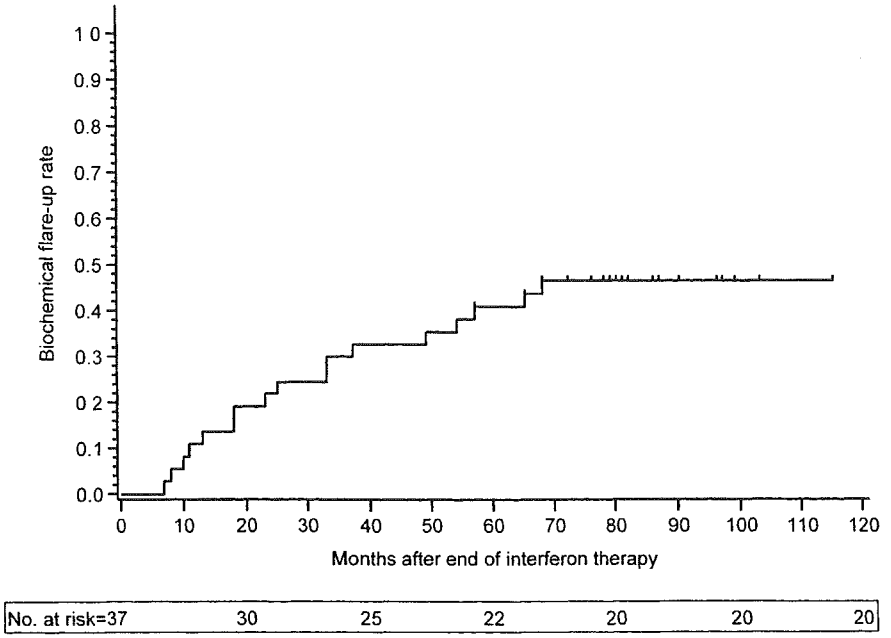

Fig. 2. Kaplan–Meier survival curve of the 37 biochemical responders (BRs) estimating time to flare-up showing ALT fluctuations of >2 × ULN.

median 1, range 0–4 at the end; $P = 0.001$) and in the BRs (median 8, range 3–11 before treatment vs. median 5, range 1–8 at the end; $P = 0.003$). The mean change was −0.74 U/year (95% CI −0.52 to −0.96) for the SVRs compared with −0.30 U/year (−0.14 to −0.46) for the BRs ($P = 0.003$). The SVRs showed improvement of each category of necroinflammation, whereas improvement was significant with respect to piecemeal necrosis and focal lytic necrosis in the BRs. However, in the absence of occult HCV and HBV infections, 13 (87%) of the SVRs retained low grade of necroinflammation, especially portal inflammation in the late convalescent phase.

The liver fibrosis score also improved in the SVRs (median 4, range 3–6 before treatment vs. median 3, range 2–6 at the end; $P = 0.007$) and was unchanged in the BRs (median 3, range 3–4 before treatment vs. median 3, range 3–6 at the end; $P = 0.480$). The mean change was −0.08 U/year (95% CI −0.03 to −0.13) for the SVRs compared with −0.01 U/year (0.04 to −0.07) for the BRs ($P = 0.066$). Of the four HCC cases with sustained virological response, the two noncirrhotic patients showed further alleviation of liver fibrosis. Patients 7 and 19 developed HCC at 4.6 and 5.5 years post-treatment and underwent final liver biopsies at 5.5 and

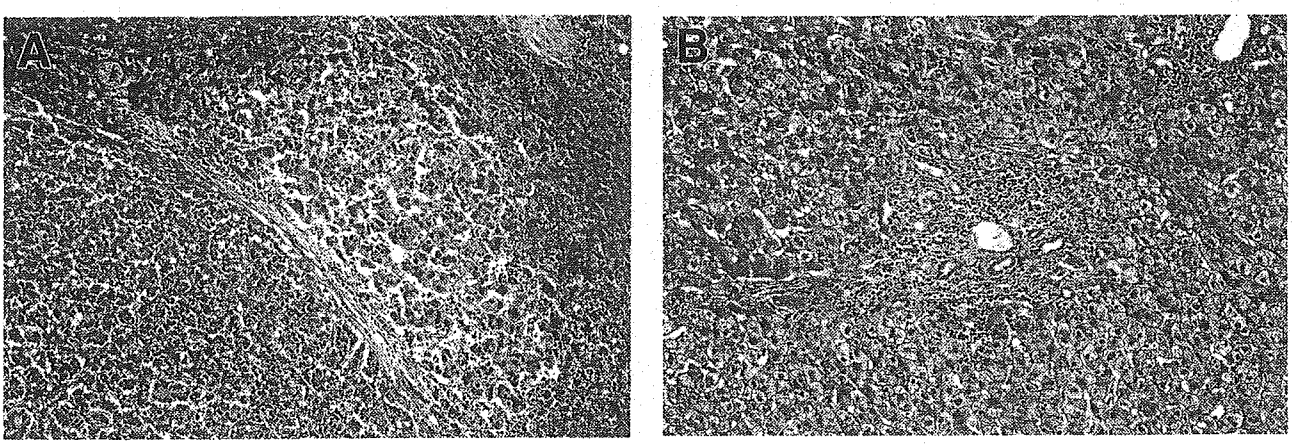

Fig. 3. Liver histology of patient 19 at the time of HCC resection. **A**: Two centimeters of moderately differentiated HCC developed after a sustained virological response; (**B**) the surrounding non-tumor liver tissue only showed slight portal inflammation without fibrosis (Ishak inflammatory grade 1 and fibrosis stage 0). Paraffin sections stained with hematoxylin–eosin. (Original magnification 100×).

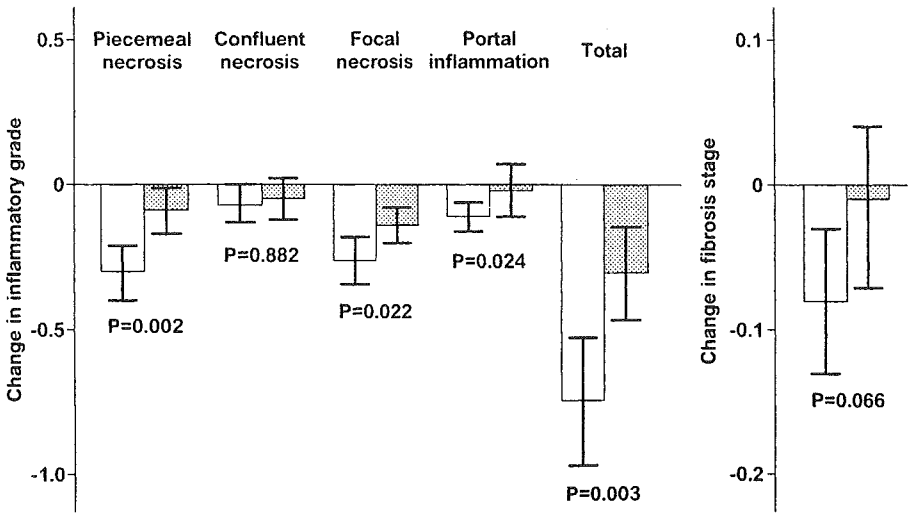

Fig. 4. Mean change (95% CI) in inflammatory grade (**left**) and fibrosis stage (**right**) between the paired liver biopsy specimens. Changes were analyzed for chronic hepatitis C patients who showed virological (open bars) or biochemical (closed bars) response at 6 months after the end of IFN therapy.

6.2 years post-treatment, respectively. Ishak scores improved in both patient 7 (inflammatory grade 8 and fibrosis stage 4 before treatment vs. 0 and 3, respectively, at the end) and patient 19 (inflammatory grade 5 and fibrosis stage 3 before treatment vs. 0 and 1, respectively, at the end).

DISCUSSION

Previous studies have shown that 91–95% of the SVRs with no serum HCV RNA at 6 months post-treatment had no detectable liver HCV RNA 1–2 years after therapy [Reichard et al., 1995; Shindo et al., 1995], whereas Marcellin et al. [1997] demonstrated that HCV eradication from the liver was sustained up to 5 years after therapy in all such patients examined. Studies on liver HCV with a longer follow-up period could shed further light on this issue but are very limited. The present study with prolonged follow-up of >5 years showed that the clearance of liver HCV RNA was sustained up to 12 years after therapy for all 15 patients. Neither positive strands nor negative strands were found in any liver biopsy specimens. Sustained reductions in HCV core antibody titers at a constant rate further corroborated complete HCV eradication. One report available in the literature showed that liver HCV RNA was not found in five SVRs 10–11 years after therapy [Lau et al., 1998]. Collectively, these findings suggest that HCV seroclearance at 6 months after IFN therapy withdrawal would usually imply virological cure.

We further investigated occult HBV infection in the liver tissue from chronic hepatitis C patients treated with IFN. Approximately half of our patients had serological evidence of previous HBV exposure, but HBV DNA was not found in the liver tissue irrespective of HCV clearance. In our local region, occult HBV infection is frequently found in the livers of untreated HCV-related chronic liver disease patients [Tamori et al., 1999]. Further studies may be necessary to examine the possible influence of IFN therapy on concomitant HBV.

Thus, the present study showed that the SVRs were completely free of occult HCV and HBV up to a decade after therapy withdrawal. When the long-term liver histological outcomes were evaluated, the SVRs showed improvement of the necroinflammatory grade and regression of the fibrosis stage. Nevertheless, mild liver inflammation persisted in the majority, the reason for which remains unclear. Intrahepatic inflammatory response triggered by HCV infection may take a very long time to cease and may continue in the absence of occult viral infections. It was also demonstrated that HCC, a late complication after sustained virological response, occurs without occult HCV or HBV infection. All HCC patients had an advanced stage of liver fibrosis before treatment, and it is conceivable that long-standing HCV-related liver injury had initiated the carcinogenesis process although persistent low-grade liver inflammation after IFN therapy may have exerted some influence on the subsequent course.

