/ALT ratio

The ratio of aspartate aminotransferase over alanine aminotransferase (AST/ALT ratio) is approximately 0.8 in normal subjects. This ratio is usually greater than 2.0 in alcoholic liver disease and less than 1.0 in patients with chronic hepatitis and chronic cholestatic syndromes. Some studies have found that a ratio greater than 1.0 suggesting the presence of cirrhosis. In the first study deriving this ratio, 177 patients suffering from various forms of nonalcoholic chronic liver disease and liver biopsy performed were included [Williams et al., 1988]. Among 100 CHB patients, the mean AST/ALT ratio was 0.59 in those without cirrhosis and 1.02 in those with cirrhosis. Furthermore, the AST/ALT ratio often rose to above 1.0 when cirrhosis first became manifest. However, there have been other inconsistent reults. On a validation study involving 316 chronic hepatitis C patients found that the AST/ALT ratio ≥1.0 had a sensitivity of identifying cirrhosis was 53% to 56% only [Imperiale et al., 2000].

5.2.3 AST to platelet ratio index (APRI)

The AST to platelet ratio index (APRI) is calculated as AST level (the times of ULN) x100 / platelet count (10^9/l). This predictive model consists of objective and readily available laboratory variables [Wai et al., 2003]. Using optimal cutoff values, the negative predictive value and positive predictive value of the APRI test for determining the presence of significant fibrosis were 86% and 88% respectively, while they were 98% and 57% for predicting cirrhosis [Wai et al., 2003]. Sensitivity, specificity, positive and negative predictive values were 80%, 78%, 31% and 97%, respectively in another study of chronic hepatitis C patients when using 1.0 as the cutoff value [Islam et al., 2005]. In another report, sensitivity, specificity, positive and negative predictive values for predicting significant fibrosis in HIV/HCV co-infected patients were 52%, 100%, 100% and 45 %, respectively when using a cutoff value of 1.5 [Al-Mohri et al., 2005].

5.2.4 Fibrotest

Fibrotest has also been evaluated in a study of 183 chronic hepatitis C patients, the AUROCs of Fibrotest combining transient elastography (Fibroscan) was 0.88 for fibrosis >F2, 0.95 for >F3 and 0.95 for >F4. When the Fibroscan and Fibrotest results agreed, liver biopsy examination confirmed them in 84% of cases for >F2, in 95 % for >F3 and in 94 % for F4 [Castera et al., 2005]. Thus, it is likely that a combination of serum biomarkers and Fibroscan will complement each other and enhance accuracy of fibrosis

detection.

ActiTest is a modification of the Fibrotest that incorporates ALT and reflects both liver fibrosis and necroinflammatory activity. ActiTest appears to improve identification of more advanced fibrosis associated with histologic inflammation [Halfon et al., 2002]. Chronic hepatitis C patients who have achieved a sustained virologic response to therapy show corresponding improvement in both ActiTest and Fibrotest scores, supporting a role in monitoring response to treatment. A meta-analysis that included a total of 1570 patients concluded that these tests were a reliable alternative to liver biopsy in chronic hepatitis C patients [Poynard et al., 2004].

5.2.5 Serum proteomics

Proteomics is the study of proteins concerning the processes of how they are modified, when and where they are expressed, how they are involved in metabolic pathways and how they interact with one another. Proteomics has also been studied as a non-invasive tool of assessing liver fibrosis. Serum proteins from a cohort of 46 CHB patients were profiled quantitatively on surface-enhanced laser desorption/ionization (SELDI) ProteinChip arrays [Poon et al., 2005]. Cross-validation showed that the artificial neural network (ANN) fibrosis indices derived from the proteomic fingerprint strongly correlated with Ishak scores (r=0.831) and were significantly different among stages of fibrosis. ROC curve areas in predicting significant fibrosis (Ishak score ≥3) and cirrhosis (Ishak score ≥5) were 0.906 and 0.921, respectively.

Inclusion of International Normalized Ratio, total protein, bilirubin, alanine aminotransferase, and hemoglobin in the ANN model improved the predictive power, giving accuracies >90% for the prediction of significant fibrosis and cirrhosis [Poon et al., 2005].

5.2.6 Glycomics

Clinical glycomics was a technology based on DNA sequencer and fragment analyzers, to generate profiles of serum protein N-glycans of liver disease patients. This technology yielded a biomarker that distinguished compensated cirrhotic from non-cirrhotic patients with 79% sensitivity and 86% specificity (100% sensitivity and specificity for decompensated cirrhosis). In combination with the clinical chemistry-based Fibrotest biomarker, compensated cirrhosis was detected with 100% specificity and 75% sensitivity [Callewaert Nat Med 2004]. This biomarker combination could eventually be used in the follow-up examinations of chronic liver disease patients to yield a warning that cirrhosis has developed and that the risk of complications (such as HCC) has increased considerably. The clinical glycomics technique can easily be implemented in existing molecular diagnostic laboratories. Its superiority alone or in combination versus Fibrotest–ActiTest is not yet demonstrated, particularly in terms of cost-utility [Callewaert Nat Med 2004].

5.3 Transient Elastography

Transient elastography (Fibroscan®, from Echosens, Paris, France), is a novel non-invasive method that has been proposed for assessment of liver fibrosis by measuring liver stiffness [Sandrin et al., 2003]. Liver stiffness measurement (LSM) with this tool has been widely studied in patients suffering from different chronic liver diseases, mostly chronic hepatitis C, in European countries.

5.3.1 Principle

An ultrasound transducer probe is mounted on the axis of a vibrator. Vibrations of mild amplitude and low frequency (50 Hz) are transmitted by the transducer, inducing a plastic shear wave that propagates through the underlying tissues. Pulse-echo ultrasound acquisition is used to follow the propagation of the shear wave and to measure its velocity, which is directly related to tissue stiffness (the elastic modulus E expressed as $E = 3\rho V^2$, where V is the shear velocity and ρ is the mass density, which is constant for tissues). The stiffer the tissue, the faster the shear wave propagates (Figure 5.1). Transient elastography measures liver stiffness in a volume that approximates a cylinder 1 cm in diameter and 4 cm in length, between 25 mm and 65 mm underneath the skin surface. This volume is at least 100 times bigger than a biopsy sample, and therefore should be more representative of the liver parenchyma.

Transient elastography has the advantages of being painless, rapid (usually less than 5 minutes) and easy to perform at the bedside or in the outpatient clinic. The examination is performed on a non-fasting patient lying supine with the right arm placed behind the head to facilitate access to the right upper quadrant of the abdomen. The tip of the probe transducer is placed on the skin between the rib bones at the level of the right lobe of the liver where liver biopsy would be performed. Once the measurement area has been located, the operator presses the button on the probe to start an acquisition. The software determines whether each measurement is successful or not. No reading would be given if a shot is unsuccessful. Results are expressed in kiloPascals (kPa) and correspond to the median of 10 validated measurements according to the manufacturer's recommendations.

5.3.2 Interpretation of results

Liver stiffness values range from 2.5 kPa to 75 kPa. The results are available immediately after measurements and are operator-independent [Fraquelli et al., 2007]. The examination can be performed by a nurse after a short learning curve (about 100 examinations) [Kettaneh et al., 2007]. The validity of LSM results also depends on two important parameters. First parameter is the success rate (the ratio of the number of successful measurements to the total number of acquisitions) should be at least 60%. Second parameter is interquartile range (IQR), which reflects the variability of the validated measures, and should not exceed 30% of the median value [Lucidarme et al.,

2007]. The clinical interpretation of transient elastography results should be always in the hands of an expert clinician and should be made having information regarding patient demographics, disease aetiology and essential laboratory parameters.

5.3.3 Reproducibility

Reproducibility of transient elastography is also an important factor for its widespread clinical application. In the initial study involving 15 patients only, reproducibility was good (with low intra- and inter-operator standardized coefficient of variation: 3.2% and 3.3%, respectively) [Sandrin et al., 2003]. Two independent groups [Fraquelli et al., 2007; Konate et al., 2006], recently addressed this issue with similar results. In one study 800 transient elastography examinations were performed by two operators in 200 patients with various chronic liver diseases, the reproducibility of LSM was excellent for both inter-observer and intra-observer agreement, with intraclass correlation coefficients (ICC) of 0.98 [Fraquelli et al., 2007]. However, interobserver agreement was significantly reduced in patients with lower degrees of liver fibrosis (ICC for F0–1 and ≥F2 were 0.60 and 0.99 respectively), with hepatic steatosis (ICC for steatosis <25% and ≥25% of hepatocytes 0.98 and 0.90 respectively) and with increased body mass index (ICC for body mass index <25 kg/m^2 and ≥25 kg/m^2 were 0.98 and 0.94 respectively). Similar results have been reported in another study involving 100 patients, suggesting that the ideal candidate for transient elastography is a lean patient with severe

fibrosis [Konate et al., 2006].

5.3.4 Limitations

LSM can be difficult in obese patients or in those with narrow intercostal space. It is impossible in patients with ascites [Sandrin et al., 2003]. Failure rates range between 2.4% and 9.4% in the different studies [Sandrin et al., 2003; Fraquelli et al., 2007; Castera et al., 2003; Ziol et al., 2005; Foucher et al., 2006a; Foucher et al., 2006b; Ganne-Carrie et al., 2006; Coco et al., 2007]. In multivariate analysis of one study, the only factor associated with failure was a body mass index above 28 (odds ratio 10.0; 95% CI 5.7–17.9) [Foucher et al., 2006b]. However, with more experience from the authors, a fatty thoracic belt rather than body mass index was more likely to be the limiting factor for the success rate, because the fatty thoracic belt attenuates both elastic waves and ultrasound making liver stiffness measurement impossible [Foucher et al., 2006b]. Specific probes are being developed for obese patients.

Three recent studies suggested that transient elastography results may be influenced by ALT flares [Coco et al., 2007; Arena et al., 2007; Sagir et al., 2007]. One study reported that a 1.3- to 3-fold increase in liver stiffness values at the time of ALT flares with a progressive return to baseline values afterwards in 10 patients with chronic viral hepatitis and acute exacerbations (9 were CHB patients) [Coco et al., 2007]. A study of 18 patients with acute

viral hepatitis without a history of chronic liver disease reported similar results, and progressive normalization of liver stiffness values was observed in parallel with the decrease of ALT levels [Arena et al., 2007]. Another study reported high liver stiffness values suggestive of cirrhosis in 15 out of 20 patients with acute liver damage without any signs or liver cirrhosis at physical examination, ultrasound examination, or liver histology (performed in 11 patients) [Sagir et al., 2007]. In six patients in whom a follow-up was available, liver stiffness values decreased to values below the cutoff for cirrhosis at the time of normalization of aminotransferase levels.

5.3.5 Normal liver stiffness values

Liver stiffness values have recently been examined in 429 healthy subjects, without overt causes of liver disease and normal liver enzymes, undergoing a medical check-up [Roulot et al., 2008]. The mean liver stiffness value in these patients was 5.5 ± 1.6 kPa. Age had no influence but as suggested previously, liver stiffness values were higher in men than in women (5.8 ± 1.5 versus 5.2 ± 1.6 kPa in men and women, respectively), and in subjects with body mass index >30 kg/m^2 (6.3 ± 1.9 versus 5.4 ± 1.5 kPa in subjects with body mass index >30 kg/m^2, and ≤30 kg/m^2, respectively) [Corpechot et al., 2006a]. However, even after adjustment for gender and body mass index, liver stiffness values remained higher in subjects with metabolic syndrome (59 patients; 13.7%) than in those without (6.5 ± 1.6 versus 5.3 ± 1.5 kPa in subjects with and without metabolic syndrome, respectively). Interestingly,

among 7 subjects with metabolic syndrome who had liver stiffness values above 8 kPa, 4 underwent liver biopsy. All had NASH with portal fibrosis but mild or absent steatosis, suggesting, in agreement with another recent study in healthy subjects who underwent a liver biopsy as potential liver donors [Kim et al., 2007], that liver stiffness values are not influenced by steatosis and that transient elastography may be a sensitive tool for the detection of liver fibrosis. Further studies are needed to determine whether the monitoring of liver stiffness values in patients with metabolic syndrome may predict the evolution towards cirrhosis.

5.3.6 Diagnostic performance

5.3.6.1 Diagnosis of significant fibrosis: F0-1 versus F2-4

The first step in evaluating this new diagnostic tool for measuring liver fibrosis is to be validated against the current clinical gold standard (liver biopsy) to determine the AUROCs, the sensitivity, specificity, positive and negative predictive values of certain cutoffs [Afdhal et al., 2007]. This has been done for transient elastography in large-scale prospective studies, initially in patients with chronic hepatitis C [Castera et al., 2003; Ziol et al., 2005]. From a recent meta-analysis, they identified 35 studies which reported data on the AUROC for significant fibrosis (≥F2) [Friedrich-Rust et al., 2008]. The mean AUROC for the diagnosis of significant fibrosis was 0.84 (95% CI, 0.82–0.86). The adjusted AUROC, which corrects for liver biopsy quality, was 0.91. The best results were shown in a study analyzing patients with chronic hepatitis C

and normal aminotransferase levels in which two thirds of patients had Metavir fibrosis stage F0-1 [Friedrich-Rust et al., 2008]. In CHB patients, LSM cutoff value of 7.0 kPa was shown to have positive and negative predictive values as 84% and 65% respectively [Marcellin et al., 2005].

5.3.6.2 Diagnosis of severe fibrosis: F0-2 versus F3-4

In the two initial studies in chronic hepatitis C patients, the performance for severe fibrosis was good, with AUROCs of 0.90 and 0.91, respectively [Castera et al., 2003; Ziol et al., 2005]. Another recent meta-analysis identified 35 studies reported data on the AUROCs for severe fibrosis (≥F3) [Friedrich-Rust et al., 2008]. The mean AUROC for the diagnosis of severe fibrosis was 0.89 (95% CI, 0.88–0.91). No significant difference in the AUROCs was found between the different underlying liver diseases, between the different countries where the studies were performed, and between abstracts and full-length articles. However, a significant reduction of heterogeneity was found when differentiating between studies using different staging systems.

Analyzing quantitative factors (where available) showed a slight significant influence of body mass index on the AUROC. No significant effect of age, percentage of male patients, length of biopsy samples, and failure rate of LSM on the AUROC was found. Cutoff values of LSM with respective sensitivity and specificity were available in 13 studies for Metavir stage ≥F3 [Friedrich-Rust

et al., 2008]. In CHB patients, LSM cutoff value of 8.1 kPa was shown to have positive and negative predictive values as 83% and 84% respectively [Marcellin et al., 2005].

5.3.6.3 Diagnosis of cirrhosis: F0-3 versus F4

LSM is a very promising tool for the early detection of cirrhosis. In the two initial studies in chronic hepatitis C patients, the best performances were observed for cirrhosis (F4), with AUROCs of 0.95 and 0.97, respectively [Castera et al., 2003; Ziol et al., 2005]. A cutoff of 12.5 kPa yielded positive and negative predictive values of 77% and 95% respectively for the diagnosis of cirrhosis; whereas a cutoff of 14.6 kPa yielded positive and negative predictive values of 78% and 97%, respectively. When compared with standard laboratory tests and non-invasive scores, LSM had the best diagnostic performance for early detection of cirrhosis in chronic hepatitis C patients, avoiding liver biopsy in 90% of cases versus 82% with platelet count, 80% with Fibrotest, 78% with prothrombin index, 76% with prothrombin time or AST/ALT ratio, 70% with APRI and 45% with Lok index, respectively [Castera et al., 2007]. In patients without clinical or biological signs suggestive of cirrhosis, diagnosis could have been made in 70% with LSM, versus 42% with Fibrotest, 24% with APRI and 8% with AST/ALT ratio and 4% with the Lok index [Castera et al., 2007].

In a recent meta-analysis based on 9 studies [Talwalkar et al., 2007], the

pooled estimates for the diagnosis of cirrhosis were excellent: sensitivity 87% (95% CI, 84%–90%), specificity 91% (95% CI, 89%–92%), positive likelihood ratio 11.7 (95% CI, 7.9–17.1), and negative likelihood ratio 0.14 (95% CI, 0.10–0.20). A cutoff effect was identified as an important cause of heterogeneity for pooled results. Indeed, an optimal LSM cutoff for cirrhosis remains debated. Reported LSM cutoffs for cirrhosis range from 10.3 kPa in CHB [Marcellin et al., 2005] to 17.3 kPa in chronic cholestatic diseases [Corpechot et al., 2006b] (Table 5.1). In the largest series involving 1007 patients with various chronic liver diseases, it has been suggested that LSM cutoff values could be optimized if specifically defined for a particular aetiology [Ganne-Carrie et al., 2006]. Because CHB is the main cause of macronodular cirrhosis, it is possible that the amount of fibrosis is lower in the cirrhotic liver of CHB patients than in that of patients with cholestatic diseases. However, it must be kept in mind that these cutoff values have been defined using ROC curves in order to maximize sensitivity and specificity. Difference between cutoff values may be simply related to difference in cirrhosis prevalence in the studied populations as recently suggested for other non-invasive methods [Poynard et al., 2007a]. Prevalence of cirrhosis in fact varied widely across the different studies ranging from 7.5% to 38.5%. Although a cutoff value defined in a given population may be relevant, it may not be applicable to another population where the prevalence is different. Furthermore, NASH or chronic hepatitis C patients with massive steatosis (particularly those infected with HCV genotype 3), steatosis might influence

the cutoff values. Thus, further studies are needed to address the effect of steatosis.

5.3.7 Comparison and combination with serum markers of fibrosis

In general, serum markers only modest accuracy to diagnose advanced liver fibrosis [Rockey 2006, Hui AJG 2005]. Furthermore, most of these tests have only been evaluated in chronic hepatitis C patients and few of them can be reproduced by other investigators. Serum proteomic and glycomic markers have also been proposed recently but validation in larger scaled studies are required [Poon 2005, Callewaert 2004, Kam 2007].

Transient elastography has certain advantages over indices based on laboratory tests, in that it provides a more direct measurement of fibrosis, is not affected by intercurrent health disorders, and is theoretically applicable to all chronic liver diseases. On the other hand, the diagnostic performance was particularly affected in patients with elevated serum ALT levels [Coco et al., 2007]. Hence a second non-invasive test independent of the serum ALT or AST levels may be a good supplementary test to LSM. Among various serum test formulae, Forns index [Forns et al., 2002] and Hui index [Hui et al., 2005] are composed of clinical parameters other than ALT or AST levels.

In a study of 183 patients with chronic hepatitis C, the combination of transient elastography and Fibrotest offered the best diagnostic performance,

both for significant fibrosis (≥F2) and for severe fibrosis (F3–4) [Castera et al., 2003]. When transient elastography and Fibrotest matched, which was the case in 70% to 80% of cases, the results also matched with those of liver biopsy in 84% of cases of significant fibrosis (≥F2), in 95% of cases of severe fibrosis (≥F3) and in 94% of cases of cirrhosis (F=4).

A clinical management algorithm, using the combination of transient elastography and Fibrotest as part of the first-line work-up, was inferred from these results. Using this algorithm, liver biopsy would have been avoided in 140 (77%) of the 183 patients. In contrast, 19 patients (10%) who qualified for treatment on the basis of liver biopsy would have been offered follow-up instead, and three patients (1.5%) would have been offered treatment instead of follow-up. However, before this algorithm can be implemented in clinical practice, the issue of discordance between LSM and Fibrotest results needs to be solved. In the two studies which addressed this problem, more false-negatives were observed with LSM than Fibrotest [Poynard et al., 2007b] and it seems that LSM more often underestimated, whereas Fibrotest more frequently overestimated liver biopsy results [Castera et al., 2005].

Combination of either LSM and serum markers [Martinez et al., 2007] or 2 non-invasive serum markers (APRI and Fibrotest) sequentially [Sebastiani et al., 2006] appears to increase diagnostic accuracy in chronic hepatitis C patients for the detection of both significant fibrosis and cirrhosis. Recently,

two algorithms (LSM combined with Fibrotest versus APRI combined with Fibrotest) in the same population of chronic hepatitis C patients were compared [Castera et al., 2007]. The results suggest that both algorithms are effective and that their use in clinical practice would result in a reduction of liver biopsies in 48% to 71% of cases for the diagnosis of significant fibrosis and in 74% to 78% of cases for cirrhosis. The combination of LSM with Fibrotest [Castera et al., 2006; Colletta et al., 2005] or other serum markers [Moreno-Otero et al., 2006] may also be of interest in chronic hepatitis C patients with normal ALT values and in CHB patients [Castera et al., 2006]. In the Bordeaux experience in 266 CHB patients, the combination of LSM and Fibrotest allowed to exclude significant fibrosis (≥F2) in nearly 80% of 100 patients in inactive carrier stage.

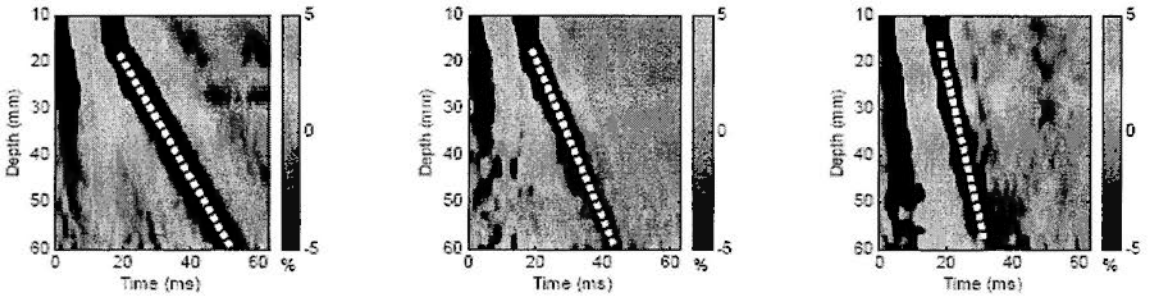
Table 5.1 Diagnostic performance of transient elastography for the diagnosis of histologic cirrhosis

Authors	Fraquelli et al. 2007	Ganne-Carrie et al. 2006	Foucher et al. 2006	Gomez-Dominguez et al. 2006	Marcellin et al. 2005	Coco et al. 2007	Ziol et al. 2005	Castera et al. 2005	de Ledinghen et al. 2006	Vergara et al. 2007	Rigamonti et al. 2008	Carrion et al. 2006	Yoneda et al. 2007	Corpechot et al. 2006
No. of biopsies	200	775	354	94	170	228	251	183	72	169	95	124	67	95
Prevalence of cirrhosis (*F4*; %)	12.0	15.5	13.3	17.0	8.0	20.2	19.0	25.0	23.6	38.5	17.0	11.0	7.5	16.0
Etiologies	All	All	All	All	HBV	HCV & HBV	HCV	HCV	HCV-HIV	HCV-HIV	HCV-LT	HCV-LT	NAFLD	PBC & PSC
Proposed cutoff values (kPa)	11.9	14.6	17.6	16.0	10.3	14.0	14.6	12.5	11.8	14.6	12.0	12.5	17.0	17.3
Sensitivity (%)	91	79	77	89	83	78	86	87	100	93	93	100	100	93
Specificity (%)	89	95	97	96	96	98	96	91	92.7	88	93	87	98	95
Negative predictive value (%)	98	96	92	98	97	82	97	95	82	94	99	100	95	99
Positive predictive value (%)	53	74	91	80	48	98	78	77	100	86	74	50	64	78
Positive LR	8.3	15.8	25.7	22.3	20.8	39.0	23.1	9.7	13.7	7.8	14.0	7.7	50.0	18.6
Negative LR	0.1	0.1	0.2	0.1	0.2	0.2	0.1	0.1	0	0.1	0.1	0	0	0.1
AUROC	0.90	0.95	0.96	0.94	0.92	0.96	0.97	0.95	0.97	0.95	0.90	0.98	0.99	0.96

Table 5.1 (continued)

HBV = hepatitis B virus infection, HCV = hepatitis C virus infection; HCV-HIV = hepatitis B virus and human immunodeficiency virus co-infection; HCV-LT = hepatitis C virus infection recurrence after liver transplantation; LR = likehood ratio; NAFLD = non-alcoholic fatty liver disease; PBC = primary biliary cirrhosis; PSC = primary sclerosing cholanigits; AUROC = Area under receiver operating characteristics curves.

