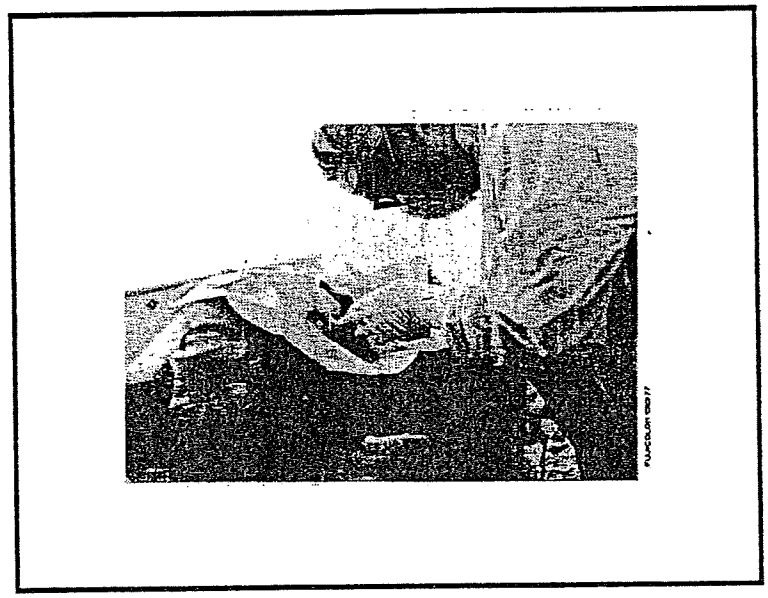
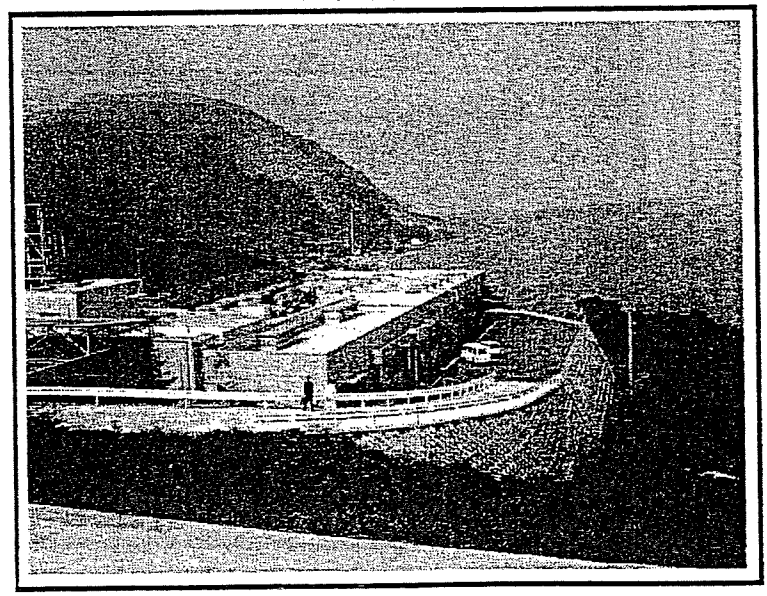
これは、1970年代に東京の真中（東京都立臨床医学総合研究所の7階）で感染実験をしていた頃の、30歳代の私です。30歳代の初めから半ば過ぎまでの6年半にわたってチンパンジーを相手に実験をしていました。

スライド24



これが現在、感染実験を委託している熊本の三角半島にあるチンパンジーのコロニーの実験棟です。感染材料は、その都度現地まで出かけて、自分で稀釈して準備し、接種以降の採血は実験担当者にお願いしています。

