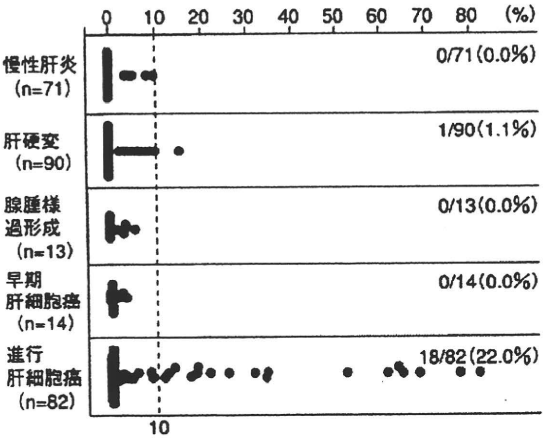

図 5-2　慢性肝疾患における AFP-L3 分画

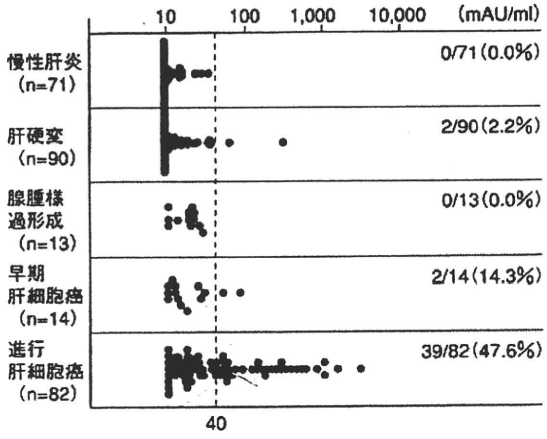

図 5-3　慢性肝疾患における PIVKA-Ⅱ

1つをとらえたものである．カットオフ値は当初15%に設定されていたが[5]，その後の検討から10%を採用することが多い[6]．慢性肝炎，肝硬変，AH，早期肝細胞癌および進行肝細胞癌の陽性率は，それぞれ 0/71(0.0%)，1/90(1.1%)，0/13(0.0%)，0/14(0.0%)および 18/82(22.0%)で，HCC を診断するうえでの感度 18.8%，特異度 99.4%であった(図 5-2)．このように特異度は高いものの感度は低く，小さな肝細胞癌の発見は単独では限界がある．しかし小さくても上昇例では進行肝細胞癌と診断できる[7]．なお，AFP-L3 分画は肝不全時に上昇することがありその解釈には注意が必要である[1]．

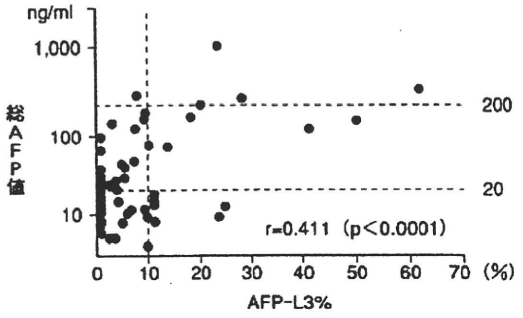

図 5-4　小肝細胞癌における AFP と AFP-L3 分画

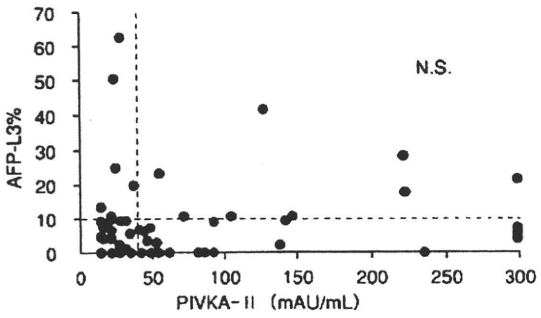

図 5-5　小肝細胞癌における PIVKA-II と AFP-L3 分画

3) PIVKA-Ⅱ

PIVKA-Ⅱは des-γ-carboxy prothrombin (DCP)とも呼ばれ，凝固活性のない異常プロトロンビンである．カットオフ値は 40 mAU/ml で，慢性肝炎，肝硬変，AH，早期肝細胞癌および進行肝細胞癌の陽性率はそれぞれ 0/71(0.0%)，2/90(2.2%)，0/13(0.0%)，2/14(14.3%)および 39/82(47.6%)で，肝細胞癌を診断するうえでの感度 42.7%，特異度 98.9%であった(図 5-3)．PIVKA-Ⅱ単独での陽性率は 3 つの腫瘍マーカーのなかで最も高く，特異性も優れていた．しかしながら，やはり早期肝細胞癌での陽性率は高いとはいえない．なお，PIVKA-Ⅱは黄疸が長期続いてビタミン K 欠乏をきたしたとき(閉塞性黄疸，肝内胆汁うっ滞など)やビタミン K サイクルを阻害するワルファリンや広域スペクトラムの抗生物質(セフェム系)を投与されたときに上昇することがあり，注意が必要である．

表 5-1　各種腫瘍マーカーの組み合わせによる陽性率(n=270)

	AFP のみ	AFP-L3 のみ	PIVKA-Ⅱのみ	AFP＋AFP-L3	AFP＋PIVKA-Ⅱ	AFP-L3＋PIVKA-Ⅱ
overall accuracy[1]	67.8%	70.7%	78.9%	68.1%	74.8%	80.0%
感度[2]	41.7%	18.8%	42.7%	42.7%	63.5%	46.9%
特異度[3]	82.2%	99.4%	98.9%	82.2%	81.0%	98.3%
PPV[4]	46.3%	74.7%	95.3%	56.9%	64.9%	93.8%
NPV[5]	71.9%	69.5%	75.8%	72.2%	80.1%	77.0%

TP：true-positive，TN：true-negative，FP：false-positive，FN：false-negative
1)overall accuracy：TP＋TN/TP＋FP＋TN＋FN，2)感度：TP/TP＋FN，3)特異度：TN/FP＋TN，4)positive predictive value (PPV)：TP/TP＋FP，negative predictive value(NPV)：TN/FN＋TN

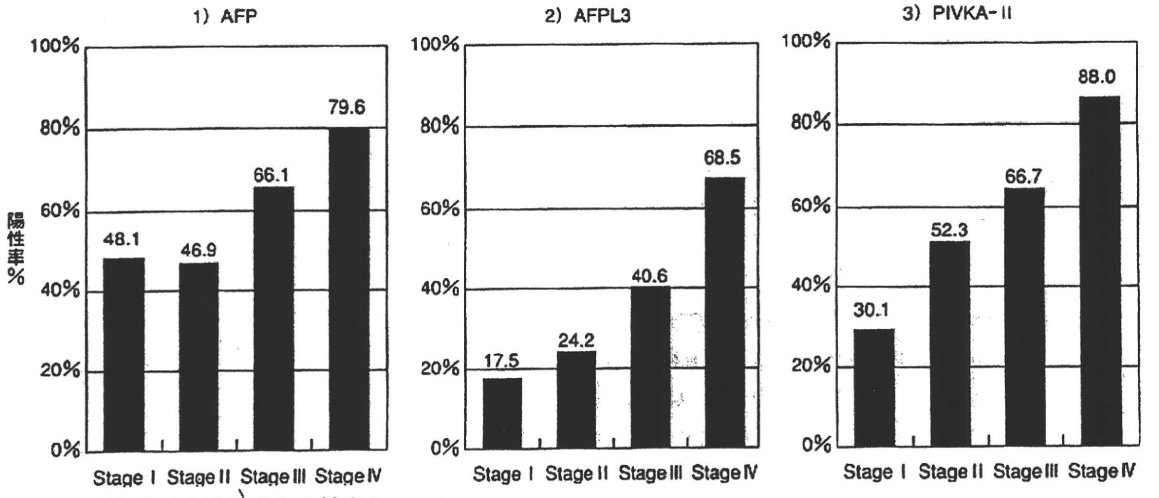

図 5-6　肝細胞癌の進行度と陽性率(n＝712)

b．組み合わせ診断の仕方

今まで述べてきたように単独での腫瘍マーカー測定での肝癌診断には限界がある．一方，わが国では診療報酬上 3 種類の腫瘍マーカーを同時に測定しても請求できない制約がある．しかし各々の腫瘍マーカーの相関は弱い(図 5-4)か認められない(図 5-5)ため，組み合わせての測定が勧められる[8,9]．3 cm 以下の肝細胞癌での組み合わせ診断の結果を表 5-1 に示す．AFP-L3 分画と PIVKA-Ⅱの組み合わせ測定が感度 46.9%，特異度 98.3%，positive predictive value(PPV)93.8%，negative predictive value(NPV)77.0%と最も良好であった．

c．悪性度評価

図 5-6 に AFP，AFP-L3 分画，PIVKA-Ⅱの同時測定を行った肝細胞癌 712 例の進行度別の各々の腫瘍マーカーの陽性率を示した．AFP での Stage Ⅰ と Ⅱ を除き各腫瘍マーカーの陽性率は Stage の進行とともに上昇した．

