FibroScan®を用いた治療後肝硬度変化の測定

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索引用語：FibroScan®，肝硬度，direct-acting antiviral agents（DAAs），HCV駆除，肝発癌

1 はじめに

C型慢性肝炎の治療効果は，高い抗ウイルス効果を持つ経口の直接作用型抗ウイルス薬（direct-acting antiviral agents：DAAs）の登場により，著明に向上している．TelaprevirやSimeprevirを含む3剤併用療法により1型高ウイルス量症例においても約80％の著効率が得られるようになった．1型高ウイルス量症例以外の症例ではすでにペグインターフェロン（PEG-IFN）・リバビリン（RBV）併用療法により約80％の著効率が得られている．開発中の経口抗ウイルス薬併用療法では，IFNを使用しないので，高齢者や肝硬変症例にも容易に使用でき，高い著効率が期待されている．

これから重要な問題はC型肝炎ウイルス（HCV）駆除後の肝発癌である．一般にHCV駆除後肝発癌率は著明に低下することが報告されている．しかし低率ではあるが，肝発癌がみられる．そのため著効になっても，線維化の進行状況に応じて年1～4回の画像診断による定期検査が推奨されている．経口抗ウイルス薬併用療法などにより，今後高齢者や肝硬変症例の著効例が増加してくると，HCV駆除後の肝発癌症例が増えてくる可能性がある．そのためHCV駆除後の肝癌スクリーニングの必要性がさらに増してくると思われる．同時にHCV駆除後の肝発癌高リスク症例を囲い込むための，発癌リスク因子についての検討が必要になってくる．

本巻の他稿「FibroScan®によるC型肝炎肝線維化評価と発癌リスク」で述べられているように，FibroScan®による肝硬度が高いほど，肝発癌率が高くなる．また本稿でこれから述べるようにHCV駆除後にはほとんどの症例で肝硬度は低下する．それではHCV駆除後に発癌する症例の肝硬度になんらかの特徴はあるのだろうか．治療前に肝硬度が高い症例はHCVが駆除されても発癌しやすいのか．HCV駆除後肝硬度が低下すれば，発癌率は低下するのか．発癌する症例はHCVが駆除

Kentaro YOSHIOKA et al：Reduction of liver stiffness after expulsion of hepatitis C virus
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されても肝硬度が下がらない症例なのか．さまざまな疑問を今後検討していかなければならない．

本稿ではこれまで行われてきたIFN単剤療法やIFN・リバビリン併用療法によりFibroScan®によって測定される肝硬度の変化について述べ，今後のHCV駆除後肝発癌の対策の参考にしていただきたい．

2 FibroScan® による肝硬度測定

肝硬度を測定する装置(Transient elastography：TE)は，2003年にフランスのEchosens社から発表され，FibroScan®として発売された．この装置は体表から肝臓に低周波弾性波を送り，その肝臓内での伝播速度を超音波により測定して肝硬度を算出する．弾性波の伝わる速度は組織の硬度に依存し，組織が硬いほど弾性波はより速く伝播する．肝硬度の測定値はキロパスカル(kPa)で表示される．

測定手技は，超音波装置と振動子を備えたプローブを肋間の体表面にあてボタンを押すのみであるが，その際Mモードとイメージを参考にして，肝臓に6 cm以上の厚さがあり(測定は2.5～4.5 cmの範囲で行われる)，大きな脈管構造がない肝臓の上にプローブを置く必要がある．10回手技を行い評価し，ディスプレイには測定中央値が自動的に表示される．

このようにFibroScan®は，①非侵襲的で無痛性，②結果がすぐに出る(1回30秒以内)，③再現性が高い，④検査範囲が肝臓全体の約1/500の範囲と大きい(肝生検の検体は約1/50,000)，⑤経時的にフォローアップできる，などの利点がある．ただし，①腹水がある症例(弾性波は液体を通過しない)，②皮下脂肪が高度な症例，③肋間の狭い症例，④肝萎縮の高度な症例，などでは再現性が低下したり，測定不能となる欠点がある．

3 肝硬度と肝線維化stageとの関係

肝生検による肝線維化評価システムと肝硬度の相関については数多くの報告があり，stageに対応するcut off値も提案されている(図1，表1)[1]．しかし肝線維化以外の炎症，うっ血，胆管閉塞などにより肝硬度が上昇することが報告されている．Cocoらは肝炎ウイルス感染患者のうち抗ウイルス治療後や自然経過によってALT値が正常化している患者では，同一の線維化stageであってもALT値が異常である患者に比べて肝硬度が低いと報告している[2]．したがって肝硬度から肝線維化を推定する時には，ALT値を考慮にいれる必要がある．

4 FibroScan® を用いた治療後肝硬度変化

C型慢性肝炎に対する抗ウイルス療法の治療効果は，ALT値やウイルス消失の有無だけでなく，肝線維化stageの改善の有無についても評価すべきである．肝生検により治療後の肝線維化stageの変化について検討した論文が報告されているが，臨床現場で侵襲のある肝生検を経時的に行うことは困難である．そこで肝線維化を非侵襲的に評価するため，肝硬度を測定することが検討されてきた．

Ogawaらは PEG-IFN・RBV併用療法を受けた145例のC型肝炎患者をFibroScan®で評価した[3]．SVR患者はnon-SVR患者に比べて，治療終了時の肝硬度が有意に低下(P=0.0127)しており，その48週後(P<0.0001)，96週後(P<0.0001)でも有意に低下していた．またnon-SVR患者の中では，biochemical responder (BR) 患者はnon-BR患者に比べて

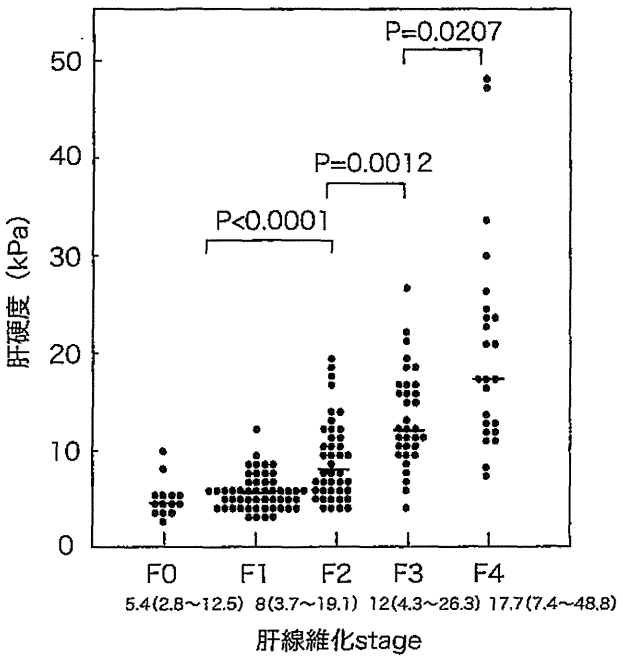


図1　C型慢性肝炎の肝線維化stageと肝硬度の関係

表1　肝線維化stage診断のための肝硬度cut off値（C型慢性肝炎）

	F2以上	F3以上	F4
Cut off value（kPa）	7.1	9.6	11.6
Positive Predictive Value（%）	86.0	72.5	41.5
Negative Predictive Value（%）	73.6	92.7	98.2
Sensitivity（%）	80.8	87.7	91.7
Specificity（%）	80.3	82.4	78.0
Positive Likelihood Ratio	4.1	5.0	4.2
Diagnostic Accuracy（%）	80.6	84.2	80.0