Even using IFN alone or in combination therapy of IFN plus ribavirin leads to at least half of the chronic hepatitis C patients showing no sustained virological response [McHutchison et al., 1998; Poynard et al., 1998]. Under these circumstances, improvement of serum ALT elevation without HCV seroclearance should be considered another favorable response to IFN therapy. At present, the definition of a biochemical response with long-term clinical benefits remains controversial. A recent European study showed that the best ALT threshold predicting significant liver injury is about two times the ULN [Pradat et al., 2002]. In the second part of the study, the long-term clinical course of a biochemical response defined as end-of-treatment ALT normalization followed by near-normal ALT levels

of $\leq 2 \times$ ULN during the subsequent 6 months was studied. The BRs were more likely to be older and have low-grade necroinflammatory reaction as compared with the SVRs, thus indicating that failure in HCV clearance may be attributable to inefficient host immune responses to HCV-infected hepatocytes.

The present study revealed that the 5-year risk of biochemical flare-up showing ALT fluctuations of $>2 \times$ ULN was 41% (95% CI 24.7–56.4) but that the flare-up could not be predicted by any clinical, virological, and histological characteristics. However, the type of IFN therapy was not uniform, and quantitative data on HCV RNA levels during IFN therapy were not available. There remains a possibility that these factors may have had relevance to the clinical course of BRs. It was shown that occult HBV was not present in the livers of BRs and did play no role in the clinical course. Occult HBV is known to be common and exert virulence in chronic hepatitis C patients, but its clinical relevance after IFN therapy may be less significant. The flare-up was easily controlled by retreatment with IFN. Paired liver biopsies showed that necroinflammation was ameliorated and that the fibrosis stage remained unchanged. These observations are compatible with a few studies on the histological outcomes of a biochemical response defined as sustained ALT normalization [Bruno et al., 2001; Shindo et al., 2001]. Although further studies with a larger number of patients are necessary, control of biochemical disease activity to near-normal levels may also confer favorable long-term histological outcomes.

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C型慢性肝炎の治療

C型慢性肝炎に対するIFN再治療の成績とその適応

Efficacy and indication of IFN retreatment for patients with chronic hepatitis C

伊与田賢也　加藤道夫

Key words：C型慢性肝炎，IFN単独再治療，IFN/リバビリン併用再治療，コンセンサスIFN

はじめに

1992年より，我が国ではC型慢性肝炎に対して，IFN治療が行われてきたが，当初，投与期間は6カ月に限られ，また，再投与も認められていなかった．2000年4月にC型慢性肝炎に対するIFN再投与が認可され，続いて2001年12月には我が国においてもIFNとリバビリンの24週併用療法が認可された．これによって，IFN単独療法で無効または再燃であった患者に新たな再治療の道が開かれた．では，実際の再治療の効果やその適応はどうかについて，本稿では述べたい．

1. IFN単独再治療の成績

リバビリンの使用が開始されるまでは，初回，IFN単独治療無効もしくは，再燃例(初回IFN治療中に血清ALT値が正常化もしくはHCV-RNAが陰性化後，再燃した症例)に対して，IFN単独再治療を行っていた．主なIFN単独再治療についての報告を表1にまとめた．

当院で2001年までにIFN単独再治療を行った75例の治療成績では，著効率(治療終了24週後におけるHCV-RNA陰性化率)は22例で29%であった．このうち，genotype 1bの高ウイルス量例(1 Meq/ml以上，100 kcopy/ml以上，300 fmol/l以上)では著効となった症例はなかった．一方，genotype 1bの低ウイルス量例に限ると，著効率は58%であった．また，これらのうち，IFN再治療の前後で肝生検を施行し得た30例中，activityは8例で，fibrosisは5例で改善していた[1]．IFN再治療によって，組織が改善するとの報告は海外でもみられる[2]．国立病院・療養所肝疾患ネットワーク参加22施設の2000年から2001年のまとめ[3]によると，IFN単独再治療例を行った76例のうち，著効例は26例(34%)であった．このうち，genotype 1bの高ウイルス量例では著効例は1例のみ(4%)であった．熊田[4]は初回IFN無効316例に対して，IFN単独再治療を行い著効例は70例(22%)であった．このうち，genotype 1bの高ウイルス量例では著効例は11例(6%)であった．また，海外からの報告[2,5-7]をみると，投与量や投与方法が異なり，著効率にややばらつきがみられるが，genotype 1以外や低ウイルス量例で著効が認められる．おおむね，初回時に比べて，IFNの投与期間が長いほど，また投与量が多いほど，高い著効率が得られている．

以上より，初回IFN無効または再燃例に対して，投与時期や期間，投与量を考慮すれば，IFN再投与により，著効となる症例が少なからず存在することが考えられる．一方で，genotype 1bの高ウイルス量例ではIFN単独で著効を得ることは極めて困難である．そこで，高

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表1 IFN単独再治療の著効率

		genotype 1bかつ高ウイルス量	genotype 1bかつ高ウイルス量以外	全例
国内	加藤ら(自験例)	0/ 30(0%)	22/ 45(49%)	22/ 75(29%)
	八橋[3]	1/ 26(4%)	25/ 50(50%)	26/ 76(34%)
	熊田[4]	11/188(6%)	59/128(46%)	70/316(22%)
海外	Davisら[5]	0/ 70(0%)	8/102(8%)	8/172(5%)
	Cammaら[6]	30/224(13%)*	35/158(22%)	73/494(15%)**
	Payenら[2]	6/ 83(7%)*	28/ 81(35%)	41/190(22%)**
	Chemelloら[7]	5/ 61(8%)*	8/ 31(26%)	13/ 92(14%)

*低ウイルス量例も含むgenotype 1全例
**ウイルス量およびgenotype未測定症例を含む

表2 IFN/リバビリン併用再治療の著効率

		genotype 1bかつ高ウイルス量	genotype 1bかつ高ウイルス量以外	全例
国内	加藤ら(自験例)	5/ 30(17%)	11/ 13(85%)	16/ 43(37%)
	八橋[3]	22/108(20%)	24/ 35(69%)	46/143(32%)
	豊田[11]	6/ 41(15%)	16/ 21(76%)	22/ 62(35%)
海外	Davisら[5]	18/ 73(25%)	66/100(66%)	84/173(49%)
	Saraccoら[8]	64/427(15%)*	43/167(26%)	107/594(18%)
	Di Bisceglieら[9]	16/ 93(17%)*	14/ 23(61%)	38/124(31%)
	Cammaら[10]	43/297(14%)	45/262(17%)	88/559(16%)

*genotype 4も含む

ウイルス量例に対するIFN再投与方法として，two-step interferon rebound therapyが考案された．これは，IFN初回投与終了後に，HCV-RNAが再上昇した後，急低下した時点を狙って，IFNを再投与する方法である．詳細は本誌，加藤の稿を参照いただきたい．

2. IFN/リバビリン併用再治療の成績

リバビリンが使用できるようになって以降，IFN/リバビリン併用療法が再治療の主流となっている．主な初回IFN再燃または無効例に対するIFN/リバビリン併用再治療についての報告を表2にまとめた．当院において今までIFN/リバビリン併用療法を行った全55例のうち，再投与症例は43例であった．著効例は16例(37%)であった．更にgenotype 1bの高ウイルス量例30例に限ると著効例は5例(17%)であった．国立病院・療養所肝疾患ネットワークにおいて，初回IFN治療後にIFN/リバビリン併用療法を再治療として行った症例は143例であった[3]．このうち，著効例は46例(32%)であった．更にgenotype 1bの高ウイルス量例108例に限ると著効例は22例(20%)であった．海外では，投与量や投与期間に差があるため，著効率にもばらつきがあるが，同様の報告がみられる[5,8-10]．Saraccoら[8]によると，IFN 3MU+ribavirin 1,000mg/dの6カ月投与群とIFN 5MU+ribavirin 1,000mg/dの12カ月投与群では著効率が11%と23%で有意差を認めており，IFN投与量を多く，ribavirin投与期間を長くすることで再投与治療の著効率を高めることが示された．

更に，IFN単独群との比較も行われている．1998年から2000年にかけて，国内11施設で行われた多施設二重盲検試験[11]において，IFN初回再燃または無効例に対するIFN再投与の著効率はIFN単独群では9%であったが，IFN/リバビリン併用群では35%であった．genotype 1bの高ウイルス量例に限ると，著効率はIFN単独群では0%であったが，IFN/リバビリン併用群

では15%であった. Davisら[5]は345例の初回IFN再燃例を対象として, IFNα-2b 3MU週3回24週投与単独群とリバビリン併用群に分けて比較検討を行った. 著効率は単独群では5%であったが, 併用群では49%であった. 更にgenotype 1bの高ウイルス量例143例に限った検討でも, 著効率は単独群では0%, 併用群では25%であった.

また, 再治療後の再燃率(治療終了時点でHCV-RNAが陰性化した症例のうち, 治療後24週以内に再陽性化した症例の割合)についても幾つか報告がみられる. 我が国における再投与症例に対するIFN単独療法とIFN/リバビリン併用療法の24週投与での比較試験において, genotype 1bの高ウイルス量例では, IFN単独群では再燃率は95%, IFN/リバビリン併用群では77%であった. それ以外の症例では再燃率は, IFN単独群では68%に対して, IFN/リバビリン併用群では15%で非常に低かった. 以上の報告から, IFN再投与にあたっては, リバビリンを併用することで, その有効性は大幅に改善すると考えられた.

3. その他のIFN再治療の成績

2001年より我が国でも使用されているコンセンサスIFNもIFN再治療に用いられる. 初回IFN再燃, 無効例に対して24週または48週コンセンサスIFNを投与したところ, 著効率は20%であった[12]. 更にIFN/リバビリン併用療法が無効であった症例に対するコンセンサスIFN投与の報告がある. BarbaroらはIFN/リバビリン併用療法が無効であったgenotype 1bの高ウイルス量例24例に対し, 著効率は33%であった[13]. IFN/リバビリン併用療法が無効であった症例にコンセンサスIFN治療の有効性が示唆されている.