腫瘍マーカーは肝細胞癌の生物学的悪性度(発育速度および浸潤・転移能)の評価に適している．組織学的には分化度が，画像的には血流動態(肝動脈血優位，門脈血欠損)が悪性度評価に使用されることが多い．図 5-7 に腫瘍の血流動態および AFP-L3 分画と PIVKA-Ⅱを同時測定した最大径 2 cm 以下の肝細胞癌 100 例での腫瘍マーカーの陽性率を示す．門脈血の減少がない時期では AFP-L3 分画と PIVKA-Ⅱも陽性とはならず，

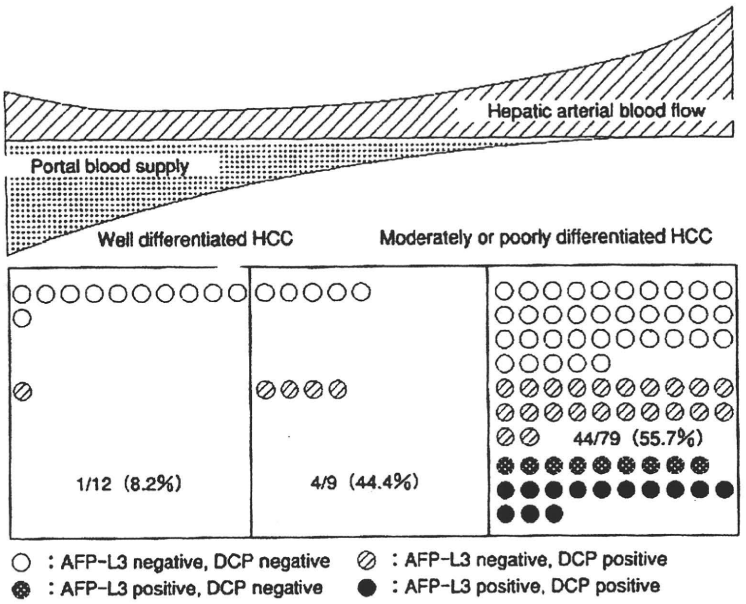

図 5-7　腫瘍の分化度と腫瘍マーカー(n = 100)

門脈血が減少すると PIVKA-Ⅱ陽性例が出現し，動脈血が増加し門脈血が欠損すると AFP-L3 もしくは両者が陽性となる．言い換えれば，腫瘍マーカーが陽性となる時期の肝細胞癌はそのサイズにかかわらず進行肝細胞癌(中・低分化)であり[7]，予後も不良となる[10]．

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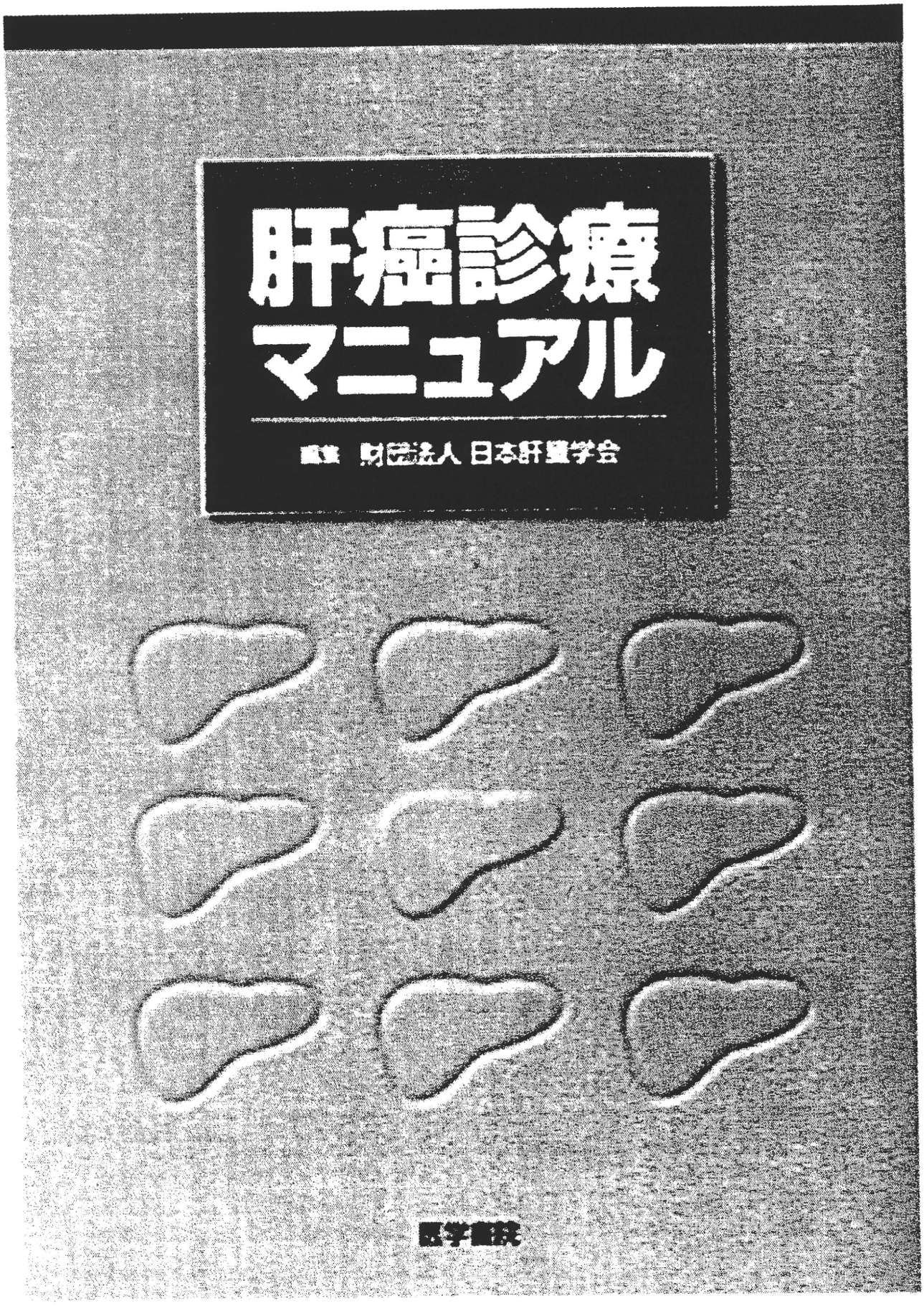
肝癌診療
マニュアル
編集 財団法人 日本肝臓学会
医学書院

D 腫瘍マーカーによる効果判定

a．治療前の腫瘍マーカーと予後

腫瘍マーカーが陽性か陰性かにより予後を検討するとAFP(カットオフ値20 ng/ml)，AFP-L3(カットオフ値10%)，PIVKA-Ⅱ(カットオフ値40 mAU/ml)のいずれも陰性例が陽性例に比して有意に長期間生存していた($p<0.0001$)(図7-4)．さらに治療前の腫瘍マーカーの陽性数と予後をみたものが図7-5である．陽性数が0個，1個，2個，3個と増加するにつれ予後は悪化した．

b．治療後の腫瘍マーカーと予後

AFP，AFP-L3およびPIVKA-Ⅱの治療前後の腫瘍マーカーの動態と予後を解析したのが図7-6である．いずれの腫瘍マーカーも治療前陽性で陰性化した群(陰性化群)，治療前陰性で治療後も陰性が持続している群(持続陰性群)，治療前陰性で治療後陽性化した群(陽性化群)，および治療前も治療後も陽性の群(持続陽性群)の4群に分類した[1,2]．なお治療後の腫瘍マーカーの測定は治療後1か月から3か月のものを採用した．AFP，AFP-L3およびPIVKA-Ⅱともに陰性化群と持続陰性群の間には差を認めず，陽性化群と持続陽性群の間にも差を認めなかった．しかし陰性化群・持続陰性群と陽性化群または持続陽性群の間にはいずれの群間でも差を認め，特にAFP-L3で顕著であった($p<0.0001$)．一方，治療後の腫瘍マーカーの陽性数と予後をみたものが図7-7である．陽性数が増加するにつれて予後は悪化し，その傾向は術前の腫瘍マーカー陽性数(図7-5)よりも顕著であった．