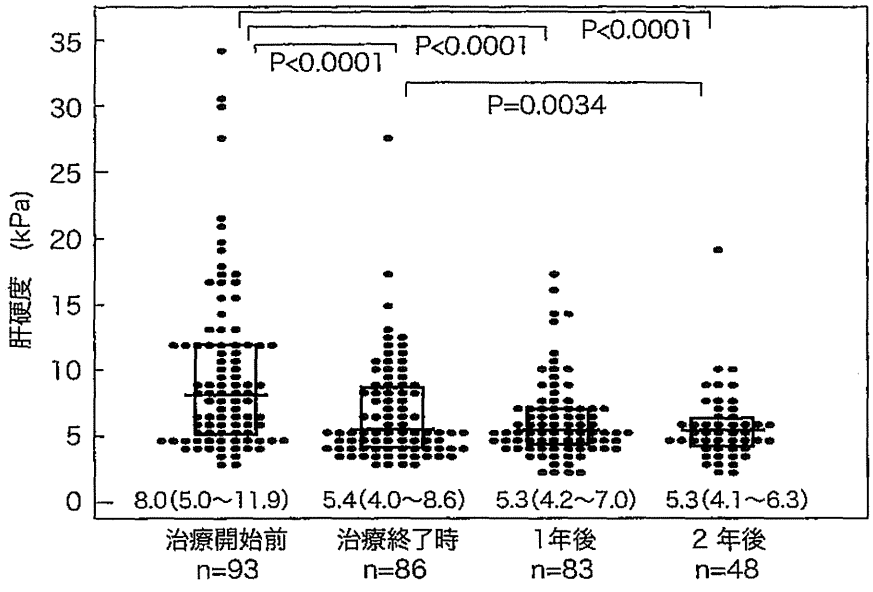


図2　C型慢性肝炎に対するIFN治療SVR例の肝硬度の低下

治療終了時(P=0.0270)，48週後(P<0.0001)，96週後(P<0.0001)とも肝硬度が有意に低下していた．

われわれもIFN単独療法あるいはIFN・RBV併用療法を受けた145例のC型慢性肝炎患者で治療前，治療終了時，治療終了1年後，2年後にFibroScan®で肝硬度を評価し，報告している[4]．SVR患者93例では，治療前と比べて治療終了時(P<0.0001)，1年後(P<0.0001)，2年後(P<0.0001)とも肝硬度は有意に低下しており，治療終了時と比べて2年後の肝硬度も有意に低下していた(P=0.0034)(図2)．relapser 28例では，治療前と比べて治療終了時(P=0.0023)，1年後(P=0.0204)とも肝硬度は有意に低下していたが，2年後は治療前と比べて有意差がな

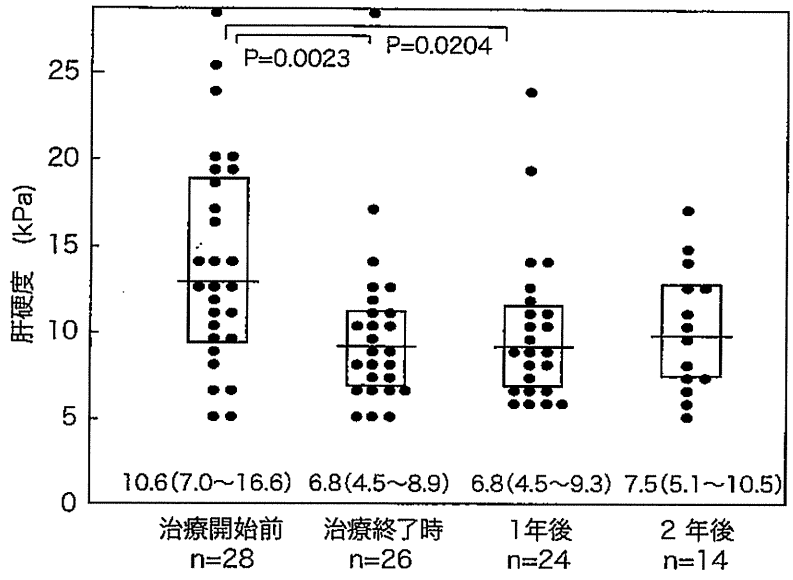


図3　C型慢性肝炎に対するIFN治療relaplar例の肝硬度の低下

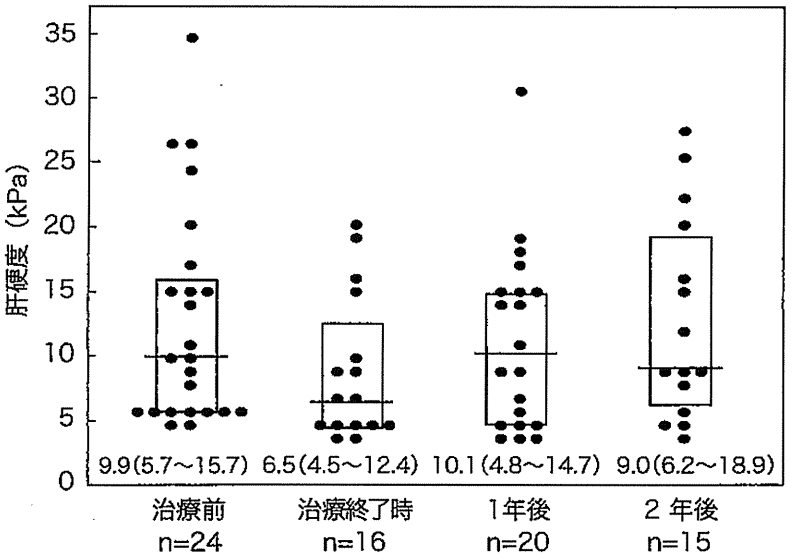


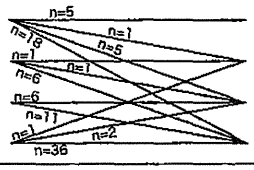
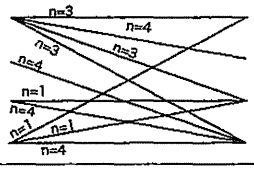
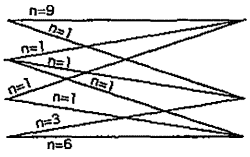
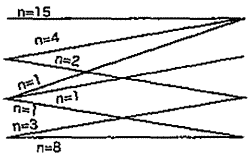
図4　C型慢性肝炎に対するIFN治療NVR例の肝硬度の変化

かった(図3). NVR例24例では治療前と比べて終了時に肝硬度が低下していたが，有意差はなかった(図4).

肝硬度からcut off値(表1)に基づいて推定される肝線維化stage（推定stage)の変化を治療効果別に検討した(表2). 治療前の推定stageがF3以上の67人の患者のうち，最終測定時に推定stageが2段階以上の改善した症例はSVR例で37例中29例(78％)，relapser例で17例中10例(59％)，NVR例で13例中2例(15％)みられた. 推定stageの2段階以上の改善に関与する因子について検討したところ，治療前の肝線維化stageが低いこと，ヒアルロン酸値が低いこと，IFN治療期間が長いこと，治療効果がSVRあるいはrelapserであること，ALT値が高いことが有意に関与していた.

Wangらは，IFN療法を受けた144例(SVR 95例，non-SVR 49例)についてFibroScan®で肝硬度を測定し，SVR患者では有意な肝

表2　肝硬度から推定される肝線維化stageの変化(治療効果別)

	肝硬度より推定される肝線維化stage	治療前		治療後
SVR patients n=93	F4	29 (31%)		5 (5%)
	F3	8 (9%)		3 (3%)
	F2	17 (18%)		14 (15%)
	F0-1	39 (42%)		71 (76%)
Relapsers n=28	F4	13 (46%)		4 (14%)
	F3	4 (14%)		4 (14%)
	F2	5 (18%)		5 (18%)
	F0-1	6 (21%)		15 (54%)
NVR patients n=24	F4	10 (42%)		11 (46%)
	F3	3 (13%)		0 (0%)
	F2	2 (8%)		5 (21%)
	F0-1	9 (38%)		8 (33%)
Patients without IFN treatment n=35	F4	15 (43%)		20 (57%)
	F3	6 (17%)		1 (3%)
	F2	3 (9%)		5 (14%)
	F0-1	11 (31%)		9 (26%)

硬度の低下(median, 0.6 ; P<0.001)を認め，non-SVR患者では肝硬度の上昇(median, 0.8 ; P=0.557)を認めた，と報告している[5]．また，SVR患者では初回の肝硬度が高いことが速やかな肝硬度低下の予測因子であったが，一方で治療前の線維化進展，高いbody mass index (BMI) などは肝硬度低下が遅いことの予測因子であったとしている．

これらの報告から，C型慢性肝炎の治療において肝硬度を測定することにより，肝線維化の改善だけでなく，肝線維化の改善に影響を及ぼす因子についても評価することが可能であるといえる．

上記3つの報告は治療後の肝生検を行っていない．そのため治療後の肝硬度の低下が，肝線維化の改善によるものであるか，炎症の改善によるものではないかという見方もある．これまで治療前後に肝生検を施行して肝線維化の改善を評価した報告がいくつかある．それらの報告では，SVR症例においてはIFN治療後それぞれ29 % の症例(前後の肝生検の間の平均期間: 1.6 年)[6]，44 % の症例(2.5 年)[7]，59 % の症例(3.7 年)[8]，82 % の症例(5.2 年)[9]で肝線維化が改善していた．Georgeらは治療前肝硬変または高度な線維化のあった患者がSVRになると5.2 年後には67 % の症例において肝線維化stageが2ポイント以上改善したことを報告している[9]．