Figure 5.1 Shear wave propagation velocity according to the severity of hepatic fibrosis (Metavir score)



F0 F2 F4

V_S = 1.0 m/s
E = 3.0 kPa

V_S = 1.7 m/s
E = 8.7 kPa

V_S = 3.0 m/s
E = 27.0 kPa

The elastic modulus E expressed as $E=3\rho V^2$, where V is the shear velocity and ρ is the mass density (constant for tissues): the stiffer the tissue, the faster the shear wave propagates. Hence, for absent fibrosis ($F0$), velocity is 1.0m/s and elasticity is 3.0 kPa, whereas for cirrhosis ($F4$) velocity is 3.0 m/s and elasticity is 27.0 kPa. Modified from Sandrin et al [Sandrin et al., 2003].

CHAPTER SIX

AIMS AND HYPOTHESIS OF THE CLINICAL STUDIES

6.1 Background of the Studies

Based on the understanding of the natural history of CHB, liver fibrosis is the important intermediate step for the progression of liver disease to cirrhosis and its various complications. Accurate assessment of liver fibrosis is of paramount importance while determining the prognosis and considering anti-viral treatment in CHB patients. LSM with transient elastography has been evaluated extensively as a non-invasive tool to assess liver fibrosis and it has high sensitivity and specificity to detect histologic liver cirrhosis [Talwalkar et al., 2007; Friedrich-Rust et al., 2008]. Despite these promising results, the use of LSM to replace liver biopsy in CHB patients has been hampered by the different cutoff values described in previous studies [Ganne-Carrié et al., 2006; Marcellin et al., 2005]. One reason for the vast difference in the LSM cutoff values might be related to the composition of patients with different ALT levels in different studies [Coco et al., 2007]. Two recent studies suggested that the degree of fibrosis could be overestimated by transient elastography in the presence of severe hepatitis [Sagir et al., 2008; Arena et al., 2008]. The optimal cutoff values for different stages of liver fibrosis for CHB patients with different ALT levels are yet to be defined.

On the other hand, the difference in the natural history of HBeAg-positive and negative CHB could be studied in more details with the transient elastography. HBeAg positivity is to be associated with more active hepatic inflammation and a higher risk of HCC in the long run [Yang et al., 2002]. While most Asian patients acquire the infection at infancy and do not have significant liver disease during the immune tolerant phase [Chan HL et al., 2002], some

patients may have abortive immune clearance resulting in significant liver fibrosis or even liver cirrhosis [Feld et al., 2006]. Differentiating these two conditions with indiscriminate liver biopsies are unlikely to gain general acceptance. The true relationship between HBV DNA, ALT and liver cirrhosis among HBeAg- positive patients, particularly the young patients in immune tolerance phase, have not been evaluated clearly in previous studies.

HBeAg seroconversion has been considered as an indicator of immune clearance, which is generally believed to reduce risk of hepatic decompensation and improved survival [Fattovich et al., 1991; Villeneuve et al., 1994]. However, 10% to 30% of patients continue to have elevated ALT and HBV DNA levels after HBeAg seroconversion [Chan et al., 2000a; Liaw et al., 1987a] and they have higher risk of developing complications of liver cirrhosis [Lok et al., 1987b; McMahon BJ et al., 2001]. Differentiation between HBeAg-negative chronic hepatitis and the inactive carrier-state is sometimes difficult as HBeAg-negative patients may have fluctuating HBV DNA and ALT levels [Sung et al., 2002; Chan et al., 2003]. The relationship between the serum ALT and HBV DNA levels with early liver cirrhosis is unclear.

Metabolic syndrome is known to be strongly associated with nonalcoholic fatty liver disease, characterized by hepatic steatosis with or without necroinflammation and fibrosis [Wong et al., 2004; Nugent et al., 2007]. It may result in histologic progression to cirrhosis, liver decompensation and liver cancer [Farrell et al., 2006; Adams et al., 2005]. The relationship between metabolic syndrome and CHB is unclear.

Therefore, a series of studies have been performed aiming to study the natural history and different clinical aspects of liver fibrosis in CHB with transient elastography. The results of these studies would be important in understanding the difference of the natural history of liver fibrosis in HBeAg-positive and negative patients, together with the effect of metabolic syndrome on liver fibrosis in CHB patients. The aims and hypothesis of this thesis can be summarized as follows.

6.2 Aims

1. To validate the accuracy of transient elastography to detect advanced liver fibrosis in CHB.
2. To study the optimal LSM cutoff values for different stages of liver fibrosis in CHB.
3. To study the clinical predictive factors of liver cirrhosis in CHB.
4. To study the effect of metabolic syndrome on liver cirrhosis in CHB.

6.3 Hypothesis

1. Transient elastography can accurately diagnose advanced liver fibrosis.
2. Different cutoff values of LSM can be defined for different stages of liver fibrosis.
3. The risk of liver cirrhosis increases with higher ALT and hepatitis B virus (HBV) DNA levels.
4. Metabolic syndrome is an independent risk factor of liver cirrhosis in CHB.

CHAPTER SEVEN

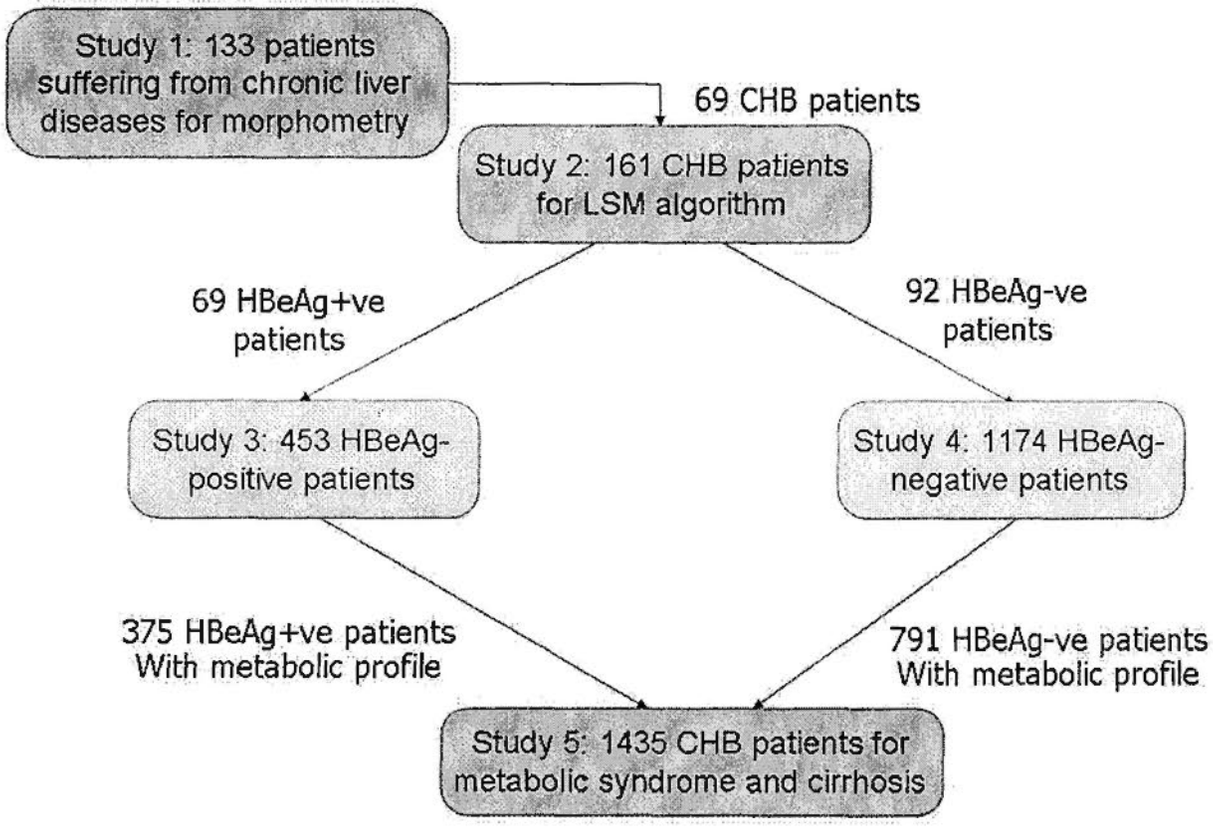
PATIENT RECRUITMENT AND INVESTIGATION METHODS

7.1 Overall Study Design

In order to provde my hypothesis of this research project, consecutive adult patients suffering from various chronic liver diseases and clinically indicated for liver biopsy examination, as well as all CHB patients fulfilling inclusion and exclusion criteria were prospectively recruited to have transient elastography. The flow diagram shown in Figure 7.1 describes the subsets of patients included in each study. First I prospectively recruited consecutive adult patients with chronic liver diseases who were clinically indicated for liver biopsy examination, in order to investigate the relationship of LSM and the distribution of liver fibrosis as determined by image and morphometric analysis (Study 1). The patients of Study 1, together with more CHB patients who underwent liver biopsy to assess of the severity of liver fibrosis and inflammation prior to treatment, were included in the analysis of Study 2 to define the optimal cutoff values of LSM for different stages of liver fibrosis in different ALT levels.

At the same time, I conducted a territory-wide screening program for treatment-naïve CHB patients from all clinics and hospitals in Hong Kong. Patients with positive HBeAg were analyzed in Study 3, while those with negative HBeAg were analyzed in Study 4, to study the clinical predictive factors of liver cirrhosis in CHB. Patients included in Study 2 formed parts of the subgroups of patients for internal validation of LSM cutoff vaules in Study 3 and 4. The patients in Study 3 and 4 with metabolic profile were analyzed in Study 5 to study the effect of metabolic syndrome on liver cirrhosis in CHB.

Figure 7.1 Flow diagram of patients included in the 5 studies



CHB: chronic hepatitis B; HBeAg: hepatitis B e antigen; +ve: positive; -ve: negative.

7.2 Patient Recruitment

In Study 1, consecutive adult patients suffering from various chronic liver diseases and clinically indicated for liver biopsy examination were prospectively recruited in Prince of Wales Hospital from July 2006 to June 2007 to validate the use of transient elastography. The indications of liver biopsy were to identify the etiology of liver disease and to assess the severity of liver fibrosis and inflammation prior to treatment. In Studies 2 to 5, adult CHB patients regardless of the disease activity were prospectively recruited for transient elastography from July 2006 to April 2008. CHB patients recruited in Study 1 were also included for analysis in Study 2 to 5 if appropriate. Referrals came from all primary care and hospital clinics in Hong Kong. Study 1 evaluated the use of transient elastography in chronic liver diseases with reference to liver histology. Study 2 evaluated the use of transient elastography in CHB patients with reference to liver histology. Study 3 evaluated the risk factors of liver fibrosis among patients with positive HBeAg. Study 4 evaluated the risk factors of liver fibrosis among patients with negative HBeAg and positive anti-HBe. Study 5 evaluated the impact of metabolic syndrome on liver fibrosis in CHB patients.

Chronic hepatitis C was diagnosed by positive serology tests for serum anti-HCV. NAFLD was diagnosed by ultrasonography and histology after exclusion of other possible etiologies of fatty liver [Chan HL et al., 2007]. Autoimmune liver disease and primary biliary cirrhosis were diagnosed with standard serological and histological criteria [Krawitt, 2006; Kaplan et al., 2005].

A group of patients belonged to a cohort of patients who were prospectively recruited and followed up every 3 to 6 months in our Hepatitis clinic since December 1997 [Chan et al., 2000b; Chan et al., 2004]. They were regularly monitored for liver biochemistry and HBeAg status. Transient elastography was performed at the time of last visit in this group of patients.

Every patient was interviewed with a standardized questionnaire concerning alcohol use. Men who consumed more than 30 grams of alcohol per week and women who consumed more than 20 grams of alcohol per week were excluded. CHB patients who received previous anti-viral or interferon treatment, and had decompensated liver disease, complications of liver cirrhosis, hepatocellular carcinoma, previous liver surgery or liver transplantation were excluded. Secondary causes of hepatic steatosis (e.g. chronic use of systemic corticosteroids and methotrexate) were also excluded. Patients with concomitant chronic liver diseases were excluded in Study 2 to 5.

7.3 Patient Evaluation

All patients received comprehensive clinical and laboratory assessment at the time of LSM, and liver biopsy was performed within 4 weeks from LSM. Anthropometric parameters including body weight, body height, hip circumference and waist circumference were measured. Body-mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Overweight was defined as body-mass index (BMI) ≥23 kg/m^2 and obesity as BMI ≥25 kg/m^2 according to the Asian and Chinese criteria [World Health Organization, 2000]. Metabolic syndrome was defined according to the International Diabetes Federation (IDF) criteria (see Chapter 2, section 2.1). [IDF 2007].

In the longitudinal part of Study 3, patients were categorized according to the ALT levels during the follow-up visits: 1. persistently ≤29 IU/l, which equals to 0.5x ULN (58 IU/l) during all the follow up visits; 2. intermittently >29 IU/l if they had fluctuating ALT levels with ALT levels >29 IU/l at some visits; and 3. persistently >29 IU/l if they had all ALT levels >29 IU/l. In the longitudinal part of Study 4, patients with ALT levels increased above ULN after normalization of ALT together with increased serum HBV DNA levels to above 2,000 IU/ml were defined as having reactivation of chronic HBV infection if other causes of elevated ALT were not identified [Hadziyannis et al., 2001]. The effect peak and trough ALT levels, the area under curve over time (AUC/t) ALT levels, duration of reactivation (in months), and the number of reactivations during follow-up were assessed.

7.4 Data Analysis

Qualitative and quantitative differences between subgroups were analyzed using Chi-square or Fisher's exact test for categorical parameters as appropriate and Student's t-test or Mann–Whitney test for continuous parameters as appropriate. Logistic regression analysis was used to identify factors associated with high LSM. Clinical parameters that were found associated with different severities of liver fibrosis on univariate analysis ($P<0.10$) were further analyzed by multivariate logistic regression model to evaluate the independent risk factors.

In Study 1, inter-observer agreement on morphometric analysis was evaluated by the intra-class correlation coefficient, which corresponded to the real agreement. Spearman's rank correlation coefficient was used to analyze the correlations between morphometric scores and the histologic fibrosis scores. Pearson's correlation coefficient was used to test the correlations between morphometric scores and LSM. Z–values calculated with the formulae by Steiger [Steiger et al., 1980] were used to compare the correlation coefficients from the analyses between morphometric scores and LSM. The overall accuracy of LSM in diagnosing histologic bridging fibrosis and cirrhosis was calculated using the receiver operating characteristics (ROC) curve and its 95 percent confidence intervals (CI). Areas under ROC were compared by DeLong test [DeLong et al., 1988].

In Study 2, optimal cutoff values for LSM were chosen either to obtain at least

90% sensitivity, at least 90% specificity, a maximum sum of sensitivity and specificity, and a maximum diagnostic accuracy (which was defined as the sum of true positives and true negatives over the total number of patients), according to the diagnostic question. The cutoff values derived by this method would be used to define insignificant fibrosis (F0-1) and advanced fibrosis (F3-4) after internally validated in the subgroup of patients with histologic evidence in Study 3. Based on the LSM distribution according to fibrosis stage and ROC curves for different ALT strata, cutoff values of associated with high sensitivity (≥90%) for histologic cirrhosis was identified to define "possible cirrhosis", such that liver cirrhosis could be confidently ruled out below these cutoff values. On the other hand, LSM cutoff values associated with high specificity (≥90%) for histologic cirrhosis was identified to define "probable cirrhosis", such that liver cirrhosis defined by these cutoff values would be very likely to be genuine cirrhosis. The cutoff values derived by this method, after an internal and external validation in the subgroups of patients with histologic evidence as well as an external validation in an indepdent cohort of CHB patients, would be used to define possible and probable cirrhosis in Study 4 and Study 5.

In Study 3 and 4, the effect of serum ALT and/or HBV DNA level on the development of advanced fibrosis, possible and probable cirrhosis were calculated as odds ratios and the 95% CI. The AUC/t ALT levels of patients who had been prospectively followed-up were calculated by trapezoidal estimation according to the ALT levels and follow-up periods. The ALT level was imputed in the Cox-regression model as a time-dependent covariate in

this cohort of patients. In Study 5, the effect of different components of metabolic syndrome on the development of liver cirrhosis was calculated as odds ratios and the 95% CI.

7.5 Serological Assays

Hepatitis B surface antigen (HBsAg) and hepatitis C antibodies (anti-HCV [third generation assay]) were tested by commercially available enzyme-linked immunosorbant assay kits (Abbott GmBH Diagnostika, Wiesbaden-Delkenheim, Germany). Hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) were measured by enzyme-linked immunosorbant assay (Sanofi Diagnostics, Pasteur, France).

7.6 HBV DNA Assays

Serum HBV DNA was quantified by the TaqMan real-time polymerase chain reaction (PCR) system (PE Biosystems, Foster City, CA) [Loeb et al., 2000; Chan et al., 2002b]. Serum samples were stored in -70°C refrigerators. One hundred μl serum was incubated with 50 μl buffer containing (final concentration) proteinase K 0.5 mg/ml, 10mM Tris-HCl pH 8.0, 20mM EDTA and 0.5% SDS at 50 °C for 2 hours. The samples were extracted by Qiaquick spin columns (Qiagen Inc., Chatsworth, CA) according to the instructions of the manufacturer. The extracted DNA was eluted with 30 μl H_2O.

PCR primers flanking HBV genome from nucleotide 1549 to 1653 (sense primer 50-CCGTCTGTGCCTTCTCATCTG and anti-sense primer 50-AGTCCAAGAGTYCTCTTATGYAAGACCTT) and a fluorescent probe (50-CCGTGTGCACTTCGCTTCACCTCTGC) were used. Each 50-ml reaction mixture contained 10 ml of DNA extract, 833 nM concentration of each primer, and 100 nM probe. After 2 min of incubation at 50°C and 2 min of denaturation at 95 °C, the mixture was subjected to 45 cycles of PCR at 95 °C for 20 seconds and 58 °C for 1 minute. The intensities of the fluorescent dyes in each reaction were read automatically during PCR cycling in a PE-Applied Biosystem Detector 7700 machine and the data was analyzed by sequence detector software (version 1.7a; Perkin Elmer, Inc., Foster City, CA). Each PCR run contained 2 negative controls and 2 positive controls, and each sample was run in duplicate. A standard curve was generated by serial 10-fold dilution of EUROHEP HBV standard (from Dr. K.H. Heerman, University of

Goettingen, Goettingen, Germany), which contained $2.7x10^9$ viral copies/ml. The range of HBV DNA detection was from 10^2-10^8 copies/ml with correlation coefficient greater routinely than 0.990. The standard curve was used to calculate the precise quantities of HBV DNA molecules for the unknown samples.

7.7 Histology

Percutaneous liver biopsy was performed using the 16G Temno needle. Liver histology was assessed by pathologists specialized in liver diseases (Dr Paul CL Choi and Dr Anthony WH Chan) without knowledge of the clinical data. Their interobserver agreement was 0.93 for fibrosis staging and 1.0 for the presence of stage 2 fibrosis or above. A liver sample was considered adequate if it was longer than 15 mm and contained 6 portal tracts or more. Liver biopsy specimens were prepared with hematoxylin and eosin stain, Masson trichrome stain, Prussian blue stain, reticulin stain, orcein stain and periodic acid Schiff stain for histologic assessment.

For patients with viral hepatitis, liver fibrosis and necroinflammatory activity were evaluated semi-quantitatively according to the Metavir scoring system as follows [Bedossa et al., 1996]: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. METAVIR activity score was defined as: A0, none; A1, mild; A2, moderate; and A3, severe. Hepatic steatosis was expressed as percentage of fat in the histology slides. The histologic grading and staging of NAFLD followed the Brunt's criteria as follows [Brunt et al., 1999]: macrovesicular steatosis was graded from 0 to 3, necroinflammatory activity was graded from 0 to 3, and fibrosis was staged from 0 to 4 (stage 0, absence of fibrosis; stage 1, pericellular or portal; stage 2, pericellular and portal/periportal; stage 3, septal or bridging fibrosis; and stage 4, cirrhosis). Cholestatic liver disease was staged according to Ludwig's score [Ludwig et al., 1978]. Hepatic

steatosis was expressed as percentage of fat in the histology slides. Bridging fibrosis was defined as Metavir or Brunt fibrosis score 3 or above, and liver cirrhosis was defined as Metavir or Brunt fibrosis score of 4.

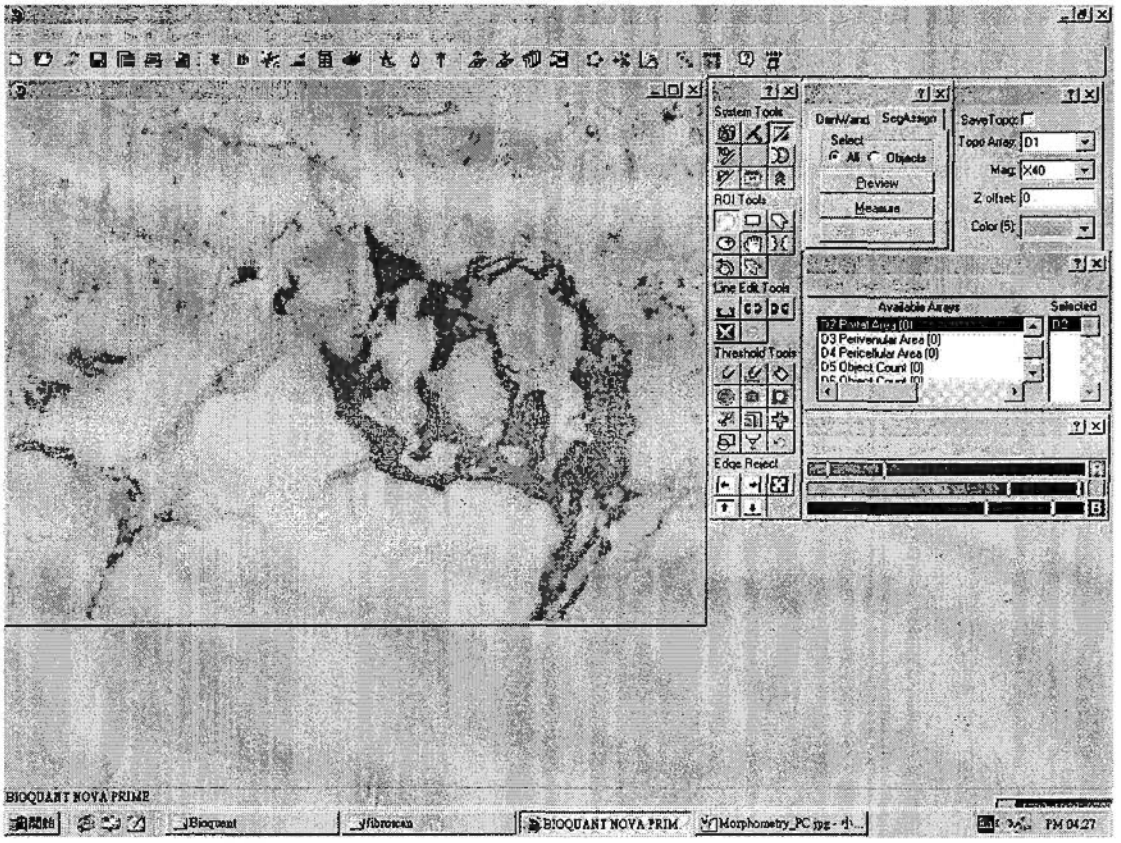
7.8 Morphometry

Morphometric analysis was used for the quantitative assessment of fibrosis in the liver biopsy [Hui et al., 2004]. Liver sections stained with 0.1% picrosirius red for collagen were subjected to morphometric analysis by three independent investigators (Mr Richard HL Chum, Mr Henry KW Chan and Mr Kenneth KK Lau) who were blind to the histologic score of the liver biopsies. We utilized a computerized image analysis system comprised of a photomicroscope (Axioplan 2) and digital camera (Axiocam) (Carl Zeiss Microscopy, Oberkochen, Germany) and the Bioquant Nova Prime software (Bioquant Image Analysis Corporation, Nashville, TN, USA). Bioquant Nova Prime was a Microsoft Windows application for semi-automated quantitative analysis of fixed histological sections. The software performed automatic measurement of areas defined using an interactive threshold editing function, which resulted in colored overlay that marked which pixels in the image were to be measured. Topographic memory function of the software ascertained that no overlapping fields were recorded. Measurement of red-stained area was carried out for each field captured. The mean area of fibrosis in mm^2 per field was calculated for each liver section.