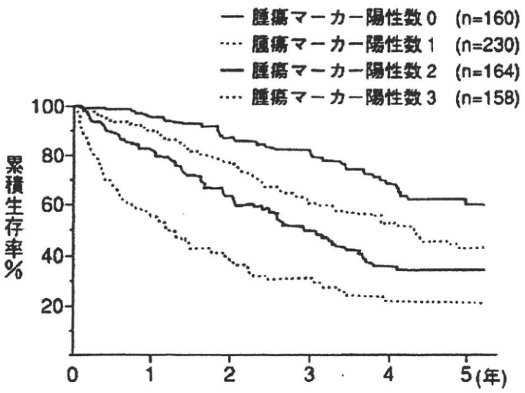

図7-5 治療前の腫瘍マーカーの陽性数と予後

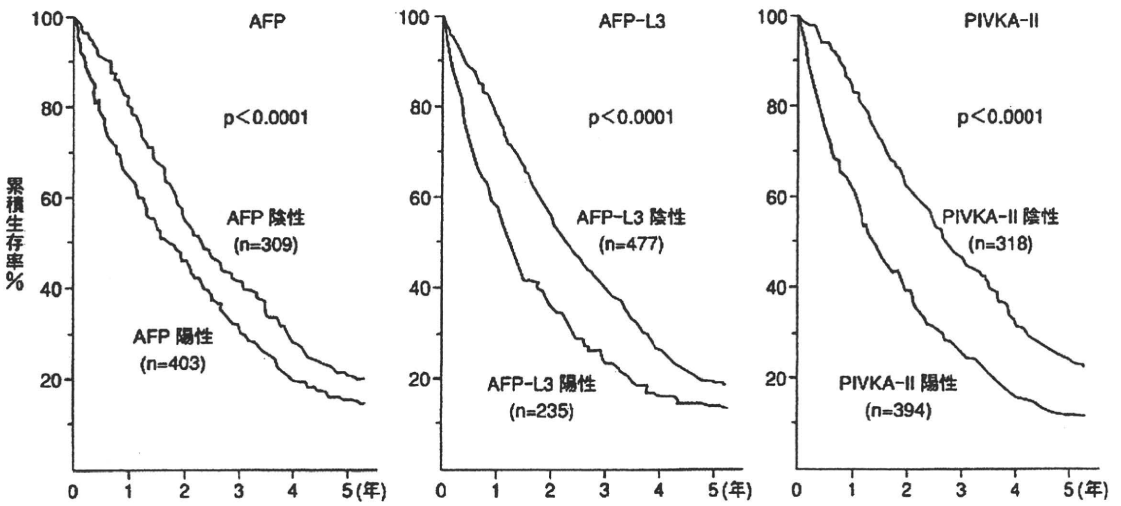

図7-4 腫瘍マーカーの陽性の有無と予後

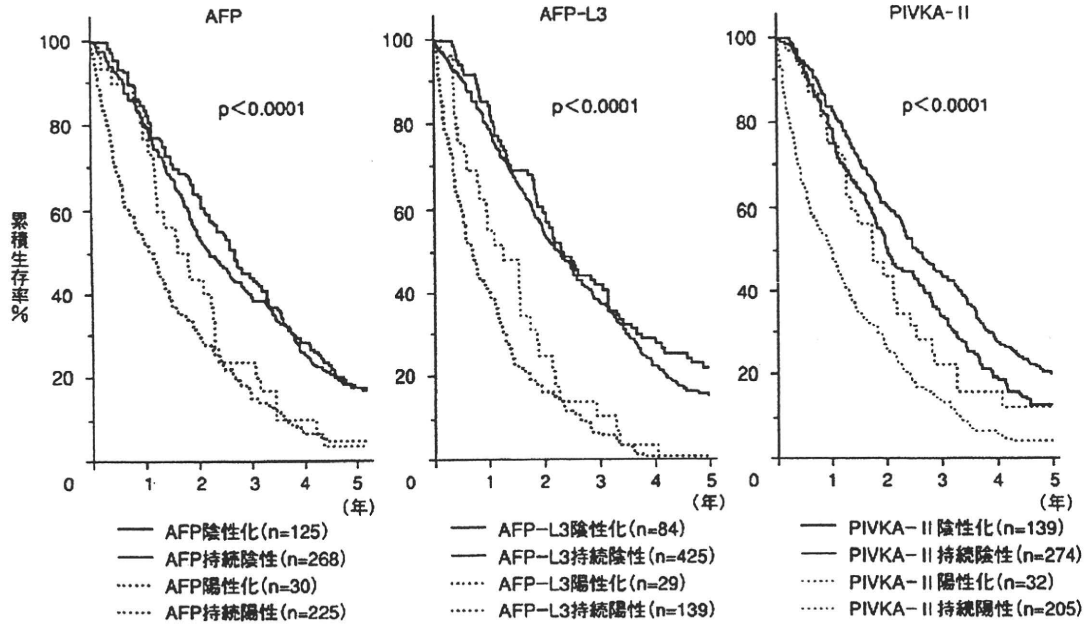

図 7-6　治療前後の腫瘍マーカーの動態と予後

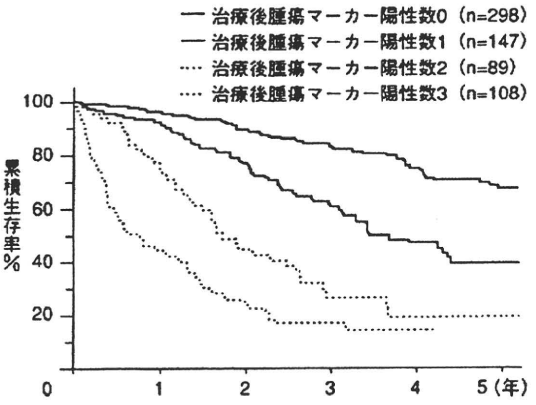

図 7-7　治療後も陰性化しない腫瘍マーカーの個数と生存率

C．治療効果判定のための腫瘍マーカーの意義

肝切除，局所治療，肝動脈塞栓術などの治療効果の判定は治療終了の可否，すなわち追加治療の要不要を判断する重要なものである．現状では画像診断による判定が主であることはいうまでもないが，画像診断法の進歩した現在においてもその検出には当然限界がある．画像上検出可能なすべての腫瘍が治療されれば治療は終了となるが，術前に陽性であった腫瘍マーカーが治療後も陽性を持続している場合，未治療肝細胞癌残存の可能性が示唆される．画像診断上描出されないものを治療することは困難であるので，より慎重な経過観察，さらに評価は定まってはいないが術後の補助化学療法の追加などを考慮する必要がある．

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(熊田　卓，豊田秀徳)

3 経過中ALTによる病態評価

Ⅰ ALTとは

①ALTはL-アラニンと2-オキソグルタール酸をピルビン酸とL-グルタミン酸に変換するアミノ酸転移反応を触媒する酵素である．一般に活性値は男性より女性が低い．

②ALTもASTと同様に細胞質に存在するs-ALTとミトコンドリアに存在するm-ALTの2つのアイソザイムが存在する．m-ALTの活性は極めて低く血清中のALTはほとんどs-ALTである．

③ALTはASTとは異なり臓器特異性が高く肝臓と腎臓に多量に含まれ，肝疾患の指標として有用である．

④血清ALTは逸脱酵素であり，細胞膜の透過性亢進あるいは細胞破壊によって血中に遊出してくる．ALTを多く含んでいる肝臓の障害で血中の活性値が上昇する．

⑤血清ALTの上昇は肝障害の指標となるが，他の酵素，AST，γ-GTP，ALP，LDHなどを組み合わせて用いて，肝疾患の鑑別，他疾患との鑑別に利用する．

Ⅱ ALTを用いたC型肝炎の経過観察

①血清ALTの基準値は各施設で異なり，個体間での変動も多い(各個人の基準値，すなわち「個性値」が判明していることが望ましい)．最近の基準値上限は男性30 IU/L，女性19 IU/Lとされている[1]．

②血清ALTが軽度上昇した場合は，BMI，脂質の測定，超音波などの画像診断を行い脂肪肝などの影響を考慮する必要がある．

③血清ALT値が異常高値を示しても，必ずしも肝障害の重症度を反映しているとは限らない．プロトロンビン時間やヘパプラスチン試験を合わせて肝実質障害に伴う蛋白合成能の低下も評価する．また，他の原因，例えば肝炎ウイルスの重複感染，薬剤(健康食品も含む)などの関与を除外する必要がある．

④血清ALTは逸脱酵素であり，その時点での状態を示しているにすぎない．ワンポイントのALT値から判断することは危険である．

⑤血清ALTの判断には経過を考慮する必要がある．例えば，各時点で測定したALTをすべて積算して測定回数で割る「算術平均値」，ALTの曲線下面積を求め経過観察期間で割る「積分平均値」，基準値を超える回数から分類する「パターン認識」などがある．

⑥血清ALTの「積分平均値」から肝発癌率を算定すると極めてよく相関し，「積分平均値」が増加すると発癌率も増加する(図1-4)[2]．多変量解析(Coxの比例ハザードモデル，変数増加法)でも，血清ALTの「積分平均値」は有意の因子の1つであった(表1-2)．