今後は, ポリエチレングリコールを付加し, 持続的に吸収されるようにすることで, 血中濃度を維持し, 週1回投与が可能となったPeg-IFNの使用が我が国でも可能となる. Peg-IFNとリバビリンを併用した場合のIFN再投与に関する著効率は無効例で34-40%, 再燃例で約60%といわれている[14]. 今後, 我が国でもIFN再治療の選択肢の一つとなることが予想される.

4. IFN再治療の適応

初回IFN治療無効, 再燃例に対しては, 原則的に全例, IFN再治療の適応があると考えられる. 再治療の方法には, 現在のところ, 大きく分けてIFN単独とIFN/リバビリン併用療法の2つがある. IFN単独再治療では, 初回再燃例で, genotype 1b以外, かつ低ウイルス量であれば, 著効例も認める.

熊田らがまとめた2003年度版のC型慢性肝炎の治療ガイドラインによれば, genotypeとウイルス量によって, その標準的治療を決めている. すなわち, 再治療でも治癒の難しいgenotype 1bで高ウイルス量例では, IFNα-2bとリバビリンの併用療法24週投与か, あるいは最大2年までのIFN長期投与となっている. 一方, 難治例以外の全例(genotype 2a, 2b全例と1bの低ウイルス量例)では, 以下の3つの方法から選択する. 一つはIFNα-2bとリバビリンの併用療法24週投与, 二つめはコンセンサスIFNの24週投与, または従来のIFN単独の24-48週投与である. 原則として, 1回目と2回目の治療で同じ方法を行うのは望ましくないとしている.

一方, 平松ら[15]は初回, IFN単独治療でウイルス学的著効が得られなかった症例では全例IFN/リバビリンの併用治療をすべきで, かつ48週の長期投与が望ましいとしている. しかし, 現在の保険診療ではIFN/リバビリンの併用は24週しか認められておらず, 今後の課題と思われる. また, リバビリンは溶血性貧血や脳出血など, IFN単独治療ではみられなかった副作用も認めることから, 症例ごとの慎重な検討が必要と思われる.

以上, まとめると, 現在可能なIFN再治療としては, ①IFN単独長期(1年以上)投与, ②コンセンサスIFN投与, ③IFN/リバビリン併用療法の24週投与, ④IFN/リバビリン併用療法の24週投与後IFN単独長期投与の4つが考えられる. しかし, 初回IFN治療の効果(再燃,

無効)などを考えて，適当な治療法を選択するべきである．実際には，(1)初回IFN治療再燃例でgenotype 2bかつ低ウイルス量例，および(2)リバビリンの副作用が問題となる症例，以外はIFN/リバビリン併用治療を選択するのがよいと考える．

おわりに

我が国におけるC型慢性肝炎患者のIFN治療後の再治療としては，現時点ではIFNとリバビリンの併用療法が最も著効率が高い．しかし，リバビリンは保険上，24週しか投与が認められておらず，長期投与への適応が今後の課題である．一方，IFN単独でも再治療によって著効を得られる症例もある．2003年12月からはPeg-IFNの単独使用が保険適応となり，将来的には，Peg-IFNとリバビリンの併用療法が我が国でも行われるようになると考えられる．IFN再治療の著効率を上げるためには，個々の症例にあわせた治療法の選択が重要となる．更に今後は，IFN/リバビリン併用療法でも著効を得られない症例に対して，どのような治療を行っていくかも課題である．

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C型慢性肝炎の治療

Two-step interferon rebound therapyとその適応

Two-step interferon rebound therapy

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Key words：難治性C型慢性肝炎，IFN治療，TIRT，リバウンド療法

はじめに

我が国のセログループ1，高ウイルスのいわゆる難治性C型慢性肝炎(難治群)に対するinterferon(IFN)治療におけるHCV-RNA持続消失(SVR)率は，IFN単独製剤治療の7%(当院)から，IFNα2b，リバビリン併用治療(24週)の20%(国内治験)まで上昇し，更にpegylated(PEG) IFN，リバビリン併用治療(48週)によって50%近くまでの向上が期待されている．しかし，それでも約半数の難治例はキャリアー状態からの脱却は困難で，更なる新たな治療法の展開が必要と考えられる．

本稿ではこれらの難治性C型慢性肝炎に対する治療手段として有用と考えられるtwo-step interferon rebound therapy(TIRT)について述べる．

1. Two-step interferon rebound therapy (TIRT)

TIRTは著者らによって発案された治療法[1]で，初回IFN(1st step IFN)投与終了後，HCV-RNA量の急増加の後のALTリバウンド時，HCV-RNA量が急激に低下した時点より再度IFN(2nd step IFN)を投与する治療法である．

これまでの検討よりIFN投与終了後にALTリバウンド(投与終了時より100以上上昇)がみられた例は約50%，HCV-RNA量が急増加後1.0Meq/mlまで低下した例(低下例)は，約35%であった．低下例はTR例およびALTリバウンド出現例に有意に高率であり，投与前のHCV-RNA量が低値であるほど[2]，また投与終了時にHCV-RNAの陰性化が認められた例ほど[3]低下例の割合が高くなる傾向が認められた．また，低下例における投与終了時よりHCV-RNA量が1.0Meq/ml未満まで低下するまでの期間と1.0Meq/ml未満が持続する期間は，それぞれ2-5カ月(平均3.5カ月)，1-4カ月(平均1.8カ月)であり，投与終了後6カ月目にはほとんどの症例でHCV-RNA量は高値に復することが判明している[4]．

難治群のSVR率を向上させるためには投与前のHCV-RNA量を低下させること，免疫を賦活させること，IFN総投与量を増加させることなどがあげられるが，TIRTは前2者の機序により高いSVR率を獲得できるものと考察している．HCV-RNA量の自然経過での急激な低下は極めてまれ[5]で，現在のところTIRTの形でのみHCV-RNA量を低下させることが可能と考えられる．

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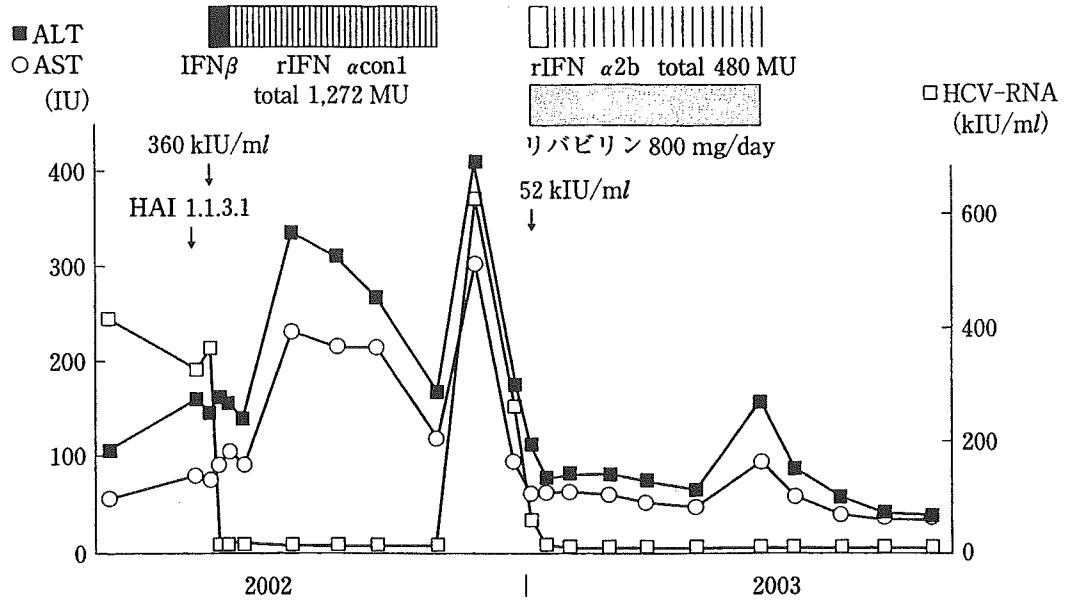

図1 2nd stepにIFNα2b，リバビリン併用治療を用いたTIRTによってSVRとなった34歳男性，セログループ1

2. 2nd stepにIFNα2b，リバビリン併用治療を用いたTIRT

現在，全国的にIFNα2b，リバビリン併用治療が行われているが，各施設における難治群に対するSVR率は国内治験と同様の20％前後という報告が多い．

当院では1st stepをIFNβ(1日2回投与)，コンセンサスIFN併用とし，このIFNα2b，リバビリン併用治療を2nd stepに用いるトライアルを行っている．TIRTによるSVR率は，1st stepのSVR率，1st step終了後のHCV-RNA急増加後1.0Meq/ml未満まで低下する割合(RNA低下率)および2nd stepのSVR率によって規定され，1st stepのSVR率を'a'，RNA低下率を'b'，2nd step投与前1.0Meq/ml未満群に対する2nd step治療によるSVR率を'c'，2nd step投与前1.0Meq/ml以上群に対する2nd step治療によるSVR率を'd'とすると，

$$a+(100-a)\times b/100\times c/100+(100-a)\times(100-b)/100\times d/100\,(\%)$$

と表される．'a'および'b'は投与前HCV-RNA量と1st stepに使用する製剤によって規定されるが，'c'は現在行われている治療法でも約80％のSVR率があり，'d'をリバビリン，IFN併用再投与例の1.0Meq/ml以上群に対する国内治験でのSVR率とすると，a，b，c，dはそれぞれ10-20％，30-60％，70-80％および15-20％となり，難治群に対するSVR率は，このTIRTプロトコールで38.4-64.8％と極めて高率になることが予測される．現在までの途中経過では1st step後のRNA低下率は43.5％，投与完了例でのSVR率が44.4％と治療効果の向上が認められている．