われわれの肝硬度に基づく推定肝線維化stageによる2ポイント以上の改善の頻度(78 %)は，Georgeらの肝生検による結果(67 %)に比較して少々高いがかけ離れた数値ではなく，肝硬度に基づく推定stageによる治療後の肝線維化stageの評価に信頼性があることを示していると考える．

5 HCV駆除後の肝硬度と発癌

自然経過ではFibroScan®による肝硬度が

高いほど，肝発癌率が高くなることが報告されている[10]．これまで述べてきたようにHCV駆除後にはほとんどの症例で肝硬度は低下する．治療後に肝硬度の低下する症例では肝発癌率はおそらく低下するものと思われる．しかし肝硬度が低下しても発癌する症例もあると思われる．HCV駆除後に発癌する症例の肝硬度になんらかの特徴はあるのだろうか．治療前に肝硬度が高い症例はHCVが駆除されても発癌しやすいのか．HCV駆除後肝硬度が低下すれば，発癌率は低下するのか．発癌する症例はHCVが駆除されても肝硬度が下がらない症例なのか．さまざまな疑問を今後検討していかなければならない．

6 おわりに

FibroScan®を使用して肝硬度を測定することにより，非侵襲的に肝線維化stageを推定することが可能となった．IFN治療によりHCVが駆除された症例では，年を経るごとに肝硬度が低下していくことが示され，これまで肝生検により示されてきたIFN治療後の肝線維化の改善が確認された．今後HCV駆除後の肝線維化の評価は非侵襲的なFibroScan®による肝硬度の測定により行われるようになると思われる．テラビック®やSimeprevirを含む3剤併用療法や経口抗ウイルス薬併用療法などにより，今後高齢者や肝硬変症例の著効例が増加し，HCV駆除後の肝発癌症例が増えてくることが危惧されている．そのためHCV駆除後の肝癌スクリーニングの必要性がさらに増してくると思われる．HCV駆除後にFibroScan®で肝硬度を測定することにより，肝発癌リスクの高い症例の絞り込みが可能となり，肝癌の早期診断に結びつくものと期待される．

文　献

1) Friedrich-Rust M, Ong MF, Martens S et al : Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 134 : 960–974, 2008
2) Coco B, Oliveri F, Maina AM et al : Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. J Viral Hepat 14 : 360–369, 2007
3) Ogawa E, Furusyo N, Toyoda K et al : The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin. Antiviral Res 83 : 127–134, 2009
4) Arima Y, Kawabe N, Hashimoto S et al : Reduction of liver stiffness by interferon treatment in the patients with chronic hepatitis C. Hepatol Res 40 : 383–392, 2010
5) Wang JH, Changchien CS, Hung CH et al : Liver stiffness decrease after effective antiviral therapy in patients with chronic hepatitis C: Longitudinal study using FibroScan. J Gastroenterol Hepatol 25 : 964–969, 2010
6) Veldt BJ, Saracco G, Boyer N et al : Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. Gut 53 : 1504–1508, 2004
7) Toccaceli F, Laghi V, Capurso L et al : Long-term liver histology improvement in patients with chronic hepatitis C and sustained response to interferon. J Viral Hepat 10 : 126–133, 2003
8) Shiratori Y, Imazeki F, Moriyama M et al : Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 132 : 517–524, 2000
9) George SL, Bacon BR, Brunt EM et al : Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. Hepatology 49 : 729–738, 2009
10) Masuzaki R, Tateishi R, Yoshida H et al : Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. Hepatology 49 : 1954–1961, 2009

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Original Article

Pegylated interferon monotherapy in patients with chronic hepatitis C with low viremia and its relationship to mutations in the NS5A region and the single nucleotide polymorphism of interleukin-28B

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Aim: Previous studies have suggested that patients with chronic hepatitis C with a low pretreatment hepatitis C virus (HCV) level have a high sustained virological response (SVR) rate, and that there would be a subpopulation of patients in which HCV can be eradicated with pegylated interferon (PEG IFN) alone without a decrease in SVR. However, the efficacy of PEG IFN monotherapy in patients with low HCV RNA levels is unclear. Several studies have reported that interferon sensitivity-determining region (ISDR) and the single-nucleotide polymorphism (SNP) of interleukin-28B (IL-28B) contribute to IFN response, but these relationships are controversial. The aim of this study was to determine whether the SNP of IL-28B (rs8099917) and amino acid substitutions in the ISDR among patients with low HCV levels affect the response to PEG IFN monotherapy.

Methods: One hundred and four patients with low-level HCV infection were studied. Low HCV level was defined as 100 KIU/mL or less.

Results: SVR was achieved in 94 patients (92.2%). HCV levels (≤50 KIU/mL) and ISDR (≥2 mutations) were associated with SVR on univariate analysis. The rates of SVR in the patients with IL-28B genotypes TT, TG and GG were 94.5%, 77.8% and 100%, respectively. The G allele tended to be associated with poor response to IFN therapy ($P = 0.0623$). On multivariate analysis, the ISDR was the factor predictive of SVR ($P = 0.004$).

Conclusion: The ISDR is significantly associated with a good response to PEG IFN monotherapy in patients with low HCV levels.

Key words: hepatitis C virus, interferon sensitivity-determining region, interferon, interleukin-28B, rapid virological response

INTRODUCTION

HEPATITIS C VIRUS (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma (HCC) that easily progresses to end-stage liver disease.[1] Because 170 000 000 persons are infected with HCV worldwide, HCV infection is a significant global health problem.

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The current recommended therapy for patients with chronic hepatitis C is a combination of pegylated interferon (PEG IFN) and ribavirin and/or telaprevir or boceprevir.[2–6] HCV RNA levels, as well as genotypes, are an important factor associated with sustained virological response (SVR) to IFN therapy.[3,4] Patients with low HCV RNA levels have a high SVR rate, and even standard IFN monotherapy is useful for eradication of HCV in patients with low viral loads.[7–9] Several studies have succeeded in reducing the duration of treatment without risk of relapse.[10,11] Although patients with low HCV RNA have higher response rates to IFN treatment, not all patients achieve SVR. Other factors for improving the prediction of SVR in patients with low HCV RNA levels are needed. The predictive factors for SVR in patients with genotype 1b and high HCV RNA levels have been investigated, and several studies have shown that the single nucleotide polymorphism of interleukin-28B (IL-28B) and amino acid substitutions in the core and NS5A region affect the response to IFN therapy.[12–16] However, the predictive factors for SVR among patients with low HCV RNA levels treated with PEG IFN monotherapy have been unclear.

Hepatitis C virus consists of three structural proteins (core, envelope 1 and envelope 2) and six non-structural proteins (NS2 to NS5). HCV NS5A protein was reported to have a domain associated with IFN response. This domain in the region of HCV genotype 1b is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR).[12,15–21] IFN acts to control replication of the virus by inducing the dsRNA-dependent protein kinase (PKR). The ISDR is located in the PKR-binding domain, is inhibited by PKR *in vitro*,[22] and is useful for prediction in patients with genotypes 2a, 2b and 3a.[23–28] Therefore, ISDR heterogeneity is an important factor that may affect response to IFN in patients with low HCV RNA levels. We hypothesized that ISDR heterogeneity could be predicted in patients with low HCV RNA levels in which HCV can be eradicated with PEG IFN-α alone without a decrease in SVR.

Not only genetic heterogeneity in the HCV genome but also host genetics contribute to IFN treatment outcomes. Therefore, several studies were performed to understand the host factors associated with IFN responsiveness; these showed that IL-28B polymorphisms are strongly associated with response to PEG IFN and ribavirin combination therapy in patients with genotype 1b and high viral load.[13,14,16,29] However, the associations between ISDR and IL-28B and the effects of PEG IFN-α monotherapy in patients with low HCV RNA levels are not well known.

The aim of the present study was to determine whether genomic heterogeneity of the ISDR and the SNP of IL-28B among patients with low HCV RNA levels affects the response to PEG IFN-α-2a monotherapy.

METHODS

A TOTAL OF 295 patients with chronic hepatitis C were treated by PEG IFN-α-2a monotherapy at Nagoya University Hospital and Affiliated Hospitals; 104 patients with low HCV RNA levels were selected for this study. The patients consisted of 62 men and 42 women with a mean age of 55.1 years (range, 19–78). All patients were positive for serum anti-HCV antibody by a commercial enzyme-linked immunosorbent assay (Dinabot, Tokyo, Japan) and for HCV RNA by a commercial polymerase chain reaction (PCR) (Roche Diagnostic Systems, Tokyo, Japan).

A low HCV level was defined as 100 KIU/mL or less, as previously reported.[4,7,9,11] No patient had hepatitis B surface antigen, co-infection with HIV, autoimmune disease or chronic alcohol abuse.