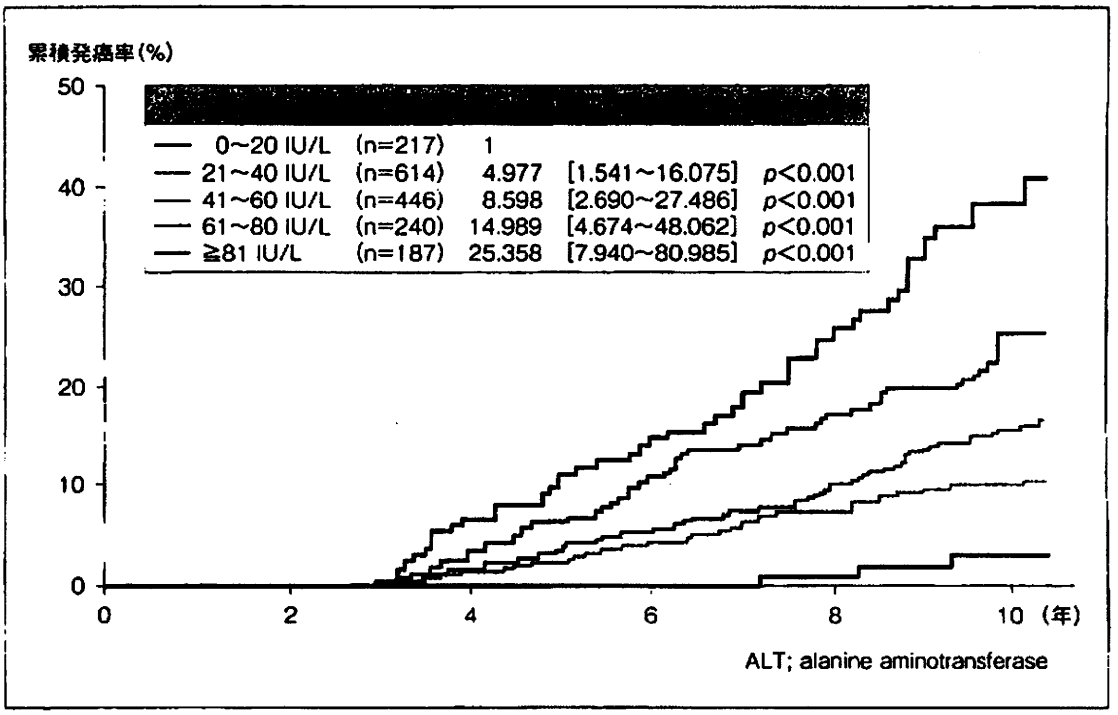

図 1-4　血清 ALT の積分平均値と肝発癌率

表 1-2　肝発癌に関与する因子(n=1,704，Cox の比例ハザードモデル)

年齢(歳)	≦65 >65	1 1.964(1.436~2.685)	<0.001
性	女性 男性	1 1.675(1.242~2.259)	0.001
ALT 積分平均値(IU/L)	0~20 21~40 41~60 61~80 ≧81	1 3.845(1.117~13.298) 4.050(1.206~13.597) 9.125(2.789~29.857) 18.838(5.735~61.881)	<0.001 0.033 0.024 <0.001 <0.001
血小板($\times 10^4/\mu L$)	≧12.0 <12.0	1 3.277(2.435~4.409)	<0.001
ALP(IU/L)	≦338 >338	1 1.590(1.167~2.166)	0.003
コリンエステラーゼ (IU/L)	≧431 <431	1 7.856(1.824~33.830)	0.006
アルブミン(g/dL)	≧3.5 <3.5	1 2.901(1.973~4.266)	<0.001
IFN 治療	無治療 非 SVR SVR	1 0.891(0.429~1.851) 0.537(0.349~0.827)	0.015 0.395 0.005

ALP; alkaline phosphatase, SVR; sustained virological response

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(熊田　卓，豊田秀徳)

【雑　　誌】

Hepatology Research 2007; 37: 994–1001 doi: 10.1111/j.1872-034X.2007.00157.x

Original Article

Liver disease in hepatitis C virus carriers identified at blood donation and their outcomes with or without interferon treatment: Study on 1019 carriers followed for 5–10 years

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Aim: To portray liver disease and project outcomes in carriers of hepatitis C virus (HCV) in the general population.

Methods: Liver disease was evaluated in 1019 individuals who were found with HCV infection at blood donation, and they were followed for 5–10 years with or without receiving interferon (IFN).

Results: At baseline, chronic hepatitis was detected in 529 (51.9%) HCV carriers and more frequently in men than in women (62.6% [299/478] *vs* 42.5% [230/541], $P < 0.01$); cirrhosis was diagnosed in five (0.5% [three men included]) and hepatocellular carcinoma (HCC) in one (0.1% [man]). Of the carriers who were followed for 5 years or longer, loss of HCV-RNA from serum was achieved in 61 (31.0%) of the 197 treated with interferon (IFN) and only one of the 211 (0.5%) without IFN ($P < 0.0001$). HCC developed in 14 carriers including six of the 211 (2.8%) without IFN and eight of the 197 (4.1%) with IFN (six non-responders included). Follow ups of the 949 carriers identified age ($P < 0.002$), male gender ($P < 0.01$) and cirrhosis at the baseline ($P < 0.0001$) as factors contributing to the development of HCC. Cumulative incidence rates of HCC during 10 years among carriers found with chronic hepatitis increased in parallel with the age at the baseline.

Conclusion: Identification of HCV carriers in the general population and treating those indicated with IFN would help decrease the development of HCC and lift its medical, as well as economic, burdens off society.

Key words: blood donation, chronic hepatitis, cirrhosis, hepatitis C virus, hepatocellular carcinoma, interferon

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INTRODUCTION

OVER THE WORLD, an estimated 170 million people are persistently infected with hepatitis C virus (HCV), most of whom are unaware of their infection.[1] The natural history of HCV infection varies widely and is influenced by many host factors including the age

at infection, gender, ethnicity, body mass index and alcohol intake, as well as virological factors such as genotypes and viral load.[2–8] It is generally accepted that HCV persists in 70–80% of individuals who have been infected with it, and causes deaths due to decompensated cirrhosis and/or hepatocellular carcinoma (HCC) in about 30% of them before they reach 70 years of age.[9] Because the time of infection is hardly specified in most HCV carriers, courses of HCV infection are defined only in recipients of transfusion[10–12] or pregnant women who received anti-D immune globulin.[13,14]

Because the time of infection is not specified in the majority of HCV carriers who contract infection sporadically and are undetected in the community, their liver disease and rate of progression to cirrhosis and HCC are not fully characterized, as yet. Individuals found with HCV infection at the time of blood donation offer a rare opportunity for clinical evaluation and prospectively following evolution of liver disease. Furthermore, they allow assessing the effects of therapeutic intervention with antiviral treatments, represented by interferon (IFN), and to devise strategies for preventing the development of HCC on a national scale.

We have identified HCV carriers at blood donation, and evaluated liver disease in them. Thereafter, they were followed for 5–10 years with or without receiving IFN treatment. Emerging results are hoped to propel screening for persistent HCV infection in the general population, and promote therapeutic intervention in those indicated to prevent the development of HCC in these patients.

METHODS

HCV carriers who were unaware of their infection

DURING AUGUST 1991 through November 2001, donated blood units were 1 925 860 in total and 3377 individuals were found with persistent HCV infection when they wished to donate blood units at the Japanese Red Cross (JRC) Hiroshima Blood Center. They were informed of their infection with HCV, and recommended to consult hepatology specialists. Of them, 1097 (32.5%) visited 20 liver clinics scattered over Hiroshima Prefecture. Clinical evaluation was feasible in 1019 of them (30.2% of the total) with the documented date of the initial visit, date of birth and the baseline liver disease or the absence thereof. The baseline diagnosis means the diagnosis at the first medical consultation.

Risk of HCC was assessed in the 949 HCV carriers for whom the date of the initial visit, date of birth and the baseline diagnosis, as well as date of the last visit and final diagnosis, had been filed. Among 1019 HCV carriers, follow ups of 5 years or longer were possible in 408 (40.0%), with date of the last visit and final diagnosis being specified, of whom 197 (48.3%) received IFN therapy.

The study design conformed to the provisions of the Declaration of Helsinki, and was approved by Ethic Committees of all institutions. Written informed consent was obtained from each HCV carrier.

Data collection

Questionnaire was distributed to hepatology specialists attending to HCV carriers in the 20 institutions. They were asked to log in the following: (i) initial diagnosis; (ii) compliance to regular visits; (iii) changes in liver pathology with time; (iv) intervention with IFN; and (v) development of HCC. These data were made anonymous on the personal identification of any HCV carrier, and analyzed collectively. The consent from HCV carriers was obtained by the family doctor.

Diagnosis of liver disease

Four clinical states were diagnosed. No changes in the liver without identifiable abnormalities connotated the following: (i) values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) within normal limits (lower than standard value in every institution); (ii) normal platelet counts; (iii) lack of abnormal pictures in those examined by imaging modalities; and (iv) no pathological findings in the liver biopsies in those who received them. Each attending specialist was asked for his/her comprehensive opinion for judging the lack of abnormality in an HCV carrier. The diagnosis of chronic hepatitis was left to the discretion of each hepatology specialist, taking into account the results of biochemical and other tests. The attending specialist made a decision on whether his/her patient should receive IFN or would be observed without it, with the consensus and endorsement of the patient.