We determined the areas of collagen in different zones of the liver by morphometric analysis (Figure 7.2). The areas of fibrosis or collagen were quantified in three different zones in mm^2: portal-periportal area (periportal), pericellular space (pericellular, which is more commonly seen in patients with alcoholic or nonalcoholic fatty liver disease) and around the centrolobular

venous area (perivenular). At least 5 periportal fields, 5 perivenular fields and 15 random fields of pericellular were captured on digitalized images at a final magnification of 400 times. Lumens, sinusoids and any other parts of the liver tissue that contained no collagen are excluded from the measurement. The mean areas per periportal, pericellular field and perivenular were calculated before further analyses. Twenty-five biopsy specimens of different fibrosis stages were randomly selected for morphometric analyses by all three investigators to assess inter-observer agreement.

Figure 7.2 Morphometric analysis with the Bioquant Nova Prime software



7.9 Transient Elastography

Liver stiffness measurement (LSM) was performed, within 4 weeks from the liver biopsy examination if histologic evidence was available, using transient elastography according to the instructions and training provided by the manufacturer (see Section 5.3.1). Three officially trained operators (Dr Grace LH Wong, Ms Angel ML Chim and Ms Karen KL Yiu) who had performed at least 50 measurements prior this study were responsible to perform the LSM, as operators with this kind of experience were found to have the best success rate and reproducibility in LSM [Fraquelli et al., 2007]. No treatment for the chronic liver disease was given during the 4-week period between liver biopsy examination and the LSM.

CHAPTER EIGHT

VALIDATION OF TRANSIENT ELGASTOGRAPHY

8.1 Transient Elastography Compared with Liver Biopsy and Morphometry (Study 1: Clin Gastroenterol Hepatol 2008;6:1027-1035)

8.1.1 Patient characteristics

One hundred and eighty-two patients suffering from chronic liver diseases underwent liver biopsy within the study period; 133 (73%) patients fulfilled the criteria for data analysis (Table 8.1). Thirty-seven patients were excluded because of inadequate liver biopsy samples size (<1.5cm and/or <6 portal tracts), 10 patients were excluded because of unsuccessful LSM (3 patients were overweight and 6 patients were obese) and 2 patients were excluded for both reasons (both patients were obese). Overall 38 out of the 182 patients (21%) had a BMI ≥28 kg/m^2, among whom 3 (8%) patients unsuccessful LSM. The clinical characteristics, disease distribution and laboratory parameters of the study cohort were well matched with the entire liver biopsy cohort. There was also no statistical difference on the severity of liver fibrosis, necroinflammation and steatosis between two cohorts.

8.1.2 Relationship between LSM and histology

Overall, LSM ranged from 3.3 kPa to 48.0 kPa (median 7.7 kPa). The mean number of measurements per patient was 12 ± 3. A total of 170 of 182 (93%) patients had 10 successful acquisitions and a success rate of at least 60% with median interquartile range 15% (1% to 30%). On morphometric analysis, the inter-observer agreement was excellent with intra-class correlation coefficients for pericellular, periportal and perivenular fibrosis 0.988, 0.990

and 0.993 respectively (all $P<0.001$) among the three independent investigators. The correlation of LSM and pericellular fibrosis on morphometry (r=0.43, $P<0.001$, Figure 8.1A) was better than that for periportal fibrosis (r=0.21, $P=0.026$; Z–value 2.2, $P=0.03$, Figure 8.1B) and perivenular fibrosis (r=0.25 $P=0.007$; Z–value 3.6, $P<0.001$, Figure 8.1C). The correlation of LSM and pericellular fibrosis was also better in patients with more severe fibrosis (F3–4; r=0.55, $P<0.001$) than that with mild fibrosis (F0–2, r=0.02, $P=0.9$; Z-value 3.6, $P<0.001$).

As pericellular fibrosis indicated more advanced fibrosis than periportal and perivenular fibrosis, we further analyzed the association of LSM with histologic bridging fibrosis and cirrhosis. The area under the ROC curves of LSM for bridging fibrosis and cirrhosis were 0.87 (95% CI 0.81–0.93, $P<0.001$) and 0.89 (95% CI 0.83–0.94, $P<0.001$), respectively (Figure 8.2). The prediction of cirrhosis by LSM seemed better than that of bridging fibrosis, though it did not reach the statistical significance (DeLong test $P=0.24$). Based on the LSM distribution according to fibrosis stage and ROC curves, a cutoff value of 8.4 kPa was associated with very high sensitivity (93%) and negative predictive value (96%) for cirrhosis (with specificity of 79% and positive predictive value of 67%). On the other hand, a LSM cutoff value of 13.4 kPa was associated with high specificity (95%) and positive predictive value (77%) for cirrhosis (with sensitivity of 41% and negative predictive value of 78%).

The effect of etiologies on the diagnostic performance of LSM was studied. Correlation of LSM with Metavir fibrosis score was good (r=0.51, $P<0.001$).

There was also a weak correlation of LSM and METAVIR activity score (r=0.22, *P*=0.01). The area under the ROC curve of LSM for cirrhosis was 0.86 (95% CI 0.78–0.94, *P*<0.001). A LSM cutoff value of 8.4 kPa was associated with 91% sensitivity and 79% specificity for cirrhosis while a cutoff value of 13.4 kPa was associated with 94% specificity and 34% sensitivity for cirrhosis.

Table 8.1 Clinical characteristics of the patients in Study 1

	All cases	Cases suitable for analysis	Cases excluded	*p* value*
Number of patients	182	133	49	
Male gender	122 (67%)	93 (70%)	29 (59%)	0.73
Age (year)	48.5 ± 10.0	48.4 ± 10.2	49.0 ± 9.5	0.85
Etiology				0.34
Chronic hepatitis B	90 (50%)	69 (52%)	21 (42%)	
Chronic hepatitis C	25 (14%)	18 (14%)	7 (14%)	
Non-alcoholic fatty liver disease	44 (24%)	34 (26%)	10 (20%)	
Others#	23 (13%)	12 (9%)	11 (22%)	
Biochemical				
Albumin (g/l)	43 ± 4	43 ± 4	43 ± 5	0.82
Bilirubin (μmol/l)	17 ± 49	14 ± 12	25 ± 93	0.20
Alkaline phosphatase (IU/l)	96 ± 63	91 ± 56	110 ± 77	0.17
Alanine aminotransferase (IU/l)	83 ± 84	86 ± 90	77 ± 64	0.95
Normal level	98 (54%)	71 (53%)	27 (55%)	
>1-5x upper limit of normal	84 (46%)	62 (47%)	22 (45%)	
Histology				
Length (mm)	16 ± 3	17 ± 2	13 ± 4	<0.001
Number of portal tracts	7 ± 3	7 ± 2	4 ± 2	<0.001
Steatosis (%)	16 ± 24	16 ± 23	17 ± 26	0.18
Bridging fibrosis	83 (45%)	63 (47%)	20 (41%)	0.27
Cirrhosis	58 (32%)	42 (32%)	16 (33%)	0.51

* The comparison was performed between the study cases and the excluded cases.

The etiologies of chronic liver diseases were classified as chronic hepatitis B, chronic hepatitis C, chronic hepatitis B and C co-infection, NAFLD, autoimmue hepatitis, primary biliary cirrhosis and cholestatic liver disease. Other etiologies included hepatitis B and C co-infection, autoimmue hepatitis, primary biliary cirrhosis and cholestatic liver disease.

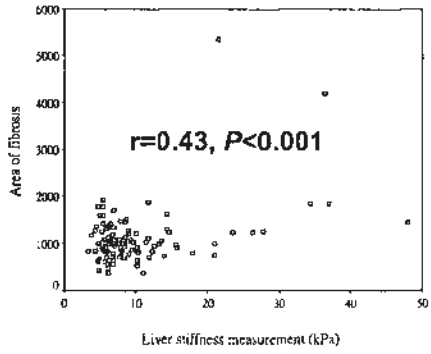
BMI = body mass index; NAFLD = non-alcoholic fatty liver disease; NI = necroinflammation; ULN = upper limit of normal.

Table 8.2 The diagnostic performances of liver stiffness measurement (LSM) for histologic cirrhosis

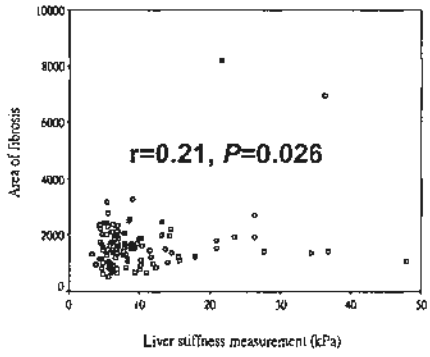
LSM cutoff values	**8.4 kPa**	**13.4 kPa**
Sensitivity (%)	93	41
Specificity (%)	79	95
Positive predictive value (%)	67	77
Negative predictive value (%)	96	78
Diagnostic accuracy (%)	83	77
Likelihood ratio for positive test	67	24

Figure 8.1 Scatter plots of the relationship between areas of fibrosis and liver stiffness measurements (LSM) in different regions: A. pericellular region, B. perivenular region, and C. periportal region.

A.



B.



C.

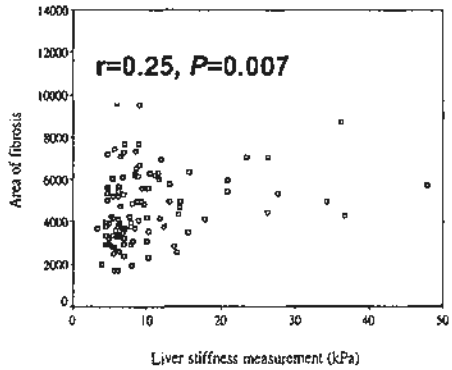
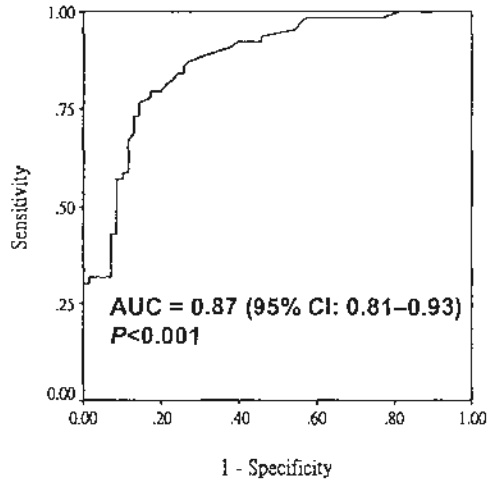
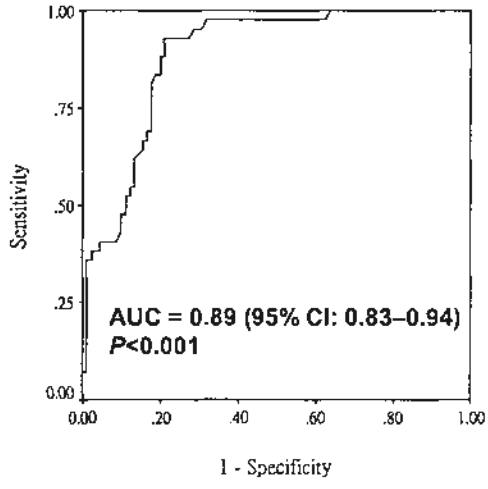


Figure 8.2 Receiver operating characteristics (ROC) curves of liver stiffness measurements (LSM) for A. bridging fibrosis and B. liver cirrhosis. AUC = area under curve; CI = confidence interval.

A.



B.



8.2 Alanine Aminiotransferase Based Algorithms (Study 2: J Viral Hepat 2009;16:36-44)

8.2.1 Patient characteristics

One hundred and eighty-six CHB patients underwent liver biopsy within the study period; 161 (87%) patients fulfilled the criteria for analysis. Twenty-two patients were excluded because of inadequate liver biopsy samples size (<1.5cm and/or <6 portal tracts), one patient was excluded because of unsuccessful LSM and two patients were excluded for both reasons. The clinical characteristics, disease distribution and laboratory parameters of the study cohort (n = 161) were well matched with the entire liver biopsy cohort (Table 8.3). These patients had been followed up in the Hepatitis clinic for median of 28 (range 4 to 92) months. Among the 58 patients with normal ALT at time of liver biopsy, 56 (97%) had normal ALT during the 6 months prior liver biopsy. Among the 98 patients with elevated ALT at time of liver biopsy, 56 (57%) had fluctuating ALT from normal to elevated ALT during the 6 months prior liver biopsy, while 42 (43%) had persistently elevated ALT during the last 6 months.

8.2.2 Relationship between LSM and histology

One hundred and eighty-three of 186 (98%) patients had 10 successful LSM acquisitions and a success rate of at least 60%. The mean number of measurements per patient was 12±2. Among the 161 patients studied, the LSM was 10.3 ± 6.4 kPa and the success rate of LSM acquisition was 90 ±

14%. The mean length of liver biopsy was 19 ± 3 mm and the number of portal tracts was 10±5. The median Metavir activity score was 2 (0–3) and fibrosis score was 2 (0–4). Nine (6%) patients had F0, 27 (17%) patients had F1, 47 (29%) patients had F2, 38 (40%) patients had F3 and 40 (25%) patients had F4 fibrosis. Bridging fibrosis (F3 and F4) was therefore present in 78 (48%) patients and cirrhosis (F4) in 40 (25%) patients. The box plots of liver stiffness for each fibrosis stage was shown in Figure 8.4. The median liver stiffness was 5.9 kPa (3.1 – 8.9 kPa) for F0, 5.9 kPa (2.5 – 10.2 kPa) for F1, 7.0 kPa (3.9 – 19.6 kPa) for F2, 8.8 kPa (4.8 – 34.3 kPa) for F3 and 14.2 kPa (8.0 – 36.9 kPa) for F4 fibrosis.

8.2.3 Effect of aminotransferase levels on LSM

Elevated ALT level was associated with higher LSM (odds ratio 2.8, 95% CI 1.6–5.0, P<0.001). Other factors including age, gender, anthropometric parameters and other biochemical parameters had no effect on LSM. Fifty-eight (36%) patients had normal ALT, 98 (61%) patients had ALT >1 to 5 times upper limit of normal (ULN), and 5 (3%) patients had ALT >5x ULN. There were similar proportions of patients with bridging fibrosis (48%, 48% and 40%, *P*=0.78) or liver cirrhosis (26%, 25% and 20%, *P*=0.65) in different ALT categories (normal, >1 to 5x ULN and >5x ULN, respectively). Owing to the small number of patients with ALT >5x ULN, these patients were excluded from the subsequent analysis on the relationship of LSM and ALT levels.

Patients with elevated ALT levels tend to have higher LSM than those with normal ALT levels at the same stage of liver fibrosis. Among patients who had no fibrosis (F0), the median LSM of patients with elevated ALT (6.3 kPa, range 3.1–8.9 kPa) had a tendency to be higher than that of patients who had normal ALT levels (4.7 kPa, range 4.0–6.7 kPa, P=0.29). This phenomenon was similar for patients who had bridging fibrosis, as the median LSM of patients with elevated ALT (12.6, range 4.8–36.9 kPa) also tend to be higher than that of patients who had normal ALT levels (10.7, range 5.2–34.3 kPa, P=0.07). Among patients with liver cirrhosis, the median LSM of patients with elevated ALT (16.6, range 8.0–36.9 kPa) was significantly higher than that of those with normal ALT levels (12.3, range 9.0–34.3 kPa, P=0.02).

The areas under the ROC curves of LSM for no fibrosis decreased from 0.88 (95% CI 0.73–1.0) to 0.76 (95% CI 0.61–0.91) as ALT levels increased from normal to elevated (P=0.01). On the other hand, the areas under the ROC curves of LSM for bridging fibrosis and cirrhosis did not decrease significantly with increasing ALT levels. The area under ROC curve for bridging fibrosis changed from 0.90 (95% CI 0.82–0.98) for normal ALT to 0.87 (95% CI 0.80–0.94) for elevated ALT (P=0.28). The area under ROC curve for liver cirrhosis changed from 0.96 (95% CI 0.92–1.00) for normal ALT to 0.94 (95% CI 0.88–0.99) for elevated ALT (P=0.53).

8.2.4 Optimal cutoff values of LSM

Four optimal cutoff values were defined to differentiate different stages of

liver fibrosis: at least 90% sensitivity, a maximum sum of sensitivity and specificity, at least 90% specificity, and a maximum diagnostic accuracy (Table 8.4). For F0 *vs.* F1–4, these optimal cutoff values ranged from 4.2 kPa to 9.0 kPa. For F0–2 *vs.* F3–4, these optimal cutoff values ranged from 6.0 kPa to 11.3 kPa. For F0–3 *vs.* F4, these optimal cutoff values ranged from 8.4 kPa to 13.4 kPa.

As patients with higher ALT levels tend to have higher LSM than those with lower ALT levels at the same stage of liver fibrosis, we further analyzed the effect of ALT levels on these optimal cutoff values (Table 8.4). For patients with normal ALT levels, the optimal cutoff values tend to be lower: ranging from 5.0–6.8 kPa for no fibrosis (F0), 6.0–9.0 kPa for bridging fibrosis (F3 and F4) and 8.4–12.0 kPa for cirrhosis (F4). For patients with elevated ALT levels, the optimal cutoff values tend to be higher: ranging from 5.0–9.0 kPa for no fibrosis (F0), 5.0–12.0 kPa for bridging fibrosis (F3 and F4) and 8.4–13.4 kPa for cirrhosis (F4).

We therefore derived an algorithm for using LSM to determine liver fibrosis in chronic hepatitis B (Figure 8.4). A LSM of less than 5.0 kPa should indicate no liver fibrosis regardless of the ALT levels. For patients with normal ALT, a LSM of 5.0–6.0 kPa should indicate insignificant fibrosis not to the stage of bridging fibrosis, a LSM of 6.0–9.0 kPa was the gray zone and fibrosis could range from nil to cirrhosis, a LSM greater than 9.0 kPa had a high chance of bridging fibrosis and that greater than 12.0 kPa had a high chance of cirrhosis.

For patients with elevated ALT levels, a LSM of 5.0–7.5 kPa indicated insignificant fibrosis not to the stage of bridging fibrosis, a LSM greater than 7.5–12.0 kPa was the gray zone and fibrosis could range from nil to cirrhosis, a LSM greater than 12.0 kPa had a high chance of bridging fibrosis and a LSM greater than 13.4 had a high chance of cirrhosis. Based on these results, the ALT-based LSM algorithm for possible cirrhosis was defined as LSM > 9.0 kPa if normal ALT and > 12.0 kPa if ALT >1-5x ULN, and probable cirrhosis as LSM > 12.0 kPa if normal ALT and > 13.4 kPa if ALT >1-5x ULN.

Based on this algorithm, 62% (36/58) of patients with normal ALT (13% could be reassured, 16% could be observed; 13% and 19% might have bridging fibrosis and cirrhosis respectively and treatment should be considered) and 58% (57/98) of patients with elevated ALT (8% could be reassured; 24% could be observed; 2% and 24% might have bridging fibrosis and cirrhosis respectively and treatment should be considered) could be exempted from liver biopsy. Twenty-two of 58 (38%) patients with normal ALT and 41 of 98 (42%) patients with elevated ALT fell in the gray zone of LSM and might required liver biopsy to stage the liver fibrosis.

8.2.5 External validation of cutoff values of LSM

An independent cohort of 82 newly recruited CHB patients who had liver biopsy performed formed the external validation set for the performance of the ALT-based LSM algorithm [Wong et al., 2010]. The area under the ROC curve of LSM in this validation cohort was 0.80 (95% confidence interval

0.68 – 0.92, $P<0.001$). The sensitivity of the cutoff values of possible cirrhosis to diagnose histologic cirrhosis was 86%; while the specificity of the cutoff values of probable cirrhosis was 81%.

Table 8.3 Clinical characteristics of the patients in Study 2

	All cases	Cases suitable for analysis	Cases excluded	*P* value*
Number of patients	186	161 (87%)	25 (13%)	
Follow-up duration (months)	27 (2 – 81)	28 (4 – 92)	25 (2 – 81)	0.86
Male gender	141 (86%)	122 (76%)	19 (76%)	0.98
Age (year)	45 ± 11	45 ± 11	47 ± 9	0.44
Biochemical				
Albumin (g/l)	43 ± 5	43 ± 5	42 ± 5	0.61
Bilirubin (μmol/l)	15 ± 13	15 ± 13	14 ± 16	0.02
Alkaline phosphatase (IU/l)	80 ± 37	80 ± 39	74 ± 22	0.49
Alanine aminotransferase (IU/l)	89 ± 75	93 ± 78	58 ± 45	0.002
Normal level	66 (35%)	58 (36%)	8 (32%)	
>1-5x upper limit of normal	114 (61%)	98 (61%)	16 (64%)	
Hepatitis B e antigen				0.33
Positive	76 (41%)	69 (43%)	7 (28%)	
Negative	110 (59%)	92 (57%)	18 (72%)	
Log_{10} [HBV DNA] (copies/ml)	6.4 ± 1.7	6.5 ± 1.7	6.0 ± 1.7	0.23
Bridging fibrosis	83 (45%)	78 (48%)	14 (56%)	0.23
Cirrhosis	58 (32%)	40 (25%)	6 (24%)	0.87

* The comparison was performed between the study cases and the excluded cases.

Data were expressed in number (percentage), mean ± standard deviation or median (range).

HBV = Hepatitis B virus; LSM = liver stiffness measurement, kPa = kiloPascal.