Schedule of IFN therapy

Patients received PEG IFN-α-2a (Pegasys Chugai-Roche, Tokyo, Japan) at a dose of 180 μg injected s.c. once per week for 24 or 48 weeks. The patients were allocated, at the discretion of the physician in charge, to a protocol lasting either 24 or 48 weeks. Laboratory tests and evaluations of adverse events were performed once per week during treatment.

The dose of PEG IFN-α-2a was reduced to 90 μg when clinically significant adverse events or laboratory abnormalities such as neutropenia (<750 cells/mm^3) or thrombocytopenia (<50 000 cells/mm^3) occurred. PEG IFN-α-2a was discontinued when neutropenia of less than 250 cells/mm^3 or a platelet count of less than 25 000 cells/mm^3 was seen.

Hepatitis C virus RNA in serum samples was examined at 4 weeks, at the end of IFN therapy, and at 6 months after the end of treatment (ETR). Serum was stored at −80°C for virological examination at pretreatment.

Patients who were persistently negative for serum HCV RNA and who had a normal serum alanine aminotransferase (ALT) level at 24 weeks after withdrawal of IFN treatment were considered to have SVR. Patients who were HCV negative at the ETR but returned to HCV

positive status after withdrawal of IFN were defined as virological relapsers. Patients who did not become HCV negative with IFN therapy were defined as non-virological responders.

This study was approved by the ethics committee of each institution involved. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virological tests

Hepatitis C virus was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions, as described previously.[30,31] Genotypes were classified according to the nomenclature proposed by Simmonds *et al.*[32]

Nested PCR analysis and direct sequencing of the NS5A-ISDR were performed as previously reported for each genotype.[15,16,27,28] In brief, RNA was extracted from 140 μL serum using a QIAamp Viral RNA Kit (Qiagen, Valencia, CA, USA) and dissolved in 50 μL diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligo and random hexamer primers with an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). NS5A-ISDR was sequenced after amplification by nested PCR as previously described.[15,16,27,28]

The primers used were as follows: NS5A-ISDR of genotype 1b, sense 5′-TGGATGGAGTGCGGTTGCACA GGTA-3′ and antisense 5′-TCTTTCTCCGTGGAGGTGGT ATTG-3′; NS5A-ISDR of genotype 2a, sense 5′-ACGTCC ATGCTAACAGACCC-3′ and antisense 5′-GGGAATCT CTTCTTGGGGAG-3′; and NS5A-ISDR of genotype 2b, sense 5′-TCTCAGCTCCCTTGCGATCCTGA-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 s, 55°C for 30 s and 72°C for 30 s in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, Foster City, CA, USA). The second PCR was done using the following sets of primers: NS5A-ISDR of genotype 1b, sense 5′-CAGGTACGC TCCGGCGTGCA-3′ and antisense 5′-GGGGCCTTGGT AGGTGGCAA-3′; NS5A-ISDR of genotype 2a, sense from the first-round PCR and a new antisense primer 5′-CGAGAGAGTCCAGAACGACC-3′; and NS5A-ISDR of genotype 2b, sense 5′-AGCTCCTCAGCGAGCCA GCT-3′ and antisense 5′-GATGGTATCGAAGGCTC-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Genomic analysis

Detection of the SNP of IL-28B (rs8099917) was done by a real-time PCR system, as previously reported.[16] In brief, genomic DNA was extracted from 15 μL of whole blood using a commercial kit (QIAamp DNA Blood mini Kit; Qiagen) and dissolved in 50 μL diethylpyrocarbonate-treated water. DNA (1 ng) was used for PCR with primers and probes of commercial kit (Taqman SNP Genotyping Assays; Applied Biosystems). The SNP of IL-28B (rs8099917) was amplified, and the results were analyzed by real-time PCR in a thermal cycler (7300 Real time PCR System; Applied Biosystems).

Statistical analysis

Data are expressed as mean ± standard deviation. A paired Student's *t*-test or Fisher's exact test were used to analyze differences in variables. $P < 0.05$ was considered significant. Multiple logistic regression models were used to identify factors predictive of SVR. Statview ver. 5.0 software (SAS Institute, Cary, NC, USA) was used for all analyses.

RESULTS

Background

PATIENTS' CLINICAL CHARACTERISTICS are summarized in Table 1. HCV genotypes 1b ($n = 34$), 2a ($n = 58$), 2b ($n = 9$) and unknown ($n = 3$) were detected.

Table 1 Clinical characteristics at pretreatment

Clinical characteristics	$n = 104$
Age (years)	55.1 ± 12.5
Sex: male/female	62/42
AST (IU/L)	50.0 ± 28.2
ALT (IU/L)	62.7 ± 47.3
Platelet count (10^4/uL)	18.4 ± 5.7
HCV RNA level (KIU/mL)	36 (1.6–100)
HCV genotype (1b/2a/2b/unknown)	34/58/9/3
IFN length (weeks) (24/48/<17)	49/45/10
Body mass index	22.7 ± 3.2

Data are expressed as mean ± standard deviation.
HCV RNA level was shown by median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon.

Table 2 Virological response in each group

(a) Virological response according to durations of IFN therapy				
	Overall ($n = 102$)	24W ($n = 48$)	48W ($n = 45$)	<17W ($n = 9$)
RVR	81.4% ($n = 83$)	87.5% ($n = 42$)	73.3% ($n = 33$)	88.9% ($n = 8$)
ETR	100% ($n = 102$)	100% ($n = 48$)	100% ($n = 45$)	100% ($n = 9$)
SVR	92.2% ($n = 94$)	93.8% ($n = 45$)	91.1% ($n = 41$)	88.9% ($n = 8$)
(b) Virological response according to HCV genotypes				
	Overall ($n = 102$)	1b ($n = 32$)	2a ($n = 58$)	2b ($n = 9$)
RVR	81.4% ($n = 83$)	81.3% ($n = 26$)	81.0% ($n = 47$)	88.9% ($n = 8$)
SVR	92.2% ($n = 94$)	87.5% ($n = 28$)	93.1% ($n = 54$)	100% ($n = 9$)

ETR, end of treatment response; HCV, hepatitis C virus; IFN, interferon; RVR, rapid virological response; SVR, sustained virological response; W, weeks.

All patients had serum HCV RNA levels of 100 KIU/mL or less, and the median HCV RNA level was 36 KIU/mL.

One hundred and four patients were initially included in this study; 49 patients were treated with PEG IFN-α-2a for 24 weeks, and 45 patients were treated for 48 weeks. Ten patients withdrew from IFN therapy within 17 weeks, and two of these 10 patients could not be followed. The reasons for discontinuing therapy were fatigue ($n = 3$), depression ($n = 1$), rash ($n = 1$), appetite loss ($n = 1$), liver failure ($n = 1$) and unknown ($n = 3$). The two patients who withdrew from follow up were excluded from the analysis, and the remaining 102 patients were followed for 6 months after the ETR.

Virological response

Virological response is shown in Table 2. Rapid virological response (RVR), which was defined as negativity for HCV after 4 weeks of treatment, for the overall group, the 48 weeks' group, the 24 weeks' group and the under 17 weeks' group was 81.4% (83/102), 73.3% (33/45), 87.5% (42/48) and 88.9% (8/9), respectively. Virological response at the ETR was 100% among all patients. Finally, 94 (92.2%) of 102 patients achieved SVR.

There was no significant difference in virological response between patients treated for 24 weeks and those treated for 48 weeks. The virological response according to HCV genotype is shown in Table 2(b). Patients with genotype 1b had a lower SVR rate than genotypes 2a and 2b, but no significant differences in genotype were noted.

Genetic heterogeneity in NS5A-ISDR and response to IFN therapy

The prevalences of the number of amino acid substitutions in ISDR according to HCV genotypes are summarized in Figure 1. The ISDR were examined by direct sequencing, and classification involved counting the number of amino acid substitutions compared to consensus strains of each genotype, as previously reported.[15,24,27,28]

Interferon sensitivity-determining region sequences were obtained in 81 patients. Five patients did not have serum at pretreatment, and 16 patients could not be amplified by PCR. Sixty-one patients (84.7%) had one mutation or more. SVR according to the ISDR is shown in Figure 2. All patients with three or more mutations in the ISDR achieved SVR, but 18 (69.2%) of 26 patients with two or less mutations in the ISDR achieved SVR. Patients with two or less mutations in the ISDR were poor responders to IFN therapy.