Markers of HCV infection

HCV-RNA was determined by polymerase chain reaction (PCR) with primers deduced from the conserved region, irrespective of genotypes, of the 5′-non-coding region of the genome.[15] Genotypes of HCV were determined by PCR with type-specific primers.[16]

Table 1 Clinical diagnosis of HCV carriers identified among blood donors at baseline

Features	Total ($n = 1019$)	Men ($n = 478$)	Women ($n = 541$)	Differences (men *vs* women)
Age	45.3 ± 11.1	43.5 ± 11.0	46.9 ± 11.4	$P < 0.01$
Liver disease				
No abnormalities	483 (47.4%)	174 (36.4%)	309 (57.1%)	$P < 0.001$
Chronic hepatitis	529 (51.9%)	299 (62.6%)	230 (42.5%)	$P < 0.001$
Treated	242 (45.7%)	136 (45.5%)	106 (46.1%)	
Followed up	222 (42.0%)	133 (44.5%)	89 (38.7%)	
Lost	65 (12.3%)	30 (10.0%)	35 (15.2%)	
Cirrhosis	5 (0.5%)	3 (0.6%)	2 (0.4%)	NS
HCC	1 (0.1%)	1 (0.2%)	0	NS
Acute hepatitis	1 (0.1%)	1 (0.2%)	0	NS

HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NS, not significant.

IFN treatment

IFN was used according to regular protocol. Types of IFN were recombinant IFN-α2a, recombinant IFN-α-2b and IFNα. For example, recombinant IFN-α2a at a daily dose of 6–9 million international units (MIU) was given during the initial 2 weeks, followed by 3 MIU three times a week until 24 weeks after the start of IFN (total dose: 300–342 MIU). Sustained virological response (SVR) to IFN was diagnosed 24 weeks after the completion of treatment by the elimination of HCV-RNA from serum detectable by PCR. Biochemical response with normalization of aminotransferases, without loss of serum HCV-RNA, was not regarded as a response to IFN.

Statistical analyses

Categorical variables were compared between groups by the χ^2 test or Fisher's exact test. Kaplan–Meier life tables were used in assessing the risk of developing HCC with reference to gender, age and liver disease at the baseline, as well as treatment with IFN, using UMP version 5 software (SAS Institute, Tokyo, Japan).

RESULTS

Liver disease in 1019 HCV carriers found at blood donation

TABLE 1 LISTS liver disease diagnosed in 1019 HCV carriers stratified by gender. Overall, they were 45.3 ± 11.1 years old and included 478 (46.9%) men. Less than half had no abnormalities in the liver (483 [47.4%]), while cirrhosis had already developed in five (0.5% [three men included]) and HCC in one (0.1% [man]); acute hepatitis C was diagnosed in one (0.1%). Less frequent were no abnormalities in the liver (36.4% *vs* 57.1%, $P < 0.01$) in men than in women, and chronic hepatitis was found more often (62.6% *vs* 42.5%, $P < 0.01$) in men than in women. Among 529 carriers who were diagnosed as chronic hepatitis, 242 carriers (45.7%) were diagnosed as requiring a treatment at the first medical consultation. There were no differences between men and women in the ratio of treated HCV carriers.

Differences were noted in the influence of age on the baseline liver disease between men and women (Table 2). Among HCV carriers aged ≤39 years and 40–49 years, chronic hepatitis was more common in men than in women ($P < 0.01$). For those aged 50–59 years and ≥60 years, however, clinical diagnoses were no different between men and women.

Comparison of clinical outcomes between HCV carriers with and without IFN treatment

Of the 1019 HCV carriers, 408 (40.0%) were followed for 5 years or longer with date of the last visit and final diagnosis being specified. Among them, 197 (48.3%) carriers received IFN therapy (one course of 24-weeks IFN). There were no significant differences in the age (46.0 ± 10.2 *vs* 47.3 ± 11.2 years) or sex (men accounting for 41% *vs* 51%) between carriers with and without IFN. However, the observation period was longer in carriers with IFN than without IFN (9.2 ± 1.7 [range: 5.0–11.7] *vs* 8.8 ± 1.9 [5.1–11.9] years, $P < 0.05$). Figure 1 compares clinical outcomes between HCV carriers with and without IFN. HCV-RNA was not detectable in serum 24 weeks after the completion of treatment in the 61 (31.0%) carriers who had received

Table 2 Baseline clinical diagnoses of the 476 men and 541 women found with serum HCV-RNA at blood donation stratified by age

Age	Men	Women	Differences
≤39 years	(n = 180)	(n = 123)	$P < 0.01$
No abnormalities	64 (35.6%)	82 (66.7%)	
Chronic hepatitis	116 (64.4%)	41 (33.3%)	
Cirrhosis	0	0	
40–49 years	(n = 146)	(n = 153)	$P < 0.01$
No abnormalities	51 (34.9%)	89 (58.2%)	
Chronic hepatitis	94 (64.4%)	64 (41.8%)	
Cirrhosis	1 (0.7%)	0	
50–59 years	(n = 111)†	(n = 199)	NS
No abnormalities	45 (40.5%)	104 (52.3%)	
Chronic hepatitis	65 (58.6%)	94 (47.2%)	
Cirrhosis	1 (0.9%)	1 (0.5%)	
≥60 years	(n = 39)‡	(n = 66)	NS
No abnormalities	14 (35.9%)	34 (51.5%)	
Chronic hepatitis	24 (61.5%)	31 (47.0%)	
Cirrhosis	1 (2.6%)	1 (1.5%)	

†Case of acute hepatitis excluded.
‡Case of hepatocellular carcinoma excluded.
HCV, hepatitis C virus; NS, not significant.

IFN, including 13 of the 48 (27.1%) without abnormalities in the liver and 48 of the 148 (32.4%) with chronic hepatitis at the baseline. In contrast, HCV-RNA disappeared in only one of the 128 (0.8%) carriers without IFN therapy; he did not have abnormalities in the liver at the baseline. Thus, HCV-RNA was cleared from serum much more frequently with than without IFN treatment (61/197 [31.0%] *vs* 1/211 [0.5%], $P < 0.0001$).

Cirrhosis developed anew in 15 carriers including five of the 211 (2.4%) without IFN and 10 of the 197 (5.1%) with IFN (Table 3). All the 10 carriers developing cirrhosis, despite receiving IFN, were non-responders (one partial responder included). Genotypes of HCV were 1b in eight of the 11 (73%) cirrhotics for whom they were determined, and 2a or 2b in the remaining three. Likewise, HCC developed in 14 carriers including six of the 211 (2.8%) without IFN and eight of the 197 (4.1%) with IFN (Table 4). Of the eight carriers developing HCC, in spite of IFN treatment, six were non-responders (one partial responder included) and one did not complete the full course of IFN. The remaining one (case 13) developed HCC 6 years after he achieved complete response to IFN; he received partial hepatectomy. Of the 10 carriers developing HCC for whom genotypes were examined, eight (80%) were infected with HCV of genotype 1b.

Factors influencing the development of HCC

Hepatocellular carcinoma developed in 14 of the 408 (3.4%) HCV carriers who had been followed for 5 years or longer, including six without and eight with IFN therapy (Table 4). As most carriers developing HCC did not respond to IFN, they were analyzed collectively with the carriers in whom HCC occurred in the absence of IFN therapy. By analysis of Kaplan–Meier life tables (Fig. 2a–c), baseline cirrhosis ($P < 0.0001$), age at the detection of HCV carrier state ($P < 0.002$) and the male gender ($P < 0.01$) enhanced the development of HCC. IFN did not significantly influence the development of HCC, however (data not shown).

Risk of developing HCC in carriers who were initially diagnosed with chronic hepatitis, stratified by the age, is shown in Figure 3. Cumulative HCC risk during 10 years was estimated at 18.7% (95% confidence interval: 1.9–35.5%) for carriers aged 60 years or older, 8.7% (0–17.5%) for those in their fifties and 7.2% (0–15.5%) for those in their forties when they presented with chronic hepatitis at blood donation.

