厚生労働科学研究費補助金

肝炎等克服緊急対策研究事業

データマイニング手法を用いた効果的なC型肝炎治療法に関する研究

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研究代表者　泉　並木

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厚生労働科学研究費補助金（肝炎等克服緊急対策研究事業）
総括研究報告書

データマイニング手法を用いた効果的なC型肝炎治療法に関する研究

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研究要旨：肝細胞癌の 70%以上わが国では C 型肝炎ウイルス持続感染者から発生するが、インターフェロンなどの抗ウイルス治療による C 型肝炎ウイルス排除により、肝癌による死亡が大幅に減少すると期待される。標準治療のペグインターフェロン・リバビリン併用療法によりウイルスが排除される確率は 50%である。データマイニング手法を用いて併用療法における治療抵抗性要因の解析と、それに基づく個別化治療法を確立し、治癒率を個々の症例で示し現在治療すべき症例を選択する根拠となるものを示すことができた。さらに肝癌を減らすために発癌リスクの高い症例を同定し、個々の症例で発癌リスクを推定でき、抗ウイルス慮法を積極的に行うことにより効率的な発癌抑止対策となる。

A．研究目的

わが国で最も患者数が多い遺伝子型1型かつ高HCVRNA量の難治性C型慢性肝炎に対するペグインターフェロン(PEGIFN)・リバビリン(RBV)併用療法における治療抵抗性要因をデータマイニングにより網羅的に解析し、個別化治療アルゴリズムを確立し、全国の拠点病院を中心に配布して各地域での治療を推進することが重要である。さらに、八橋班との共同研究によりアルゴリズムの妥当性を検証し、普遍性を担保することにより、他施設でも臨床応用可能な有用性の高いアルゴリズムを全国に配布して治療を受ける機会を増加させることが必要である。また、インターフェロン治療を受けなかった場合の肝発癌率を個々の症例で予測をして治療を受ける機会を増やすことも重要である。個々の症例での治療アルゴリズムと肝発癌アルゴリズムを構築することにより、科学的根拠に基づいて治療効果を予測し、最適な治療法を選択することが可能となる。データマイニングを用いてこれらの課題を解決し、全国でC型慢性肝炎に対する標準治療を推進し、治療均てん化を目的とした。

B．研究方法

1年目に班員施設から1530例のデータを収集し、分析用データベースを構築した。2年目にはさらに症例数を増やし、2117例からなる分析用データベースを構築した。データマイニング解析により、治療開始前に治療効果を予測するアルゴリズムを構築した。一般臨床医の使用を考慮し、一般検査のみで治療効果を予測するアルゴリズムを作成し、八橋班の外部データで検証した。さらに、肝臓専門医による使用を目的とし、HCV遺伝子変異(NS5A, Core)や肝生検を含む治療効果予測モデルを作成した。また、HCV陰性化時期に応じた治療効果予測アルゴリズムを構築し効果的な治療を行う指標を作成した。貧血は最も頻度が高い副作用であるが、これを予測するアルゴリズムを作成した。さらに、個々の症例で発癌リスクを同定するアルゴリズムを構築した。個々の症例毎の5年間の肝発癌リスクが判定できるため、科学的根拠に基づくインフォームドコンセントが得られ、治療を受ける機会が増加する。個別研究では、C型肝炎のウイルス排除に関わる因子の解析や、副作用対策を検討した。

(倫理面への配慮)

ヒトの遺伝子（DNA）に係わる実験・解析は行わない。臨床データのデータベース構築においては、氏名、年齢など個人情報を連結可能匿名化する。臨床試験の目的・方法、治療の副作用、患者に関する個人情報の守秘義務、患者の権利保護等について十分な説明を行い同意を得たうえで臨床試験を遂行した（新GCPに遵守）。既に医療保険が認められている治療法においても上記に準じて同意書を得ている。本研究の遂行においては各研究施設において必要な申請を行い、各種倫理規定を遵守した

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分担研究報告書

C．研究結果

(1) 班員施設から1530例の情報を収集した。治療開始後24週以内に中止した症例を除外し、また一般検査データに欠損がある症例を除外した。通常の多変量解析では、性、年齢、血小板数とγGTPが有意であった。治療開始前に、ウイルス学的著効（ウイルス排除）を予測する2種類のアルゴリズムを構築した。一般臨床医の使用を考慮し、一般検査のみを用いたウイルス学的著効予測モデル(Journal of Gastroenterology)(図１)は、通常の日常診療で行われる血液検査と性や年齢

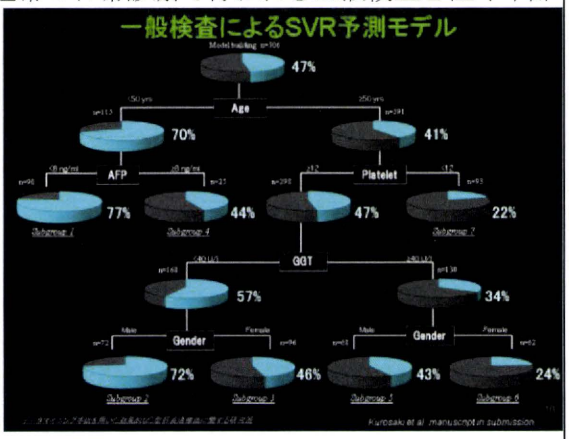


図1.一般検査によるSVR予測モデル

のみで構築し、非専門医で用いることができるものが得られた。

(1) 一般検査成績によるSVR予測モデル：年齢、血小板、AFP、GGT、性別が予測因子として同定された。これらの因子の組み合わせにより、予測著効率が22%から77%の7グループが同定された(図1)。

High Probability groupのSVR率は①50歳未満でAFPが8未満では77%、②50歳以上だが、血小板が12万以上、GGTが40未満の男性では71%、Low Probability groupのSVR率は①50歳以上で血小板が12万未満では30%、②50歳以上で、血小板が12万以上だが、GGTが40以上の女性では17%、Intermediate Probability groupのSVR率は①50歳未満だがAFPが8以上では44%、②50歳以上で、血小板数が12万以上で、GGTが40未満の女性では55%③50歳以上で、血小板が12万以上で、GGT40以上の男性では41%であった。モデル作成群と内部検証群のそれぞれのグループにおけるSVR率を比較したところ、両者には強い相関関係が見られ、本モデルの安定性が証明された(図2)。