Table 8.4 Optimal cutoff values for different degrees of liver fibrosis with respective to different alanine aminotransferase levels

Fibrosis stage*	Category#	Cutoff (kPa)	Sn (%)^	Sp (%)^	PPV (%)^	NPV (%)^	LR(+)^	LR(-)^	DA (%)^
All cases									
F0	Sn	5.0	92 (89–97)	40 (14–73)	96 (91–98)	25 (8–53)	1.5 (1.0–2.6)	0.20 (0.09–0.46)	89 (86–94)
	Sn + Sp	6.8	72 (64–79)	80 (44–96)	98 (93–100)	16 (7–30)	3.6 (1.0–12.5)	0.35 (0.26–0.47)	73 (65–79)
	Sp	9.0	46 (38–54)	100 (66–100)	100 (93–100)	11 (5–20)	infinite	0.54 (0.47–0.63)	49 (41–65)
	DA	4.2	98 (94–99)	20 (4–56)	95 (90–98)	40 (7–83)	1.2 (0.9–1.7)	0.10 (0.02–0.67)	93 (90–95)
F3	Sn	6.0	96 (88–99)	37 (27–48)	58 (49–67)	91 (75–98)	1.5 (1.3–1.8)	0.11 (0.03–0.33)	65 (56–74)
	Sn + Sp	8.4	84 (74–91)	76 (65–85)	77 (66–85)	84 (74–91)	3.5 (2.4–5.3)	0.20 (0.12–0.35)	80 (10–89)
	Sp	11.3	55 (43–66)	95 (88–98)	91 (78–97)	70 (60–78)	11.5 (4.3–30.4)	0.47 (0.37–0.61)	76 (66–85)
	DA	8.4	84 (74–91)	76 (65–85)	77 (66–85)	84 (74–91)	3.5 (2.4–5.3)	0.20 (0.12–0.35)	80 (10–89)
F4	Sn	8.4	98 (85–100)	62 (53–71)	46 (35–57)	99 (92–100)	2.6 (2.0–3.2)	0.04 (0.01–0.28)	71 (62–77)
	Sn + Sp	9.0	98 (85–100)	75 (66–82)	57 (44–68)	98 (93–100)	1.0 (0.93–1.00)	0.01 (0–0.07)	81 (70–86)
	Sp	13.4	60 (43–75)	93 (87–97)	75 (56–88)	88 (80–93)	9.1 (4.4–18.6)	0.43 (0.29–0.63)	85 (74–95)
	DA	13.4	60 (43–75)	93 (87–97)	75 (56–88)	88 (80–93)	9.1 (4.4–18.6)	0.43 (0.29–0.63)	85 (74–95)
Normal alanine aminotransferase levels									
F0	Sn	5.0	91 (79–97)	75 (22–99)	98 (88–100)	38 (10–74)	3.6 (0.7–19.9)	0.12 (0.05–0.34)	90 (71–100)
	Sn + Sp	5.0	91 (79–97)	75 (22–99)	98 (88–100)	38 (10–74)	3.6 (0.7–19.9)	0.12 (0.05–0.34)	90 (71–100)
	Sp	6.8	65 (51–77)	100 (40–100)	100 (88–100)	17 (6–40)	infinite	0.35 (0.25–0.50)	67 (46–78)
	DA	5.0	91 (79–97)	75 (22–99)	98 (88–100)	38 (10–74)	3.6 (0.7–19.9)	0.12 (0.05–0.34)	90 (71–100)
F3	Sn	6.0	93 (75–99)	47 (29–65)	62 (46–76)	88 (60–98)	1.7 (1.2–2.5)	0.15 (0.04–0.53)	69 (51–81)
	Sn + Sp	9.0	71 (51–86)	100 (86–100)	100 (80–100)	79 (62–90)	infinite	0.29 (0.16–0.41)	86 (70–94)
	Sp	9.0	71 (51–86)	100 (86–100)	100 (80–100)	79 (62–90)	infinite	0.29 (0.16–0.41)	86 (70–94)
	DA	9.0	71 (51–86)	100 (86–100)	100 (80–100)	79 (62–90)	infinite	0.29 (0.16–0.41)	86 (70–94)
F4	Sn	8.4	100 (75–100)	77 (61–88)	60 (39–78)	100 (87–100)	4.3 (2.5–7.4)	0	83 (72–90)
	Sn + Sp	9.0	100 (75–100)	88 (74–96)	75 (51–90)	100 (89–100)	8.6 (3.8–19.6)	0	91 (72–96)
	Sp	12.0	60 (33–83)	95 (83–99)	82 (48–97)	87 (74–95)	12.9 (3.1–53.1)	0.42 (0.23–0.78)	86 (67–98)
	DA	9.0	100 (75–100)	88 (74–96)	75 (51–90)	100 (89–100)	8.6 (3.8–19.6)	0	91 (72–96)

Table 8.4 (continued)

Fibrosis stage*	Category#	Cutoff (kPa)	Sn (%)^	Sp (%)^	PPV (%)^	NPV (%)^	LR(+)^	LR(-)^	DA (%)^
Alanine aminotransferase levels > 1 to 5 times upper limit of normal									
F0	Sn	5.0	92 (84–97)	17 (1–64)	94 (87–98)	13 (1–53)	1.1 (0.77–1.6)	0.46 (0.04–4.76)	88 (76–98)
	Sn + Sp	9.0	50 (39–61)	100 (51–100)	100 (90–100)	11 (5–24)	infinite	0.50 (0.41–0.61)	53 (31–59)
	Sp	9.0	50 (39–61)	100 (51–100)	100 (90–100)	11 (5–24)	infinite	0.50 (0.41–0.61)	53 (31–59)
	DA	5.0	92 (84–97)	17 (1–64)	94 (87–98)	13 (1–53)	1.1 (0.77–1.6)	0.46 (0.04–4.76)	88 (76–98)
F3	Sn	7.5	96 (84–99)	59 (44–72)	68 (55–79)	94 (78–99)	2.3 (1.7–3.2)	0.07 (0.02–0.29)	77 (64–85)
	Sn + Sp	8.4	87 (74–95)	69 (54–80)	72 (58–83)	85 (70–94)	2.8 (1.8–4.2)	0.19 (0.09–0.40)	78 (64–97)
	Sp	12.0	51 (36–66)	98 (88–100)	96 (78–100)	68 (56–79)	26.0 (3.7–185)	0.50 (0.37–0.67)	76 (64–84)
	DA	8.4	87 (74–95)	69 (54–80)	72 (58–83)	85 (70–94)	2.8 (1.8–4.2)	0.19 (0.09–0.40)	78 (64–97)
F4	Sn	8.4	96 (77–100)	54 (42–55)	40 (28–54)	98 (86–100)	2.1 (1.6–2.7)	0.07 (0.01–0.54)	63 (43–65)
	Sn + Sp	12.0	79 (57–92)	92 (83–97)	76 (54–90)	93 (84–97)	9.8 (4.4–21.6)	0.23 (0.10–0.50)	89 (74–98)
	Sp	13.4	75 (53–89)	93 (84–97)	78 (56–92)	92 (83–97)	11.1 (4.6–26.7)	0.27 (0.13–0.54)	89 (74–98)
	DA	13.4	75 (53–89)	93 (84–97)	78 (56–92)	92 (83–97)	11.1 (4.6–26.7)	0.27 (0.13–0.54)	89 (74–98)

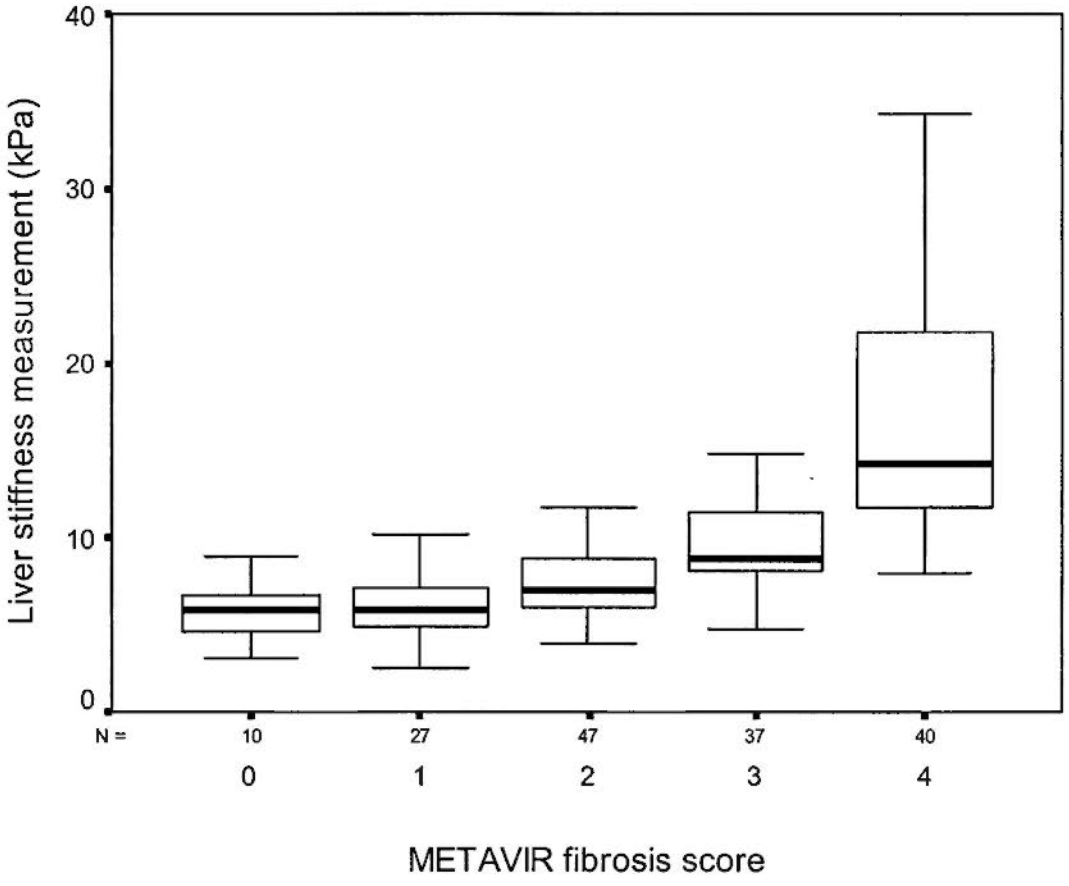
Fibrosis stage*: F0 = F0 *vs.* F1–4; F3 = F0–2 *vs.* F3–4; F4 = F0–3 *vs.* F4.

Category#: Sn = at least 90% sensitivity; Sn + Sp = a maximum sum of sensitivity and specificity; Sp = at least 90% specificity; DA = a maximum diagnostic accuracy.

^95% confidence intervals were shown in blankets.

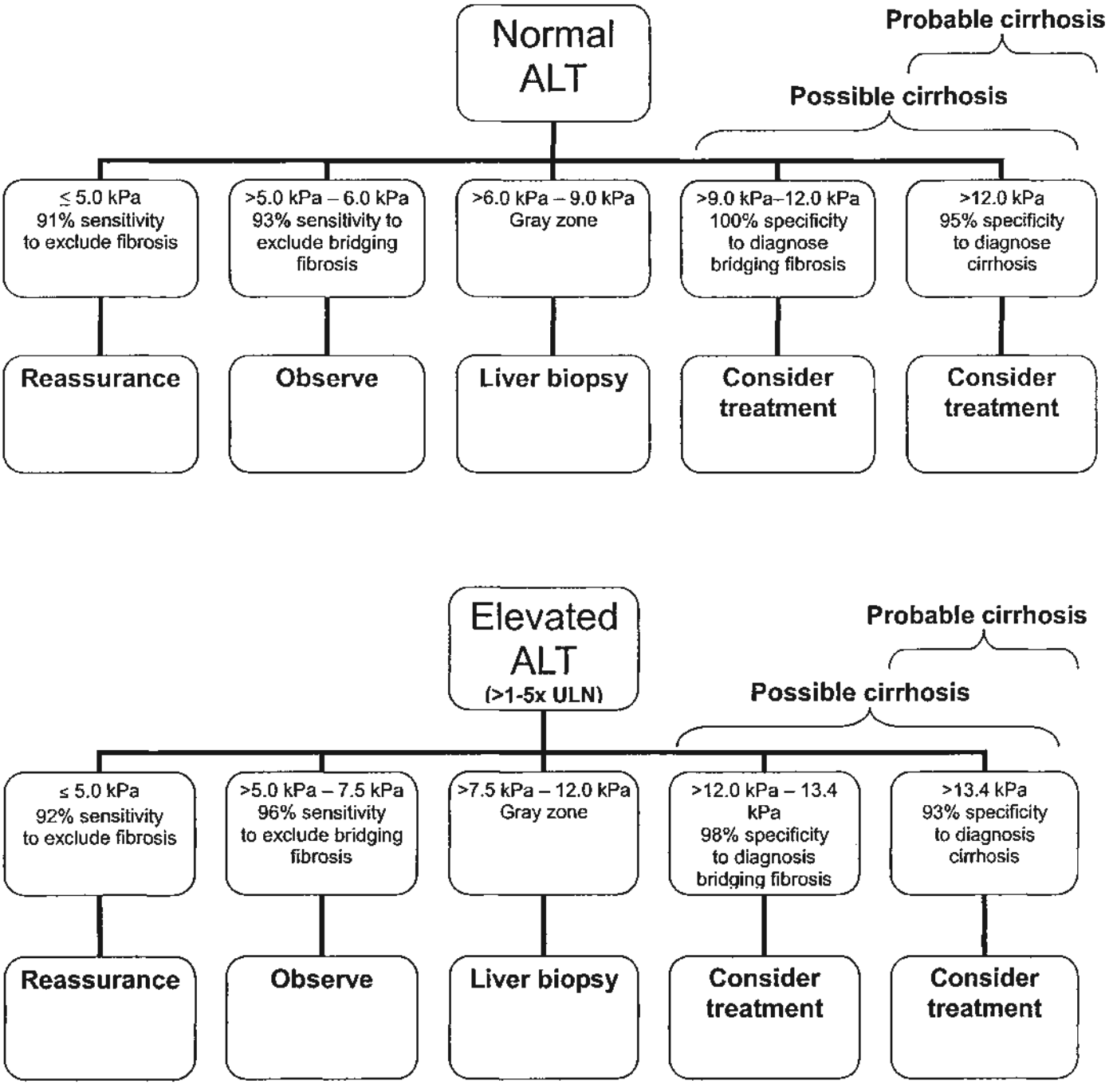
kPa = kilopascal; Sn= sensitivity, Sp = specificity, PPV = positive predictive value, NPV = negative predictive value; LR(+) = positive likelihood ratio; LR(-) = negative likelihood ratio; DA = diagnostic accuracy.

Figure 8.3 Liver stiffness values for each fibrosis stage (F0 to F4) in chronic hepatitis B patients



The top and bottom of the boxes are the first and third quartiles. The length of the box represents interquartile ranges, within which are located 50% of the values. The lines through the middle of the boxes represent median values.

Figure 8.4 Recommended clinical algorithm of liver stiffness measurement for patients with normal and elevated ALT (>1-5x ULN) levels



ULN = upper limit of normal; ALT = alanine aminotransferase.

CHAPTER NINE

FACTORS ASSOCIATED WITH LIVER FIBROSIS IN HEPATITIS B E ANTIGEN-POSITIVE CHRONIC HEPATITIS B

9.1 Cross-sectional Study of Clinical Factors and Liver Fibrosis (Study 3: Clin Gastroenterol Hepatol 2009;7:227-233)

9.1.1 Patient characteristics

Four hundred and eighty-seven HBeAg-positive CHB patients underwent LSM within the study period. After excluding 11 patients who failed transient elastography because of obesity, 6 patients with unreliable LSM (success rate below 60%), and 17 patients with ALT >5x ULN, 453 (93%) patients were included in the analysis of Study 3 (Table 9.1). The median success rate of LSM was 100% (interquartile range 81%, 100%).

Eighty patients had both transient elastography and liver biopsies; 74 (93%) patients fulfilled the criteria for data analysis. Five patients were excluded because of inadequate liver biopsy size (<1.5cm and/or <6 portal tracts) and one patient was excluded because of unsuccessful LSM. The clinical characteristics, disease distribution and laboratory parameters of this biopsy group were representative of the entire study cohort (Table 9.1). Seventeen patients (23%), 36 patients (49%) and 21 patients (28%) had histologic insignificant fibrosis (F0-1), mild-to-moderate fibrosis (F2) and advanced fibrosis or possible cirrhosis (F3-4) respectively.

The cutoff values proposed by Study 2 were prospectively validated in this

group of patients with liver biopsies performed. At the LSM cutoff values for insignificant fibrosis (≤6.0 kPa for normal ALT and ≤7.5 kPa for ALT >1-5x ULN), the sensitivity to exclude fibrosis ≥F2 was 90%. The cutoff values for advanced fibrosis or possible cirrhosis (>9.0 kPa for normal ALT and >12.0 kPa for elevated ALT) was associated with specificity of 95%.

Patients were divided into three groups according to the LSM cutoff values for normal and elevated ALT: insignificant fibrosis (216 patients, 48%); mild-to-moderate fibrosis (135 patients, 30%); and possible cirrhosis (102 patients, 22%). One hundred twenty-four patients (27%) had LSM indicative of insignificant fibrosis and normal ALT levels, and these patients were likely in the immune tolerant phase. Patients who had more severe liver fibrosis were older, had lower serum albumin, higher serum total bilirubin, alkaline phosphatase, ALT and alpha-fetoprotein levels. On the other hand, serum HBV DNA level decreased with increasing severity of liver fibrosis (Table 9.1).

9.1.2 Relationship of patient age and liver fibrosis

Age had positive correlation with LSM (r=0.30, P<0.001) (Figure 9.1). Probability of insignificant fibrosis decreased with age, while those of possible cirrhosis increased with age (Figure 9.2, P<0.001). To determine the optimal age from which assessment of liver fibrosis was warranted, we used receiver operating characteristics (ROC) analysis for age versus LSM cutoff values for possible cirrhosis. Age of 35 has a high specificity for the LSM cutoff values

for possible cirrhosis s (91%).

9.1.3 Relationship of ALT and liver fibrosis

The probability of insignificant fibrosis decreased while that of possible cirrhosis increased with increasing ALT level (Figure 9.3, $P<0.001$). We further studied the effect of ALT by stratifying patients into five groups according to the ALT levels: ≤0.5x ULN (16%); >0.5–1x ULN (38%); >1–2x ULN (33%); and >2–5x ULN (13%). The odds ratio of possible cirrhosis increased with increasing ALT level strata (Table 9.2). Patients who had ALT >0.5–1x ULN started to have significantly increased risk of possible cirrhosis (odds ratio 5.0, 95% CI 1.9–13.1, $P<0.001$) as compared to patients with ALT≤0.5x ULN. We also tested the relationship of ALT and possible cirrhosis by stratifying patients into four groups using the new ALT cutoff values proposed by Prati and colleagues (30 IU/l for men and 19 IU/l for women) [Prati et al., 2002]: <1x ULN (18%); 1–2x ULN (28%); 2–5x ULN (43%); and >5x ULN (11%). Similarly, the odds ratio of possible cirrhosis increased with increasing ALT level strata.

9.1.4 Relationship of HBV DNA and liver fibrosis

There was a bell-shaped relationship between LSM and serum HBV DNA as illustrated by the box-plot (Figure 9.4). Patients with very high and very low HBV DNA tended to have lower LSM, while patients with intermediate HBV DNA levels (around 5–7 $\log_{10}$ copies/ml) tended to have higher LSM.

9.1.5 Independent factors associated with liver fibrosis

On multivariate analysis, older age (>35 years) and higher ALT level (>0.5x ULN) were independently and consistently associated with possible cirrhosis (Table 9.3). Male gender, obesity and serum HBV DNA level did not affect the severity of liver fibrosis.

Among the 47 patients who were aged 35 years or below and ALT ≤0.5x ULN, 39 (83%) had LSM indicative of insignificant fibrosis, and only one patient (2%) had LSM indicative of possible cirrhosis. On the other hand, among the 217 patients who were above 35 years with ALT >0.5x ULN, 80 patients (37%) had LSM indicative of possible cirrhosis (Table 9.4). The number of patients needed to be biopsied to detect one case of possible cirrhosis was 47 for patients aged≤35 years and ALT ≤0.5x ULN, while it was 3 for patients older than 35 years and ALT >0.5x ULN.

Table 9.1 Clinical characteristics of the patients according to the severity of liver fibrosis

	All cases	Biopsy group	Different severities of liver fibrosis by liver stiffness measurement (LSM)			
Fibrosis			**Insignificant**	**Mild-to-moderate**	**Advanced fibrosis (Possible cirrhosis)**	***P* value**
LSM cutoff values for						
Normal ALT			**≤6.0 kPa**	**>6.0-9.0 kPa**	**>9.0 kPa**	
Elevated ALT (>1-5xULN)			**≤7.5 kPa**	**>7.5-12.0 kPa**	**>12.0 kPa**	
Number of patients	453	74	216 (48%)	135 (30%)	102 (22%)	
Male gender	270 (60%)	55 (74%)	121 (57%)	85 (63%)	64 (63%)	0.38
Age (year)	37 (30-48)	38 (29-47)	32 (28-40)	41(32-48)	48 (38-56)	<0.001
Body mass index (kg/m^2)	22.4 (19.8-24.9)	23.8 (21.7-26.0)	21.7 (19.5-24.0)	23.0 (20.3-25.4)	23.5 (20.7-26.1)	0.001
Albumin (g/l)	44 (42-46)	44 (41-46)	44 (43-46)	44 (42-46)	42 (39-44)	<0.001
Bilirubin (μmol/l)	12 (9-17)	13 (10-17)	12 (9-17)	13 (10-17)	13 (10-18)	0.26
Alkaline phosphatase (IU/l)	69 (57-85)	71 (58-95)	65 (55-75)	71 (57-86)	85 (67-99)	<0.001
Alanine aminotransferase (IU/l)	55 (35-85)	83 (66-127)	52 (30-78)	56 (38-82)	60 (43-96)	0.001
Alpha-fetoprotein (μg/l)	3 (2-5)	4 (2-7)	2 (2-3)	3 (2-5)	6 (3-13)	<0.001
Log_{10} [HBV DNA] (copies/ml)	7.4 (6.3-8.6)	8.1 (6.7-8.7)	8.2 (6.9-8.7)	7.1 (6.1-8.3)	6.7 (6.0-7.7)	<0.001
Liver stiffness measurement (kPa)	6.8 (5.2-10.1)	8.7 (6.3-13.6)	5.1 (4.4-5.9)	8.1 (6.9-9.0)	14.7 (12.1-22.5)	<0.001

Table 9.1 (continued)

ALT = alanine aminotransferase; kPa = kiloPascal.

Remarks: categorical variables were presented as number (percentage in parentheses), while continuous variables were presented as median (interquartile range in parentheses).

Definition of liver fibrosis by liver stiffness measurement (LSM):

1. Insignificant fibrosis (F0-1): LSM ≤6.0 kPa for ALT ≤ ULN and ≤7.5 kPa for ALT >1-5x ULN;
2. Mild-to-moderate fibrosis (F2): LSM >6.0-9.0 kPa for ALT ≤ ULN and >7.5-12.0 kPa for ALT >1-5x ULN;
3. Advanced fibrosis or possible cirrhosis (F3-4): LSM >9.0 kPa for ALT ≤ ULN and >12.0 kPa for ALT >1-5x ULN.

Table 9.2 Risks of possible cirrhosis according to different serum alanine aminotransferase levels based on our cutoff values (58 IU/l) and gender-specific cutoff values

ALT levels	N (%)*	n/N (%)#	Odds ratio (95% CI)	*P* value
Our upper limit of normal (ULN) = 58 IU/l for both gender				
≤0.5x ULN	73 (16.1%)	5/73 (6.8%)	Referent	
>0.5–1x ULN	172 (38.0%)	46/172 (26.7%)	5.0 (1.9 – 13.1)	<0.001
>1–2x ULN	150 (33.1%)	33/150 (22.0%)	3.8 (1.4 – 10.3)	0.005
>2–5x ULN	58 (12.8%)	31/58 (31.0%)	6.1 (2.1 – 17.8)	<0.001
Gender-specific upper limit of normal (ULN) = 30 IU/l for men and 19 IU/l for women				
≤1x ULN	80 (18%)	8 (10%)	Referent	
>1–2x ULN	127 (28%)	25 (20%)	2.2 (0.9 – 5.2)	0.06
>2–5x ULN	196 (43%)	50 (26%)	3.1 (1.4 – 6.9)	0.004
>5 x ULN	50 (11%)	18 (36%)	5.0 (2.0 – 12.8)	<0.001

N (%)*: numbers (and percentage) of patients that particular ALT level among the whole cohort.

n (%)#: numbers (and percentage) of patients suffering from possible cirrhosis among that particular ALT level.

ALT = Alanine aminotransferase; CI = confident interval; ULN = upper limit of normal, 58 IU/l for both gender.