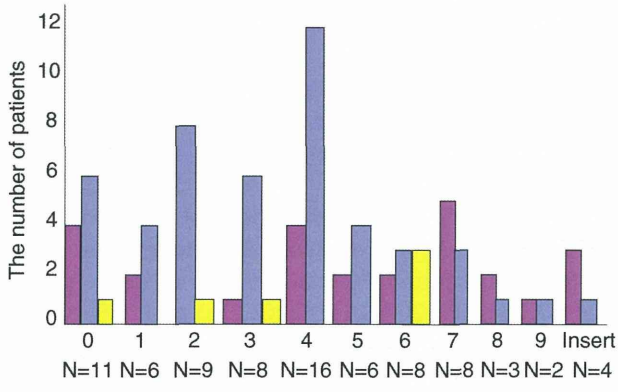

Figure 1 Number of amino acid substitutions in interferon sensitivity-determining region (ISDR) according to hepatitis C virus (HCV) genotypes. ■, HCV genotypes 1b; ■, HCV genotypes 2a; ■, HCV genotypes 2b.

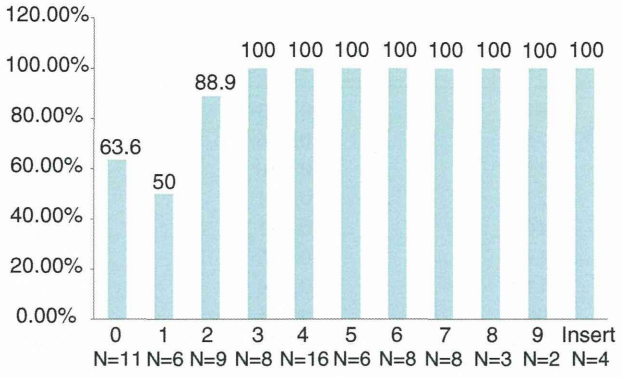

Figure 2 Sustained virological response (SVR) according to the number of amino acid substitutions in interferon sensitivity-determining region (ISDR).

Prevalence of the SNP of IL-28B (rs8099917) T (major allele) and G (minor allele) and response to IFN therapy

The frequencies of the IL-28B genotypes were: major homozygotes (TT), 73; heterozygotes (TG), 18; and minor homozygotes (GG), two. The rates of SVR in the patients with TT, TG and GG were 94.5% (69/73), 77.8% (14/18) and 100% (2/2), respectively. The SVR rate of patients with G allele of the IL-28B genotype was 80.0% (16/20), and that with T allele was 94.5% (69/73). Patients with T allele of the IL-28B genotype had a slightly higher SVR rate than did those with G allele, but there were no significant differences ($P = 0.0623$).

Analysis for factors predictive of SVR

The results of univariate analysis for factors predictive of SVR are shown in Table 3. HCV RNA levels were lower in patients with SVR than in those without SVR ($P = 0.0154$). SVR was achieved in 41.2% of patients with less than two mutations in the ISDR and 98.4% of patients with two or more mutations in the ISDR ($P = 0.0001$). HCV RNA levels and ISDR were associated with SVR on univariate analyses.

Results of multivariate analyses of factors predictive of SVR are shown in Table 4. Variables were recorded categorically as ordinal data. Background factors were age (<60 vs ≥60 years), sex (male vs female), platelet count ($<15 \times 10^4/mm^3$ vs $\geq 15 \times 10^4/mm^3$), HCV RNA level (<50 vs ≥50 KIU/mL), ALT levels (<70 vs ≥70 IU/L), aspartate aminotransferase (AST) levels (<60 vs ≥60 IU/L), HCV genotype (1 vs 2), ISDR (<2 vs ≥2 mutations), IL-28B (TT vs TG and GG) and RVR (yes vs no). As can be seen in Table 4, factors such as age, sex, platelet count, HCV RNA level, ALT levels, AST levels, HCV genotype, IL-28B and RVR did not have any effect on SVR. In contrast, the ISDR was the most influential factor.

DISCUSSION

THE HCV RNA level is one of the most important factors affecting response to IFN therapy. Patients with high HCV RNA levels respond poorly to IFN therapy, whereas patients with low HCV RNA levels have a high SVR rate to IFN therapy. Thus, most patients with low HCV RNA levels have achieved SVR, but other therapeutic options for patients who fail IFN therapy are needed. Several studies have attempted to reduce the duration of treatment, reduce the dose of IFN and/or ribavirin, or use standard IFN without risk of relapse.[8–10] The present study confirmed the high SVR rate (92.2%) in patients with low HCV RNA levels (≤100 KIU/mL)

Table 3 Univariate analysis: factors predictive of SVR

Factors	SVR ($n = 94$)	Non-SVR ($n = 8$)	*P*-value
Age (years)	54.6 ± 12.6	57.4 ± 8.8	0.5528
Sex: male/female	58/36	2/6	0.0619
ALT (IU/L)	63.2 ± 48.3	56.3 ± 32.5	0.7126
AST (IU/L)	50.7 ± 28.6	41.4 ± 21.6	0.4043
PLT ($\times 10^4/mm^3$)	18.5 ± 5.8	18.0 ± 5.0	0.8292
HCV RNA level (KIU/mL)	42.5 ± 34.8	75.0 ± 45.7	0.0154
HCV genotype: 1/2	29/63	4/3	0.4337
ISDR: <2/≥2	10/63	7/1	0.0001
IL-28B: TT/TG, GG	69/16	4/4	0.0623
RVR: yes/no	78/16	5/3	0.1661

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin-28B; ISDR, interferon sensitivity-determining region; PLT, platelets; RVR, rapid virological response; SVR, sustained virological response.

Table 4 Multivariate analysis: factors predictive of SVR

Factors	*P*-value	Risk ratio	95% CI	
Age: <60 years	0.4556	2.837	0.183	43.891
Sex: male	0.8712	0.756	0.026	22.166
AST: <60 IU/L	0.7806	2.131	0.010	438.334
ALT: <70 IU/L	0.6063	0.239	0.001	55.563
Platelet count: $<15 \times 10^4$/uL	0.6873	0.463	0.011	19.680
HCV RNA: <50 KIU/mL	0.1046	13.170	0.585	296.318
Genotype: 2	0.1693	14.110	0.324	614.872
ISDR: <2	0.0074	0.004	0.001	0.235
IL-28B: TT	0.2684	5.978	0.252	141.852
RVR: yes	0.7495	1.756	0.055	55.696

95% CI, 95% confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL-28B, interleukin 28B; ISDR, interferon sensitivity-determining region; RVR, rapid virological response; SVR, sustained virological response.

treated by PEG IFN-α-2a monotherapy. Although the effects of shortened treatment duration of PEG IFN-α with ribavirin for patients with low HCV RNA levels are unclear, PEG IFN-α-2a monotherapy could reduce the cost and adverse events of ribavirin while maintaining a high SVR rate. This treatment would be a good therapeutic option for patients with low HCV RNA levels. However, selection by HCV RNA level alone was insufficient to predict IFN responsiveness completely, and other factors would be necessary to improve the positive predictive values for SVR in patients infected with low HCV RNA levels.

Hepatitis C virus genotype is another major factor, in addition to HCV RNA levels, that is associated with response to IFN therapy. In the present study, the SVR rates of genotypes 1 and 2 were 87.5% and 94.0%, respectively. Patients infected with genotypes 2 had a slightly higher SVR rate than did those with genotype 1, but there were no significant differences in our small study. The difference in SVR according to genotype may exist, but HCV genotype did not have enough power to be a determinant of IFN response completely among patients with low HCV RNA levels because of the bias for HCV RNA levels. However, patients infected with low HCV RNA levels respond differently to IFN therapy, suggesting that an additional factor associated with resistance to IFN exists.

The heterogeneity of the HCV NS5A region is an important factor that may affect response to IFN in patients with HCV genotype 1b and was named the ISDR.[17] Mutations in the ISDR affect the interaction with PKR and may inhibit viral replication. Therefore, ISDR of other HCV genotypes, in addition to 1b, could be used as predictors of IFN responsiveness.[23–28] In the present study, it was hypothesized that the amino acid substitutions in the ISDR would explain differences in IFN resistance in patients infected with low HCV RNA levels. Therefore, the utility of substitutions of amino acids in the ISDR for predicting IFN responsiveness was investigated. The ISDR was the most influential factor for SVR on multivariate analyses. All patients with three or more mutations in the ISDR achieved SVR, and 18 of 26 patients with less than three mutations in the ISDR achieved SVR. Thus, patients with less than three mutations in the ISDR would be resistant to PEG IFN-α-2a monotherapy and may need to receive much more powerful treatment, even if they have low HCV RNA levels. The ISDR system could be used as a diagnostic tool to predict SVR in patients infected with low HCV RNA levels. An individualized treatment strategy based on HCV RNA levels and the ISDR in patients with chronic hepatitis C would be an important consideration to achieve optimal therapy and avoid unnecessary treatment.