本モデルの安定性、再現性をさらに検証するために、八橋班と連携し外部検証を行った。八橋班の症例524例をモデルに当てはめて分類し、それぞれのグループのSVR率を算出した。

その結果、High Probability groupのSVR率は①50歳未満でAFPが8未満では70%、②50歳以上だが、血小板が12万以上、GGTが40未満の男性では59%、Probability groupのSVR率は①50歳以上で血

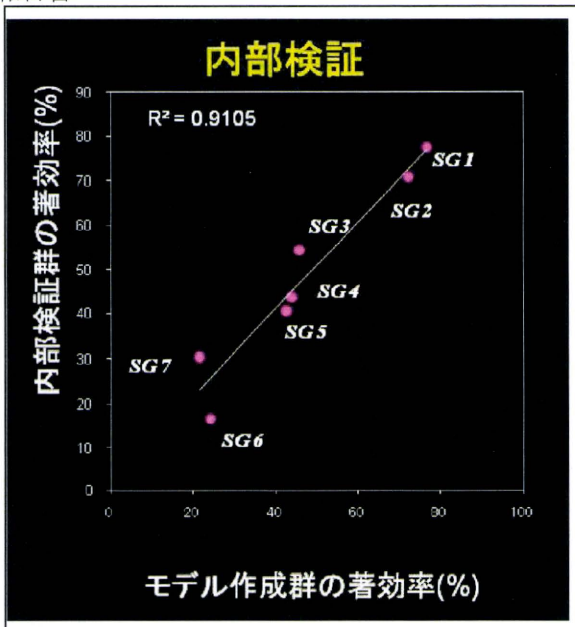
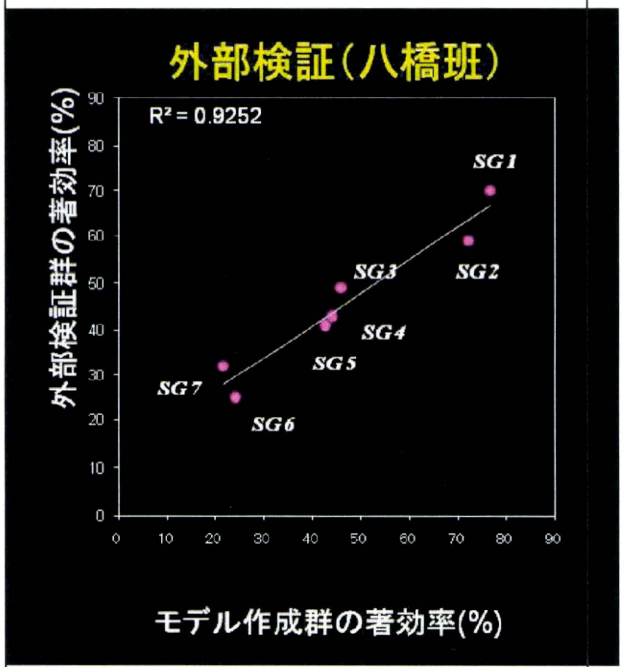


図 2.一般検査モデルの内部検証

小板が12万以上だが、GGTが40以上の女性では25%、Intermediate Probability groupのSVR率は①50歳未満だがAFPが8以上では43%、②50歳以上で、血小板数が12万以上で、GGTが40未満の女性では49%③50歳以上で、血小板が12万以上で、GGT40以上の男性では41%であった。モデル作成群と外部検証群のそれぞれのグループにおけるSVR率を比較したところ、両者には強い相関関係が見られた。このように、内部検証、および外部検証において、本モデルの安定性が証明されたため、本モデルには普遍性があると考えられる。



(2)遺伝子変異検査、肝生検を加味したSVR予測アルゴリズム：NS5A-ISDR変異数、Core70変異、年齢、LDL-Chol、肝線維化がSVR規定因子として選択された。これらの因子の組み合わせにより、予測著効率が31-83%の5グループが同定された(図4)。以下の2グループにおいてはSVR率が80%以上であった①NS5A-ISDR変異数が2個以上(83%)、②NS5A-ISDR変異数が0-1でも、年齢が60歳未満、Core70野生型、LDL-cholesterol120以上(83%)。一方、以下の3グループではSVR率が30%台であった①NS5A-ISDR変異数が0-1個で60歳以上(31%)、②NS5A-ISDR変異数が0-1個で60歳未満だが、Core70が変異型(36%)、③NS5A-ISDR変異数が0-1個で60歳未満、Core70野生型だが、LDL-cholesterol120未満で、肝線維化が以上(32%)。

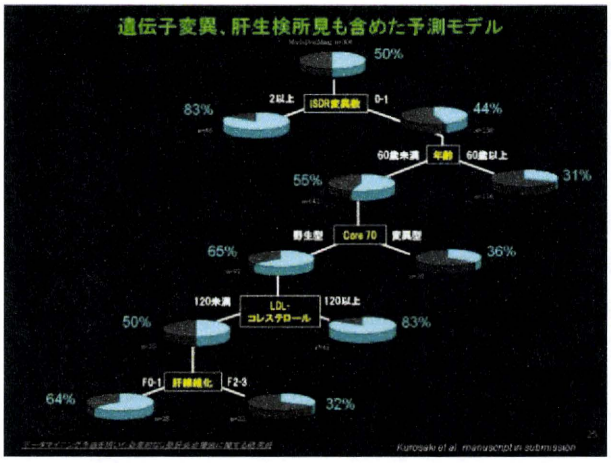


図4. 遺伝子変異検査、肝生検を加味したSVR予測モデル

このモデルの安定性について内部検証を行った結果、SVR率は以下のとおりであった：①NS5A-ISDR変異数が2個以上(75%)、②NS5A-ISDR変異数が0-1でも、年齢が60歳未満、Core70野生型、LDL-cholesterol120以上(71%)。このように、HCV遺伝子変異を検討することにより、治療効果の得られやすい症例をさらに精密に診断することが可能であった。本モデルの安定性については、さらに症例数を重ねて検証する予定である。