Table 9.3 Multivariate logistic regression analysis on factors associated with different severities of liver fibrosis

		Insignificant fibrosis, N = 216			Possible cirrhosis, N = 102		
Parameters	**N (%)**	**Odds ratio**	**95% CI**	***P* value**	**Odds ratio**	**95% CI**	***P* value**
Male gender	270 (60%)	1.0	0.9–1.0	0.08	0.9	0.5–1.4	0.45
Age >35 years	243 (54%)	0.4	0.2–0.9	0.03	4.7	2.7–8.3	<0.001
Obesity (BMI ≥25 kg/m^2)	104 (23%)	0.5	0.3–0.9	0.02	1.6	0.9–2.8	0.08
ALT>0.5xULN	380 (84%)	0.4	0.2–0.8	0.006	3.7	1.4–9.9	0.006
HBV DNA >5 $\log_{10}$ copies/ml	417 (92%)	1.2	0.6–2.6	0.62	0.5	0.2–1.1	0.10

CI = confident interval; ALT = alanine aminotransferase; BMI = body mass index.

Definition of liver fibrosis by liver stiffness measurement (LSM):

1. Insignificant fibrosis (F0-1): LSM ≤6.0 kPa for ALT ≤ ULN and ≤7.5 kPa for ALT >1-5x ULN;
2. Possible cirrhosis (F3-4): LSM >9.0 kPa for ALT ≤ ULN and >12.0 kPa for ALT >1-5x ULN.

Table 9.4 Distribution of the severities of liver fibrosis according to the age and alanine aminotransferase level

Age (years)	ALT levels (x ULN)	Insignificant fibrosis, n (%)	Mild-to-moderate fibrosis, n (%)	Advanced fibrosis or possible cirrhosis, n (%)	Number-needed-to-biopsy*
≤ 35	≤ 0.5 (N = 47)	39 (83%)	7 (14%)	1 (2%)	47
	> 0.5 (N = 163)	102 (63%)	44 (27%)	17 (10%)	10
> 35	≤ 0.5 (N = 26)	14 (54%)	8 (31%)	4 (15%)	7
	> 0.5 (N = 217)	61 (28%)	76 (35%)	80 (37%)	3

Number-needed-to-biopsy*: numbers of patients needed to be biopsied to detect one case of possible cirrhosis.

ALT = Alanine aminotransferase; ULN = upper limit of normal, 58 IU/l for both gender.

Figure 9.1 Box-plot of liver stiffness measurements against age

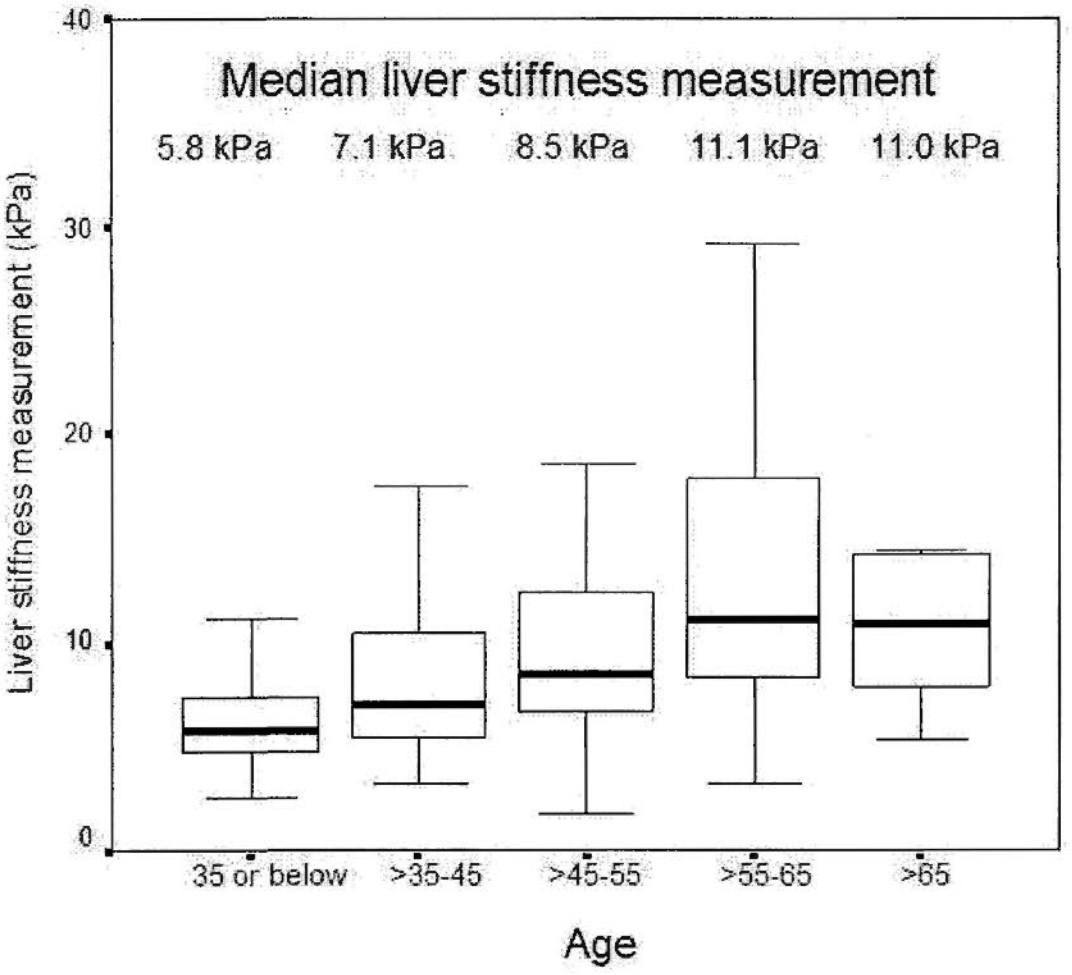


Figure 9.2 Bar charts on the prevalence of different severities of liver fibrosis in different age groups

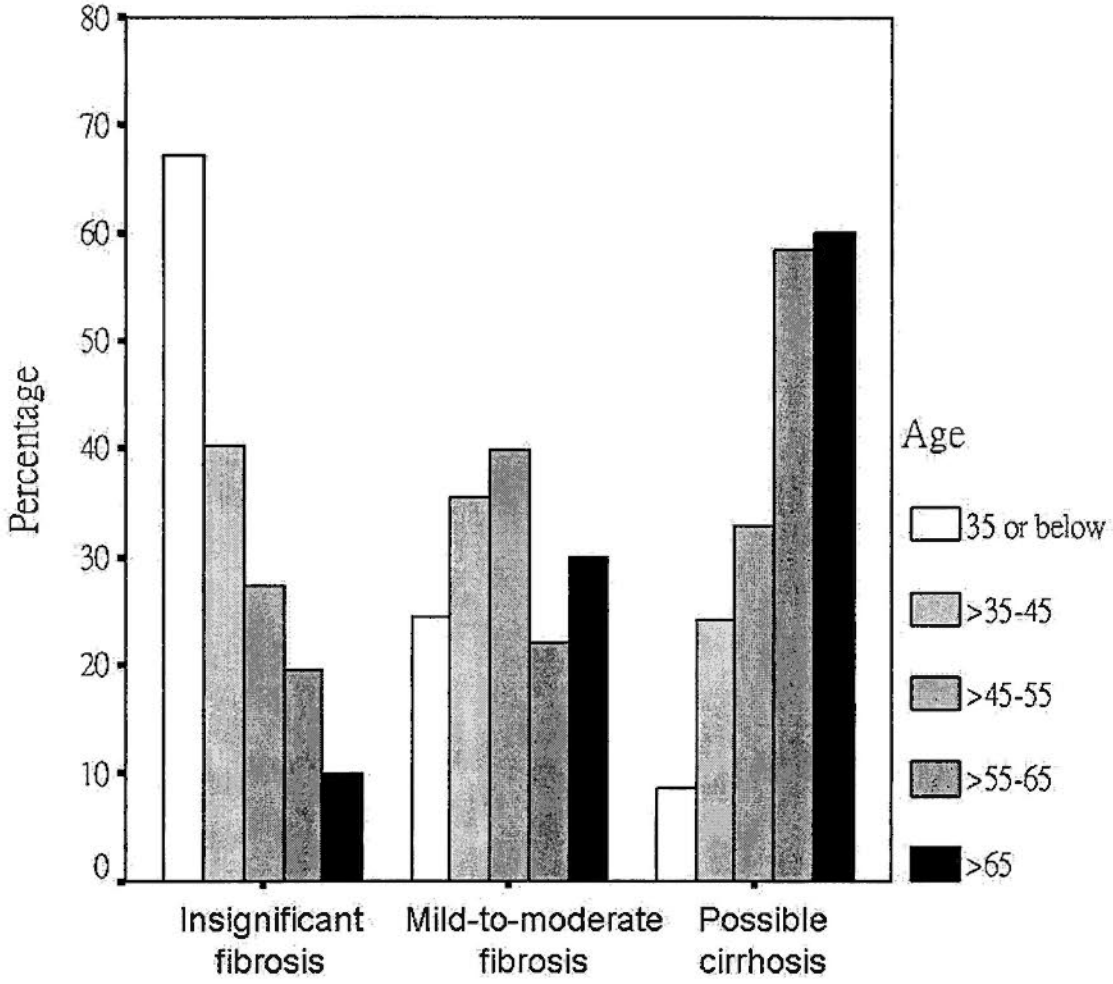
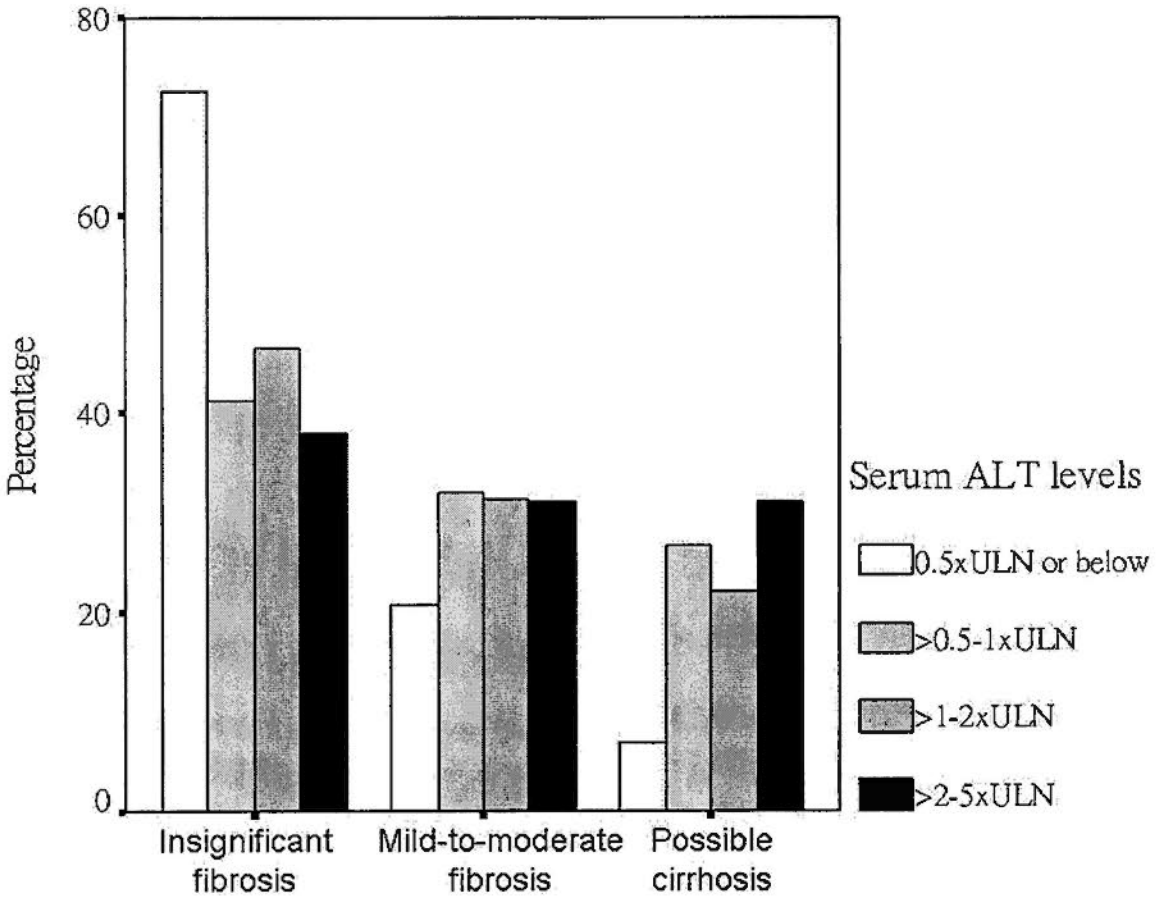
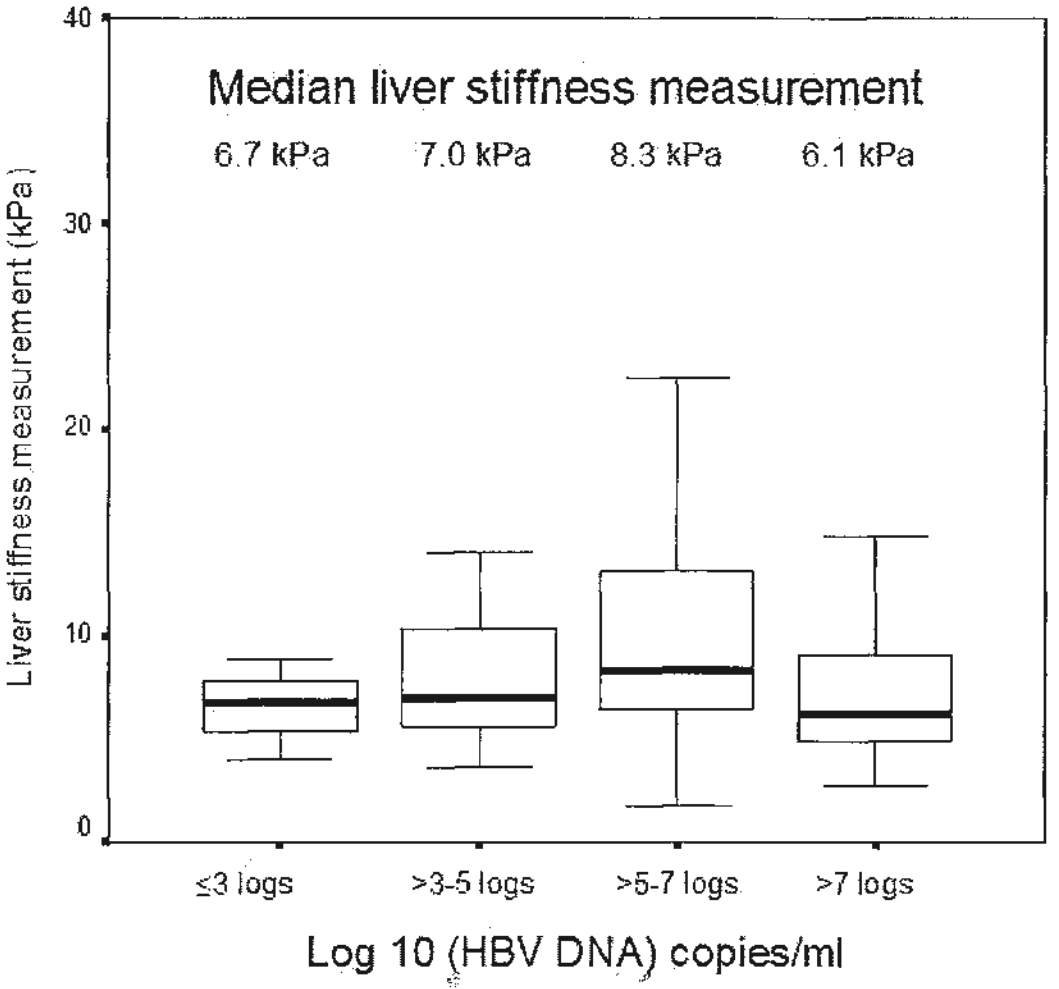


Figure 9.3 Bar charts on the prevalence of different severities of liver fibrosis in different serum alanine aminotransferase (ALT) levels



ULN = upper limit of normal

Figure 9.4 Box-plot of liver stiffness measurements against serum HBV DNA levels



9.2 Longitudinal Study of Alanine Aminotransferase and Liver Fibrosis (Study 3: Clin Gastroenterol Hepatol 2009;7:227-233)

9.2.1 Pattern of ALT and liver fibrosis

Twenty-eight patients who were prospectively followed up for a median of 102 (range 95-110) months were further studied. All patients had at least 5 (median 18) readings of ALT levels during the follow-up period. Sixteen (57%), 8 (29%) and 4 patients (14%) had LSM indicative of insignificant fibrosis, mild-to-moderate fibrosis, and possible cirrhosis respectively. Nine, 7 and 12 patients had ALT persistently ≤29 IU/l, intermittently >29 IU/l and persistently >29 IU/l respectively according to the definitions. More patients with ALT persistently ≤29 IU/l had LSM indicative of insignificant fibrosis than those with ALT intermittently >29 IU/l and persistently >29 IU/l [100% (9/9 patients) *vs.* 57% (4/7 patients) and 25% (3/12 patients); P=0.003] and fewer of these patients tended to have LSM indicative of possible cirrhosis [0% *vs.* 14% (1/7 patients) and 25% (3/12 patients); P=0.27].

9.2.2 Independent factors associated with liver fibrosis in longitudinal study

Age of patient had significant effect of liver fibrosis from this longitudinal study. Among the 22 patients who were aged ≤35 years at the time of LSM, 16 (73%) had LSM indicative of insignificant fibrosis and none had LSM indicative of possible cirrhosis. In contrast, none of the 6 patients who were

older than 35 years had LSM indicative of insignificant fibrosis (P=0.01 when compared with younger patients) while 4 (67%) of them had LSM indicative of possible cirrhosis at the time of LSM (P<0.001 when compared with younger patients).

CHAPTER TEN

FACTORS ASSOCIATED WITH LIVER CIRRHOSIS IN HEPATITIS B E ANTIGEN-NEGATIVE CHRONIC HEPATITIS B

10.1 Cross-sectional Study of Clinical Factors and Liver Fibrosis (Study 4: Am J Gastroenterol 2008;103:3071–3081)

10.1.1 Patient characteristics

One thousand two hundred and sixty-nine HBeAg-negative CHB patients were referred from different hospitals and clinics for LSM within the study period. One thousand one hundred and seventy-four (93%) patients who had reliable LSM and ALT levels within 5x ULN were included for analysis in Study 4. Twenty-three patients were excluded because of ALT > 5x ULN. Among the 72 patients who were excluded because of unsuccessful LSM, they were older (53.5 ± 11.7 years *vs.* 48.0 ± 11.5 years, $P<0.001$), had higher waist circumference (94 ± 12 cm *vs.* 85 ± 1 0cm, $P=0.001$), hip circumference (102 ± 9 cm *vs.* 96 ± 7 cm, $P<0.001$) and BMI (26.7 ± 4.3 kg/m^2 *vs.* 23.6 ± 4.0 kg/m^2, $P<0.001$). Most patients (89%) were referred from hepatitis specialty clinics; other patients were referred from general medical specialty clinics (6%) and general practitioners (5%). Patients who were referred from hepatitis speciality clinics tend to have higher proportion of liver cirrhosis. Three hundred and forty-seven (29%) patients had elevated ALT levels and 703 (61%) patients had HBV DNA higher than 10,000 copies/ml.

10.1.2 Factors associated with possible and probable cirrhosis

One hundred and seventeen patients underwent liver biopsy; 100 (85%)

patients fulfilled the criteria for data analysis to internally validate the cutoff values proposed in Study 2. Sixty-four (64%) of the 100 patients in Study 4 had elevated ALT, while 46 (46%) had normal ALT. The number of histologic cirrhosis increased with ALT levels: ≤0.5x ULN (1/16, 6%); >0.5–1x ULN (3/20, 14%); >1–5x ULN (15/62, 24%); and >5x ULN (1/2, 50%). The ALT-based LSM cutoff values of possible cirrhosis were associated with satisfactory sensitivity (84%) and negative predictive value (83%) for cirrhosis (with specificity of 81% and positive predictive value of 57%); while the ALT-based LSM cutoff values of probable cirrhosis were associated with satisfactory specificity (87%) and positive predictive value (74%) for cirrhosis (with sensitivity of 77% and negative predictive value of 89%).

Three hundred and five patients (26%) and 141 (12%) were defined as possible and probable cirrhosis according to ALT-based LSM cutoff values respectively. Patients who had possible and probable liver cirrhosis were predominantly male, older and had higher BMI. Biochemically, cirrhotic patients had lower serum albumin, higher serum bilirubin, alkaline phosphatase, ALT and alpha-fetoprotein. Their serum HBV DNA levels were also higher. On multivariate logistic regression analysis, older age, lower albumin, higher alkaline phosphatase, ALT, alpha-fetoprotein and HBV DNA were found independently associated with both possible and probable cirrhosis. Other factors including gender, obesity and total bilirubin did not have consistent effect on the risk of possible and probable liver cirrhosis.

10.1.3 Effect of ALT and gender on development of liver cirrhosis

We stratified patients into three groups according to the ALT levels: ≤0.5x ULN (36%); >0.5–1x ULN (37%); and >1–5x ULN (27%). The odd ratios of the risk for development of possible and probable cirrhosis increased with increasing ALT levels (Table 10.2 and Figure 10.1A). The risk of liver cirrhosis increased with the ALT level strata. Patients who had ALT levels of >0.5–1x ULN started to have significantly increased risk of both possible and probable cirrhosis compared with patients with ALT levels ≤0.5x ULN.

A recent study suggested that the ULN for ALT should be decreased to 30 IU/l for men and 19 IU/l for women [Prati et al., 2002]. Therefore we also analyzed the effect of gender on ALT levels. The area under the ROC curve of ALT for possible and probable cirrhosis was 0.70 (95% CI 0.65–0.74, $P<0.001$) and 0.68 (95% CI 0.62–0.74, $P<0.001$) respectively for male patients, and 0.77 (95% CI 0.70–0.83, $P<0.001$) and 0.83 (95% CI 0.75–0.91, $P<0.001$) respectively for female patients.

The performance of ALT levels to predict possible and probable cirrhosis was significantly better in female than male (DeLong test $P=0.02$ and $P<0.001$, respectively). In male patients, the newly recommended normal value for ALT (30 IU/l) was very close to 0.5x ULN (29 IU/l). If an ALT cutoff value of 30 IU/l was used to define normal instead of the current laboratory ULN (58 IU/l),

the sensitivity was increased from 52% to 91% to detect possible cirrhosis (while the specificity, positive predictive value and negative predictive value changed from 72%, 49% and 74% to 32%, 41% and 87%, respectively) and increased from 55% to 93% to detect probable cirrhosis (while the specificity, positive predictive value and negative predictive value changed from 67%, 19% and 91% to 27%, 15% and 96%, respectively). In female patients, if the newly recommended normal ALT cutoff value (19 IU/l) was used instead of the current laboratory ULN (58 IU/l), the sensitivity was increased from 35% to 97% to detect possible cirrhosis (while the specificity, positive predictive value and negative predictive value changed from 90%, 44% and 86% to 30%, 23% and 98%, respectively) and increased from 58% to 100% to detect probably cirrhosis (while the specificity, positive predictive value and negative predictive value changed from 89%, 28% and 97% to 27%, 9% and 100%, respectively).

Patients were then stratified into four groups according to ALT levels using this new ULN (men 30 IU/l; women 19 IU/l): ≤1x ULN (25%); >1–2x ULN (41%); >2–5x ULN (27%); and >5x ULN (7%) (Table 10.3 and Figure 10.2). Patients with ALT levels of >1x ULN already had a significantly increased risk of both possible and probable cirrhosis compared with patients with normal ALT levels.