Some studies of SVR to PEG IFN-α-2b and ribavirin and/or telaprevir combination therapy for chronic hepatitis C patients with genotype 1 and high viral load identified genetic variation near the IL-28B gene associated with IFN responsiveness.[13,14,16] However, the effects of genetic variation near the IL-28B gene on SVR in patients with low HCV RNA levels treated with PEG IFN monotherapy are unknown. Therefore, the utility of the SNP of IL-28B for predicting IFN responsiveness was investigated. Patients with IL-28B (rs8099917) genotypes TG and GG had a lower SVR rate than genotype TT, but no significant differences in genotype were found in this study. The SNP of IL-28B would be associated with the response to IFN, especially for poor responders, and

was partially associated with SVR in a study of patients with HCV genotype 2 who were treated with PEG IFN-α-2b and ribavirin.[13,14,16,33,34] The clear suggestion of a correlation between the SNP of IL-28B with IFN responsiveness would not be supported in patients with low HCV RNA levels because of the high SVR rate and predominant genotype 2.

Viral factors associated with SVR have been studied, and several regions, including 5′-untranslated region, core, E2, NS5A and NS5B, have been suggested to play important roles in IFN responsiveness.[14,16,35–38] Further studies need to investigate whether these other viral factors, especially interferon and ribavirin resistance-determining region of NS5A and core amino acid substitutions, among patients with low HCV RNA levels affect the response to PEG IFN monotherapy.

Hepatitis C virus RNA levels could be easy to measure using commercial kits and would be useful for clinical practice, but sequencing analysis, which involves much effort and cost, would be needed to characterize the ISDR. SVR was achieved in 95.1% of patients with lower HCV RNA levels (<50 KIU/mL) and 98.4% of patients with mutant type. ISDR was a better factor, but HCV RNA level might be used as a predictive factor instead of measurement of ISDR.

The definition of the low HCV RNA level that was related to a good response to IFN therapy has varied widely, from 100–600 KIU/mL.[7,9–11] Zeuzem *et al.* reported that 24 weeks of therapy with PEG IFN-α-2b plus ribavirin is insufficient for the treatment of patients with HCV genotype 1 and a HCV RNA level of 600 KIU/mL or less.[10] They suggested that patients with HCV RNA of 250 KIU/mL or less would have a good response to PEG IFN-α-2b and ribavirin combination therapy for 24 weeks. Most reports from Japan defined 100 KIU/mL as the cut-off level for low HCV levels and used standard IFN monotherapy.[4,7,9,11] The outcome that would maximize the efficacy of IFN therapy would depend on the relationships between the cut-off HCV RNA level and therapeutic regimens. The optimal cut-off level for low HCV levels and the matching therapeutic regimens are not well understood, and further studies are needed to clarify these issues.

Based on the SVR in patients receiving therapy for 24 weeks compared to those treated for 48 weeks, there was no difference in IFN responsiveness by duration in this small study. However, this study was not a randomized study. Further studies are needed to investigate the optimal duration of PEG IFN-α-2a monotherapy for patients with low HCV RNA levels.

Pascu *et al.* performed a meta-analysis for the correlation between SVR and ISDR in patients with HCV genotype 1b infection who received standard IFN therapy.[19] They found that 11 of 21 European patients with mutant type ISDR and HCV RNA levels of less than 6.6 log copies/mL achieved SVR, but 67 of 69 Japanese patients with mutant type ISDR and HCV RNA levels of less than 6.6 log copies/mL achieved SVR. The mode of HCV infection and geographical and racial differences would have effects on the prediction of SVR by ISDR.[39,40] As a result, the ISDR system is more suitable for predicting SVR in Asian than in European patients. Although validation of these observations in larger cohorts is required, mutations in the ISDR were useful for predicting the response to PEG IFN-α-2a monotherapy in patients with low HCV levels.

In conclusion, in patients with HCV infection, low HCV levels and more than two mutations in the ISDR are significantly associated with a good response to PEG IFN-α-2a monotherapy. An individualized treatment strategy based on HCV RNA levels and the ISDR in patients with chronic hepatitis C would be useful in clinical practice.

REFERENCES

1 Seeff LB. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35–46.
2 Hoofnagle JH, Seeff LB. Peginterferon and ribavirin for chronic hepatitis C. *N Engl J Med* 2006; **355**: 2444–51.
3 Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335–74.
4 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 8–13.
5 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827–38.
6 Kwo PY, Lawitz EJ, McCone J *et al.* Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705–16.
7 Yokosuka O, Iwama S, Suzuki N *et al.* High sustained virologic response rate after interferon monotherapy in Japanese hepatitis C patients with a low HCV RNA titer and/or HCV genotype 2. A prospective study. *Intervirology* 2004; **47**: 328–34.

8 Tabaru A, Narita R, Hiura M, Abe S, Otsuki M. Efficacy of short-term interferon therapy for patients infected with hepatitis C virus genotype 2a. *Am J Gastroenterol* 2005; **100**: 862–7.
9 Kawamura Y, Arase Y, Ikeda K *et al.* The efficacy of short-term interferon-beta therapy for chronic hepatitis C patients with low virus load. *Intern Med* 2008; **47**: 355–60.
10 Zeuzem S, Buti M, Ferenci P *et al.* Efficacy of 24 weeks treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. *J Hepatol* 2006; **44**: 97–103.
11 Arase Y, Suzuki F, Akuta N *et al.* Combination therapy of peginterferon and ribavirin for chronic hepatitis C patients with genotype 1b and low-virus load. *Intern Med* 2009; **48**: 253–8.
12 Okanoue T, Itoh Y, Hashimoto H *et al.* Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. *J Gastroenterol* 2009; **44**: 952–63.
13 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105–9.
14 Akuta N, Suzuki F, Hirakawa M *et al.* Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. *Hepatology* 2010; **52**: 421–9.
15 Hayashi K, Katano Y, Ishigami M *et al.* Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy. *J Viral Hepat* 2011; **18**: 280–6.
16 Hayashi K, Katano Y, Honda T *et al.* Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy. *Liver Int* 2011; **31**: 1359–65.
17 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; **334**: 77–81.
18 Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T. Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? *J Hepatol* 1999; **30**: 1014–22.
19 Pascu M, Martus P, Höhne M *et al.* Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* 2004; **53**: 1345–51.
20 Yen YH, Hung CH, Hu TH *et al.* Mutations in the interferon sensitivity-determining region (nonstructural 5A amino acid 2209-2248) in patients with hepatitis C-1b infection and correlating response to combined therapy of pegylated interferon and ribavirin. *Aliment Pharmacol Ther* 2008; **27**: 72–9.
21 Muñoz de Rueda P, Casado J, Patón R *et al.* Mutations in E2-PePHD, NS5A-PKRBD, NS5A-ISDR, and NS5A-V3 of hepatitis C virus genotype 1 and their relationships to pegylated interferon-ribavirin treatment responses. *J Virol* 2008; **82**: 6644–53.
22 Gale M Jr, Blakely CM, Kwieciszewski B *et al.* Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: molecular mechanisms of kinase regulation. *Mol Cell Biol* 1998; **18**: 5208–18.
23 Sáiz JC, López-Labrador FX, Ampurdanés S *et al.* The prognostic relevance of the nonstructural 5A gene interferon sensitivity determining region is different in infections with genotype 1b and 3a isolates of hepatitis C virus. *J Infect Dis* 1998; **177**: 839–47.
24 Murakami T, Enomoto N, Kurosaki M, Izumi N, Marumo F, Sato C. Mutations in nonstructural protein 5A gene and response to interferon in hepatitis C virus genotype 2 infection. *Hepatology* 1999; **30**: 1045–53.
25 Sarrazin C, Kornetzky I, Rüster B *et al.* Mutations within the E2 and NS5A protein in patients infected with hepatitis C virus type 3a and correlation with treatment response. *Hepatology* 2000; **31**: 1360–70.
26 Dal Pero F, Tang KH, Gerotto M *et al.* Impact of NS5A sequences of Hepatitis C virus genotype 1a on early viral kinetics during treatment with peginterferon- alpha 2a plus ribavirin. *J Infect Dis* 2007; **196**: 998–1005.
27 Nagase Y, Yotsuyanagi H, Okuse C *et al.* Effect of treatment with interferon alpha-2b and ribavirin in patients infected with genotype 2 hepatitis C virus. *Hepatol Res* 2008; **38**: 252–8.
28 Hayashi K, Katano Y, Honda T *et al.* Mutations in the interferon sensitivity-determining region of hepatitis C virus genotype 2a correlate with response to pegylated-interferon-alpha 2a monotherapy. *J Med Virol* 2009; **81**: 459–66.
29 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399–401.
30 Otagiri H, Fukuda Y, Nakano I *et al.* Evaluation of a new assay for hepatitis C virus genotyping and viral load determination in patients with chronic hepatitis C. *J Virol Methods* 2002; **103**: 137–43.
31 Hayashi K, Fukuda Y, Nakano I *et al.* Prevalence and characterization of hepatitis C virus genotype 4 in Japanese hepatitis C carriers. *Hepatol Res* 2003; **25**: 409–14.
32 Simmonds P, Bukh J, Combet C *et al.* Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology* 2005; **42**: 962–73.
33 Mangia A, Thompson AJ, Santoro R *et al.* An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010; **139**: 821–7.