(3)再燃の予測

12週以内にHCVRNAが陰性化した場合(cEVR)のSVR率は76%と高率であった。しかしながら24%は治療終了後にHCVRNAが再出現(再燃)した。12週時点でHCVRNAが陰性化した場合には、再燃のリスクを考慮した治療計画を構築する必要があるために、再燃を予測するモデルを構築した。治療期間が48週以内の標準治療例で、12週以内にHCVRNAが陰性化した809例を対象とした。その結果、再燃と最も関連する因子は、既報のとおりHCVRNA陰性化時期であり、4週以内にHCVRNAが陰性化するRVRからの再燃率は11%、5-8週でHCVRNAが陰性化したcEVRからの再燃は24%、9-12週でHCVRNAが陰性化したcEVR-12からの再燃は37%であった。cEVR-8の中でも、RBV総投与量が3360mg未満では再燃リスクが高く(32%)、またRBV総投与量が3360mg以上でも60歳以上の高齢者では再燃率が28%であった。cEVR-12では、年齢60歳以上で再燃率が高く、特に女性では再燃率が65%であった。男性でもRBV総投与量が3024mg未満では再燃率が44%であった。この結果から、cEVR-8、cEVR-12では、薬剤adherenceを維持するか治療期間を延長してRBV総投与量3360mg以上を確保することが重要であり、また特に60歳以上の高齢者や女性では治療期間の延長が必要であることが示された。本検討は48週以内の標準治療を行った症例での検討であるため、来年度は延長治療を行った症例も含めて解析することにより、cEVRにおける目標薬剤投与量、最適治療期間を明らかとしたい。

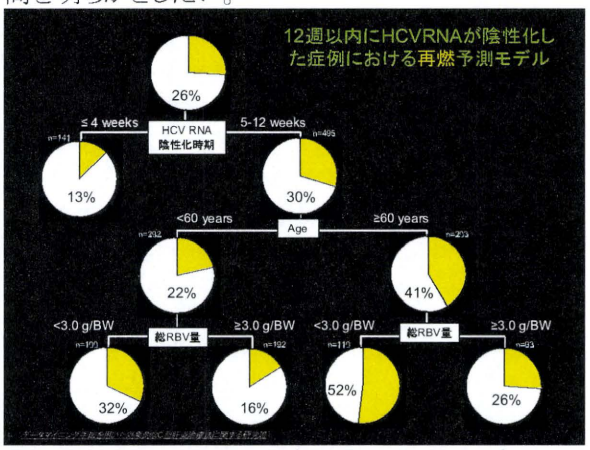


図5.HCVRNAが12週で陰性化したcEVR症例の再燃予測アルゴリズム

(4)重症貧血の予測アルゴリズム

PEGIFNとRBV併用療法を行うにあたって、副作用で最も重要であるのは貧血である。貧血によって途中で治療が中止になると、著効が得られにくい。そこで、中止基準であるヘモグロビン(Hb)が8.5g/dl未満に至る症例のアルゴリズムを構築した。全体で3.3%の症例が貧血によって治療を中止していたが、貧血リスク予測に最も重要であったのは治療前Hb値が14g/dl以上か未満であった。Hbが14g/dl未満であった場合にはクレアチニンクリアランス(Ccr)が80ml/分未満の場合に重症貧血のリスクが高く、Ccrが80以上であった場合には治療開始2週間目のHbが2g以上低下する場合に重症貧血のリスクが高かった。治療前のHbが14g/dl以上であった場合には、治療開始2週間目に2g/dl以上低下すると重症貧血になるリスクが高かった。このアルゴリズムを参考にして、治療が中止とならないようにRBVの投薬量を調節して治療を行うことが著効率を向上させ、長期的に肝癌を減少させることに役立つと考えられる。このアルゴリズムについて2/3の691症例でモデルを作成し、1/3の390例で検証した。両者がきわめてよく一致したため、このモデルに汎用性があると考えられた（図6）。

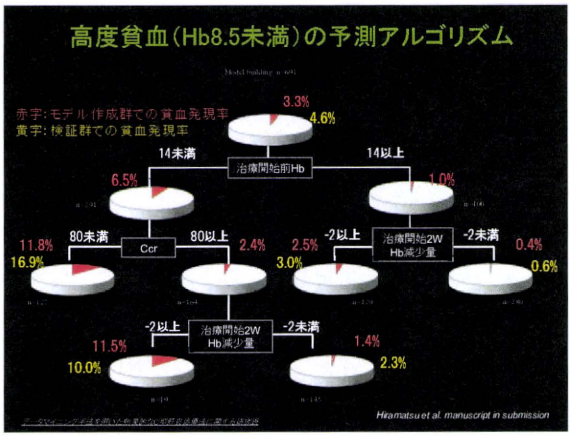


図6. 高度貧血(Hb 8.5/dl未満)の予測アルゴリズムとその検証

(5)肝発癌リスクのアルゴリズム

C型慢性肝炎においては長期経過の中で肝発癌にいたることが最も予後規定因子となる。そこで適切な治療を推進するにあたって、個々の症例での肝発癌リスクを予測することがインフォームドコンセントに重要となる。そこで当院でIFN治療を受けて非著効であった1,194例について、5年以内に発癌した例と5年以上観察して発癌がない症例を集積し、データマイニング解析を行った。武蔵野赤十字病院の865例でアルゴリズムを構築し、班員の施設で経過観察している329例で検証を行った。全体で6%の症例で5年以内に肝発癌がみられたが、発癌リスク予測に最も重要であったのは血小板数であり、15万/μl未満が11%、15万/μl以上が1%であった。血小板低下例では、アルブミン値4.0g/dl未満の例が18%の発癌リスクであり、4g/dl以上が5%であった。アルブミン低値例ではγGTPが40 IU/L以上の発癌リスクが21%と高く、40 IU/L未満では15%であった。一方、血小板低下例でアルブミン値4.0g/dl以上の例では5年間の肝発癌率は5%であり、この中では血清AST値30 IU/L以上の群が7%、30 IU/L未満では0%であった。血小板が15万/μl以下の例ではアルブミン値が3.75g/dl未満例で5年発癌率が7%、3.75g/dl以上では5年発癌率は1%であった。