10.1.4 Effect of serum HBV DNA levels on development of liver cirrhosis

The serum HBV DNA levels were higher for patients with possible and probable cirrhosis when compared to non-cirrhotic patients. We stratified patients into seven groups according to the serum HBV DNA levels (copies/ml): ≤3 $\log_{10}$ (22%); >3–4 $\log_{10}$ (20%); >4–5 $\log_{10}$ (21%); >5–6 $\log_{10}$ (16%); >6–7 $\log_{10}$ (13%); >7 $\log_{10}$ (8%) (Table 10.3 and Figure 10.1B). The odd ratios for development of possible and probable cirrhosis increased with increasing HBV DNA levels. The odds ratios for cirrhosis started to increase significantly when serum HBV DNA was >4 $\log_{10}$ copies/ml.

10.1.5 Combined analysis of serum HBV DNA and ALT

As serum HBV DNA and ALT were independent risk factors associated with increased risk of liver cirrhosis, we analyzed the relationship of these two variables and their combined effect on the risk of liver cirrhosis. First of all, we found that serum HBV DNA and ALT were correlated to each other (Pearson correlation coefficient r=0.29, $P<0.001$). The correlation was better in patients with ALT levels ≤2x ULN (r=0.53, $P<0.001$) than those with ALT levels >2x ULN (r=0.02, $P=0.85$). Among patients with ALT levels ≤2x ULN, 19% (82/442) and 6% (27/442) with HBV DNA ≤4 $\log_{10}$ copies/ml had possible and probable cirrhosis, which was lower than patients with HBV DNA >4 $\log_{10}$ copies/ml, among whom 36% (214/602, $P<0.001$) and 13% (80/602,

P<0.001) had possible and probable cirrhosis respectively. Among patients with ALT levels >2x ULN, 50% (3/6) and 0% (0/6) with HBV DNA ≤4 $\log_{10}$ copies/ml had possible and probable cirrhosis, while 58% (56/101, *P*=0.79) and 27% (25/101, *P*=0.02) with HBV DNA >4 $\log_{10}$ copies/ml had probable cirrhosis (Table 10.4).

Then we tried to determine the risk of cirrhosis for patients who fulfilled both the criteria of ALT ≤0.5x ULN and HBV DNA ≤4 $\log_{10}$ copies/ml. Among this low risk group of patients, 9% (26/283) and 2% (7/283) had possible and probable cirrhosis respectively, which were significantly lower when compared to 31% (279/891, *P*<0.001) and 15% (134/891, *P*<0.001) of patients who did not fulfill the low-risk criteria, respectively. If the new gender-specific ULN together with HBV DNA <4 $\log_{10}$ copies/ml was used to defined the low risk group, 8% (17/206) and 3% (7/206) had possible and probable cirrhosis, which were also significantly lower when compared to 30% (288/968, *P*<0.001) and 14% (134/968, *P*<0.001) of patients not fulfilling the low-risk criteria, respectively. Similar results were obtained from the subgroup of patients with liver biopsy performed. More patients not fulfilling the low-risk criteria had histologic cirrhosis than those fulfilling the low-risk criteria using the usual [28% (18/64) *vs.* 6% (2/36), *P*<0.001] or new gender-specific ALT cutoff value [29% (20/69) *vs.* 0% (0/31), *P*<0.001].

Table 10.1 Clinical characteristics of the patients in Study 4

	All cases	Biopsy group	Possible cirrhosis			Probable cirrhosis		
			Yes	No	P value	Yes	No	P value
Number of patients	1174	100	305 (26%)	869 (74%)		141 (12%)	1033 (88%)	
Male gender	763 (65%)	78 (78%)	241 (79%)	522 (60%)	<0.001	110 (78%)	653 (63%)	0.002
Age (year)	48.0 ± 11.4	49.0 ± 8.5	51.3 ± 10.2	46.4 ± 11.6	<0.001	54.4 ± 10.0	47.2 ± 11.4	<0.001
Biochemical								
Albumin (g/l)	44 ± 3	43 ± 4	43 ± 4	45 ± 3	<0.001	42 ± 4	44 ± 3	<0.001
Bilirubin (μmol/l)	15 ± 12	12 ± 6	17 ± 18	14 ± 7	<0.001	19 ± 15	14 ± 10	<0.001
Alkaline phosphatase (IU/l)	74 ± 29	77 ± 25	85 ± 35	70 ± 24	<0.001	98 ± 44	72 ± 25	<0.001
Alanine transaminase (IU/l)	66 ± 131	74 ± 47	198 ± 182	48 ± 70	<0.001	131 ± 287	56 ± 86	<0.001
Log_{10} [HBV DNA] (copies/ml)	4.6 ± 1.7	5.9 ± 1.6	5.3 ± 1.7	4.3 ± 1.6	<0.001	5.5 ± 1.7	4.5 ± 1.7	<0.001
LSM (kPa)	8.1 ± 6.0	8.9 ± 4.9	14.5 ± 7.7	5.6 ± 1.4	<0.001	20.8 ± 9.2	6.4 ± 2.3	<0.001
Success rate of acquisition (%)	89 ± 13	88 ± 13	90 ± 13	88 ± 13	0.01	90 ± 12	89 ± 13	0.01

LSM = liver stiffness measurement.

Table 10.2 Risks of possible or probable cirrhosis according to different serum alanine aminotransferase and HBV DNA (log_{10} copies/ml) levels

		Possible cirrhosis			Probable cirrhosis		
	N (%)*	n (%)#	Odd ratio (95% CI)	*P* value	n (%)#	Odd ratio (95% CI)	*P* value
ALT levels							
≤0.5x ULN	418 (36%)	51 (12%)	Referent		17 (4%)	Referent	
>0.5–1x ULN	432 (37%)	122 (28%)	3.0 (2.1 – 4.2)	<0.001	51 (12%)	3.2 (1.7 – 6.0)	<0.001
>1–5x ULN	324 (27%)	132 (41%)	5.8 (3.9 – 10.1)	<0.001	73 (23%)	6.3 (3.1 – 11.9)	<0.001
HBV DNA levels (log_{10} copies/ml)							
≤3 log	248 (21%)	45 (18%)	Referent		15 (6%)	Referent	
>3–4 log	230 (20%)	40 (17%)	1.0 (0.6 – 1.5)	0.89	16 (7%)	1.2 (0.6 – 2.5)	0.67
>4–5 log	246 (21%)	56 (23%)	1.5 (1.1 – 2.3)	0.03	23 (9%)	1.5 (0.8 – 2.9)	0.15
>5–6 log	188 (16%)	46 (24%)	1.9 (1.2 – 2.8)	<0.001	28 (14%)	2.6 (1.4 – 5.0)	0.006
>6–7 log	155 (13%)	65 (42%)	3.1 (2.0 – 5.2)	<0.001	30 (19%)	3.7 (1.8 – 7.1)	<0.001
>7 log	107 (9%)	53 (50%)	5.5 (2.9 – 10.8)	<0.001	29 (25%)	5.4 (2.2 – 11.6)	<0.001

N (%)*: numbers (and percentage) of patients that particular ALT or HBV DNA level among the whole cohort.
n (%)#: numbers (and percentage) of patients suffering from possible or probable cirrhosis among that particular ALT or HBV DNA level. ALT = Alanine aminotransferase; ULN = upper limit of normal, 58 IU/l for both gender.

Table 10.3 Risks of possible and probable cirrhosis according to different alanine aminotransferase levels based on the gender-specific cutoff values (30 IU/l for men and 19 IU/l for women)

		All (n=1174)			Men (n=763)			Women (n=411)		
ALT levels	N (%)*	n (%)#	Odd ratio (95% CI)	*P* value	n (%)#	Odd ratio (95% CI)	*P* value	n (%)#	Odd ratio (95% CI)	*P* value
A. Possible cirrhosis										
≤1x ULN	307 (26%)	29 (9%)	Referent		26 (13%)	Referent		3 (3%)	Referent	
>1–5x ULN	809 (69%)	240 (30%)	3.8 (2.1 – 5.5)	<0.001	193 (36%)	2.9 (2.0 – 4.1)	<0.001	47 (17%)	6.3 (2.0 – 10.3)	0.005
>5 x ULN	58 (5%)	36 (62%)	7.9 (4.6 – 11.9)	<0.001	27 (66%)	5.6 (3.5 – 9.1)	<0.001	9 (38%)	13.2 (4.5 – 48.8)	
B. Probable cirrhosis										
≤1x ULN	307 (26%)	10 (3%)	Referent		9 (5%)	Referent		1 (1%)	Referent	
>1–5x ULN	809 (69%)	105 (13%)	4.6 (2.5 – 9.5)	0.001	79 (16%)	3.7 (1.8 – 8.1)	<0.001	26 (9%)	9.8 (1.6 – 46.5)	0.002
>5x ULN	58 (5%)	26 (45%)	15.5 (7.7 – 24.6)	<0.001	19 (32%)	8.4 (3.5 – 18.5)	<0.001	7 (27%)	25.8 (7.5 – 85.2)	<0.001

N (%)*: numbers (and percentage) of patients that particular ALT level among the whole cohort.

n (%)#: numbers (and percentage) of patients suffering from possible or probable cirrhosis among that particular ALT level.

ALT = Alanine aminotransferase; ULN = upper limit of normal, 30 IU/l for men and 19 IU/l for women; N/A: not applicable.

Table 10.4 Risks of possible or probable cirrhosis according to different levels of serum alanine aminotransferase and HBV DNA (log_{10} copies/ml) levels

		≤3 log	>3–4 log	>4–5 log	>5–6 log	>6–7 log	>7 log
ALT levels	**N(%)* / n(%)#**	253 (22%)	232 (20%)	248 (21%)	188 (16%)	153 (13%)	100 (8%)
A. Possible cirrhosis							
≤0.5x ULN	418 (36%)	12/155 (8%)	14/128 (11%)	16/101 (16%)	8/31 (26%)	0/2 (0%)	1/1 (100%)
<0.5-1x ULN	432 (37%)	16/66 (24%)	19/85 (22%)	35/106 (33%)	24/100 (24%)	23/59 (39%)	5/16 (31%)
>1–5x ULN	324 (27%)	10/32 (32%)	5/19 (26%)	13/41 (32%)	25/57 (44%)	37/92 (40%)	42/83 (51%)
B. Probable cirrhosis							
≤0.5x ULN	418 (36%)	3/155 (2%)	4/128 (3%)	3/101 (3%)	5/31 (16%)	1/2 (50%)	1/1 (100%)
<0.5-1x ULN	432 (37%)	6/66 (9%)	7/85 (8%)	13/106 (12%)	13/100 (13%)	5/59 (8%)	7/16 (44%)
>1–5x ULN	324 (27%)	5/32 (16%)	4/19 (21%)	7/41 (17%)	12/57 (21%)	21/92 (23%)	24/83 (29%)

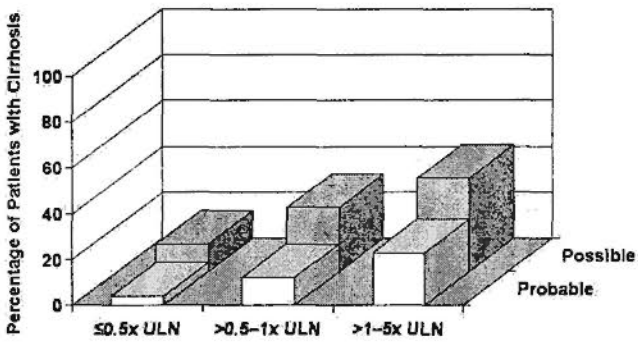
N (%)*: numbers (and percentage) of patients that particular ALT or HBV DNA level among the whole cohort.

n (%)#: numbers (and percentage) of patients suffering from possible or probable cirrhosis among that particular ALT level.

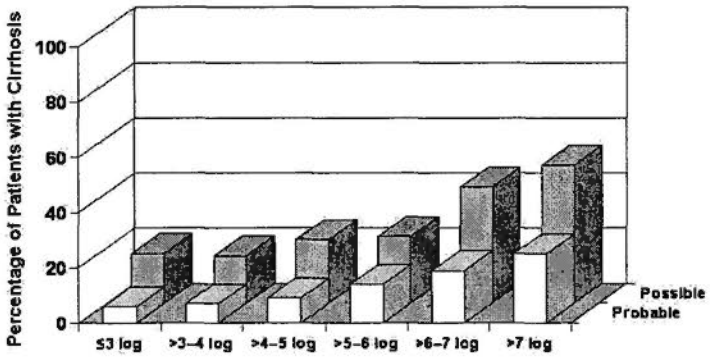
ALT = Alanine aminotransferase; ULN = upper limit of normal, 58 IU/l for all patients; N/A: not applicable.

Figure 10.1 Bar charts showing the percentages of possible or probable cirrhosis

A. According to serum alanine aminotransferase levels



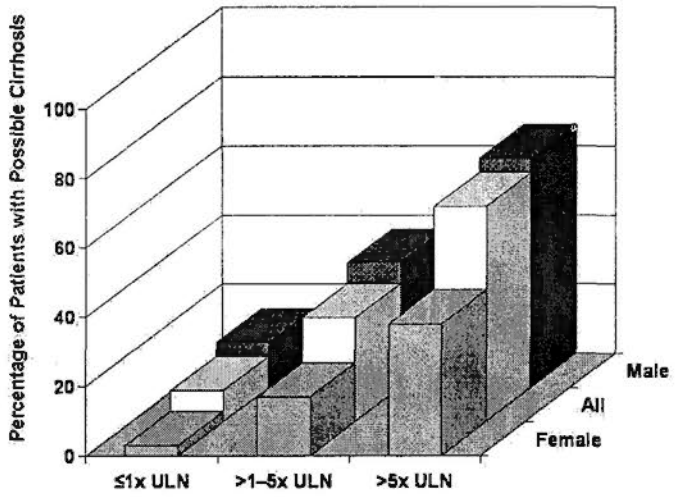
B. According to HBV DNA (log_{10} copies/ml) levels



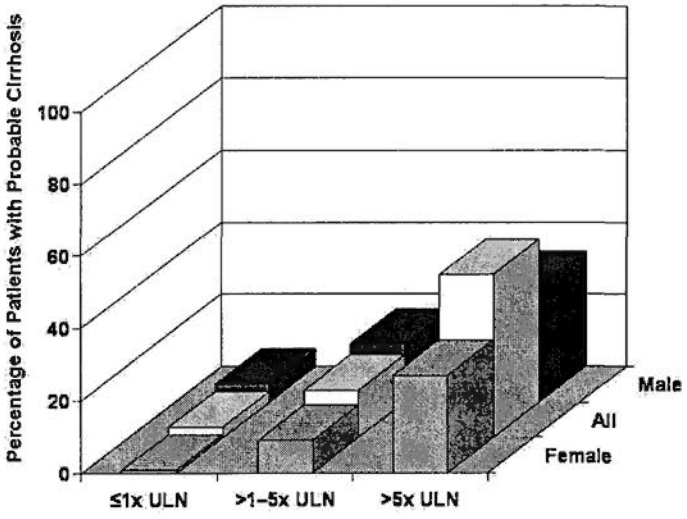
ULN = upper limit of normal; log = log_{10} copies/ml.

Figure 10.2 Bar charts showing the percentages of cirrhosis according to different alanine aminotransferase levels based on the gender-specific cutoff values (30 IU/l for men and 19 IU/l for women)

A. Possible cirrhosis



B. Probable cirrhosis



ULN = upper limit of normal

10.2 Longitudinal Study of Alanine Aminotransferase and Liver Fibrosis (Study 4: Am J Gastroenterol 2008;103:3071–3081)

Seventy-two patients who were prospectively followed up for 91.2 ± 14.6 months were further studied. All patients had at least 5 readings of ALT levels during the follow-up period. LSM was performed at the time of last visit. Sixteen patients (22%) had LSM defined as possible liver cirrhosis. Comparing patients with and without possible cirrhosis, higher peak ALT levels (205 ± 114 IU/l *vs.*140 ± 130 IU/l, P=0.03) and higher AUC/t ALT levels (67 ± 34 IU/l *vs.* 45 ± 23 IU/l, P=0.01) were associated with higher risk of possible cirrhosis. However, the trough ALT levels were similar among patients with and without possible (28 ± 10 IU/l *vs.* 25 ± 11 IU/l, P=0.31) cirrhosis. The ALT level at the time of LSM was slightly higher in patients without possible cirrhosis (53 ± 121 IU/l) than those without possible cirrhosis (51 ± 27 IU/l, P = 0.03). A Cox proportional hazards model using the AUC/t ALT level as a time-dependent covariate showed that association was noted between ALT levels and possible cirrhosis (P=0.03). As only 2 patients (3%) had probable cirrhosis, we could not analyze on the relationship of ALT levels and probable cirrhosis in this longitudinal cohort.

The effect of reactivation on the development of cirrhosis was analyzed with respect to the number of episodes and duration of reactivation. The numbers

of episodes of reactivation were higher for patients with possible cirrhosis (3.0 ± 1.7 episodes *vs.* 1.5 ± 1.5 episodes, P=0.005) when compared to non-cirrhotic patients. On the other hand, the duration of reactivation tended to be longer among patients with possible cirrhosis than those without possible cirrhosis (40 ± 30 months *vs.* 25 ± 28 months, P=0.06) without reaching statistical significance.

CHAPTER ELEVEN

METABOLIC SYNDROME AND CHRONIC HEPATITIS B

11.1 Effect of Metabolic Syndrome on Liver Histology (Study 5: Gut 2009;58:111–117)

One hundred sixty-seven CHB patients underwent liver biopsy and 134 (80%) had adequate liver biopsy samples for histologic assessment. The liver biopsy specimens were 19 ± 4mm in length containing 11 ± 5 portal tracts. The interobserver agreement was 0.93 for fibrosis staging and 1.00 for the presence of F3-4 fibrosis. Sixty-one (46%) patients had F3-4 disease (bridging fibrosis), and 32 (24%) had F4 disease (cirrhosis). The mean LSM score was 8.3 ± 6.3 kPa. Correlation of LSM with METAVIR fibrosis score was good (r=0.59, $P<0.001$). The area under the ROC curves of LSM for cirrhosis (F4 fibrosis) was 0.89 (95% CI 0.83–0.94, $P<0.001$). The ALT-based LSM cutoff values of possible cirrhosis were associated with satisfactory sensitivity (86%) and negative predictive value (89%) for histologic cirrhosis (with specificity of 77% and positive predictive value of 59%). The ALT-based LSM cutoff values of probable cirrhosis were associated with high specificity (90%) and positive predictive value (72%) for cirrhosis (with sensitivity of 70% and negative predictive value of 88%).

Histologic liver cirrhosis, which was present in 24% (32/134) of patients underwent liver biopsy, was associated with male gender, higher BMI, greater waist and hip circumference, higher blood pressure, presence of metabolic

syndrome, higher ALT and higher alpha-fetoprotein levels. The distribution of the histologic liver cirrhosis across different age groups was 0%, 16%, 31% and 11% for patients of age 0–20, 21–40, 41–60 and >60 respectively. Liver cirrhosis was commoner among patients who had metabolic syndrome (P<0.001, Figure 11.1). When the different components of metabolic syndrome were independently analyzed, histologic liver cirrhosis was associated with hypertension and diabetes mellitus. On the other hand, liver fibrosis was more severe in patients who had metabolic syndrome (median METAVIR fibrosis stage = 4) than those who did not have metabolic syndrome (median METAVIR fibrosis stage = 2, P=0.002). Severity of steatosis (median Kleiner grade 1 *vs.* 0, P<0.001, Table 11.2), but not necroinflammation (median METAVIR Activity score 4 *vs.* 4, P=0.6), was also significantly more severe in patients with metabolic syndrome than those without. By logistic regression analysis, histologic cirrhosis was independently and significantly associated with male gender, obesity (BMI ≥25 kg/m^2), metabolic syndrome, and hypoalbuminemia (serum albumin <40 g/l, Table 11.3).

Table 11.1 Clinical characteristics of the patients underwent liver biopsy in Study 5

	Histologic cirrhosis in biopsy group		
	Yes	**No**	**P value**
Number of patients	32 (24%)	102 (76%)	
Male gender	29 (91%)	74 (73%)	0.03
Age, year	46 ± 8	43 ± 12	0.13
Body weight, kg	70 ± 11	66 ± 11	0.11
Body height, cm	166 ± 10	167 ± 8	0.76
Body mass index (BMI), kg/m^2	26 ± 4	24 ± 3	0.03
Overweight (BMI≥23kg/m^2)	23 (72%)	61 (60%)	0.22
Obesity (BMI≥25kg/m^2)	15 (47%)	32 (32%)	0.11
Waist circumference, cm	89 ± 15	82 ± 9	0.01
Hip circumference, cm	98 ± 8	94 ± 7	0.01
Systolic blood pressure, mmHg	131 ± 15	122 ± 15	0.002
Diastolic blood pressure, mmHg	74 ± 11	69 ± 10	0.03
Metabolic syndrome	12 (38%)	11 (11%)	<0.001
Central obesity	15 (47%)	32 (31%)	0.10
Raised triglycerides	6 (19%)	16 (16%)	0.68
Reduced HDL-cholesterol	4 (13%)	10 (10%)	0.66
Hypertension	18 (56%)	32 (31%)	0.01
Diabetes mellitus	15 (47%)	26 (26%)	0.02
Biochemical			
Albumin, g/l	42 ± 4	43 ± 5	0.02
Bilirubin, μmol/l	17 ± 24	15 ± 9	0.32
Alkaline phosphatase, IU/l	81 ± 26	79 ± 45	0.31
Alanine aminotransferase, IU/l	74 ± 100	65 ± 79	0.39

Table 11.3 (continued)

Hepatitis B e Antigen			0.81
Positive	59 (45%)	46 (45%)	
Negative	75 (55%)	56 (55%)	
Log_{10} [HBV DNA] log_{10} copies/ml	5.7 ± 1.5	6.3 ± 2.0	0.18
Liver stiffness measurement, kPa	14.5 ± 7.7	8.8 ± 5.2	<0.001
Interquartile range/LSM, %	17 ± 9	20 ± 14	0.01
Success rate of acquisition, %	90 ± 14	89 ± 15	0.83

Data were expressed in number (percentage) or mean ± standard deviation.

HBV = Hepatitis B virus; LSM = liver stiffness measurement, kPa = kiloPascal.

Table 11.2 Histopathologic grading of liver biopsies in patients according to the presence of the metabolic syndrome

	All cases	Metabolic syndrome		*P* value
		Yes	No	
Number of patients (%)	134	23	111	
Length of biopsy, mm	19 ± 4	19 ± 3	19 ± 4	0.98
Number of portal tracts	11 ± 5	10 ± 5	11 ± 5	0.34
Steatosis (Kleiner grade)	0 (0 – 3)	1 (0 – 3)	0 (0 – 2)	<0.001
METAVIR Activity score,	2 (1 – 3)	2 (1 – 3)	2 (1 – 3)	0.6
METAVIR fibrosis score	2 (0 – 4)	4 (0 – 4)	2 (0 – 4)	0.002
F0	9 (7%)	4 (17%)	5 (5%)	
F1	23 (17%)	2 (9%)	21 (19%)	
F2	41 (30%)	3 (13%)	38 (34%)	
F3	29 (22%)	2 (9%)	27 (24%)	
F4	32 (24%)	12 (52%)	20 (18%)	
Bridging fibrosis	61 (46%)	14 (61%)	47 (42%)	0.10
Cirrhosis	32 (24%)	12 (52%)	20 (18%)	<0.001

Data were expressed in number (percentage), mean ± standard deviation or median (range).