34 Akuta N, Suzuki F, Seko Y *et al.* Association of IL28B genotype and viral response of hepatitis C virus genotype 2 to interferon plus ribavirin combination therapy. *J Med Virol* 2012; **84**: 1593–9.
35 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; **48**: 372–80.
36 Watanabe K, Yoshioka K, Yano M *et al.* Mutations in the nonstructural region 5B of hepatitis C virus genotype 1b: their relation to viral load, response to interferon, and the nonstructural region 5A. *J Med Virol* 2005; **75**: 504–12.
37 Katano Y, Hayashi K, Ishigami M *et al.* Association with 5′-untranslated region and response to interferon in chronic hepatitis C. *Hepatogastroenterology* 2007; **54**: 854–7.
38 El-Shamy A, Nagano-Fujii M, Sasase N *et al.* Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; **48**: 38–47.
39 Layden-Almer JE, Kuiken C, Ribeiro RM *et al.* Hepatitis C virus genotype 1a NS5A pretreatment sequence variation and viral kinetics in African American and white patients. *J Infect Dis* 2005; **192**: 1078–87.
40 Jenke AC, Moser S, Orth V, Zilbauer M, Gerner P, Wirth S. Mutation frequency of NS5A in patients vertically infected with HCV genotype 1 predicts sustained virological response to peginterferon alfa-2b and ribavirin combination therapy. *J Viral Hepat* 2009; **16**: 853–9.

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EDITORIAL

What is the benefit of computer-assisted image analysis of liver fibrosis area?

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Liver fibrosis is usually semiquantitatively assessed in liver biopsy specimens by the numerical system of Scheuer [1], the Metavir group [2], or Ishak [3]. Fibrosis is staged as F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis and few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis. Staging mainly depends on the degree of the architectural changes of liver structure.

Computer-assisted image analysis of the stained fibrosis area in liver biopsy specimens is a method for quantitatively measuring the amount of liver fibrosis [4]. It is not used for the clinical assessment of liver fibrosis in general, but is often used in the assessment of fibrosis in animal models. Its low popularity in clinical practice may be attributed to the complexity of the method.

The fibrosis stage as determined by the numerical systems and the relative area of fibrosis measured by computer-assisted image analysis usually correlate well to each other. However, discrepancy between the two sometimes occurs. Which of the two is more useful in clinical practice may depend on the objectives of assessing liver fibrosis.

The current study by Isgro et al. showed that collagen proportionate area (CPA) has a better relationship with liver stiffness measurement (LSM) and with hepatic venous pressure gradient (HVPG) compared with the Ishak stage. They also reported that CPA at 1-year post-transplantation in hepatitis C virus-infected patients predicts subsequent clinical decompensation more accurately than Ishak stage or HVPG [5]. They conclude that CPA should be the histological parameter with which to compare LSM and other non-invasive fibrosis markers and also be used to subclassify cirrhosis.

Nitta et al. [6] also reported the good correlation between LSM and fibrosis area measured by image analysis in the patients with chronic hepatitis C, while LSM and Metavir score yielded better correlation. Xie et al. [7] reported that fibrosis area measured by image analysis significantly correlated with model for end-stage liver disease score, serum bilirubin levels and prothrombin time in the patients with hepatitis B virus-related decompensated cirrhosis.

Arima et al. [8] reported that 42 % of chronic hepatitis C patients with pretreatment F3-4 who obtained sustained virological response by interferon (IFN) therapy had decreased fibrosis assessed by the numerical staging system, while the fibrosis area measured by image analysis decreased in 92 %. Thus the computer-assisted image analysis of liver fibrosis is more sensitive to measure the reduction of liver fibrosis after IFN treatment than the numerical system.

In conclusion, the relative fibrosis area measured by computer-assisted image analysis is suitable for the comparison with newly developing non-invasive methods for fibrosis assessment, such as LSM. It is also useful to assess the degree of severe fibrosis in cirrhosis for predicting prognosis and to assess the change of fibrosis after antiviral treatment or in natural courses. It is better to add computer-assisted image analysis to the interpretation of liver biopsy in order to obtain valuable quantitative information in the specimens. The standardization and simplification of the method is needed in order that computer-assisted image analysis of fibrosis area will be widely used.

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References

1. Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol. 1991;13:372–4.
2. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. The French METAVIR Cooperative Study Group. Hepatology 1994;20:15–20.
3. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22:696–9.
4. Goodman ZD, Becker RL Jr, Pockros PJ, Afdhal NH. Progression of fibrosis in advanced chronic hepatitis C: evaluation by morphometric image analysis. Hepatology. 2007;45:886–94.
5. Manousou P, Dhillon AP, Isgro G, Calvaruso V, Luong TV, Tsochatzis E, et al. Digital image analysis of liver collagen predicts clinical outcome of recurrent hepatitis C virus 1 year after liver transplantation. Liver Transpl. 2011;17:178–88.
6. Nitta Y, Kawabe N, Hashimoto S, Harata M, Komura N, Kobayashi K, et al. Liver stiffness measured by transient elastography correlates with fibrosis area in liver biopsy in patients with chronic hepatitis C. Hepatol Res. 2009;39:675–84.
7. Xie SB, Ma C, Lin CS, Zhang Y, Zhu JY, Ke WM. Collagen proportionate area of liver tissue determined by digital image analysis in patients with HBV-related decompensated cirrhosis. Hepatobiliary Pancreat Dis Int. 2011;10:497–501.
8. Arima M, Terao H, Kashima K, Arita T, Nasu M, Nishizono A. Regression of liver fibrosis in cases of chronic liver disease type C: quantitative evaluation by using computed image analysis. Intern Med. 2004;43:902–10.

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Editorial

How to adjust the inflammation-induced overestimation of liver fibrosis using transient elastography?

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Impact of mild to moderate elevations of alanine aminotransferase on liver stiffness measurement in chronic hepatitis B patients during antiviral therapy
Li-Bo Yan, Xia Zhu, Lang Bai, Ling-Bo Liang, En-Qiang Chen, Ling-Yao Du, Li-Chun Wang, Li-Yu Chen and Hong Tang

Transient elastography (TE) is a non-invasive reproducible method for measurement of liver stiffness (LSM). LSM correlates well with liver fibrosis stage. Thus, it has been hoped that TE will be able to replace liver biopsy which is problematic in its invasive nature, risk of complications and technical limitations derived from small sample sizes or interpretation variability. TE has been reported to provide useful clinical information based on the close correlation between LSM and liver fibrosis stage, such as appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis.[1]

However, LSM does not give us a completely corresponding estimation of fibrosis stage with liver biopsy. One of the reasons for this discrepancy is that LSM is affected by the histological findings other than liver fibrosis; such as edema, steatosis, inflammation or necrosis. Especially, inflammation has been reported to affect LSM in many reports. Acute or chronic inflammation can result in high LSM indicating the presence of falsely higher fibrosis stages than real fibrosis stages.[2–6] Thus, we have to establish a method to adjust the inflammation-induced overestimation of liver fibrosis using TE.

The current study by Yan *et al.* highlights some of the difficulties in adjusting the overestimation of fibrosis stage using TE in patients with liver inflammation. The authors studied the changes of LSM of the chronic hepatitis B patients with mild-to-moderate elevation of baseline alanine aminotransferase (ALT) levels (2–10-times the upper limit of normal [ULN]) treated with nucleoside/nucleotide analogs. This study shows that LSM decreased in parallel with the decline of ALT levels. The authors described that the decrease of LSM values is attributed mainly to amelioration of inflammation and maybe partially to regression of fibrosis. They report that pretreatment fibrosis stages of liver biopsies corresponded with LSM after normalization of ALT levels at a significantly higher rate than with baseline LSM in the patients of F0–1 (12/27 vs 23/25, $P < 0.001$), but not in those of F2–3 (4/12 vs 7/12, $P = 0.414$). They concluded that LSM became more accurate for the assessment of fibrosis stage after elevated ALT levels have been reduced to normal levels in chronic hepatitis B.