1例を呈示する．34歳男性，セログループ1，2002年5月13日より1st stepを開始，2カ月後にHCV-RNA(−)となり終了時まで持続したが，終了後1カ月目にHCV-RNA 630kIU/mlに上昇，ALTも416IUとリバウンドを認めた．その1.5カ月後にHCV-RNA 52kIU/mlまで低下したためIFNα2b，リバビリン併用の2nd stepを施行しSVRが得られた(図1)．

3. PEG-IFN治療再燃例に対するTIRT

2003年12月よりPEG-IFNα2aが保険適用となった．国内治験の成績では難治群に対するSVR率は16％とそれほど高くはないが，週1回投与という利便性のメリットは大きく，当面は需要が大きいことが予測される．このPEG-

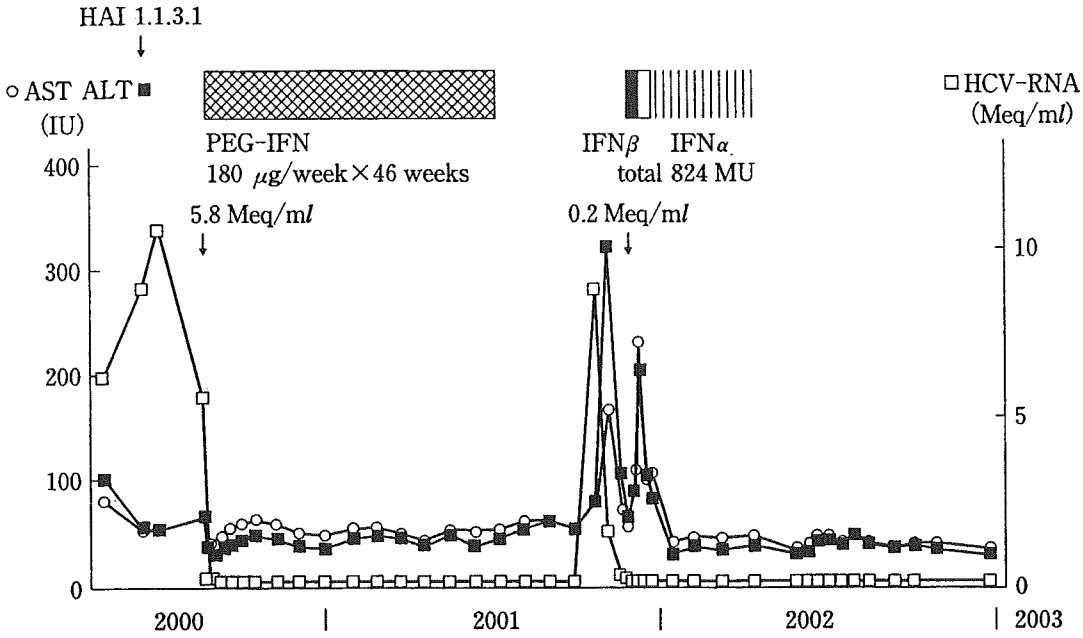

図2 PEG-IFN治療再燃例に対するTIRTによってSVRとなった53歳女性，セログループ1

IFNα2a単独投与の難治群に対するSVR率を向上させる目的にもTIRT療法は応用可能であり，再燃例に対するPEG-IFNα2a単独あるいは現在治験中のPEG-IFN，リバビリン併用治療を2nd stepに用いることによってSVR率を向上させることができると考えられる．

PEG-IFNα2a単独治療再燃例に対してTIRTが奏効した1例を呈示する．53歳女性，セログループ1，2000年8月，HCV-RNA 5.8 Meq/mlの時点よりPEG-IFNα2a 180 μg/週46週投与を行ったが，TRに終わった．投与終了後2カ月目まではHCV-RNA陰性であったが，3カ月目にHCV-RNA陽性となり，その1カ月後にHCV-RNAは9.2 Meq/mlに上昇，その2週後にALTが315 IUにリバウンドした．その3週間後0.2 Meq/ml未満の状態より2nd stepを開始し，従来のIFN製剤の単独治療24週投与でSVRとなった．この症例はリバビリン併用例にみられるのと同様に，1st step終了後長期間のHCV-RNA陰性化の後，再陽性化が認められた(図2)．

4. リバウンド時におけるサイトカインの動態

IFN投与終了後の血中サイトカインの動態を検討すると，Th1系のIFNγは投与終了時に比し終了1カ月後有意($p<0.01$)に増加し，その後もとのレベルに復することがわかった．一方，Th2系のサイトカインであるIL-10についてみると，IFN投与終了後いったん減少した後，ALTのリバウンド上昇時に有意($p<0.02$)に増加した．両者の検討よりTh1/Th2はIFN投与終了後いったんTh1側にシフトし，ALTのリバウンド上昇時にTh2優位の状態になると考えられる(図3)．貝沼ら[6]もリバウンド時にCD19+リンパ球数の著明な増加を認め，液性免疫の賦活化が示唆されるとしている．また，武井ら[7]はIFNα投与終了後3カ月目のIFNγとIL-4を測定し，終了時と比較してIFNγは有意に増加し，IL-4は無効例で有意に低下したと報告している．投与終了後3カ月目はALTのリバウンド後HCV-RNA量が減少する時期にあたり，Th1系へのシフトが2nd stepの有効性の向上に関与しているとも考えられる成績である．

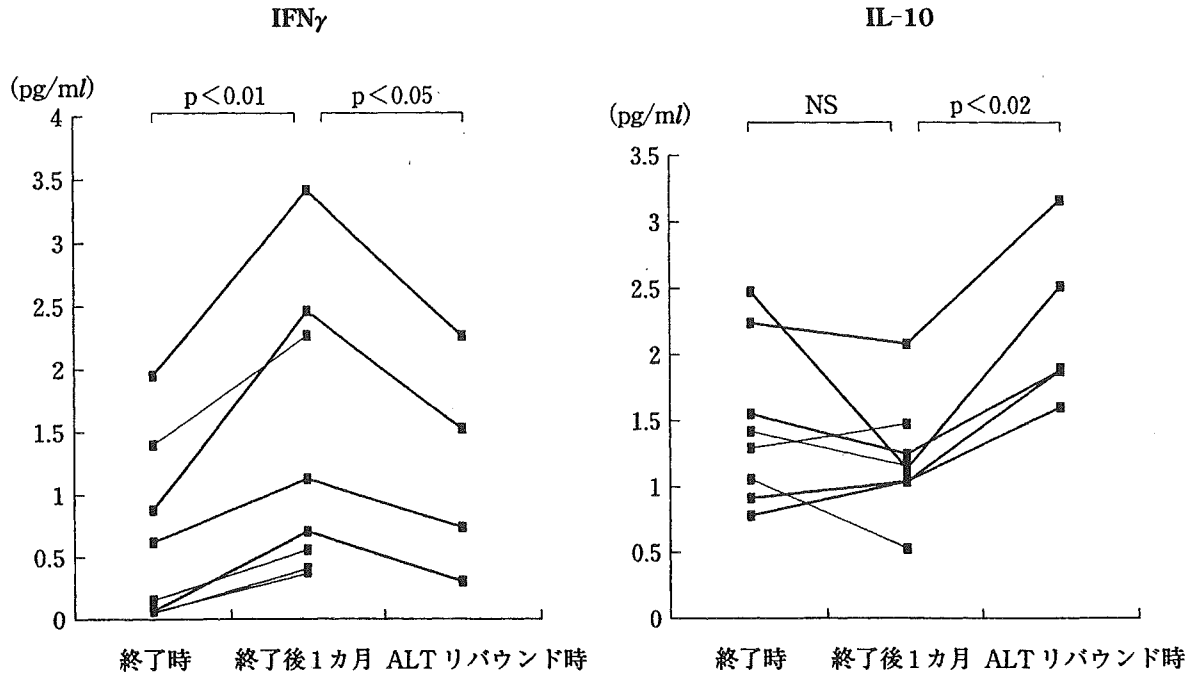

図3 IFN投与終了後の血中Th1およびTh2サイトカインの変動

5. TIRT治療効果向上のためのポイント

a. 1st stepに用いる製剤の選択

1st stepに用いる製剤の選択に関しては二とおりの考え方がある．一つはその時期に保険診療で使用できる最もSVR率が高い治療法を用いる方法，他の一つは副作用が軽度で6カ月間確実に治療が持続できる製剤を用いる方法である．前者の利点は1st stepで高いSVR率を得ることができる点であるが，脱落例や2nd step導入が困難な例も多くなることが予想され，また，1st step再燃例に2nd stepとして1st stepと同じ治療が保険適用になるかという問題もある．一方，後者では1st stepでのSVR率は低いが，再燃後のALTリバウンド発現例ではリバウンド後のHCV-RNA量低下時より，またALTリバウンドが発現しない症例でも2nd stepとして最もSVR率が高い治療法を用いることができるため，トータルでみると後者の方がSVR率が高くなるのではないかと推察している．1st stepでの脱落例と2nd step導入が困難な例をできるだけ少なくすることがTIRT治療効果を向上させるうえで大きなポイントになると考える．

b. 1st step終了後のHCV-RNA量の経時的測定

1st step終了後のHCV-RNA量の経時的測定は通常1カ月1回の間隔でよいが，終了後2カ月を過ぎALT上昇，HCV-RNA量の低下傾向がみられる時期になると，できるだけ最下降点より2nd stepを開始するためにHCV-RNA量の2週や週1回の測定が必要な場合がある．しかし，このような頻回のHCV-RNA定量(PCR法，プローブ法)は保険適用とならないし，結果が判明するまでに時間がかかりすぎる．TIRTにおける最も重要で，かつ最も煩雑な作業はこの1st step終了後のHCV-RNA量のfollow upであるが，HCV-RNAと相関の高いHCVコア抗原検査の開発により頻回のチェックが可能となった．HCVコア抗原は保険点数が低く，測定時間も2時間以内と極めて短時間であるため，外来にてのリアルタイムのfollow upが可能となり，HCV-RNA量の低値を確認後速やかに2nd stepに入ることができると考えられる．