Table 11.3 Multivariate logistic regression analysis on factors associated with histologic cirrhosis

Parameters	**Odds ratio**	**95% confidence interval**	***P* value**
Male gender	9.1	1.7–47.1	0.009
Obesity (body mass index ≥25 kg/m^2)	1.5	1.1–2.0	0.04
Metabolic syndrome	5.1	1.6–16.5	0.006
Albumin <40 g/l	3.9	1.1–13.9	0.04

11.2 Metabolic Syndrome and Risk of Cirrhosis (Study 5: Gut 2009;58:111–117)

11.2.1 Patient characteristics

One thousand five hundred and thirty-two CHB patients were referred for transient elastography within the study period. On thousand four hundred and thirty-five (94%) patients with reliable LSM and ALT levels within 5x ULN were included for analysis. Nine hundred and eighteen patients (64%) were male and the mean age was 46 ± 12 years. Mean BMI was 23 ± 3 kg/m^2 (Table 11.4). The overall prevalence of metabolic syndrome was 13% (186/1435). The prevalence of metabolic syndrome increased with age (0%, 7%, 15% and 21% for patients of age 0–20, 21–40, 41–60 and >60 respectively, P<0.001; Figure 11.2). Six hundred and three (42%) patients had central obesity, 196 (14%) had hypertriglyceridemia, 256 (18%) had reduced HDL cholesterol level, 654 (46%) had high blood pressure, and 205 (14%) had impaired fasting glucose or diabetes. Three hundred and fifty-four (25%) patients and 393 (27%) were overweight and obese, respectively. Sixty-two (4%) patients were moderately obese, and 42 (3%) patients had severe obesity.

11.2.2 Metabolic syndrome and risk of possible and probable cirrhosis

Four hundred and two patients (28%) and 201 (14%) were defined as

possible and probable cirrhosis according to ALT-based LSM cutoff values respectively. Patients who had possible and probable liver cirrhosis were predominantly male, older, had higher BMI and blood pressure. Metabolic syndrome and its components were more prevalent in patients with possible and probable cirrhosis. The prevalence of possible and probable cirrhosis was significantly higher in the presence of metabolic syndrome at different age groups (Figure 11.1). Biochemically, cirrhotic patients had lower serum albumin, higher serum bilirubin, higher alkaline phosphatase and higher ALT. Their serum HBV DNA levels were also higher. On multivariate logistic regression analysis, presence of metabolic syndrome, together with male gender, age above 40 years, lower serum albumin, higher serum alkaline phosphatase and higher ALT were independently associated with both possible and probable cirrhosis. Other factors including obesity, serum total bilirubin and serum HBV DNA level did not have consistent effect on the risk of possible and probable cirrhosis. Metabolic syndrome was more likely to be associated with cirrhosis independent of ALT level, and the effect of metabolic syndrome on cirrhosis was even more apparent in patients with normal ALT (Figure 11.3).

Patients with more components of metabolic syndrome according to the IDF criteria were at higher risk of cirrhosis. The odds ratios for development of possible and probable cirrhosis had an increasing trend with increasing numbers of components of metabolic syndrome (Table 11.5). The risk of

histologic liver cirrhosis did not show a consistent increasing trend, probably due to relative small numbers of patients in all groups (Table 11.5).

Table 11.4 Clinical characteristics of all patients in Study 5

	All cases	Possible cirrhosis			Probable cirrhosis		
		Yes	No	P value	Yes	No	P value
Number of patients	1435	402 (28%)	1033 (72%)		201 (14%)	1234 (86%)	
Male gender	918 (64%)	307 (76%)	611 (59%)	<0.001	154 (77%)	764 (62%)	<0.001
Age, year	46 ± 12	49 ± 12	44 ± 12	<0.001	51 ± 12	45 ± 12	<0.001
Body weight, kg	65 ± 12	67 ± 12	64 ± 12	<0.001	68 ± 12	64 ± 12	<0.001
Body height, cm	166 ± 12	166 ± 15	167 ± 10	0.58	166 ± 21	167 ± 8	0.79
Body mass index (BMI), kg/m^2	23 ± 3	24 ± 4	23 ± 3	<0.001	24 ± 4	23 ± 3	<0.001
Overweight (BMI≥23kg/m^2)	747 (52%)	241 (60%)	506 (49%)	<0.001	124 (62%)	623 (50%)	0.001
Obesity (BMI≥25kg/m^2)	393 (27%)	148 (37%)	245 (24%)	<0.001	78 (39%)	315 (26%)	<0.001
Waist circumference, cm	84 ± 10	86 ± 10	83 ± 10	<0.001	87 ± 11	83 ± 10	<0.001
Hip circumference, cm	96 ± 26	96 ± 8	96 ± 31	0.05	97 ± 7	96 ± 28	0.16
Systolic blood pressure, mmHg	128 ± 19	133 ± 20	125 ± 18	<0.001	136 ± 21	127 ± 18	<0.001
Diastolic blood pressure, mmHg	76 ± 12	78 ± 13	75 ± 11	<0.001	79 ± 13	76 ± 12	<0.001
Metabolic syndrome	186 (13%)	95 (24%)	91 (9%)	<0.001	49 (24%)	137 (11%)	<0.001
Central obesity	603 (42%)	213 (53%)	390 (38%)	0.01	98 (49%)	505 (41%)	0.01
Raised triglycerides	196 (14%)	78 (19%)	118 (11%)	0.002	35 (17%)	161 (13%)	0.07
Reduced HDL-cholesterol	256 (18%)	100 (25%)	156 (15%)	0.003	50 (25%)	206 (17%)	0.005
Hypertension	654 (46%)	262 (65%)	392 (38%)	<0.001	125 (62%)	529 (43%)	<0.001
Diabetes mellitus	205 (14%)	115 (29%)	90 (9%)	<0.001	58 (29%)	147 (12%)	<0.001

Table 11.4 (continued)

Biochemical							
Albumin, g/l	44 ± 4	42 ± 5	45 ± 3	<0.001	41 ± 6	44 ± 3	<0.001
Bilirubin, µmol/l	15 ± 12	17 ± 18	14 ± 7	<0.001	20 ± 17	14 ± 10	<0.001
Alkaline phosphatase, IU/l	75 ± 30	85 ± 36	70 ± 23	<0.001	95 ± 37	72 ± 26	<0.001
Alanine aminotransferase, IU/l	67 ± 112	96 ± 109	52 ± 74	<0.001	103 ± 116	60 ± 87	<0.001
Hepatitis B e Antigen				0.001			0.009
Positive	359 (25%)	116 (29%)	243 (24%)		64 (32%)	295 (24%)	
Negative	1076 (75%)	286 (71%)	790 (76%)		137 (68%)	939 (76%)	
Log_{10} [HBV DNA] log_{10} copies/ml	5.0 ± 1.9	5.5 ± 1.8	4.7 ± 1.9	<0.001	5.8 ± 1.6	4.9 ± 1.9	<0.001
Liver stiffness measurement, kPa	8.3 ± 6.3	14.6 ± 7.8	5.4 ± 1.4	<0.001	21.3 ± 9.0	6.6 ± 2.4	<0.001
Interquartile range/LSM, %	19 ± 16	20 ± 14	18 ± 17	<0.001	22 ± 16	18 ± 16	<0.001
Success rate of acquisition, %	89 ± 13	90 ± 13	89 ± 13	0.01	91 ± 13	89 ± 13	0.03

Data were expressed in number (percentage) or mean ± standard deviation.

HBV = Hepatitis B virus; LSM = liver stiffness measurement, kPa = kiloPascal.

Table 11.5 Risks of histologic, possible or probable cirrhosis according to the numbers of the five components of the metabolic syndrome present

	Histologic cirrhosis			Possible cirrhosis			Probable cirrhosis		
Numbers of components*	**n/N (%)#**	**OR (95% CI)**	***P* value**	**n/N (%)#**	**OR (95% CI)**	**P value**	**n/N (%)#**	**OR (95% CI)**	**P value**
0	8/54 (15%)	Referent		72/390 (18%)	Referent		30/390 (8%)	Referent	
1	15/56 (27%)	2.1 (0.8–5.5)	0.12	112/511 (22%)	1.4 (0.9–1.9)	0.15	53/511 (10%)	1.3 (0.9–2.1)	0.18
2	4/25 (16%)	1.1 (0.3–4.0)	0.89	120/330 (36%)	2.1 (1.6–3.1)	<0.001	59/330 (18%)	2.4 (1.4–4.0)	<0.001
3	9/17 (53%)	6.5 (1.9–21.8)	0.001	59/136 (43%)	2.8 (1.8–4.0)	<0.001	37/136 (27%)	3.9 (2.1– 6.7)	<0.001
4	4/8 (50%)	5.8 (1.2–27.8)	0.02	27/51 (53%)	4.5 (2.6–7.8)	<0.001	16/51 (31%)	4.1 (1.9–8.4)	<0.001
5	1/4 (25%)	1.9 (0.2–20.8)	0.59	12/17 (71%)	8.7 (3.2–23.9)	<0.001	6/17 (35%)	5.2 (1.6–14.2)	<0.001

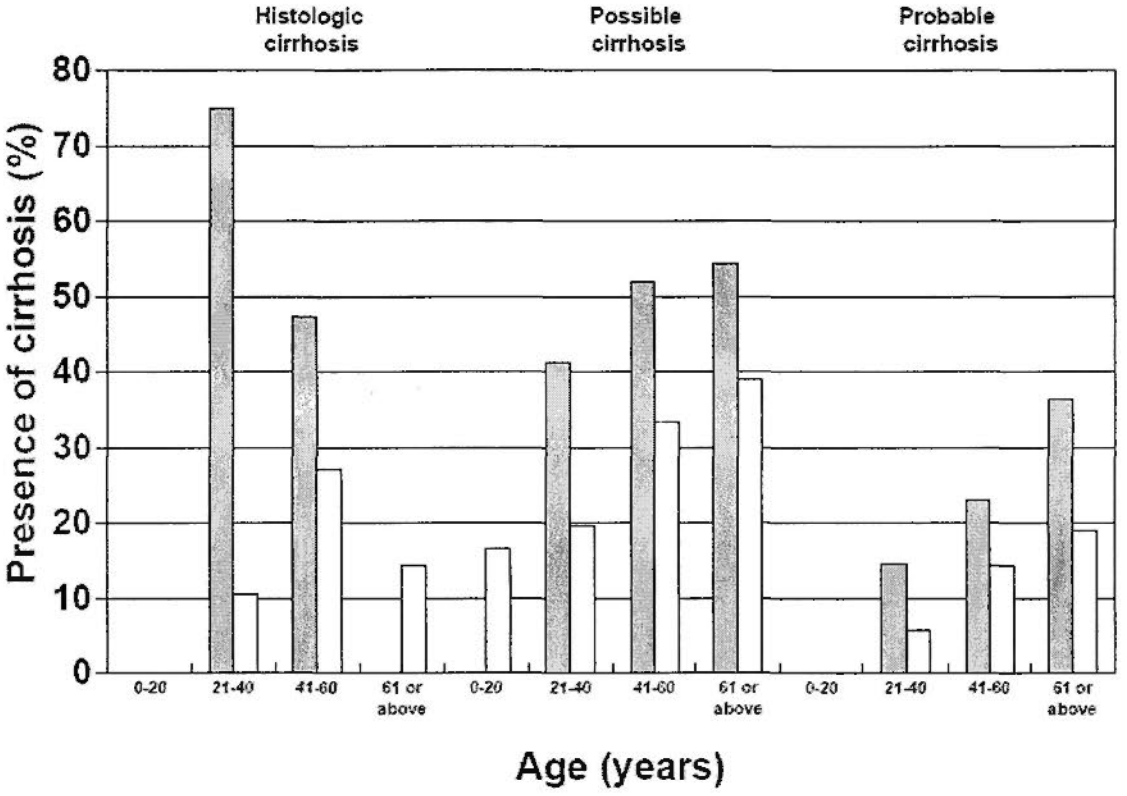
Numbers of components*: numbers of the five components of the metabolic syndrome present.

n: numbers of patients suffering from histologic possible or probable cirrhosis in that particular group of patients.

N: numbers of all patients in that particular group of patients.

OR: odds ratio. CI: confidence interval.

Figure 11.1 Bar charts on the prevalence of histologic, possible and probable cirrhosis in different age groups



Grey bars represent patients with metabolic syndrome; white bars represent patients without metabolic syndrome.

Figure 11.2 Bar charts on the prevalence of the metabolic syndrome in different age groups

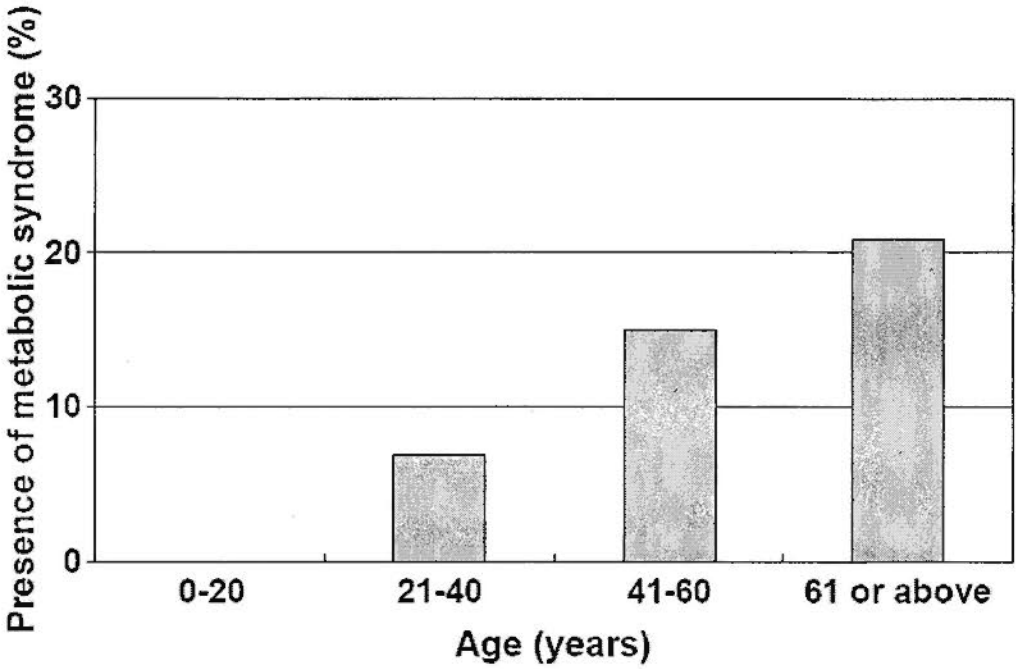
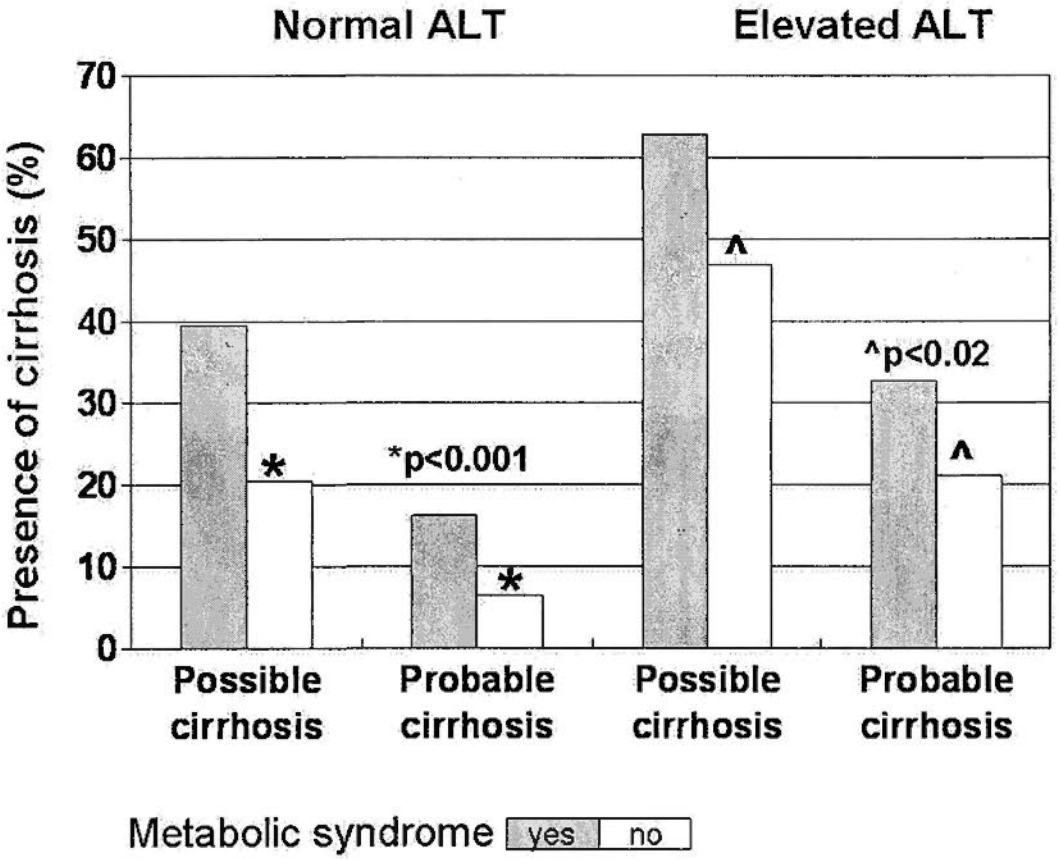


Figure 11.3 Bar charts on the prevalence of the metabolic syndrome in normal and elevated alanine aminotransferase levels



ALT: alanine aminotransferase.

CHAPTER TWELVE

DISCUSSIONS

12.1 Validation of Transient Elastography

12.1.1 Validation of transient elastography to detect advanced liver fibrosis in chronic hepatitis B

In Study 1, LSM with transient elastography was found to have good correlation with histologic liver fibrosis in patients with chronic liver diseases. Using morphometric analysis, LSM was shown to be better correlated with pericellular fibrosis than periportal or perivenular fibrosis. This phenomenon may explain why LSM is accurate in detecting bridging fibrosis and cirrhosis but not liver fibrosis of milder degree.

In previous studies among patients with viral hepatitis, the diagnostic performance of LSM was best for histologic cirrhosis and less satisfactory for earlier stages of liver fibrosis [Talwalker et al., 2007]. In Study 1 including mostly patients with viral hepatitis B or C, the diagnostic accuracy of LSM for liver cirrhosis seemed higher than that for bridging fibrosis. On morphometric analysis, which provided a objective and quantitative assessment of the distribution of liver fibrosis in different zones, LSM was found to be correlated better with pericellular than periportal and perivenular fibrosis. This observation may explain the fact that the diagnostic performance of LSM for fibrosis was inferior in earlier stages, in which most of the fibrous tissue deposits in portal area (viral hepatitis) or perivenular area (NAFLD). Fibrous tissue starts to deposit in pericellular space when the disease advances to

bridging fibrosis or cirrhosis.

Different LSM cutoff values have been proposed by different investigators for various stages of liver fibrosis. The optimal LSM cutoff values for cohorts with predominant chronic hepatitis C patients was between 10.4 kPa and 17.6 kPa, and the area under ROC for liver cirrhosis was 0.93 to 0.96 [Fraquelli et al., 2007; Ganne-Carrie et al., 2006; Foucher et al., 2006; Castera et al., 2003]. In Study 1, no single optimal LSM cutoff values for liver cirrhosis could be identified among patients with viral hepatitis. The relatively less satisfactory performance of LSM in our patients was likely due to the predominance of CHB. Previous reports have shown the area under ROC for histologic cirrhosis among CHB patients was approximately 0.90, which tend to be lower than that of patients with other liver etiologies [Ganne-Carrie et al., 2006; Marcellin et al., 2005]. In contrast, the performance of LSM in NAFLD patients appears better. Among 775 patients with chronic liver disease undergoing liver biopsies and LSM with transient elastography, the overall accuracy of LSM for the diagnosis of histologic cirrhosis was 90% for CHB and 96% for alcoholic or NAFLD [Ganne-Carrie et al., 2006]. However, only 47 patients in that study had NAFLD, and only 3 had NASH cirrhosis. In another study on 67 Japanese NAFLD patients (5 had cirrhosis), the area under ROC curve of LSM to diagnosis cirrhosis was 99.7% [Yoneda et al., 2007]. In view of small numbers of NAFLD patients and NAFLD-related cirrhosis, subgroup analysis was not performed in Study 1. More definitive studies are warranted to investigate the

different cutoff values of LSM for NAFLD and viral hepatitis in defining liver cirrhosis.

The lower LSM cutoff value for histologic cirrhosis among this cohort of viral hepatitis patients was in agreement with previous CHB cohorts where the optimal cutoff value was approximately 10.3 kPa [Ganne-Carrie et al., 2006; Marcellin et al., 2005]. It is not certain whether there is any relationship between ethnicity and the LSM cutoff values for liver cirrhosis, but apparently the accuracy of LSM is not affected by the BMI of the patients. Another factor affecting predictive value by LSM is the prevalence of histologic cirrhosis in the study population [Poynard et al., 2007]. Forty-two (31.6%) patients in our cohort had histologic cirrhosis, which was higher than the rate of 15.4–26.8% previously reported [Ganne-Carrie et al., 2006; Foucher et al., 2006; Marcellin et al., 2005; Ziol et al., 2005; Castera et al., 2003; Corpechot et al., 2006b]. This might also explain the slightly lower area under ROC of predicting histologic cirrhosis in our study when compared to the previous studies.

12.1.2 The optimal LSM cutoff values for different stages of liver fibrosis in chronic hepatitis B - alanine aminiotransferase based algorithms

In Study 2, LSM with transient elastography was again found to have good correlation with bridging fibrosis and liver cirrhosis in CHB patients. Patients with elevated serum ALT levels had higher LSM values despite the same

degrees of liver fibrosis. Elevated serum ALT levels posed most serious influence on the diagnostic performance of LSM for no fibrosis (F0 *vs.* F1–4) while that for bridging fibrosis and cirrhosis were less seriously affected. HBeAg status did not significantly affect the diagnostic performance of LSM after taking ALT into account. We also defined different optimal cutoff values for LSM with respective to the ALT level and a practical algorithm for using LSM to substitute liver biopsy was recommended.

Different LSM cutoff values have been proposed by different investigators for various stages of liver fibrosis [Ganne-Carrie et al., 2006; Marcellin et al., 2005]. In Study 2, instead of proposing a single optimal LSM cutoff for each degree of liver fibrosis, we defined different optimal cutoff values for LSM according to different diagnostic questions, and also according to different ALT levels. One of the advantages of this approach is the flexibility of applying LSM in different clinical scenarios. The LSM cutoff value of high sensitivity would be useful to identify patients who have the lowest risk of having advanced fibrosis or cirrhosis while the cutoff values of high specificity would be useful to confirm the presence of advanced fibrosis or cirrhosis. Using these LSM cutoff values, patients with very low LSM can be reassured and patients with high LSM can be considered for anti-viral treatment without the need of liver biopsy. The cutoff values with high diagnostic accuracy would allow the best guess for a correct diagnosis.

Essentially, this proposed clinical algorithm for CHB concurs with what Afdhal and Curry suggested for chronic hepatitis C [Afdhal et al., 2007]. In our algorithm, patients of low LSM value below 5.0 kPa could be reassured regardless of the ALT level. High LSM values with LSM >9.0 kPa for normal ALT and LSM >12.0 kPa for elevated ALT should be considered for treatment particularly if HBV DNA is elevated. In this way, liver biopsy can be avoided in approximately 62% of patients with normal ALT and 58% of patients with elevated ALT from liver biopsy. However, as some patients who have ALT levels higher than 2 times ULN will be treated without liver biopsy [Lok et al., 2007; Liaw et al., 2008], the number of liver biopsies exempted among patients who have elevated ALT levels should be an underestimate in Study 2.