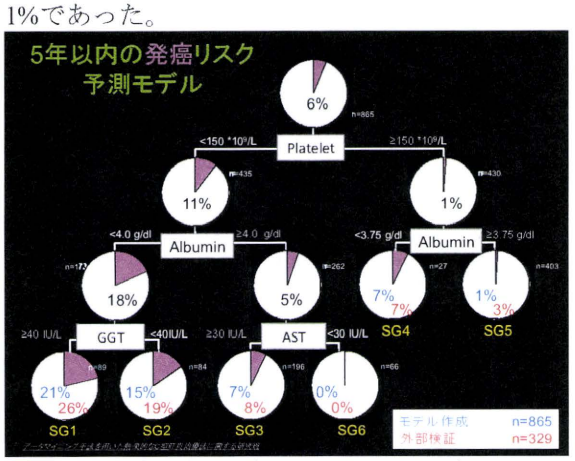
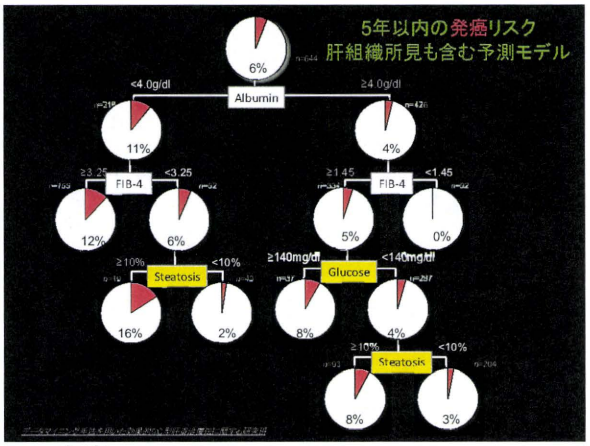


図7. 5年以内の肝発癌予測アルゴリズム

このアルゴリズムを、班員施設329例で検証した結果、両者はきわめてよく一致していた。さらに、5年発癌率が高いグループでは、10年間の発癌率も高かったため、臨床的に有用なモデルと考えられる。

肝生検を施行した症例644例において5年間の肝発癌率のデータマイニング解析を行うと、アルブミン4.0g/dlが最も重要であり、アルブミン値が低い例では血小板数、性、年齢、ALT値で算出されるFIB-4 indexが3.25以上の例では5年間で12%の肝発癌がみられたのの対し、3.25未満では6%で、この群では肝組織の脂肪化が10%以上みられた場合に16%の発癌がみられ、10%未満の脂肪化であった場合には2%の肝発癌リスクであった。アルブミン値が4.0g/dl以上であった例では、FIB-4 indexが1.45未満なら肝発癌は0%であったが、1.45以上だと5%の発癌率であった。この群の中では随時血糖値が140mg/dl以上の例で8%に肝発癌がみられるが、140mg/dl未満だと4%の発癌率であり、さらにこの中で肝組織脂肪化が10%以上みられた場合には5年間発癌率は8%であるが、10%未満の場合には3%であった（図8）.



D．考察

C型慢性肝炎は肝発癌リスクが高い疾患であり、これを防止するために適切な抗ウイルス療法を推進することが重要である。日常診療の中で治療効果を予測し、肝発癌率の両面からインフォームドコンセントを行うことが必要となる。さらに、個々の症例で副作用対策を講じることや、効果的な治療を行うための治療中の指標が必要である。

一般検査のみから治療開始前に効果を予測するモデルを作成した。内部検証に加え、八橋班との連携により精度の高い外部検証を行うことにより、このモデルの再現性、一般性が確認できた。本解析により従来は注目されなかったAFP、GGT、が治療効果と密接に関連することが明らかとなり、これらを測定することで一般検査からでも治療効果を予測することが可能であることを示した。この情報を、一般臨床医に広く周知することにより、従来は治療効果が明確でなかったために治療が導入されなかった患者についても、科学的根拠に基いた治療効果予測を説得力のあるインフォームドコンセントの一助として使用することにより、患者の治療機会増加に寄与できると期待する。紹介を受け

た肝臓専門医が、さらに精密に治療効果を予測する上で、HCV遺伝子解析や肝生検の情報が重要であることも示した。NS5A-ISDR、Core70は治療効果予測において大きなインパクトを有することを示した。SVRの確率を予測することは、治療の費用対効果を考慮する上でも重要である。

さらに、治療中の反応性によって再燃するか否かを予測することも重要である。12週以内にHCVRNAが陰性化した場合でも、再燃しやすい例を同定し、薬剤投与量を確保したり治療期間を延長することによって、効果を改善できる可能性がある。実際に治療を担当する医師にとって、重要な治療指針となりえる。

また、副作用を予知して、それを防止する対策が重要である。ペグインターフェロンとリバビリン併用療法における副作用で最も高頻度で、中止の原因となる高度貧血のリスクのアルゴリズムを作成できた。これを用いることによって、治療途中での中止を防ぎ、薬剤投与量を調節するなどの工夫によって完遂率を向上させることによってSVR率が改善することが期待される。

C型慢性肝炎においては、肝癌を合併することが予後に最も重要である。個々の症例で、治療しなかった場合の肝発癌率を予測することは、患者が治療を受ける機会を逸しないために重要である。それぞれの症例での5年以内の肝発癌を科学的なデータを示して、インフォームドコンセントを行うことによって、適切なインターフェロン治療機会を提供することが重要である。SVRが得られた場合には、肝発癌率が明らかに低下し、またSVRが得られない場合でも肝発癌を遅延させることが認められているため、患者の同意を得るために重要な根拠を示せるものと期待される。

E．結論

C型慢性肝炎の患者におけるペグインターフェロンとリバビリン併用によるウイルス排除率を個々の症例で治療前に予測できるアルゴリズムを、一般検査と専門的検査の両者で作成した。内部と外部の検証で一致率が高かったため、国内で普遍的に用いることができる。治療中の再燃予測や重篤な副作用予測のアルゴリズムを作成できたため、治療効果を改善させられる。また、個々の症例で、治療を受けなかった場合の肝発癌リスクのアルゴリズムを作成できたため、インフォームドコンセントが推進され、適切な治療を行うことによって肝発癌の低減につながる。

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H. 知的財産権の出願・登録状況(予定を含む。)

今回の研究においてはとくになし。

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Special Report

Management of hepatitis C; Report of the Consensus Meeting at the 45th Annual Meeting of the Japan Society of Hepatology (2009)

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The consensus meeting for the diagnosis, management and treatment for hepatitis C was held in 45th annual meeting for the Japan Society of Hepatology (JSH) in June 2009 where the recommendations and informative statements were discussed including organizers and presenters. The Several important informative statements and recommendations have been shown. This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. Thus, this is the first report of JSH consensus of hepatitis C. The rate of development of hepatocellular carcinoma (HCC) in HCV-infected patients in Japan is higher than in the USA, because the average age of the HCV-infected patients is greater and there are more patients with severe fibrosis of the liver than in the USA. In Japan, more than 60% of HCV-infected patients are genotype 1b infection, and they show lower response to perinterferon and ribavirin combination treatment. To improve the response rate is also an important issue in our country. To establish the original recommendations and informative statements to prevent the development of HCC is a very important issue in Japan.