おわりに

PEG-IFN，リバビリン併用治療の難治群に対するSVR率が極めて高率になることが予測されている．しかし，それでもウイルスの消失しない例も多く，それらの'超難治群'に対する対策としてTIRTは重要な手段である．PEG-IFN，リバビリン併用治療を1st stepに用い，再燃例で低ウイルスとなった例にIFN再投与を行うか，侵襲の軽度なIFN製剤を1st stepに用い，HCV-RNA量を低下させた後にPEG-IFN，リバビリン併用治療を2nd stepとして用いることが考えられる．いずれの場合もIFN治療後は常にTIRTを念頭に置いた，慎重な経過観察が必要である．

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Significance of liver negative-strand HCV RNA quantitation in chronic hepatitis C

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***Background/Aims*: Liver negative-strand hepatitis C virus (HCV) RNA is the most direct indicator of active viral replication but has only been examined in a few semiquantitative studies.**

***Methods*: Positive- and negative-strand HCV RNA in the right (R) and left (L) liver lobes was quantified by rTth-based strand-specific real-time polymerase chain reaction for 48 chronic hepatitis C patients.**

***Results*: Close correlations between lobes were seen for positive- and negative-strand amounts ($r=0.950$; $P<0.001$ and $r=0.920$; $P<0.001$, respectively). The ratio of negative to positive strands (median, 0.14 for R and 0.13 for L) varied by 2 log directly in relation to HCV replication assessed by liver negative strands but had no relation to liver positive strands and circulating HCV. Only negative-strand quantitation was inversely correlated with age ($r=-0.322$; $P=0.026$ for R and $r=-0.340$; $P=0.018$ for L), while liver tissues with hepatitis B virus DNA contained larger amounts of each strand. In 27 patients treated with enhanced interferon monotherapy, the amounts of liver negative strands (<4 log copies/100 ng RNA) were the only independent predictor of a sustained virologic response.**

***Conclusions*: Negative-strand quantitation is uniform in the liver and bears distinct relevance to the disease.**

***Keywords*: Negative-strand HCV RNA; HCV replication; Chronic hepatitis C**

1. Introduction

Hepatitis C virus (HCV) replication, like that of other single-strand, positive-sense RNA viruses, is presumably preceded by the synthesis of negative-strand RNA. Thus, the amounts of negative-strand RNA-replicative intermediates in liver tissues should serve as a more reliable marker of active viral replication than positive-strand HCV RNA in the liver or in circulation. Serum HCV loads are affected by replication within the liver and extrahepatic sites and by immunologic clearance of the virus. The detection of liver positive-strand (genomic) HCV RNA can simply imply contamination by such circulating virions. Thus far, only a few semiquantitative studies have been done on the clinical relevance of liver negative-strand HCV [1–4], and controversy remains. Patients with chronic hepatitis C can show uneven distribution of liver injury, but intrahepatic variation of HCV replication also remains to be clarified. To further address these issues, we quantitatively analyzed positive- and negative-strand HCV RNA in each liver lobe by strand-specific real-time polymerase chain reaction (PCR) using rTth.

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2. Patients and methods

2.1. Patients

Forty-eight patients with chronic hepatitis C underwent laparoscopic liver biopsies. All patients were positive for serum HCV RNA (Amplicor HCV Test, Roche Diagnostics K.K., Tokyo, Japan). No confounding etiology of liver disease was found in any patient. They were negative for hepatitis B surface antigen in the serum. The group was comprised of 25 men and 23 women ranging in age from 33 to 70 years (median, 57 years). Sixteen (33%) patients had a history of blood transfusion 8–52 years (median, 36 years) earlier. Biopsies were performed using 13-gauge Tru-Cut needles (Hakko Medical Co., Ltd, Nagano, Japan), and liver tissues sufficient for histologic and virologic evaluation were obtained from the anterior segment of the right lobe and the lateral segment of the left lobe. Specimens 15 mm long and 2 mm wide were embedded in paraffin for histopathological study. The remaining portions were immediately frozen and then stored at −80 °C until PCR testing. With one patient, the specimen from the left lobe was subjected to only virologic evaluation due to its limited size. Paired serum samples were obtained from all patients at laparoscopy and stored at −80 °C without thawing until virologic tests. Of the 48 patients, 27 (Table 1) were treated with enhanced interferon (IFN) monotherapy. After laparoscopy, 3 MU of IFN-β (Feron, Toray Co., Tokyo, Japan) was administered twice a day for 2 weeks followed by 9 MU of IFN-α (Sumiferon, Sumitomo Pharm. Co., Osaka, Japan) daily for 2 weeks and thrice weekly for 20 weeks. The study was approved by the local research ethics committee in accordance with the 1975 Declaration of Helsinki, and all patients provided written informed consent.

2.2. Virologic testing

Circulating HCV genomic RNA was quantified by a PCR assay (Amplicor HCV Monitor Test version 2.0, Roche Diagnostics K.K.). HCV RNA of ≧6.4 log copies/mL was measured after serum dilution. HCV genotypes were determined by a PCR genotyping system [5].

2.3. Positive- and negative-strand HCV RNA quantitation by rTth-based strand-specific real-time reverse-transcription polymerase chain reaction (RT-PCR)

Strand-specific TaqMan RT-PCR was designed to quantify the 5′ untranslated region of the HCV genome using a thermostable enzyme, rTth (Applied Biosystems, Foster City, CA). Total hepatic RNA, 100 ng, was added to an RT reaction mixture containing 2 μL of 10× RT buffer (Applied Biosystems), 20 nmol of $MnCl_2$, 5 U of rTth, 24 U of RNasin (Promega, Madison, WI), 4 nmol of each dNTP, and 10 pmol of sense primer HCV-20F (5′-CGACACTCCACCATGAATCACT-3′) for the negative-strand assay or antisense primer HCV-114R (5′-GAGGCTG-CACGACACTCATACT-3′) for the positive-strand assay. The RT reaction was performed in a final volume of 20 μL at 70 °C for 60 min. The reaction

Table 1
Baseline patient characteristics before IFN therapy

		n
Age	<50	11 (41%)
	≥50	16 (59%)
Sex	Male	16 (59%)
	Female	11 (41%)
Transfusion history	+	9 (33%)
	−	18 (67%)
ALT	<2×ULN	17 (63%)
	≥2×ULN	10 (37%)
Liver histology		
Grading score[a]	<7	17 (63%)
	≥7	10 (37%)
Staging score[a]	<4	19 (70%)
	≥4	8 (30%)
Between-lobe grade discrepancy	+	10 (38%)
	−	16 (62%)
Between-lobe stage discrepancy	+	9 (35%)
	−	17 (65%)
HCV genotype	1	21 (78%)
	2	6 (22%)
Serum HCV RNA	<5.4 log copy/mL	6 (22%)
	≥5.4 log copy/mL	21 (78%)
+Strand[a]	<5 log copy/100 ng RNA	6 (22%)
	≥5 log copy/100 ng RNA	21 (78%)
−Strand[a]	<4 log copy/100 ng RNA	9 (33%)
	≥4 log copy/100 ng RNA	18 (67%)
−/+ Strand ratio[a]	<0.1	14 (52%)
	≥0.1	13 (48%)
Between-lobe+strand discrepancy	+	4 (15%)
	−	23 (85%)
HBV antibody (anti-HBc and/or anti-HBs)	+	7 (26%)
	−	20 (74%)
Liver HBV DNA	+	2 (7%)
	−	25 (93%)

[a] Mean values of right and left liver lobes.

was then treated with 5 μL of 10× chelating buffer (Applied Biosystems), 75 nmol of $MgCl_2$, 10 nmol of each dNTP, 10 pmol of antisense primer HCV-114R for the negative-strand assay or sense primer HCV-20F for the positive-strand assay, and 5 pmol of TaqMan probe HCV-P43 (5′FAM-CCCTGTGAGGAACTACTGTCTTCAC-GCAGATAMRA3′). The final volume was adjusted to 50 μL. The samples were promptly set in an ABI PRISM 7700 Sequence Detection System (Applied Biosystems) and incubated at 70 °C for 2 min and then at 94 °C for 2 min. Real-time PCR amplification and data analysis were subsequently performed for 45 cycles (94 °C for 20 s and 62 °C for 1 min). Copy numbers of the 95-base target sequence were determined using the standard curve based on measurements of serial 10-fold dilutions of synthetic positive- and negative-strand HCV RNA. The sensitivity was 2 log copies/reaction for the positive-strand assay and 3 log copies/reaction for the negative-strand assay. The dynamic ranges were 2–7 log copies/reaction and 3–7 log copies/reaction, respectively. In each assay, false detection of an incorrect strand occurred when the amount of incorrect strand added reached 7 log copies. The positive- and negative-strand quantitation before normalization was ≦6.4 and ≦5.9 log copies/100 ng liver RNA, respectively, in this study. Thus, the strand-specificity was unlikely to be affected by an excess of incorrect strands. Self-priming or endogenous priming was ruled out by the lack of amplification product following RT-PCR of total hepatic RNA without primers in the RT mixture. All assays were done in duplicate, and the mean values were obtained. Hepatic RNA samples from the same liver were always measured in the same run.

The HCV-specific primers and probe used are conserved among genotypes. To verify that HCV genotypes 1b, 2a and 2b could be quantified with similar efficiency, high-concentration serum samples of each genotype were obtained from eight patients and diluted to 4.4 log copies/reaction by Amplicor HCV Monitor version 2.0, which is known to equally amplify all genotypes. The positive-strand HCV quantitation by the TaqMan RT-PCR was the same for genotypes 1b (5.3±0.7), 2a (5.5±0.4) and 2b (4.9±0.5 log copies/reaction) ($P=0.141$ by one-way analysis of variance).