12.1.3 Effect of alanine aminotransferase on liver stiffness measurement

Higher ALT level was found to be associated with higher LSM scores in previous studies [Foucher et al., 2006; Coco et al., 2007]. Elevated ALT level usually reflects increased histologic necroinflammation, which is a logical cause of increased liver fibrosis. In Study 2, however, increased ALT was associated with more severe necroinflammation but not histologic liver fibrosis. This phenomenon may be related to the confounding effect of duration of immune clearance. Older patients with longer duration of abortive immune clearance should have more severe fibrosis, and the degree of liver damage

could not be fully represented by a snapshot of liver biochemistry or histologic necroinflammation [Lok et al., 2007]. One possible explanation for the association of ALT and LSM was a stiffer liver during active inflammation. In a previous report among hepatitis patients with flare-ups, the LSM was increased by 1.3 to 3 folds during the ALT flares and decreased to baseline values thereafter [Coco et al., 2007]. Patients with higher ALT levels, which were associated with increased necroinflammation, tend to have higher LSM despite the same fibrosis staging in Study 2. The diagnostic performance of LSM for no fibrosis (F0 *vs.* F1–4) was most seriously significantly decreased when ALT was elevated, while that for bridging fibrosis and liver cirrhosis was less seriously affected. Among patients who had normal ALT levels, the overlap of the optimal ranges of LSM cutoffs for no fibrosis, bridging fibrosis and cirrhosis was very small. In contrast, among patients who had elevated ALT levels, the optimal range of LSM for no fibrosis fell within the range of bridging fibrosis, while the difference between the LSM ranges between bridging fibrosis and cirrhosis was still satisfactory.

Based on these results, patients only having mild fibrosis would be misdiagnosed to have moderate-to-severe fibrosis and even cirrhosis if they had raised ALT levels. It is almost impossible to differentiate the stages of fibrosis below F3 using LSM in the presence of raised ALT levels. The diagnostic performance of a lower cutoff value of LSM for cirrhosis was therefore less reliable when ALT was higher than the ULN and a higher LSM

cutoff value may be needed. As ALT flare is common in CHB, the impact of ALT levels may contribute to the relatively less satisfactory prediction of LSM for liver fibrosis in this condition than in other liver diseases.

In Study 2, the diagnostic performance of LSM in patients with ALT levels ≥5x ULN could not be assessed appropriately due to the small number of patients. In fact, use of LSM to diagnose liver cirrhosis might be inappropriate as LSM values would be very much elevated during acute hepatitis and then gradually decreased with the resolution of hepatitis [Sagir et al., 2007; Arena et al., 2007]. Hence the use of transient elastography to determine liver fibrosis at the time of severe acute exacerbation of CHB is not recommended. These patients should have their LSM performed after the normalization of ALT in order to accurately assess the severity of liver fibrosis or to diagnose liver cirrhosis.

12.2 Clinical Predictive Factors of Liver Cirrhosis in Chronic Hepatitis B

12.2.1 Factors associated with liver fibrosis in hepatitis B e antigen-positive chronic hepatitis B

The true prevalence of different severities of liver fibrosis and the immune tolerance phase has been very difficult to study, as the indication of liver biopsy in HBeAg positive patients with normal liver biochemistry is weak. With the availability of transient elastography together with optimal cutoff values according to ALT levels derived in Study 2, different severities of liver fibrosis could be defined with high specificity. In Study 3, we have demonstrated that the prevalence of immune tolerance phase and advanced fibrosis (or possible cirrhosis) was 27% and 22% respectively. Age of patients and serum ALT level were the independent predictive factors of severities of liver fibrosis.

In Asian patients who acquire the hepatitis B infection at infancy, there is usually minimal liver damage in the immune tolerance phase. Patients in the immune clearance are usually older with elevated ALT [Lok et al., 1988]. In Study 3, we have identified that the risk of cirrhosis, which was the end-product of immune clearance, started to increase after age of 35 and was reflected by ALT >0.5x ULN. Only 2% of patients of age ≤35 and ALT ≤0.5x ULN had possible cirrhosis. The diagnosis of immune tolerance was more certain if these patients had persistently low normal ALT levels (≤0.5x ULN) as

illustrated by our longitudinal cohort. These findings were in line with an Indian series in which 44 of the 73 (60%) HBeAg-positive patients with persistently normal (<40 IU/l) ALT had minimal to mild histologic liver fibrosis (<F2) [Kumar et al., 2008]. Therefore, liver biopsy should seldom be indicated in patients who have favorable age and ALT levels.

Patients who remained HBeAg-positive after the age of 35 yet still had ALT >0.5x ULN might reflect an abortive immune clearance as reflected by the results of our study (37% of these patients had possible cirrhosis). Patients who had persistently elevated ALT levels (>0.5x ULN) had highest risk of liver cirrhosis, which echoed the results of another study which showed ALT <0.5x ULN was associated with the lowest risk of complications [Yuen et al., 2005b]. Therefore, patients who have positive HBeAg after the age of 35, particularly if they have ALT >0.5x ULN, should be carefully assessed for liver fibrosis and considered for treatment if necessary. Nonetheless, LSM has not been shown to reflect immue response to HBV. Longitudinal study of natural history of CHB involving larger numbers of patients will be useful to study the use of LSM to reflect host immune response to the virus.

Most previous studies found that higher HBV DNA was associated with increased risk of liver cirrhosis [Iloeje et al., 2006, Yuen et al., 2005b] and HCC [Chen et al., 2006b, Chan et al., 2008]. In these studies, HBeAg-negative patients were the predominant population. Among HBeAg-negative patients

whom should have undergone the stage of immune clearance, failure of clearing the virus will be accompanied by persistently hepatitis activities and more severe histologic damage [Chan et al., 2000]. However, this phenomenon cannot be extrapolated to and can be potentially misleading among the young HBeAg-positive patients in the immune tolerance phase whose HBV DNA is usually very high but the liver damage is minimal. Patients with more successful immune clearance (and possibly almost having HBeAg seroconversion) might have lower HBV DNA and also minimal fibrosis. In contrast, advanced fibrosis mainly occurred in patients with intermediate HBV DNA levels, as they might had less successful immune clearance of the virus and thus more liver damage. Hence a bell-shaped rather than a linear relationship was present between LSM and HBV DNA levels among HBeAg-positive patients.

Our findings posed a few important clinical implications. First, our results supported the findings of previous studies that the current definition of normal ALT was too high and cannot differentiate patients with and without liver cirrhosis in CHB. The use of 0.5x ULN or the newly gender-specific ULN would be a more appropriate representation of the normality of ALT [Prati et al., 2002]. Second, we have confirmed that young HBeAg-positive patients of age below 35 and ALT ≤0.5x ULN were likely in the immune tolerance phase and had mild liver fibrosis. However, older age and higher ALT levels in the presence of positive HBeAg might indicate abortive immune clearance and

hence a higher risk of liver cirrhosis. Current guidelines on the assessment of liver fibrosis among HBeAg-positive patients may need to be loosened as early anti-viral treatment among patients with advanced fibrosis may retard the development of cirrhotic complications. Transient elastography can be considered to supplement liver biopsy in the future.

12.2.2 Factors Associated with Liver Cirrhosis in Hepatitis B e Antigen-Negative Chronic Hepatitis B

Similar to the case of HBeAg-positive CHB, the true prevalence of liver cirrhosis among HBeAg-negative CHB patients has been difficult to study, as it is impossible to perform liver biopsy in a large cohort of HBeAg negative patients who have normal liver biochemistries. With the availability of transient elastography, we can determine early liver cirrhosis with a high degree of certainty [Talwalkar et al., 2007]. In Study 4, we have demonstrated that early liver cirrhosis was not uncommon among HBeAg-negative patients in Hong Kong, probably ranging from 12% (probable cirrhosis) to 26% (possible cirrhosis). Elevated serum ALT and HBV DNA were independent risk factors associated with liver cirrhosis. Our findings were in concordance with previous case histology series that serum ALT and HBV DNA could reflect histologic necroinflammation and fibrosis [Chan et al., 2002c; ter Borg et al., 1998]. Based on our large cohort of patients undergoing transient elastography, a high normal ALT based on the current laboratory cutoff value has already an increased risk of liver cirrhosis.

Among Asian patients who acquire the HBV infection at infancy, most patients do not have significant liver disease in the immune tolerance phase despite a very high HBV DNA level [Chan, 2002a]. Hepatic necroinflammation and liver damage starts to occur at immune clearance and prolonged abortive immune clearance increases the risk of liver cirrhosis. Among patients who have active liver disease after HBeAg seroconversion, liver cirrhosis will continue to develop or worsen. In a Taiwanese cohort, 7.8% of patients developed liver cirrhosis after a median follow-up of 8.6years after spontaneous HBeAg seroconversion [Hsu et al., 2002]. Hence appropriate investigation for liver cirrhosis is warranted for HBeAg-negative patients, even if their ALT levels are normal or slightly elevated as antiviral may be indicated for these patients.

We showed that the risk of liver cirrhosis increased with ALT levels from as low as 0.5x ULN. This level was in line with previous findings in a longitudinal study that ALT level >0.5x ULN was associated with increased risk of hepatic complications [Yuen et al., 2005b]. Based on the current guidelines, anti-viral treatment is recommended for HBeAg-negative patients with ALT >2x ULN and liver biopsy should be considered when ALT is between 1 and 2x ULN [Lok et al., 2007; Liaw et al., 2008]. Using the current laboratory normal, some patients who have advanced liver disease will be missed. In a recent report among 192 CHB patients in America, advanced liver fibrosis and necroinflammation was found in 37% of patients with persistently normal ALT

particularly when the age was older than 40 and ALT was at the high normal range [Lai et al., 2007]. Therefore, we support the recommendation that the current the laboratory normal for ALT should be revised to a lower level [Prati et al., 2002].

HBeAg-negative patients tend to have fluctuating ALT levels [Chan et al., 2000; Liaw et al., 2004a; Hadziyannis et al., 2001]. Some patients may have normalization of ALT levels after transient elevation. In the past, we have shown that a single elevation of ALT can predict biochemical relapse in a longitudinal follow-up of 3 years [Chan et al., 2000]. Based on the longitudinal cohort in Study 4, the peak ALT and the AUC/t ALT levels were better associated with the risk of liver cirrhosis than the trough ALT levels. In other words, it is probably the cumulative effect of hepatic necroinflammation that causes hepatic injury and liver fibrosis. Transient remission of biochemical activity may not indicate a recovery. Therefore, treatment should be considered for HBeAg-negative patients who have intermittent elevation of ALT levels regardless of the transient biochemical remission.

In line with the Taiwanese experience, the risk of liver cirrhosis started to increase when HBV DNA was higher than 4 $\log_{10}$ copies/ml in our patients [Iloeje et al., 2006]. However, approximately 5% (20/381) and 4% (7/166) of our patients who had serum HBV DNA <4 $\log_{10}$ copies/ml and <2 $\log_{10}$ copies/ml (*i.e.* undetectable) were found to have probable liver cirrhosis

respectively. This echoed the previous findings that there is no cutoff value of serum HBV DNA levels to differentiate between patients with or without possible cirrhosis in HBeAg-negative patients [Chan et al., 2003; Chu et al., 2002a]. One possible explanation is that these patients have previous prolonged hepatic necroinflammation and development of liver fibrosis, which persisted even after immune control took over at the time of assessment. Fluctuating viral activity and hepatitis will be another possibility to explain this phenomenon. We found that the use of combined ALT and HBV DNA can more accurately predict the presence of liver cirrhosis. Only 2% of patients who had ALT ≤0.5x ULN and HBV DNA ≤4 $\log_{10}$ copies/ml had probable cirrhosis. Our results echoed that of a recent longitudinal study in Canada that HBeAg-negative patients with normal ALT level tend to have inactive disease in the subsequent year [Feld et al., 2007].

12.3 Metabolic Syndrome and Liver Cirrhosis

In study 5, metabolic syndrome was found to be strongly associated with increased risk of severe fibrosis and cirrhosis in CHB patients. There may be additive effect of individual different components of metabolic syndrome on the risk of liver cirrhosis. This is the first prospectively population-based study reporting the association of metabolic syndrome, using recently proposed Asian-specific definitions, with liver cirrhosis in CHB. The increased risk found in the study was independent of other important and well-studied risk factors such as age, gender, ALT levels, HBV DNA, and HBeAg status [Fattovich, 2003].

Findings of Study 5 echoed the results from a recent study involving 317 patients suffering from chronic viral hepatitis (95 and 176 patients suffering from CHB and CHC, respectively) and NAFLD [Tsochatzis et al., 2008]. Independent association of metabolic syndrome with severe fibrosis was noted in both chronic viral hepatitis and NAFLD. This study was limited by the heterogeneous patient population with a minority suffered from CHB. The relatively small number of CHB patients also did not allow any meaningful statistical adjustment of the potential confounding factors for liver cirrhosis.

The underlying mechanism of the progression of fibrosis in relation to metabolic syndrome could be a direct stimulation of liver stellate cells by

hyperinsulinemia and hyperglycemia, resulting in increased production of the connective tissue growth factor and subsequent accumulation of extracellular matrix [Paradis et al., 2001]. Mechanisms linking steatosis and liver fibrosis are probably related to the oxidative stress generated from fat accumulation within hepatocytes, with subsequent secretion of inflammatory cytokines and activation of stellate cells [Asselah et al., 2006]. These processes may contribute to further hepatic injury from other common factors such as hepatitis B virus [Lonardo et al., 2004]. However, metabolic syndrome but not steatosis was independently associated with liver cirrhosis in our cohort. One possible hypothesis would be insulin resistance as the major driving force for liver fibrosis progression. This would be in line with evidence from CHC patients, as previous studies found that steatosis was not associated with severe liver fibrosis [Asselah et al., 2003; Hui et al., 2003]. On the other hand, HBV does not seem to induce insulin resistance, as insulin resistance was more frequent in CHC patients than in a group of matched CHB patients [Poynard et al., 2003].

The prevalence of metabolic syndrome in this cohort was 13%. This was comparable with the results of a recent cross-sectional study of 7,473 subjects, in which the age-standardized prevalence of metabolic syndrome was 13.9% in Hong Kong [Ko et al., 2007]. Recent surveys across the Asian-Pacific region showed a consistent increase in the prevalence of metabolic syndrome [Nestel et al., 2007]. As CHB is also highly prevalence in the Asia-Pacific region, the

additive effect of metabolic syndrome and CHB on liver fibrosis poses significant risk on patients suffered from both conditions. Early recognition and management of metabolic syndrome in CHB patients may have a positive effect on their long term. Lifestyle modifications including physical activity [Centers for Disease Control 1996], weight loss [World Health Organisation 2000], and diet [Vessby et al., 2001; Summers et al., 2002] favorably affect the various components of metabolic syndrome, at least in the short term. These lifestyle modifications may have potential therapeutic or preventive role for liver fibrosis in CHB patients, particularly in CHB patients suffering from metabolic syndrome. The treatment strategies for CHB patients might also be altered by the presence of metabolic syndrome because of the increased risk of cirrhosis. A lower threshold for initiating anti-viral therapy might be considered.

Using transient elastography, a validated and non-invasive tool to diagnose liver cirrhosis, has facilitated us to investigate the effect of metabolic syndrome in a general CHB population. This has been a no-mans-land in the past when liver biopsy was the only tool for fibrosis assessment but deemed not ethical and feasible for a large number of patients with relatively normal liver function tests. Transient elastography also has the theoretical advantage of measuring a volume of liver tissue at least 100 times bigger than a biopsy sample, even though liver biopsy is still the gold standard for liver fibrosis assessment [Sandrin et al., 2003]. In Study 5, the use of transient

elastography has been internally validated by liver histology and two LSM cutoff values were to use define possible and probable cirrhosis as derived in Study 1 to improve the reliability of our results. The approach was different from that of Study 3 as we aimed to identify patients in the immune tolerance with minimal liver fibrosis. Hence the algorithm derived in Study 2 with ALT incorporated into the equation was sensitive to detect patients with insignificant liver fibrosis. As Study 4 and 5 were aimed to identify liver cirrhosis, we used the two cutoff values of which one was sensitive and one was specific to detect patients with liver cirrhosis.

In the present study, 4% of patients were excluded from analysis due to failed LSM, while 91.4% and 99.0% of patients with and without central obesity have reliable LSM results, respectively. Most previous study of transient elastography reported a failure rate of 5% to 8% due to obesity and other reasons [Talwalkar et al., 2007; Malik et al., 2007], and these patients were more likely have high BMI and central obesity. Hence central obesity might pose a potential limitation for the use LSM to detect metabolic syndrome in patients with fatty liver disease. We expect the failure rate of LSM among Caucasian patients who are in general more obese than Asians may be higher.

12.4 Limitations

One of the common limitations in the 5 studies was that liver biopsy was used as the gold standard in all these studies despite its limitation of sampling bias, similar to previous studies. The mean length of liver biopsies ranged from 17mm to 19mm, which was still shorter than the optimal length of 25mm as suggested by Bedossa *et al.* [Bedossa et al., 2003]. In that study 65% of biopsies measured 15 mm in length were categorized correctly according to the reference value by using the Metavir scoring system. This increased to 75% for a 25-mm liver biopsy specimen without any substantial benefit for longer biopsy specimens. The slightly shorter length of the liver biopsies in my studies might limit the accuracy of histologic assessment of necroinflammation and fibrosis. As transient elastography measures a volume of liver tissue at least 100 times bigger than a biopsy sample, there might be a possibility that the performance of LSM has been underestimated [Sandrin et al., 2003]. The upper limit of LSM (48 kPa) in my studies was lower than that (75 kPa) in previous reports. This was probably because we excluded patients who had decompensated liver disease or complications of liver cirrhosis. Hence patients suffering from more severe form of liver cirrhosis, who might have higher LSM score, were excluded.

In Study 1 and 2, 27% and 13% of the original patient cohort was excluded from analysis because of inadequate liver biopsy or unreliable LSM results

respectively. However, its effect on the results should be modest as the clinical and histological characteristics were similar between the original and the study cohorts. The relatively small sample size and heterogeneous etiologies in Study 1 was another limitation. The small number of patients suffering from NAFLD especially severe steatohepatitis renders our study insufficient to make any recommendation among these patients.

In Study 3 to 5, transient elastography was used instead of liver biopsy as the standard for liver fibrosis, which is not 100% accurate in differentiating different fibrosis stages. The accuaracy of transient elastography has been questioned in elevated ALT levels as it was found to yield pathologically high LSM values in patients with minimal liver fibrosis but acute liver damage due to various etiologies (acute viral hepatitis, drug-induced hepatitis and autoimmune hepatitis) [Sagir et al., 2007]. LSM values decreased to values below the cutoff value for liver cirrhosis after the acute hepatitic phase. In another cohort of patients suffering from acute viral hepatitis with minimal liver fibrosis, LSM was found to be unreliable to detect cirrhosis [Arena et al., 2007]. Unlike patients from these two studies, the majority of the patients in Study 1 to 5 had normal or only mildly elevated ALT. Although LSM is overestimated during acute hepatitis, there is currently no evidence to suggest that LSM is inaccurate in patients with chronic viral hepatitis with ALT in the range of the current study. Another study showed that the use of a high cutoff value may ensure a high specificity of liver cirrhosis for patients

with elevated ALT levels [Coco et al., 2007]. To overcome this limitation, patients with histologic proof from liver biopsy were included in Study 3 to 5 to internally validate the LSM cutoff values used. Cutoff values with high specificities were chosen such that the different severities of liver fibrosis would be diagnosed with high certainty according to the results of Study 1 and 2.

Another limitation was that Study 3 to 5 were cross-sectional studies, therefore the impact of disease fluctuation on liver fibrosis cannot be assessed. The impact of variations ALT and HBV DNA on LSM is clinically important. A recent study conducted our team showed that LSM may misdiagnose liver cirrhosis in patients suffering from severe acute exacerbation of chronic hepatitis B [Wong et al., 2009]. Hence these patients should have their LSM performed at least 3 months after the normalization of ALT in order to accurately assess the severity of liver fibrosis or to diagnose liver cirrhosis. Unfortunately, the information concerning the ALT or HBV DNA levels during the 3-6 months prior LSM were not available for all the patients included in the studies. This potential bias can be partly compensated by the large number of patients studied and the inclusion of patients who have been longitudinally followed up in Study 3 and 4. The possibility of referral bias that may overestimate the prevalence of advanced fibrosis could not be excluded. With the open invitation policy, a significant proportion of patients were referred from primary care physicians. Furthermore, over 40% of patients in

our specialist clinics were referred from community-based screening programs of asymptomatic subjects [Chan et al., 2000]. In Study 4, 39% of our patients had HBV DNA less than 4 log copies/ml and 72% of patients had normal ALT levels; while in Study 5, 39% of our patients had HBV DNA less than 4 log copies/ml and 66% of our patients had normal ALT levels. Therefore the results could likely be generalized to other CHB patients either under the care of hepatology specialists or general practitioners.

In Study 5, 4% of patients were excluded from analysis due to failed LSM, while 91.4% and 99.0% of patients with and without central obesity have reliable LSM results, respectively. Most previous study of transient elastography reported a failure rate of 5% to 8% due to obesity and other reasons [Talwalkar et al., 2007; Malik et al., 2007], and these patients were more likely have high BMI and central obesity. Hence central obesity might pose a potential limitation for the use LSM to detect metabolic syndrome in patients with fatty liver disease. We expect the failure rate of LSM among Caucasian patients who are in general more obese than Asians may be higher. Last, information on the ultrasonograhic evidence of fatty change in Study 5 was not available for analysis. Nonetheless, ultrasonography can only pick up fatty change when there is more than 33% fat on histology and it is not a very sensitive measure for fatty liver disease [Chan HL et al., 2007].

CHAPTER THIRTHEEN

CONCLUSIONS

Results from my studies suggest that LSM using transient elastography is an accurate non-invasive tool to diagnose bridging fibrosis and cirrhosis because of its good correlation with pericellular fibrosis. Using different LSM cutoff values, advanced liver fibrosis or cirrhosis can be diagnosed or excluded with high certainty, as a result liver biopsy may be obviated in a significant proportion of patients. As liver fibrosis tends to be overestimated by LSM when ALT levels were elevated, higher LSM cutoff values may be needed to diagnose different degrees of liver fibrosis in patients with elevated ALT levels. Different LSM cutoff values and algorithms were derived for patients with normal and elevated ALT levels.

The prevalence of immune tolerance phase and advanced fibrosis in my cohort of HBeAg-positive CHB patients was 27% and 22% respectively. The prevalence of advanced liver fibrosis in HBeAg-positive CHB patients started to increase as age above 35 and ALT above 0.5x ULN. These patients should be carefully evaluated for the need of anti-viral treatment. Future studies should be conducted to address the efficacy and cost-effectiveness of anti-viral therapy for HBeAg-positive patients who have ALT above 0.5x ULN in the prevention of liver cirrhosis and its related complications.

Liver cirrhosis was found to be common among HBeAg-negative CHB patients. Insidious and continuous liver damage due to persistent viremia and biochemical activity can lead to liver cirrhosis. The current laboratory normal

ALT is too high to stratify the risk of liver cirrhosis. The use of a lower ALT cutoff value (0.5x ULN) or the newly recommended gender-specific upper limits of normal for ALT levels (men 30 IU/l and women 19 IU/l) by Prati et al. is probably more sensitive to identify patients at risk of developing cirrhosis.

Metabolic syndrome was independently associated with liver cirrhosis in CHB. Therefore hepatology specialists or general practitioners taking care of CHB patients should proactively look for the evidence of metabolic syndrome and its components, apart from the standard investigations and monitoring for the disease. As CHB patients with metabolic syndrome are more likely to harbor early cirrhosis, diagnostic workup for cirrhosis is warranted in these cases because of the important therapeutic and surveillance implications.

In all, the results of these 5 studies are important in the understanding of the natural history of CHB. They also provide important data concerning the risk of developing advanced liver fibrosis in CHB, which may direct future follow-up and treatment strategies among CHB patients.

CHAPTER FOURTEEN

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CHAPTER FIFTEEN

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CHAPTER SIXTEEN

LIST OF PUBLICATIONS OF MY WORK USED IN THIS THESIS

STUDY 1

Wong GL, Wong VW, Choi PC, Chan AW, Chum RH, Chan HK, Lau KK, Chim AM, Yiu KK, Chan FK, Sung JJ, Chan HL.
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Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B.
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STUDY 3

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Clinical Factors Associated with Liver Stiffness in Hepatitis B e Antigen-Positive Chronic Hepatitis B Patients.
Clin Gastroenterol Hepatol 2009;7:227-233
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