Key words: chronic hepatitis C, peginterferon, ribavirin, fibrosis of the liver, hepatocellular carcinoma, HCV mutation

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a major public health problem and a leading course of death from liver disease in Japan. Two million people are infected, and more than 30 000 patients die from hepatocellular carcinoma (HCC) and/or liver cirrhosis every year. HCC is the fourth leading cause of death from malignant neoplastic disease, and prevention of the development of HCC is an urgent issue in Japan. The purpose of this consensus is to provide clinicians with consensus-based approaches to diagnosis and treatment of HCV infection.

The consensus meeting for the diagnosis, management and treatment for hepatitis C was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M. Kudo), where the recommendations and informative statements were discussed and compared with AASLD practice guidelines which has been published in *Hepatology*.[1] This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. This is the first report of the JSH consensus of hepatitis C. Established information regarding the pathogenesis and contributing factors for disease

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Table 1 Grading system for recommendations

	Description
Classification	
Class I	Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation procedure or treatment is beneficial, useful and effective
Class II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a diagnostic evaluation, procedure or treatment
Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
Class IIb	Usefulness/efficacy is less well established by evidence/opinion
Class III	Conditions for which there is no evidence and/or general agreement that a diagnostic evaluation, procedure/ treatment is not useful/effective and in some cases may be harmful
Level of evidence	
Level A	Data derived from multiple randomized clinical trials or meta-analysis
Level B	Data derived from a single randomized trial, or non-randomized studies
Level C	Only consensus opinion of experts, case studies or standard of care

progression which were agreed by the organizers and presenters are shown as informative statements, and clinically useful consensuses are shown as "Recommendations". The rate of development of HCC in HCV-infected patients in Japan is higher than that in the USA, because the average age of the patient is greater and there are more patients with severe fibrosis of the liver than in the USA. To establish original recommendations and informative statements to prevent the development of HCC is a very important issue in our country. The quality of recommendations or informative statements is required to show a "class" (reflecting benefit vs risk) and "level" (assessing strength or certainty) of evidence according to AASLD practice guidelines (Table 1).[1,2]

PATHOGENESIS OF HEPATITIS C

HEPATITIS C VIRUS infection causes acute and chronic hepatitis (CH), cirrhosis and HCC. The severity and rate of progression of the disease are highly variable and may reflect both host and viral factors, but the mechanisms of pathogenesis are incompletely understood. Thus, understanding the mechanisms of HCV pathogenesis is an important goal of HCV research.

Entry pathway of HCV

For the virus, the first step in propagation is enter into hepatocytes. A decade ago, the HCV envelop protein E2 was shown to bind human CD81, a tetraspanin expressed on various cell types including hepatocytes and B lymphocytes.[3] Next, two other essential proteins, scavenger receptor class B type I (SR-B1)[4] and claudin-1 (CLDN1),[5] and potentially additional accessory factors such as glycosaminoglycans and low-density protein receptors[6] were identified as receptors involved in HCV entry. Finally, the crucial factor was identified as the tight junction protein occludin (OCLN).[7] Interestingly, both CLDN1 and OCLN are components of tight junctions which are structures forming firm seals between adjacent cells. The initial adhesion of HCV to hepatocytes may be mediated by accessory factors and/or direct interaction with SR-B1 and CD81 proteins. On transfer to a tight junction complex, HCV may interact directly with CLDN1 and/or OCLN, allowing viral uptake into the cell.

Hepatitis C virus infects only humans and chimpanzees. Once these HCV entry factors were identified, the next concern was to determine which factors dictate species-specific tropism. CD81 proteins from other mammals, such as the mouse, are used inefficiently by HCV.[8] Although HCV does not discriminate between human and mouse SR-B1 and CLDN1, mouse OCLN like CD81 cannot substitute for the related human protein in aiding viral entry. These findings indicate that CD81 and OCLN represent minimal human-specific entry factors.

Informative statement: CLDN1 and OCLN in addition to CD81 and SR-B1 are required for entering of HCV into hepatocytes, and especially CD81 and OCLN represent minimal human-specific entry factors. (Grade A.)

Evasion of intracellular host defense by HCV

One of the mechanisms by which HCV infection is likely to lead to be persistent is evasion of intracellular host defense through a complex combination of processes that include interference of interferon (IFN) signaling, modulation of its effectors and continual viral genetic variation. The HCV genome contains pathogen-associated molecular pattern (PAMP) signatures which

are recognized by the retinoic-inducible gene I (*RIG-I*) and specific Toll-like receptors when introduced into naïve cells.[9–11] Viral signaling through *RIG-I* and its adaptor protein, IFN promoter-stimulator 1 (IPS-1), activates IFN regulatory factor-3 (IRF-3) and the host IFN-α/β response that limits virus infection.[12,13] HCV NS3/4A protease cleaves IPS-1, releasing IPS-1 from the mitochondrial membrane.[14] Cleavage results in subcellular redistribution of IPS-1 and loss of interaction with *RIG-I*, thereby preventing downstream activation of IRF-3 and induction of IFNβ.[15]

Secreted IFNβ engages the local tissue through the autocrine and paracrine processes of binding the IFN-α/β receptors. This results in activation of the Jak-signal transducer and activator of transcription (STAT) pathway, in which the receptor-associated Jak and Tyk1 protein kinases catalyze the phosphorylation of STAT proteins. The resulting IFN-stimulated gene factor-3 (ISGF3) transcription factor complex localizes in the cell nucleus, where it binds to the IFN-stimulated response element (ISRE) within the promoter/enhancer region of IFN-stimulated genes (ISG). Jak-STAT signaling leads to a second wave of transcriptional activity stimulating ISG expression in the infected cells. Expression of the HCV core protein has been associated with increased expression levels of suppressor of cytokine signaling (SOCS)-3.[16] The SOCS proteins are known for their role as negative regulators and inhibitors of Jak-STAT signaling, where they mediate a classic negative feedback loop on IFN-α/β receptor signaling events.[17] The HCV NS5A protein has been shown to induce interleukin (IL)-8 production leading to partial inhibition of the IFN-induced antiviral response, probably through the alteration of ISG expression.[18] The HCV NS5A and E2 proteins also bind double-strand RNA-activated protein kinase (PKR) and inhibit its catalytic activity,[19,20] which allows HCV to evade in part the translational-suppressive actions of IFN. Thus, HCV evasion of the host response includes various strategies directed by viral proteins to control IFN signaling, ISG expression or function.