2.4. Normalization of hepatic HCV RNA amounts and criteria for between-lobe discrepancies

GAPDH mRNA in total hepatic RNA, 100 ng, and control total RNA (Raji cell line), 100 ng, was also quantified by real-time RT-PCR, and copy numbers were determined using the standard curve (Human GAPD Endogenous Control, Applied Biosystems). Hepatic HCV RNA and GAPDH mRNA quantitation, which were performed in separate tubes, showed a linear relationship with the amounts of target RNA (Fig. 1). The HCV RNA copy number was divided by the ratio of the sample GAPDH mRNA amounts to the TaqMan control value. Thus, normalized hepatic HCV RNA amounts were obtained and used for data analysis. In preliminary experiments, assay variance for the $\log_{10}$ transformed HCV RNA quantitation before normalization was evaluated based on five measurements of 10 liver samples (intra-assay coefficients of variation (CVs)=0.88–2.85% and inter-assay CVs=1.19–6.91% for the positive-strand assay; intra-assay CVs=2.27–9.72% and inter-assay CVs=1.52–18.11% for the negative-strand assay). Assay variance was greater for the negative-strand assay, which may be attributable to interfering factor(s) such as a large amount of positive strands in the RT reaction. The mean SDs of intra-assay variance were 0.106 and 0.081 for <5 and ≧5 log copies, respectively, in the positive-strand assay, whereas they were 0.374, 0.256 and 0.158 for <4, 4–5 and ≧5 log copies, respectively, in the negative-strand assay. The HCV RNA quantitation was assumed to vary within twice these SDs. Between-lobe HCV RNA differences were considered significant when the normalized HCV RNA amounts differed by more than the estimated variance for normalized values. All discrepancies were confirmed by repeating the assays.

2.5. Detection of liver hepatitis B virus (HBV) DNA by nested PCR

Total hepatic DNA, 100 ng, was subjected to nested PCR to amplify HBV DNA. The primers were set in the surface region (outer sense 5′-TCGTGTTACAGGCGGGGTTT-3′; outer antisense 5′-CGAACCACT-GAACAAATGGC-3′; inner sense 5′-CAAGGTATGTTGCCCGTTTG-3′; inner antisense 5′-GGCACTAGTAAACTGAGCCA-3′) and the X region (outer sense 5′-GCATGGAGACCACCGTGAA-3′; outer antisense 5′-CAGACCAATTTATGCCTACAG-3′; inner sense 5′-TACATAAGAG-GACTCTTGGACT-3′; inner antisense 5′-CAGACCAATTTATGCCTA-CAG-3′). PCR products (233 and 151 bp, respectively) were visualized by 3% agarose electrophoresis and ethidium bromide staining. All assays were done in duplicate. The sensitivity was 1 copy/100 ng liver DNA for each primer set. To avoid contamination in all PCR assays, the contamination avoidance measures [6] were strictly applied throughout the study, and positive and negative controls were used.

2.6. Histologic evaluation

After routine staining with hematoxylin–eosin, all liver biopsy specimens were examined by the same experienced pathologist without knowledge of their source. Biopsy specimens were semiquantitatively

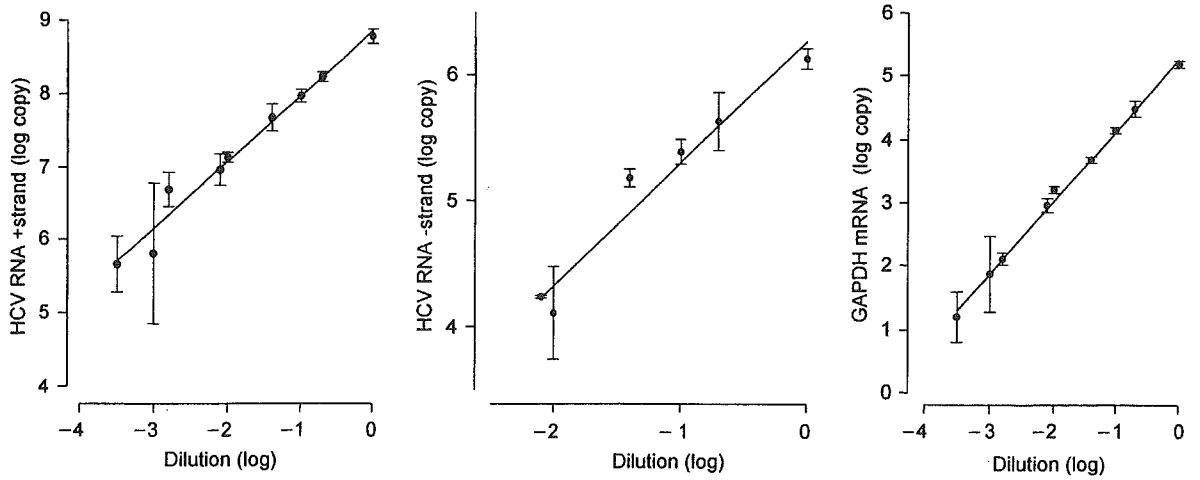

Fig. 1. Changes in hepatic HCV RNA and GAPDH mRNA quantitation in separate tubes according to the amounts of target RNA. Serial dilutions of total hepatic RNA, 100 ng, were subjected to real-time RT-PCR, and copy numbers were determined using the standard curve. The copy number (log) showed a linear relationship ($P<0.001$) with the amounts of target RNA expressed as dilution (log) ($y=8.86+0.90x$ [$n=9$, $r=0.985$] for positive-strand HCV RNA, $y=6.27+0.96x$ [$n=6$, $r=0.976$] for negative-strand HCV RNA and $y=5.27+1.13x$ [$n=9$, $r=0.998$] for GAPDH mRNA). Data are the mean±2SD of triplicate measurements. Pearson's correlation test was performed to examine the relationship.

evaluated using the modified histologic activity index described by Ishak et al. [7].

2.7. Statistical analysis

Viral load was $\log_{10}$ transformed to obtain a more symmetrical distribution without outliers. An arbitrary value of 0 log copy/100 ng liver RNA was attributed to the liver tissues negative by PCR. Data on continuous variables were presented as mean ± SD unless otherwise stated. Statistical analysis for group comparisons was performed using the Wilcoxon nonparametric test. Correlations between the variables were calculated using Spearman rank order correlations. To assess variables potentially related to virologic and histologic between-lobe discrepancies and responses to IFN, stepwise multivariate logistic regression models were used. All analyses were done with SAS (version 8.02) (SAS Institute, Inc., Cary, NC). A P value of less than 0.05 (2-tailed) was considered to indicate significance.

3. Results

3.1. Amounts of positive- and negative-strand HCV RNA in right (R) and left (L) liver lobes

Normalized positive-strand HCV loads in the right liver lobe (median, 5.9; range, 2.5–8.5 log copies/100 ng liver RNA) showed a correlation with those in the left liver lobe (median, 6.0; range, negative to 6.8 log copies/100 ng liver RNA) ($r=0.950$; $P<0.001$) (Fig. 2A). Six (13%) of the 48 patients had a between-lobe discrepancy of 0.3–2.2 log. The discrepancy was related to gender (6 [26%] of 23 women vs. none of 25 men) (odds ratio 10.9 [95% CI 1.3–90.9], $P=0.027$). Fig. 2B shows a correlation between normalized negative-strand HCV loads in the right lobe (median, 4.9; range, negative to 7.2 log copies/100 ng liver RNA) and the left lobe (median, 5.0; range, negative to 6.3 log copies/100 ng liver RNA) ($r=0.920$; $P<0.001$). A discrepancy of 2.0 log was seen in one (2%) patient (Table 2).

In 38 patients with detectable levels of positive and negative strands in each liver lobe, the ratio of negative- to positive-strand HCV (median, 0.14; range, 0.01–0.81 for R and median, 0.13; range, 0.03–0.45 for L) increased according to negative-strand liver HCV ($r=0.282$; $P=0.086$ for R and $r=0.441$; $P=0.006$ for L) (Fig. 3). The ratio showed no correlation with positive-strand liver HCV ($r=-0.192$; $P=0.248$ for R and $r=-0.097$; $P=0.564$ for L) and circulating HCV ($r=0.154$; $P=0.355$ for R and $r=0.106$; $P=0.527$ for L). Serum HCV RNA loads ranged between 3.1 and 7.6 log copies/mL (median, 6.1), and displayed a relation to the positive-strand liver HCV quantitation ($r=0.604$; $P<0.001$ for R and $r=0.634$; $P<0.001$ for L) and the negative-strand liver HCV quantitation ($r=0.632$; $P<0.001$ for R and $r=0.609$, $P<0.001$ for L).

3.2. Determinants of positive- and negative-strand HCV RNA amounts in the liver

The amounts of positive- and negative-strand HCV in each liver lobe were correlated with patient characteristics including age, gender, mode of infection, duration of infection estimated from years after blood transfusion, serum alanine aminotransferase (ALT) levels, histologic grade and stage, HCV genotypes and detection of HBV DNA in the corresponding liver lobe. An inverse correlation was found between the negative-strand liver HCV