DISCUSSION

IN PREVIOUS STUDIES, we have identified HCV carriers at the time of blood donation in the Japanese

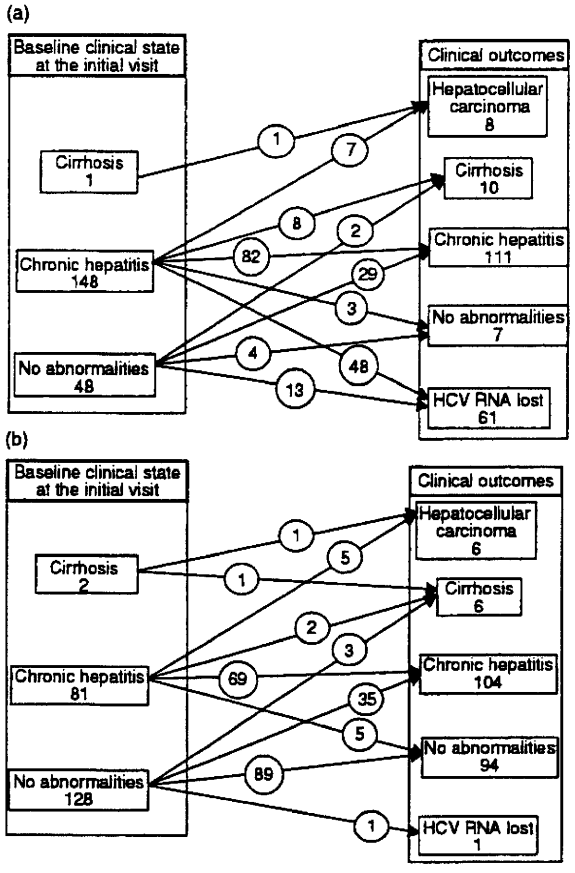

Figure 1 (a) Clinical outcomes of the 197 hepatitis C virus (HCV) carriers with interferon (IFN) therapy during follow ups of 5 years or longer stratified by the baseline clinical state. (b) Clinical outcomes of the 211 carriers without IFN therapy during follow ups of 5 years or longer stratified by the baseline clinical state.

Red Cross (JRC) Hiroshima Blood Center and followed them for evolution of liver disease at local hepatology centers.[17–19] Here we report the final results on 1019 HCV carriers compiled during the past 15 years. Such attempts may shed light on the morbidity of HCV carriers in the community who have no symptoms or knowledge of their infection. Unlike patients with HCV infection who visit hospitals[10,11] or pregnant women accidentally infected by contaminated anti-D gammaglobulin,[13,14] who account for only a minority of total HCV infection, asymptomatic carriers represent the great majority of HCV infection in the community. Asymptomatic HCV carriers were incidentally identified at blood donation; the present study clarified the clinical natural course of HCV carriers who were found without symptoms at the occasion of blood donation. This study is a population-based study by contrast with a hospital-based case study.

First, more than half of the 1019 HCV carriers (mean age 45.3 ± 11.1 years) identified at blood donation had liver disease; no abnormalities in the liver were diagnosed in 483 (47.4%) of them. Chronic hepatitis was more frequent in men than in women (62.6% [299/478] *vs* 42.5% [230/541], $P < 0.01$). Cirrhosis had developed already in three (0.6%) men and two (0.4%) women, and HCC in one (0.1%) man. Subjectively, they were healthy enough to offer blood donation. Liver disease would have progressed to chronic hepatitis insidiously in many HCV carriers in their forties. Distribution of chronic hepatitis was age dependent. Among HCV carriers aged ≤39 and 40–49 years, it was more frequent in men than in women (64.4% *vs* 38.0% and 64.4% *vs* 41.8%, respectively, $P < 0.01$ for each); gender differences were smaller in those aged 50–59 and ≥60 years (58.6% *vs* 47.2% and 61.5% *vs* 47.0%, respectively), however.

Second, IFN can improve the course of asymptomatic HCV infection. HCV-RNA was cleared from the circulation in 61 of the 197 (31.0%) carriers who had received IFN, as against only one of the 211 (0.5%) who had not. Responders to IFN included 48 of the 148 (32.4%) carriers with chronic hepatitis and 13 of the 48 (27.0%) without abnormalities in the liver at the baseline. It remains debatable whether or not IFN should be given to HCV carriers without liver disease.[20–23] Loss of HCV infection would be auspicious, by any standard, and may deserve consideration unless IFN is contra-indicated.

Third, cirrhosis developed in 15 (3.6%) and HCC in 14 (3.4%) of the 408 HCV carriers during follow ups for 5 years or longer; genotype 1b predominated in carriers who developed with cirrhosis (8/11 [73%]) or HCC (8/10 [80%]). Carriers who developed cirrhosis or HCC despite receiving IFN were largely non-responders. During the study period, the standard IFN for 24 weeks was approved in Japan. With recent remarkable advances in antiviral therapy, represented by pegylated IFN combined with ribavirin, the response has been improved to 50% even in patients infected with HCV of genotype 1b.[24,25] There is a possibility, therefore, that cirrhosis and HCC in the 29 HCV carriers would have been prevented, at least in part, should they have received sophisticated antiviral treatment.

Fourth, risk factors for the development of HCC were identified in the followed HCV carriers. The initial diag-

Table 3 Development of cirrhosis in 15 HCV carriers during follow ups for 5 years or longer

Case no.	Age/Sex	Baseline diagnosis	Cirrhosis (years elapsed)	IFN therapy	HCV genotype
1	52/M	No abnormalities	4	No	1b
2	50/F	No abnormalities	8	No	1b
3	49/F	Chronic hepatitis	10	No	1b
4	63/F	Chronic hepatitis	3	No	1b
5	59/M	No abnormalities	9	No	1b
6	36/M	Chronic hepatitis	10	Yes (NR)	1b
7	40/M	Chronic hepatitis	6	Yes (NR)	ND
8	41/M	Chronic hepatitis	5	Yes (NR)	ND
9	42/M	No abnormalities	5	Yes (NR)	ND
10	45/F	Chronic hepatitis	3	Yes (NR)	2b
11	48/F	Chronic hepatitis	3	Yes (NR)	2a
12	51/F	Chronic hepatitis	0	Yes (NR)	1b
13	55/F	Chronic hepatitis	5	Yes (PR)	ND
14	56/M	Chronic hepatitis	7	Yes (NR)	1b
15	59/F	No abnormalities	7	Yes (NR)	2a

HCV, hepatitis C virus; IFN, interferon; ND, not determined; NR, no response to IFN; PR, partial response with the normalization of alanine aminotransferase without loss of HCV-RNA.

nosis of cirrhosis, old age at detection of HCV-RNA and male risk factor were significant risk factors for HCC, in corroboration with previous reports.[5,26] Among carriers presenting with chronic hepatitis, the cumulative incidence of HCC during 10 years was significantly higher ($P < 0.01$) in those aged ≥60 years at the baseline (18.7% [95% confidence interval: 1.9–35.5%]) than in those aged 50–59 years (8.7% [0–17.5%]) or 40–49 years (7.2% [0–13.3%]) by analysis in Kaplan–Meier life tables. Hence, it would be imperative to detect HCV infection early in life and treat liver disease before it has progressed, especially in men.

Finally, the present results would justify the campaign for preventing HCC arising in asymptomatic carriers. Encouraged by these results on HCV carriers identified at blood donation, the Japanese government started a 5-year program since the fiscal year 2002 to identify ongoing HCV infection in the recipients of health check-

Table 4 Development of HCC in 14 HCV carriers during follow ups of 5 years or longer

Case no.	Age/Sex	Baseline diagnosis	Years until HCC	IFN therapy	HCV genotype
1	46/M	Chronic hepatitis	7	No	1b
2	41/M	Chronic hepatitis	19	No	1b
3	58/M	Chronic hepatitis	4	No	ND
4	62/F	Chronic hepatitis	5	No	1b
5	61/M	Cirrhosis	7	No	ND
6	60/M	Chronic hepatitis	11	No	1b
7	40/M	Chronic hepatitis	10	Yes (NR)	2b
8	52/M	Cirrhosis	9	Yes (NR)	1b
9	53/M	Chronic hepatitis	10	Yes (PR)	ND
10	52/F	Chronic hepatitis	10	Yes (NR)	1b
11	59/M	Chronic hepatitis	5	Yes (NR)	1b
12	61/M	Chronic hepatitis	7	Yes†	1b
13	63/M	Chronic hepatitis	8	Yes (CR)	2b
14	65/F	Chronic hepatitis	10	Yes (NR)	ND

†Did not receive the full course of interferon (IFN).
CR, complete response with the loss of HCV-RNA from serum; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ND, not determined; NR, no response to IFN; PR, partial response with the normalization of alanine aminotransferase without loss of HCV-RNA.