Informative statement: HCV evades intracellular host defenses through a complex combination of processes that include IFN signaling, modulation of its effectors and continual viral genetic variation. These mechanisms include cleavage of IPS-1 by the NS3/4A protease, inhibition of Jak-STAT signaling by HCV-induced SOCS3, inhibition of the IFN-induced antiviral response by NS5A-induced IL-8, and/or inhibition of catalytic activity of PKR by the NS5A and E2 proteins. (Grade A.)

Oxidative stress induced by HCV

Oxidative stress is well known to be present in CH-C to a greater degree than in other inflammatory liver diseases. Although the mechanisms underlying oxidative stress induced by HCV have not been elucidated fully, there are several lines of evidence suggesting that HCV directly generates reactive oxygen species (ROS) *in vitro* and *in vivo*. Hepatic ROS production is significantly higher in HCV core transgenic mice than in normal control mice in the absence of hepatic inflammation.[21] HCV core protein also produces ROS in human hepatoma Huh-7 cells and HeLa cells.[22] Analysis of the interaction of HCV core protein with mitochondria in transgenic mice and direct interaction of recombinant core protein and isolated mitochondria indicated oxidation of the mitochondrial glutathione pool and an increase in ROS production by the mitochondrial electron transport complex I, suggesting that direct interaction of core protein with mitochondria is an important cause of the oxidative stress seen in CH-C.[23]

Informative statement: Mitochondrial dysfunction induced by HCV leads to ROS generation that causes the oxidative stress seen in CH-C. (Grade A.)

Metabolic disorders caused by HCV

Epidemiological studies have suggested a link between type 2 diabetes and chronic HCV infection, which implies HCV-induced insulin resistance. A high level of tumor necrosis factor (TNF)-α and disturbance of tyrosine phosphorylation of the insulin receptor substrate (IRS)1 by TNF-α has been demonstrated in HCV core transgenic mice.[24] Another possible mechanism is that HCV core-induced SOCS3 promotes proteosomal degradation of IRS1 and IRS2 through ubiquitination.[25] Hepatic steatosis is one of the histopathological features in CH-C. HCV core protein inhibits microsomal triglyceride transfer protein activity and secretion of very low density lipoprotein.[26] HCV core protein also upregulates the sterol regulatory element binding protein (SREBP)1c promoter activity through the enhanced binding of the LXRα/RXRα to LXR-response element,[27] which leads to an increase in transcription of genes involved in hepatic fatty acid synthesis. Hepatic iron accumulation is also a histopathological feature of CH-C, even though its levels are not extremely high. HCV-induced ROS down-regulates the transcriptional activity of hepcidin, a negative regulator in iron homeostasis, in transgenic mice expressing the HCV polyprotein[28] and in HCV replicon cells[29] (Fig. 1).

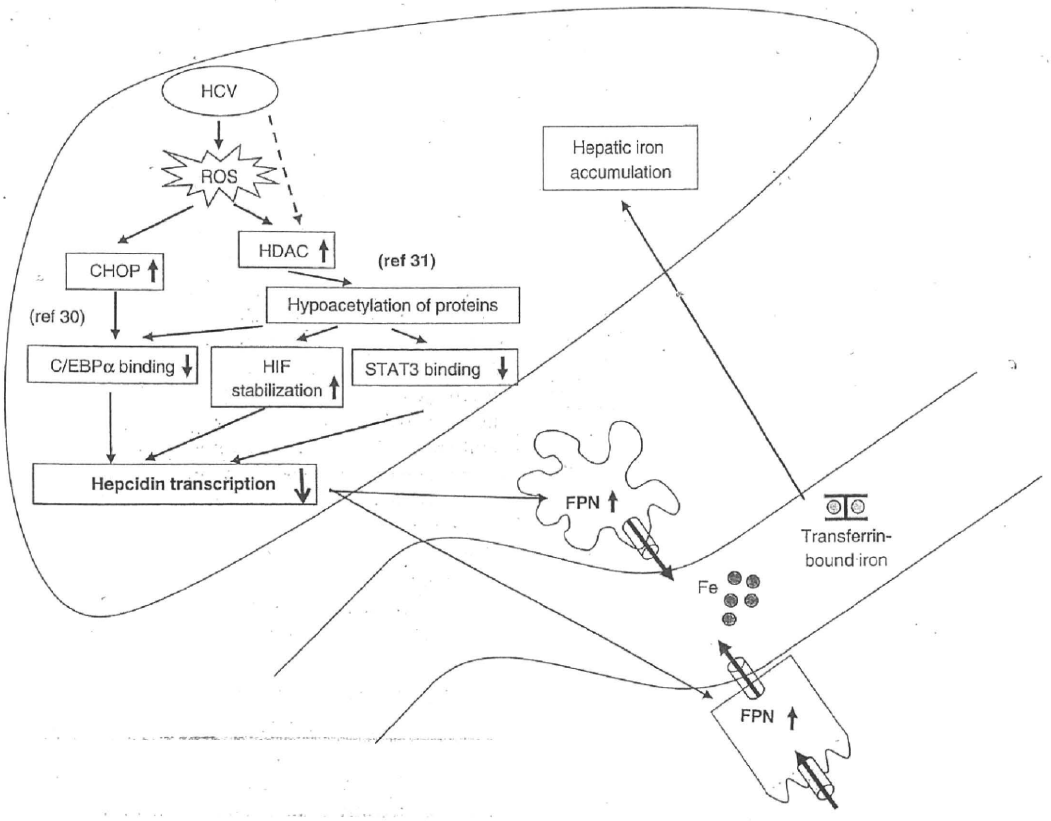

Figure 1 Schematic diagram depicting the mechanisms underlying the hepatic iron accumulation induced by HCV. HCV-induced ROS reduces hepcidin transcription through the inhibited binding of CHOP and/or STAt3 to the hepcidin promoter, and/or stabilization of HIF that is negative hepcidin regulator. C/EBP, CCAAT/enhancer-binding protein; CHOP, C/EBP homology protein; FPN, ferroportin; HCV, hepatitis C virus; HDAC, histone deacetylase; HIF, hypoxia inducible factor; ROS, reactive oxygen species; STAT, signal transducer and activation of transcription.