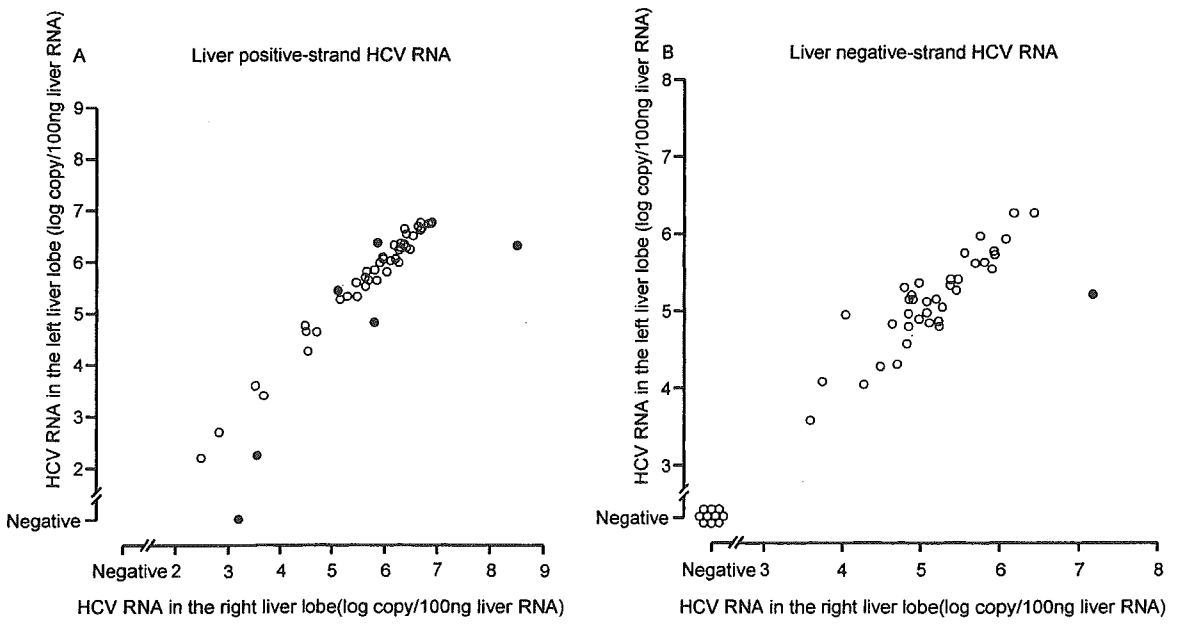

Fig. 2. Correlations between positive-strand HCV RNA levels of the right and left liver lobes ($r=0.950$; $P<0.001$) (A) and between negative-strand HCV RNA levels of each liver lobe ($r=0.920$; $P<0.001$) (B) in the 48 chronic hepatitis C patients. Between-lobe discrepancy of the viral loads was found in six (13%) patients for positive-strand HCV RNA and one (2%) patient for negative-strand HCV RNA (closed circles).

Table 2
Normalized positive- and negative-strand HCV RNA amounts in the right and left liver lobes in 48 chronic hepatitis C patients

Patient	Age (years)	Positive-strand HCV RNA[a]		Negative-strand HCV RNA[a]		HBV DNA[b]	
		Right	Left	Right	Left	Right	Left
1	62	5.96	6.08	5.23	4.80	–	–
2	47	6.88	6.73	6.18	6.26	–	–
3	66	4.48	4.74	3.59	3.58	–	–
4	52	6.68	6.60	5.90	5.53	–	–
5	68	6.38	6.62	5.28	5.04	–	–
6	49	6.04	5.79	4.98	4.89	–	–
7	64	6.11	6.00	4.99	5.36	–	–
8	49	6.54	6.48	5.94	5.72	–	–
9	54	8.51	6.30[c]	7.18	5.20[c]	–	–
10	46	6.30	6.26	5.23	4.86	–	–
11	67	4.49	4.63	4.28	4.04	–	–
12	57	5.80	5.83	5.08	4.97	–	–
13	53	4.53	4.25	–	–	–	–
14	48	5.69	5.62	4.83	4.57	–	–
15	48	5.97	6.04	4.91	5.15	–	–
16	57	5.48	5.30	4.71	4.30	–	–
17	33	6.89	6.76	6.08	5.92	–	+
18	57	5.65	5.79	4.64	4.83	–	–
19	69	3.68	3.38	–	–	–	–
20	64	6.41	6.26	5.11	4.84	–	–
21	59	6.20	6.04	5.38	5.40	–	–
22	56	5.86	6.36[c]	5.76	5.97	–	–
23	67	5.45	5.57	4.86	5.15	–	–
24	38	6.28	6.20	5.81	5.63	–	–
25	48	2.49	2.18	–	–	–	–
26	60	2.83	2.68	–	–	–	–
27	48	5.28	5.30	4.04	4.95	–	–
28	68	5.80	4.80[c]	–	–	–	–
29	44	5.62	5.68	4.85	4.96	–	–
30	43	3.20	–[c]	–	–	–	–
31	58	6.70	6.64	5.46	5.26	–	–
32	56	6.63	6.67	6.43	6.26	–	–
33	45	6.28	5.97	5.20	5.15	–	–
34	50	6.36	6.32	5.93	5.77	+	+
35	63	5.11	5.43[c]	4.49	4.28	–	–
36	70	4.71	4.62	–	–	–	–
37	52	6.83	6.72	4.80	5.30	–	–
38	41	3.56	2.23[c]	–	–	–	–
39	51	6.41	6.52	5.70	5.62	+	+
40	67	5.64	5.51	–	–	–	–
41	61	3.52	3.57	–	–	–	–
42	52	5.91	5.97	5.08	5.11	–	–
43	59	6.18	6.30	5.38	5.32	–	–
44	57	6.48	6.23	5.48	5.40	–	–
45	67	5.15	5.26	3.75	4.08	–	–
46	58	6.68	6.73	5.56	5.75	+	+
47	62	6.30	6.34	4.89	5.20	–	–
48	66	5.85	5.63	4.85	4.79	–	–

[a] Hepatic HCV RNA amounts were normalized to GAPDH mRNA amounts and expressed as log copy/100 ng liver RNA.
[b] Liver HBV DNA was detected by nested PCR using two sets of primers in the surface and X regions, respectively.
[c] Between-lobe differences in the normalized HCV RNA amounts were considered significant according to the intra-assay variance-based criteria.

quantitation and age ($r=-0.322$; $P=0.026$ for R and $r=-0.340$; $P=0.018$ for L). The positive-strand liver HCV quantitation, however, had no relation to age ($r=-0.237$; $P=0.104$ for R and $r=-0.216$; $P=0.140$ for L) (Fig. 4). The amounts of positive- and negative-strand liver HCV did not differ between 38 patients with HCV genotype 1b and 10 patients with genotype 2 (seven with genotype 2a and three with genotype 2b), but were affected by concomitant liver HBV. By using X primers, HBV DNA was detected in both liver lobes in three patients and only in the left lobe in another patient. None of the patients tested positive for liver HBV DNA using surface primers. The four

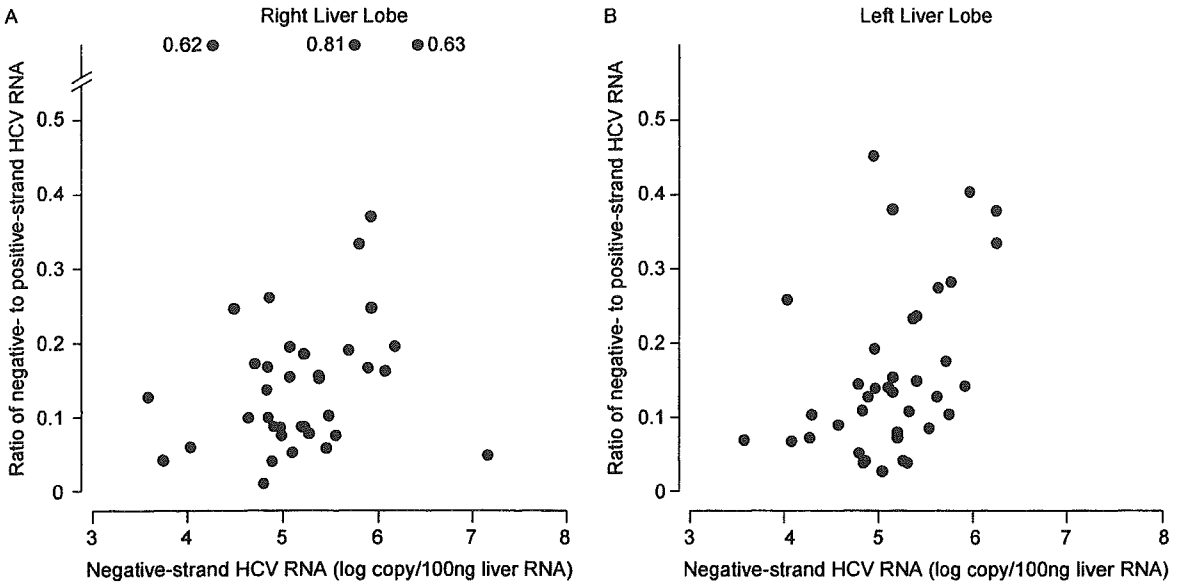

Fig. 3. Direct variation of ratios of negative- to positive-strand HCV RNA in liver tissues in relation to HCV replication, assessed by negative-strand HCV RNA in the right liver lobe ($r=0.282$; $P=0.086$) (A) and the left liver lobe ($r=0.441$; $P=0.006$) (B).

HBV DNA-positive liver tissue samples from the left lobe contained larger amounts of positive and negative strands than the 44 HBV DNA-negative tissues (6.6 ± 0.2 vs. 5.4 ± 1.4; $P=0.007$ and 5.8 ± 0.1 vs. 3.9 ± 2.2 log copies/100 ng liver RNA; $P=0.006$, respectively). For the right liver lobe, the positive- and negative-strand liver HCV quantitation also tended to be high in the three HBV DNA-positive liver tissues (6.5 ± 0.2 vs. 5.6 ± 1.2; $P=0.081$ and 5.7 ± 0.2 vs. 4.0 ± 2.3 log copies/100 ng liver RNA; $P=0.049$, respectively). None of the patient characteristics examined showed a relationship to the ratio of negative- to positive-strand HCV and serum HCV RNA load.

3.3. Histologic variation between right and left liver lobes

The total necroinflammatory grade ranged between 2 and 10 (median 7) in each liver lobe ($P=0.295$ by signed rank test). The fibrosis stage ranged from 1 to 6 (median 4) in the right lobe and from 2 to 6 (median 3) in the left lobe ($P=0.614$). Fig. 5 shows the histologic between-lobe variation among the 47 patients studied. Eleven (23%) patients showed differences of the necroinflammatory grade defined as a difference of $\geqq 2$ points, and 19 (40%) patients of the fibrosis stage defined as difference of $\geqq 1$ point. The between-lobe variation in the HCV quantitation had no impact on the histologic variation. The mean grading score of the right and left liver lobes was <7 in 10 (91%) out of the 11 patients with a grade difference compared with 16 (44%) out of the 36 patients without it (odds ratio 6.5 [95% CI 1.3–33.3], $P=0.025$). The difference in the fibrosis stage, however, had no relation to any of the patient characteristics examined.