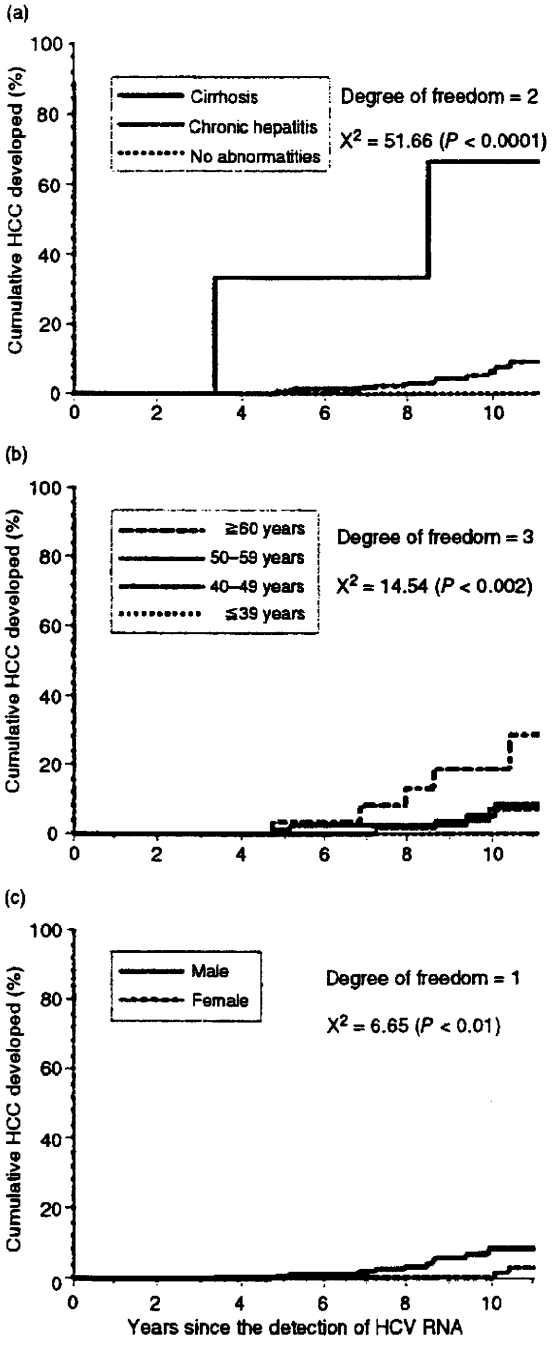

Figure 2 Risk of hepatocellular carcinoma (HCC) increasing with time in the individuals ($n = 949$) found with hepatitis C virus (HCV) infection on blood donation. Influence of liver disease at the (a) baseline, (b) age at the diagnosis of HCV infection and (c) genders is shown.

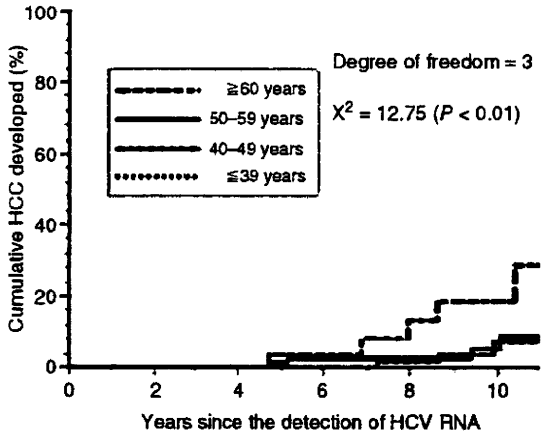

Figure 3 Risk of hepatocellular carcinoma (HCC) increasing with time in the HCV carriers ($n = 498$) identified at blood donation who were diagnosed with chronic hepatitis at the baseline.

ups offered to individuals older than 40 years at a 5-year interval until they enter their seventies.[18] In the present study, however, only 1097 of the 3377 (32.5%) individuals found with HCV infection at blood donation visited hepatology specialists in Hiroshima Prefecture during 1991–2001. Further efforts are crucial for orienting the general population to take tests for HCV infection, and when found with it, consult specialists and receive IFN treatment as required. The results of the present study promise that such endeavors are rewarding, by deceasing morbidity and mortality of persistent HCV infection, along with lessened economic burdens on the nation.

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Epidemiological survey of oral lichen planus among HCV-infected inhabitants in a town in Hiroshima Prefecture in Japan from 2000 to 2003

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Abstract. The objective of our study was to evaluate the natural history of oral lichen planus (OLP) and other extrahepatic manifestations in the inhabitants of an area in Japan that is hyperendemic for hepatitis C virus (HCV) infection. Over 4 years, 224 adult inhabitants with HCV infection were examined for OLP by a single oral surgeon. All subjects were interviewed regarding the natural history of other extrahepatic manifestations they had developed. The antibodies to HCV (anti-HCV) and serum HCV RNA were determined. Anti-HCV were detected in sera from 224 subjects (100%); HCV RNA in 210 (93.8%). Of the 224, 88 had at least 1 oral examination for OLP during the 4-year period. In 2000, 2001, 2002 and 2003, OLP was observed in 8.5 (5/59), 14.8 (8/54), 20 (11/55) and 21.4% (12/56) of subjects, respectively. OLP prevalence increased as the subjects grew older. The incidence of OLP over the 4 years among all subjects with HCV infection was 17.0% (15/88, 2 men and 13 women). None experienced natural healing or the development of malignant transformations. Between 2000 and 2003, there was an increase in the prevalence of type 2 diabetes mellitus (DM), thyroid dysfunction, skin disease, renal disease and hypertension. Screening for extrahepatic manifestations should be conducted in patients with risk factors for HCV infection.

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Abbreviations: HCV, hepatitis C virus; OLP, oral lichen planus; HCC, hepatocellular carcinoma; anti-HCV, antibodies to HCV; DM, diabetes mellitus

Key words: lichen planus, hepatitis C virus, extrahepatic manifestations

Introduction

Hepatitis C virus (HCV) infection is a major health problem in Japan. It is highly prevalent in subjects with chronic liver disease and is strongly associated with hepatocellular carcinoma (HCC). HCV-related HCC accounts in large part for the recent increase in HCC and now constitutes about 80% of all HCC cases in Japan. HCV also incites many extrahepatic manifestations (1,2) of which lichen planus is the most common (3,4). Other associated diseases include cryoglobulinaemic nephropathy and glomerulonephritis (5), thyroid dysfunction (6), porphyria cutanea tarda (7) and type 2 diabetes mellitus (DM) (8).

We previously reported that the incidence of oral lichen planus (OLP) in subjects with HCV infection was significantly higher than in those without HCV. We reached this conclusion by mass screening 685 inhabitants of a hyperendemic area, H town, located in the Fukuoka prefecture of Northern Kyushu, Japan (Fig. 1) for HCV infection (9). The prevalence of other extrahepatic manifestations in subjects with antibodies to HCV (anti-HCV) was higher than in those without HCV (10).

We also conducted an epidemiological study of another HCV hyperendemic area, O town, in the northwest of the Hiroshima prefecture in Honshu, Japan (Fig. 1). The presence of HCV-associated extrahepatic manifestations was found in 66.1% (39/59) of those screened (11). These findings suggest that the high prevalence of various extrahepatic manifestations among HCV-infected subjects is not unique to specific areas.

In the present investigation, we annually examined extrahepatic manifestations in the inhabitants of O town from 2000 to 2003. The aim of this study was to evaluate the natural history of OLP and other extrahepatic manifestations in individuals with HCV infections.

Patients and methods

Patients. From 2000 to 2003, we studied a total of 224 adult inhabitants of O town, a hyperendemic area of HCV infection. All were HCV carriers, though the causes of viral transmission were unknown. In 2000, 2001, 2002 and 2003,

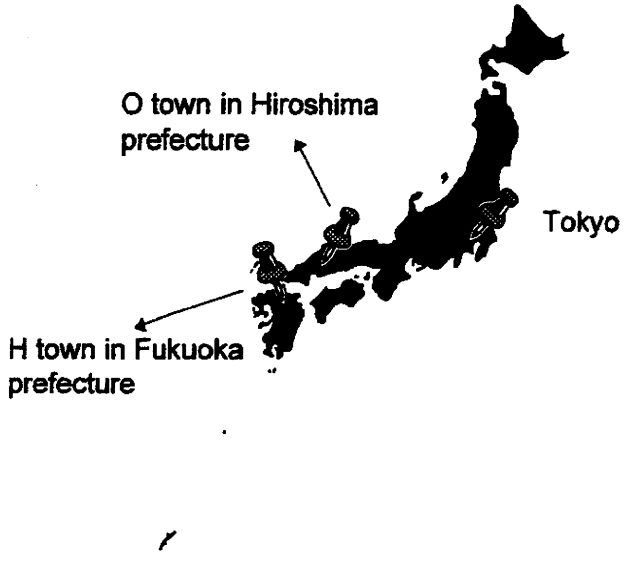

Figure 1. The research area. The location of O town in the northwest region of the Hiroshima prefecture in Honshu, Japan.

we examined 59, 54, 55 and 56 inhabitants, respectively (Table I). A single oral surgeon examined subjects for oral membrane diseases. A topographic classification of the oral mucosa, with location codes indicated, is shown in Fig. 2 (12). The diagnosis of OLP was made based on clinical and histopathological features.

All subjects were interviewed in person by 2 trained interviewers. We inquired about the following: cigarette smoking habits, present health condition and the presence of extrahepatic manifestations of HCV infection such as type 2 DM, rheumatoid arthritis, thyroid dysfunction, skin disease, renal disease, hypertension and extrahepatic malignant tumors.

Informed consent was obtained from all subjects once the purpose and methods of the study were explained.