Metabolic disorders caused by HCV such as insulin resistance, hepatic steatosis and iron accumulation are clinically important in terms of amplification of oxidative stress and involvement in hepatocarcinogenesis in CH-C.[30–33] In addition, these metabolic disorders are related to the response to antiviral therapy. Insulin resistance[34] and hepatic steatosis[35] seem to be negatively correlated with response to antiviral therapy in CH-C.

Informative statement: HCV induces insulin resistance, hepatic steatosis, and/or hepatic iron accumulation, which is associated with hepatocarcinogenesis in CH-C. (Grade A.)

Recommendation 1: Insulin resistance and hepatic steatosis seem to be negatively correlated with response to antiviral therapy in CH-C, whereas it remains controversial whether hepatic iron accumulation is related to a poor response to therapy. (Level 2a, Grade C.)

Liver biopsy for evaluating pathogenesis of hepatitis C

Assessment of the extent of liver fibrosis is still of great importance in terms of predicting the response to antiviral therapy and hepatocarcinogenesis in CH-C. It is also apparent that as many as a quarter of CH-C patients with persistently normal aminotransferase values have significant fibrosis.[36] The recently developed transient elastography that uses ultrasound and low-frequency elastic waves to measure liver elasticity has

Table 2 Definitions of virological responses to interferon therapy

RVR (rapid virological response)	Undetectable HCV RNA at week 4
cEVR (complete early virological response)	Undetectable HCV RNA at week 12
pEVR (partial early virological response)	Two log drop of HCV RNA without undetectable level at week 12
LVR (late virological response)	Undetectable HCV RNA between week 13 and 24 week
NVR (null virological response)	Positive HCV RNA during treatment
Relapse	Undetectable HCV RNA at end of treatment followed by detectable level after treatment
SVR (sustained virological response)	Undetectable HCV RNA at 24 weeks after treatment

improved the ability to define the extent of fibrosis without a liver biopsy, particularly when combined with other non-invasive markers,[37] but it is not yet ready to replace liver biopsy. Among the pathological features, steatosis and excess hepatocellular iron that affect disease progression and possibly impede treatment response are difficult to diagnose without liver biopsy. Thus, a liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment.

Recommendation 2: A liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment. (Level 1, Grade C.)

VIRAL LOAD, GENOTYPE, VIRAL MUTATIONS

DEFINITIONS OF VIROLOGICAL responses to IFN therapy are summarized in Table 2.

HCV RNA assay and genotype

In clinical practice, the usual approach is to test initially for antibodies to HCV (anti-HCV), then to use HCV RNA to document viremia. The quantity of HCV RNA is useful to know before providing and monitoring HCV treatment. For HCV RNA determination, quantitative tests based on target amplification (reverse transcriptase polymerase chain reaction [RT–PCR]) and signal amplification (branched DNA [bDNA]) techniques with differing sensitivity and linear measuring ranges are commercially available. The COBAS Amplicor HCV Monitor Test v2.0 (Roche Molecular Systems, Branchburg, NJ, USA), however, requires sample dilutions for accurate quantification of high-titer specimens. In addition, the assay displays relatively low sensitivities of approximately 600 IU/mL. Recently, the COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems) and AccuGene m-HCV (Abbott Molecular, Des Plaines, IL, USA) have become available. These meet the requirements for highly sensitive detection and reliable quantification of HCV in clinical samples.

There are six major HCV genotypes. Genotype specificity predicts the likelihood of treatment response and determines the duration of treatment. Therefore, HCV genotype should be determined in all HCV-infected persons prior to treatment in order to determine the duration of therapy and likelihood of response.[38]

Many reports showed that sustained virological response (SVR) rates in IFN monotherapy and IFN plus ribavirin (RBV) combination therapy were higher in patients who had lower pretreatment RNA levels and genotype 2 infections.[39–41]

Recommendation 3: HCV RNA level and genotype should be determined in all HCV-infected persons prior to treatment in order to predict the efficacy of response of therapy. SVR rate in IFN therapy are higher in patients who had lower pretreatment RNA levels and genotype 2 HCV infections in IFN therapy. (Level 1, Grade A.)

HCV mutation

IFN sensitivity determining region (ISDR)

Enomoto *et al.* were able to demonstrate a strong correlation between the number of mutations within the carboxy terminal region of the NS5A gene, the ISDR spanning codons 2209–2248, and response to IFN therapy.[42] Thus, no patient infected with HCV with a wild-type ISDR sequence (identical to the prototype Japanese HCV strain [HCV-J]) responded to IFN therapy whereas all patients infected with the "mutant type", defined by four or more amino acid substitutions in this region, showed an SVR.[43] These initial findings have been confirmed by other Japanese studies but controversial data were reported from other parts of the world, particularly from Europe and the

USA. This may indicate that geographical factors account for different sensitivities of HCV genotype 1b infection to antiviral therapy. Pascu *et al.* reported that the distribution of wild-, intermediate- and mutant-type ISDR sequences differed significantly between Japanese ($n = 655$) (44.1%, 37.6% and 18.3%, respectively) and European patients ($n = 525$) (24.8%, 63.4% and 11.8%, respectively; $P = 0.001$). However, there was a significant positive correlation between the number of ISDR mutations and SVR rate, irrespective of geographical region.[44]

Moreover, Shirakawa *et al.* reported that a logistic regression model that includes the sequence of ISDR of HCV, and other factors (T-helper cell [Th]1/Th2 ratio, bodyweight and neutrophil count) can be useful for accurately predicting accurately the SVR rate before pegylated (PEG)-IFN and RBV combination therapy.[45]

Recommendation 4: The ISDR should be evaluated before IFN treatment in order to predict the response to treatment. (Level 2b, Grade B.)

IFN/RBV resistance-determining region (IRRDR)

El-Shamy *et al.* have reported recently that a high degree of sequence variation in the V3 and the pre-V3 regions (amino acid [aa]2334-2355) of NS5A, which they refer to collectively as the IRRDR (aa2334-2379), was closely correlated with virological response in HCV-1b-infected patients treated with PEG-IFN and RBV.[46] A high degree (>6 aa substitutions) of sequence variation in the IRRDR should be a useful marker for predicting SVR, whereas a less diverse (<5) IRRDR sequence predicts non-SVR.