3.4. Factors influencing the efficacy of IFN treatment

Eighteen (67%) out of the 27 patients were negative for serum HCV RNA at the end of treatment, and eight (30%) patients displayed sustained HCV clearance over 6 months posttreatment. The end-of-treatment virologic response was independently associated with an absence of between-lobe discrepancy of the necroinflammatory grade (odds ratio 0.2 [95% CI 0–0.9], $P=0.042$). However, the amounts of negative-strand HCV RNA in the liver were identified as the only independent predictor of a sustained virologic response. The mean negative-strand quantitation of the right and left liver lobes was <4 log copies/100 ng liver RNA in all sustained virologic responders (SVRs) compared with 1 (5%) of the 19 non-SVRs (odds ratio 85.4 [95% CI 5.4–999], $P=0.002$).

4. Discussion

Little has been known about the clinical significance of quantifying negative-strand RNA-replicative intermediates in the liver. The present study analyzed the ratio of liver negative- to positive-strand RNA. This ratio is the most reliable parameter since it does not depend on genotypes or normalization to the cellular GAPDH mRNA quantitation. For each liver lobe, the median ratio of 0.1 was similar to that found with cell-based HCV replicon systems [8]. Importantly, it was disclosed that the ratio was not constant but varied by 2 log values in relation to the intrahepatic HCV-replicative status. These observations suggest that the negative-strand quantitation is not merely a reflection of

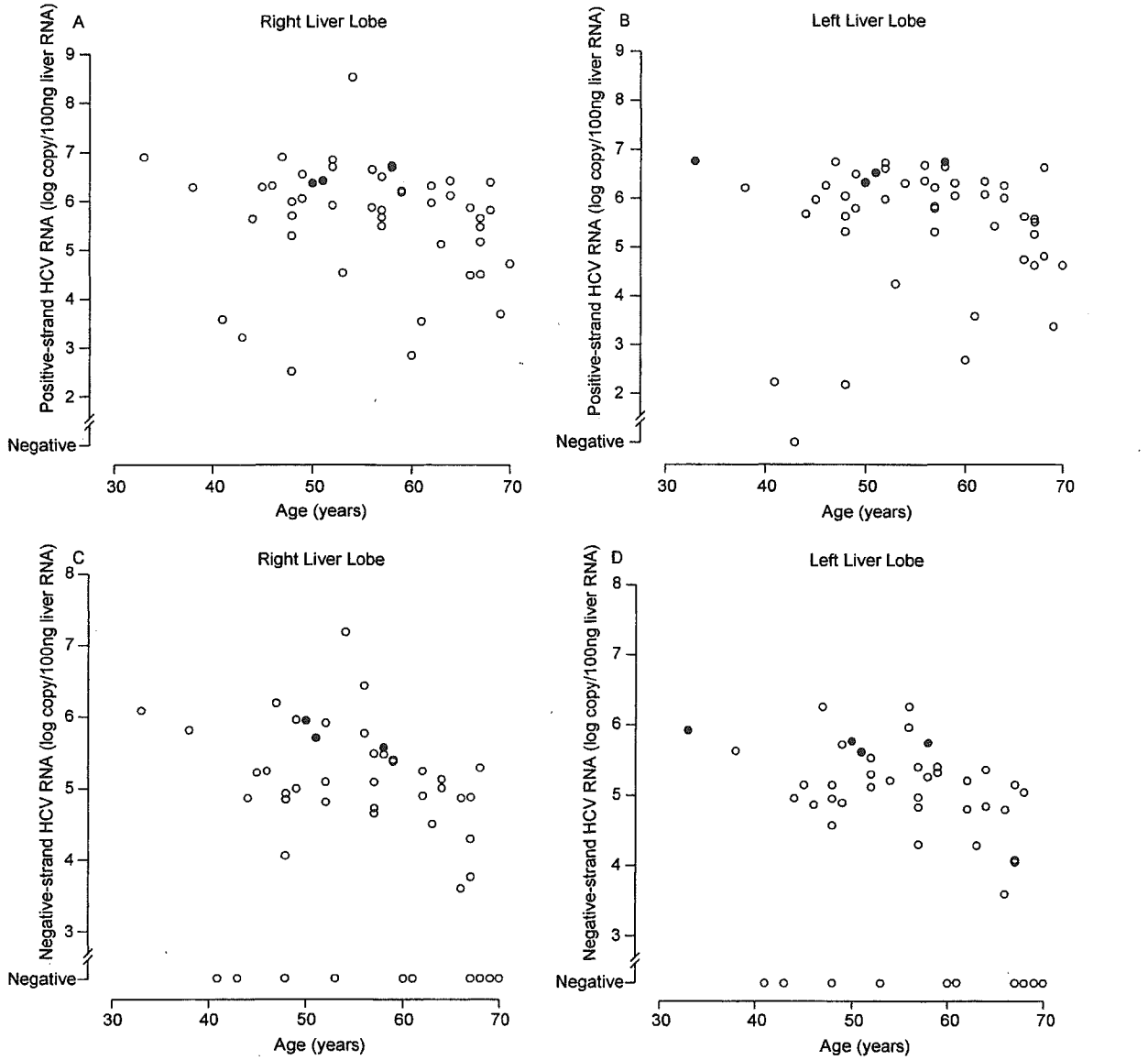

Fig. 4. Liver HCV RNA levels in relation to age and occult HBV infection in 48 chronic hepatitis C patients. No relationship was evident between age and positive-strand HCV RNA levels of the right liver lobe ($r=-0.237$; $P=0.104$) (A) and the left liver lobe ($r=-0.216$; $P=0.140$) (B), whereas inverse correlations were found between age and negative-strand HCV RNA levels of the right liver lobe ($r=-0.322$; $P=0.026$) (C) and the left liver lobe ($r=-0.340$; $P=0.018$) (D). HBV DNA-positive liver tissues (closed circles) contained higher levels of positive-strand HCV RNA ($P=0.081$ for the right liver lobe and $P=0.007$ for the left liver lobe) and negative-strand HCV RNA ($P=0.049$ for the right liver lobe and $P=0.006$ for the left liver lobe).

liver positive strands but should serve as a distinct HCV replicative marker.

Chronic hepatitis C is known as a disease with uneven distribution of lesions in the whole liver [9]. Previous studies have shown a correlation between positive-strand HCV RNA levels of the right and left liver lobes [9,10]. The present study demonstrated a close correlation between lobes not only for positive strands but also for negative strands. Thus, HCV replication within the liver was shown to be uniform, and a single biopsy seemed generally representative of the whole liver. Although the between-lobe variation of HCV RNA loads should be interpreted with caution when the difference is small, it was only found in women, raising a possibility that sex hormone(s) and sex-linked genetic factor(s) are involved in the heterogeneity of HCV replication. In the present study, the amounts of positive- and negative-strand HCV and the ratio of negative to positive strands showed no correlation with the necroinflammatory grade and the fibrosis stage. However, we must stress the possibility that the HCV replication level, especially that assessed by negative strands, may have some relevance to histologic features such as steatosis [4].

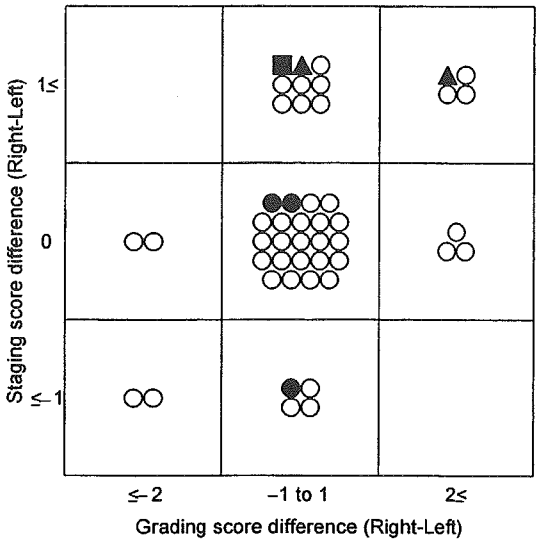

Fig. 5. Histologic and virologic discrepancies between the right and left liver lobes in chronic hepatitis C patients. ○, no between-lobe HCV RNA discrepancy; ●, larger positive-strand amounts in the right lobe; ■, larger positive- and negative-strand amounts in the right lobe; ▲, larger positive-strand amounts in the left lobe.

Factors affecting HCV replication within the liver have been the subject of controversial discussions from the standpoint of the liver and circulating positive strands. Based on the negative-strand level, HCV replication in each liver lobe was shown to be inversely correlated with age. The efficiency of negative-strand RNA synthesis can be influenced by various host factors at multiple levels [11]. The data obtained raise the possibility that some age-related factor(s) may be involved in the regulation of HCV replication within the liver. The present study further showed that liver tissues with concomitant occult HBV contained larger amounts of negative- and positive-strand HCV RNA. Among HCV patients, those carrying occult HBV can manifest severer liver disease and display a poor response to IFN [12]. Occult HBV may also have relevance for hepatocarcinogenesis [13], although the mechanism remains to be clarified. Although further studies are necessary, the data obtained raise the possibility that occult HBV exerts virulence partly by enhancing HCV replication.

As for IFN-based therapy, only limited data are available on the significance of the liver negative-strand HCV RNA quantitation. In a previous semiquantitative study, the negative-strand levels were not related to the outcomes of short-term IFN-α therapy (3 MU thrice weekly for 10 weeks) [2]. Our patients were treated with 6-month enhanced IFN monotherapy [14]. A sustained virologic response was only associated with small amounts of liver negative-strand HCV RNA (<4 log copies/100 ng liver RNA). Based on these preliminary data, further studies are warranted in populations treated with the currently standard regimen of peginterferon and ribavirin.

In conclusion, our findings combined indicate that liver negative-strand HCV RNA quantitation offers clinically relevant information distinct from that available from positive strands within the liver and in the circulation.

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