Examination for anti-HCV and HCV RNA in serum. Sera were examined for the presence or absence of HCV. Anti-HCV were measured by a second-generation, enzyme-linked immunosorbent assay (Abbott HCV PHA 2nd Generation, Dainabot Co., Ltd., Tokyo, Japan). HCV RNA in the sera was detected using the Amplocore HCV test (Nippon Roche, Tokyo, Japan).

Examination of the prevalence of extrahepatic manifestations from 2000 to 2003. We have previously reported on the prevalence of extrahepatic manifestations in HCV infection, including OLP, for inhabitants of the same town (11). We now examined the prevalence of these extrahepatic manifestations from 2000 to 2003.

Results

Anti-HCV were detected in the sera of 224 subjects (100%) and HCV RNA in 210 subjects (93.8%), as shown in Table I. Of the 224, 88 had at least 1 oral examination over the course of the 4 years of the study (34 men and 54 women).

Table I shows the prevalence of OLP in all subjects. In 2000, 2001, 2002 and 2003 it was 8.5 (5/59), 14.8 (8/54), 20 (11/55) and 21.4% (12/56), respectively. The prevalence of OLP in HCV RNA positive subjects in 2000, 2001, 2002 and 2003 was 8.8 (5/57), 16 (8/50), 21.6 (11/51) and 23.1% (12/52), respectively. The prevalence of OLP increased with age. The incidence of OLP among all subjects with HCV infection over the 4-year period was 17.0% (15/88, 2 men and 13 women). A history of smoking was found in 1/15 OLP cases (6.7%) among inhabitants. Of the 15 cases, 2 had medical checkups once a year, 3 had them 3 times a year, 9 had them twice a year and 1 had a checkup just once in the 4-year period from 2000 to 2003 (Table II). No one had visited a clinic for the treatment of their OLP prior to our discovery of their OLP lesions. By far the most common site for OLP was the buccal mucosa. The predominant type in 53.3% of the 15 cases (8/15) was the reticular form of the disease. In 46.7% (7/15) it was the erosive form. Fig. 3 shows the erosive form (inhabitant No. 6 in Table II). Reticular lesions were generally asymptomatic. Two of the 15 cases had aggravated oral symptoms during the 4-year period. None experienced natural healing or developed malignant transformation.

From 2000 to 2003, there was an increase in the prevalence of type 2 DM, thyroid dysfunction, skin disease, renal disease and hypertension (Table I).

Discussion

HCV carriers in Japan are presumed to number 2 million (13). The growing incidence of HCC is expected to reach a plateau by around the year 2015. However, there are many people who are not aware that they are infected, some of whom will advance to liver cirrhosis or HCC (14). The incidence of HCC varies greatly among different regions. Epidemiological studies conducted by the Japanese Ministry of Health, Labour and Welfare showed that the mortality rate associated with HCC was high in several prefectures in Western Japan. Areas with high rates of anti-HCV, such as the Saga prefecture (3.9%), Hiroshima (1.8%), Fukuoka (1.7%) and Kagawa (1.7%), had high death rates for primary liver cancer of 43.1, 39.6, 39.8 and 31.9 per 100,000 people, respectively. These rates were higher than the national average (15).

HCV is associated with a wide range of extrahepatic manifestations. Zignego *et al* classified the extrahepatic manifestations of HCV into 4 main categories (16). The first category (A) includes extrahepatic manifestations characterised by a very strong association to HCV and supported by both epidemiological and pathogenetic evidence. Category A comprises mixed cryoglobulinaemia. The second category (B) includes disorders which are significantly associated with HCV infection, supported by adequate data. Category B comprises B-cell non-Hodgkin's lymphoma, monoclonal gammopathies, porphyria cutanea tarda and lichen planus. The third category (C) includes manifestations whose association with HCV still requires confirmation and/or a more detailed characterisation of similar pathologies of different aetiology or idiopathic nature. Finally, the fourth category (D) includes only anecdotal observations.

Lichen planus is a chronic inflammatory disease of the skin and mucous membranes that frequently involves the oral mucosa. In Japan, the age-adjusted incidence rate of OLP is 59.7 per 100,000 males and 188.0 per 100,000 females (17).

Table I. Prevalence of extrahepatic manifestations in adult inhabitants with HCV infection.

	2000	2001	2002	2003
Subjects	59	54	55	56
Age (mean years ± SD)	70.7±7.2	71.2±7.2	72.0±6.5	73.4±6.8
Sex (M/F)	21/38	22/32	23/32	24/32
% with history of smoking	18.6 (11/59)	11.1 (6/54)	12.7 (7/55)	14.3 (8/56)
% positive for anti-HCV	100 (59/59)	100 (54/54)	100 (55/55)	100 (56/56)
% positive for HCV RNA	96.6 (57/59)	92.6 (50/54)	92.7 (51/55)	92.9 (52/56)
Extrahepatic manifestations				
% positive for oral lichen planus	8.5 (5/59)	14.8 (8/54)	20.0 (11/55)	21.4 (12/56)
Age (mean years ± SD)	74.8±5.2	74.3±5.7	73.1±5.1	74.7±5.8
Sex (M/F)	1/4	2/6	2/9	2/10
% positive for anti-HCV	8.5 (5/59)	14.8 (8/54)	20.0 (11/55)	21.4 (12/56)
% positive for HCV RNA	8.8 (5/57)	16.0 (8/50)	21.6 (11/51)	23.1 (12/52)
% positive for DM	15.3 (9/59)	24.1 (13/54)	20.0 (11/55)	19.6 (11/56)
Age (mean years ± SD)	67.9±7.2	68.8±7.9	69.5±7.9	68.6±7.4
Sex (M/F)	5/4	10/3	8/3	7/4
% positive for anti-HCV	15.3 (9/59)	24.1 (13/54)	20.0 (11/55)	19.6 (11/56)
% positive for HCV RNA	14 (8/57)	20.0 (10/50)	15.7 (8/51)	15.4 (8/52)
% positive for rheumatoid arthritis	1.7 (1/59)	1.9 (1/54)	5.5 (3/55)	5.4 (3/56)
Age (mean years ± SD)	67.9±7.2	70.0±0	70.0±0.8	73.0±2.9
Sex (M/F)	5/4	0/1	1/2	1/2
% positive for Anti-HCV	15.3 (9/59)	1.9 (1/54)	5.5 (3/55)	5.4 (3/56)
% positive for HCV RNA	14 (8/57)	2.0 (1/50)	5.9 (3/51)	5.8 (3/52)
% positive for thyroid dysfunction	0	3.7 (2/54)	3.6 (2/55)	8.9 (5/56)
Age (mean years ± SD)	-	67.0±1.0	68.0±1.0	72.0±3.3
Sex (M/F)	-	1/1	1/1	1/4
% positive for anti-HCV	-	3.7 (2/54)	3.6 (2/55)	8.9 (5/56)
% positive for HCV RNA	-	4.0 (2/50)	3.9 (2/51)	9.6 (5/52)
% positive for skin disease	5.1 (3/59)	11.1 (6/54)	7.3 (4/55)	16.1 (9/56)
Age (mean years ± SD)	70.3±7.3	71.3±5.8	70.8±5.2	74.1±5.9
Sex (M/F)	0/3	1/5	1/3	4/5
% positive for anti-HCV	5.1 (3/59)	11.1 (6/54)	7.3 (4/55)	16.1 (9/56)
% positive for HCV RNA	5.3 (3/57)	12.0 (6/50)	7.8 (4/51)	15.4 (8/52)
% positive for renal disease	1.7 (1/59)	5.6 (3/54)	0	1.8 (1/56)
Age (mean years ± SD)	76.0±0	76.0±2.2	-	86.0±0
Sex (M/F)	1/0	1/2	-	1/0
% positive for anti-HCV	1.7 (1/59)	5.6 (3/54)	-	1.8 (1/56)
% positive for HCV RNA	1.8 (1/57)	6.0 (3/50)	-	1.9 (1/52)
% positive for hypertension	28.8 (17/59)	40.7 (22/54)	43.7 (24/55)	55.4 (31/56)
Age (mean years ± SD)	71.0±6.9	70.9±6.3	72.6±6.0	74.4±6.7
Sex (M/F)	6/11	7/15	10/14	13/18
% positive for anti-HCV	28.8 (17/59)	40.7 (22/54)	43.6 (24/55)	55.4 (31/56)
% positive for HCV RNA	26.3 (15/57)	42.0 (21/50)	41.8 (13/51)	57.7 (30/52)
% positive for extrahepatic malignant tumor	11.9 (7/59)	13 (7/54)	9.1 (5/55)	7.1 (4/56)
Age (mean years ± SD)	74.4±3.5	76.3±3.7	77.2±4.2	79.3±2.2
Sex (M/F)	2/5	3/4	3/2	3/1
% positive for anti-HCV	11.9 (7/59)	13 (7/54)	9.1 (5/55)	7.1 (4/56)
% positive for HCV RNA	12.3 (7/57)	14 (7/50)	9.8 (5/51)	7.7 (4/52)