Although the approach of this study to adjust the inflammation-induced overestimation of fibrosis stage using TE is interesting and probably useful, its weakness is that the number of the patients studied is small; 25 patients of F0–1, 12 of F2–3 and four of F4. Additionally, in four of 12 patients of F2–3, fibrosis stages were underestimated by LSM both in pretreatment and after normalization of ALT levels. This finding indicates two facts. One is that we have to invent another method to adjust the overestimation of fibrosis stages for F2–3. The other is that there is also a problem of underestimation of liver fibrosis using TE. The mechanism of underestimation has not yet been elucidated. In addition, we need to know the correct fibrosis stages from LSM for determining how to treat the patients before treatment, but this study provides us the method to establish the fibrosis stage only after treatment.

It is important to determine the optimal time for restoring the reliability of LSM for assessing liver fibrosis after antiviral therapy or natural amelioration. Park *et al.* examined the decline of LSM and ALT levels in patients with chronic hepatitis B experiencing acute exacerbation.[7] Three months after acute exacerbation, ALT levels had decreased below two times the ULN and stabilized (medians, 522, 43, 21, 19, 18 and and 16 IU/L at baseline, 1, 3, 6, 9 and 12 months, respectively). However, LSM required 3 months more (6 months after exacer-

bation) for stabilization (median, 15.1, 10.0, 7.4, 7.1, 6.3 and 5.8 kPa at baseline, 1, 3, 6, 9 and 12 months, respectively). The authors conclude that LSM should be postponed for at least 3 months after stabilization of ALT levels below two times the ULN to restore the reliability of LSM in assessing liver fibrosis. Whether LSM at stabilization actually indicates the correct liver fibrosis stage should be also further studied.

Several reports suggested using different cut-off values for normal and elevated ALT levels in order to adjust the inflammation-induced overestimation of fibrosis stage using TE. As patients with higher ALT levels tend to have higher LSM than those with lower ALT levels at the same stage of liver fibrosis, the optimal cut-off values of LSM tend to be lower for the patients with normal ALT levels than those with elevated ALT levels. Chan *et al.* derived an algorithm using LSM to determine liver fibrosis in chronic hepatitis B.[8] An LSM of less than 5 kPa should indicate F0 regardless of ALT levels. For patients with normal ALT levels, an LSM of 5–6 kPa would indicate F0–2, an LSM of 6–9 kPa (gray zone) F0–4 (liver biopsy is recommended), an LSM of more than 9 kPa F3–4 and an LSM of more than 12 kPa F4. For patients with elevated ALT levels (>1–5-times ULN), an LSM of 5–7.5 kPa would indicate F0–2, an LSM of 7.5–12 kPa (gray zone) F0–4, an LSM of more than 12 kPa F3–4 and an LSM of more than 13.4 kPa F4. Chan *et al.* suggest that, based on these algorithms, liver biopsy can be avoided in 62% and 58% of normal and elevated ALT levels, respectively.

Kim *et al.* reported that the cut-off LSM values for F2 or higher, F3 or higher and F4 were 6.0, 7.5 and 10.1 kPa, respectively, in chronic hepatitis B patients with normal ALT levels, whereas they were 8.9, 11.0 and 15.5 kPa, respectively, in those with elevated ALT levels (>1–2-times ULN).[9]

Kim *et al.* also developed an LSM-based prediction model for cirrhosis using different cut-off values according to ALT levels in chronic hepatitis B.[10] They proposed LSM spleen diameter to platelet ratio index (LSPI): LSM × spleen diameter / platelet count × 100. In the whole cohort, LSPI cut-off values of 39 and 77 provided a negative predictive value (NPV) of 96.1% and positive predictive value (PPV) of 95.8%, respectively. 74.8% of the patients with LSPI of less than 39 or more than 77 could be diagnosed as non-cirrhotic or cirrhotic and avoid liver biopsy. In the patients with normal ALT levels, LSPI cut-off values of 38 and 62 provided an NPV of 95.7% and PPV of 95.5%, respectively. In those with elevated ALT levels, LSPI cut-off values of 42 and 94 provided an NPV of 95.1% and PPV of 96.4%, respectively. With this method, 76.7% of the patients can be diagnosed as non-cirrhotic or cirrhotic and avoid liver biopsy.

Acoustic radiation force impulse (ARFI) elastography can measure LSM and be used to assess the liver fibrosis stage as well as TE. Yoon *et al.* reported that the optimum cut-off values for ARFI elastography were 1.13 m/s for F2 or more and 1.98 m/s for F4; these decreased to 1.09 m/s for F2 or more and 1.81 m/s for F4 in patients with normal ALT levels.[11]

To adjust the inflammation-induced overestimation of liver fibrosis, two methods have been proposed. One is to wait until inflammation and its effect on LSM subside naturally or by antiviral treatment. This method has some problems; when is the optimal time for measurement of LSM and whether LSM at the point of normal ALT levels actually indicates real fibrosis stages. The other method is to use different cut-off values for the patients with normal ALT levels and those with elevated ALT levels. This method also has some problems. We should consider the degrees of ALT elevation. The cut-off values may have to differ according to the degrees of elevation of ALT levels. For example, the cut-off values should differ between the patients with ALT levels of 1–2-times ULN and those of 2–5-times ULN. There is also a question whether the degree of elevation of ALT levels actually correlate with elevation of LSM. Vigano *et al.* reported that LSM correlated significantly with bilirubin only and that the decline of LSM significantly correlated with bilirubin in acute hepatitis B.[12] In addition, the degrees of inflammation-induced overestimation differ among the different fibrosis stages. Nitta *et al.* reported that LSM significantly correlated with ALT levels only in F2, but not in the other fibrosis stages.[5] Arena *et al.* also reported that the presence of inflammation significantly affected LSM in patients who did not have cirrhosis.[6]

In conclusion, TE is a non-invasive and reproducible method for measuring LSM and assessing liver fibrosis stages, which may hopefully replace liver biopsy. Inflammation can result in high LSM values indicating the presence of falsely higher fibrosis stages than real fibrosis stages. Several studies have recommended methods to adjust the inflammation-induced overestimation of liver fibrosis using TE, but the results are not yet satisfactory to use them in clinical practice. Thus, further studies are needed.

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REFERENCES

1 Yoshioka K, Hashimoto S. Can non-invasive assessment of liver fibrosis replace liver biopsy? *Hepatol Res* 2012; **42**: 233–40.
2 Coco B, Oliveri F, Maina AM *et al.* Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; **14**: 360–9.
3 Oliveri F, Coco B, Ciccorossi P *et al.* Liver stiffness in the hepatitis B virus carrier: a non-invasive marker of liver disease influenced by the pattern of transaminases. *World J Gastroenterol* 2008; **14**: 6154–62.
4 Tapper EB, Cohen EB, Patel K *et al.* Levels of alanine aminotransferase confound use of transient elastography to diagnose fibrosis in patients with chronic hepatitis C virus infection. *Clin Gastroenterol Hepatol* 2012; **10**: 932–7.
5 Nitta Y, Kawabe N, Hashimoto S *et al.* Liver stiffness measured by transient elastography correlates with fibrosis area in liver biopsy in patients with chronic hepatitis C. *Hepatol Res* 2009; **39**: 675–84.
6 Arena U, Vizzutti F, Abraldes JG *et al.* Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. *Gut* 2008; **57**: 1288–93.
7 Park H, Kim SU, Kim D *et al.* Optimal time for restoring the reliability of liver stiffness measurement in patients with chronic hepatitis B experiencing acute exacerbation. *J Clin Gastroenterol* 2012; **46**: 602–7.
8 Chan HL, Wong GL, Choi PC *et al.* Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B. *J Viral Hepat* 2009; **16**: 36–44.
9 Kim SU, Kim Y, Park JY *et al.* How can we enhance the performance of liver stiffness measurement using FibroScan in diagnosing liver cirrhosis in patients with chronic hepatitis B? *J Clin Gastroenterol* 2010; **44**: 66–71.
10 Kim BK, Han KH, Park JY *et al.* A novel liver stiffness measurement-based prediction model for cirrhosis in hepatitis B patients. *Liver Int* 2010; **30**: 1073–81.
11 Yoon KT, Lim SM, Park JY *et al.* Liver stiffness measurement using acoustic radiation force impulse (ARFI) elastography and effect of necroinflammation. *Dig Dis Sci* 2012; **57**: 1682–91.
12 Vigano M, Massironi S, Lampertico P *et al.* Transient elastography assessment of the liver stiffness dynamics during acute hepatitis B. *Eur J Gastroenterol Hepatol* 2010; **22**: 180–4.