Amino acid substitutions in the HCV core region

Akuta *et al.* identified pretreatment substitutions of aa70 and aa91 in the core region as independent and significant pretreatment factors associated with virological non-response, based on 48-week combination therapy of IFN plus RBV.[47] Moreover, they identified aa70 and aa91 substitutions in the core region as predictors of response to PEG-IFN-RBV therapy in Japanese patients infected with HCV genotype 1b[48] (Table 3). Donlin *et al.* reported sequencing the complete pretreatment genotype 1 HCV open reading frame using samples from 94 participants in the Virahep-C study to assess the effects of viral diversity on response to therapy.[49] Genotype 1b sequences from patients with more than 3.5 log declines in viral RNA levels by day 28 (marked responders) were more variable than those from patients with declines of less than 1.4 log (poor responders) in core and NS3. Moreover, arginine (R) at aa70 in the core region was related to a marked response.

Recently evaluations were made of the impact of aa substitutions in HCV core region on hepatocarcinogenesis. Akuta *et al.* reported that cumulative hepatocarcinogenesis rates in double wild-type (arginine at aa70/leucine at aa91) of the HCV core region were significantly lower than those in the non-double wild type in CH-C patients.[50] Moreover, another report showed that a logistic regression model developed

Table 3 Factors associated with sustained virological response to 48-week pegylated interferon plus ribavirin combination therapy in patients infected with hepatitis C virus genotype 1b, identified by multivariate analysis ($n = 114$) 52)

Factor	Category	Risk ratio (95% confidence interval)	*P*
Amino acid substitution in core region	1: double wild	1	
	2: non-double wild	0.102 (0.022–0.474)	0.004
Low-density lipoprotein cholesterol (mg/dL)	1: <86	1	
	2: ≥86	12.87 (2.177–76.09)	0.005
Sex	1: male	1	
	2: female	0.091 (0.017–0.486)	0.005
ICG R15 (%)	1: <10	1	
	2: ≥10	0.107 (0.017–0.678)	0.018
γ-Glutamyltransferase	1: <109	1	
	2: ≥109	0.096 (0.0011–0.819)	0.032
Ribavirin dose (mg/kg)	1: <11.0	1	
	2: ≥11.0	5.173 (1.152–23.22)	0.032

through analysis of full-length core gene sequences identified seven polymorphisms significantly associated with increased HCC risk (36G/C [aaK12N], 209A [aaR70Q], 271U/C [aaL91M], 309A/C, 435A/C, 481A and 546A/C).[51] HCV core gene sequence data might provide useful information about HCC risk.

Recommendation 5: Amino acid substitutions in the HCV core region (aa70 and aa91) should be determined before IFN treatment in order to predict the response to treatment. (Level 2b, Grade B.)

NS3 protein secondary structure

Recently, Ogata *et al.* reported that HCV-1b strains can be classified into different groups based on the secondary structure of an amino-terminal portion of the NS3 protein and that specific strains are more prevalent among patients with HCC.[52] Moreover, the cumulative incidence of HCC was highest among patients infected with specific group HCV-1b, in whom the risk of HCC significantly increased compared with that among patients infected with another group (hazard ratio = 4.95 [95% confidence interval = 1.43–17.11]) after adjustment for age and histological stage.[53]

Informative statement: NS3 protein secondary structure may be related to hepatocarcinogenesis. (Grade B.)

NATURAL HISTORY OF CH-C

Progression to cirrhosis and HCC

PREVIOUS PUBLICATIONS REPORTED that approximately 60–80% of patients with acute hepatitis C develop chronic infection in the natural course.[54–57] Because it is difficult to ascertain precisely when the HCV infection occurred except for patients who had blood transfusions, and because chronic infection progresses slowly and asymptomatically, the natural entity of the disease has not been elucidated fully. Seeff *et al.* compared the long-term prognosis of HCV antibody-positive and -negative young men and reported that liver disease-related death was very rare in HCV antibody-positive patients.[58,59] Kenny-Walsh studied the liver histology of 363 young women 17 years after HCV infection and showed that 83% had no or mild hepatic fibrosis whilst 2% had liver cirrhosis.[60] These results demonstrate that progression to serious liver disease is a rare event two decades after infection of young people with HCV.

On the other hand, in blood transfusion-associated CH-C patients the mean interval to liver cirrhosis is estimated to be approximately 20–30 years and that to HCC approximately 30–40 years.[61,62] Because HCC is the most serious complication of HCV-infected people, it is desirable to predict the overall incidence of HCC in each patient. Up to now, many investigators have reported a close relationship between the stage of hepatic fibrosis and incidence of HCC. According to reports from Japan, the annual incidence of new HCC in liver cirrhosis is estimated to be approximately 5–8%.[63–65]

Informative statement: The natural history of CH-C is highly variable. HCV infection does not have much impact on the overall mortality of all the infected people, whereas progression to liver cirrhosis is observed 20–30 years and to HCC 30–40 years after infection. In Japan, the annual incidence of HCC in liver cirrhosis is estimated to be 5–8%. (Level 2b, Grade B.)

Recommendation 6: Treatment of HCV-infected people should be determined in consideration of the higher annual incidence of HCC in patients with liver cirrhosis in Japan as compared to Western countries. (Level 2b/3, Grade B.)

Progression of fibrosis

The rate of progression of fibrosis varies among patients with CH-C. Poynard *et al.*[66] calculated the average progression rate of hepatic fibrosis in CH-C to be 0.133 fibrosis units/year. In Japan, Shiratori *et al.*[67] reported this to be 0.10 fibrosis units/year. In HCV carriers with persistently normal aminotransferase levels (PNALT), progression of hepatic fibrosis is slower. Persico *et al.*[68] reported that median histological scores did not differ after 5 years of follow up in PNALT and Okanoue *et al.*[69] calculated the average progression rate of hepatic fibrosis in PNALT to be 0.05 fibrosis units/year.

Informative statement: On average, progression of hepatic fibrosis in CH-C is 0.10–0.13 fibrosis score units/year. The hepatic stage/grade score of HCV carriers with PNALT *are generally low and the progression of hepatic fibrosis is slow. Excessive alcohol intake, insulin resistance and hepatic steatosis are the major factors which induce the progression of hepatic fibrosis. (Level 2b, Grade B.)*

Alanine aminotransferase (ALT) levels

Alanine aminotransferase is an easy tool to evaluate hepatocellular damage in liver diseases. In the past, a higher incidence of HCC was reported in liver cirrhotic patients with elevated ALT levels.[70] The normal range of serum ALT level varies according to the institutions or hospitals, but it is likely to be located between 30 IU/L