スライド25

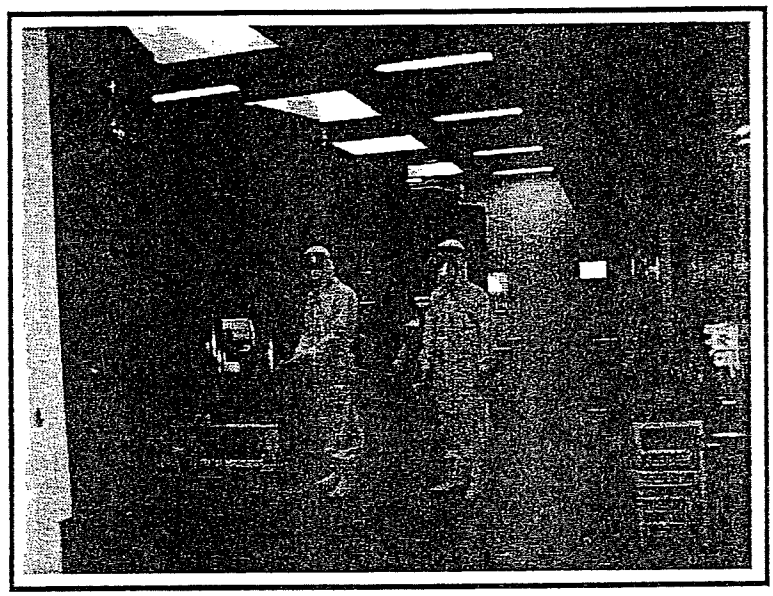


実験棟の中の、感染実験を行なっている室の様子です。

1970年当時の私達の実験室と違って広々としており、実験環境は大分よくなっています。

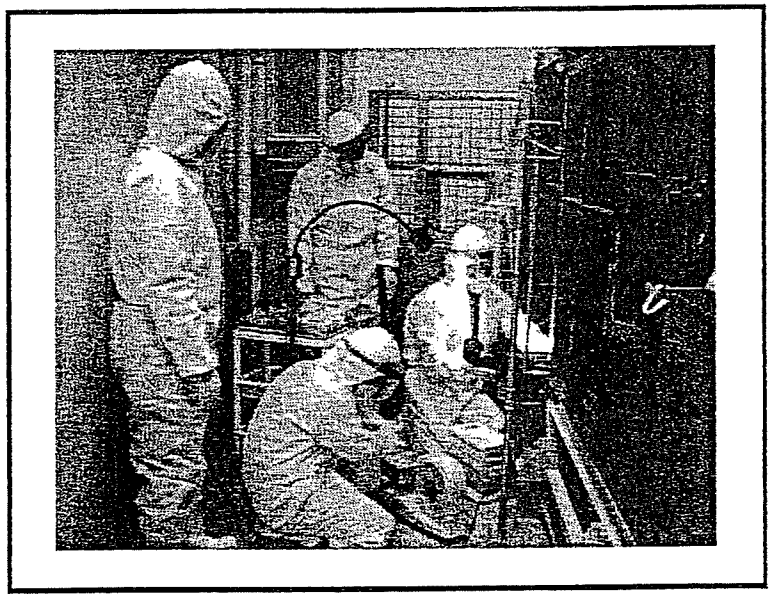
実験担当者はこのように完全武装の状態で、実験者自身の安全性の確保はむろんのこと、感染個体から別の個体への汚染の伝播（carry over）も完璧に抑えた状態で実験を行ないます。

スライド26



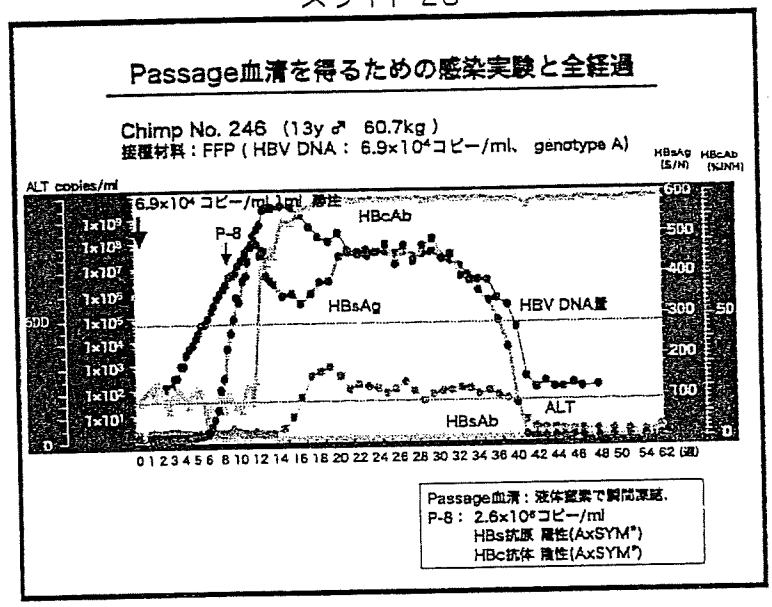
いまや、私はただ立って見ているだけの人になってしまったわけです。これは実験担当者が3人で手分けして、ヒトと同じ方式で、血液バックを使って採血をしているところです。

スライド27



まず、実験は感染性の減弱を最少限に抑えた「HBV 感染早期」の感染材料を入手することから始めます。このために、過剰の HBV を接種して感染を成立させた後に、宿主の HBc 抗体が出現する前の段階で、かつ、HBV DNA 量ができるだけ多くなった時期（この実験では接種後８週目）の血液を採取し、すみやかに血清を分離、少量（1.1ml）ずつに分注した後に、液体窒素にて瞬間凍結し、そのまま－80℃のディープフリーザー中に保存しました。

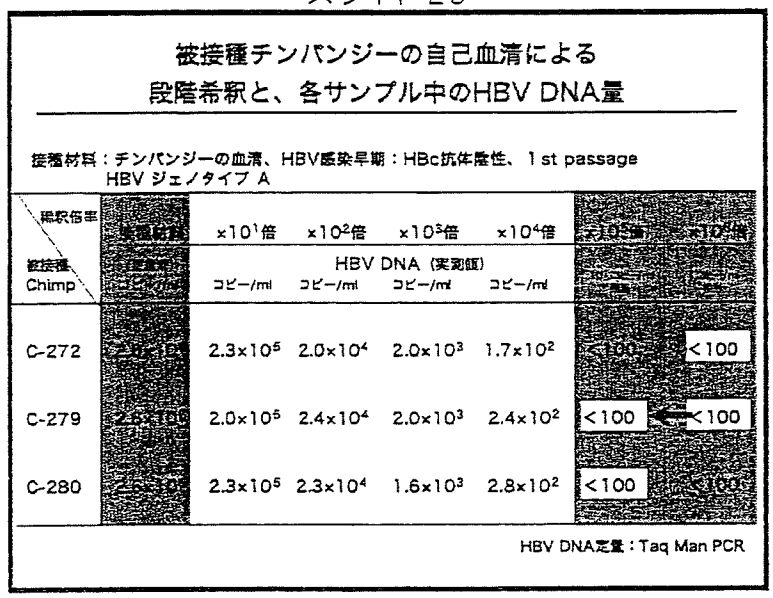
スライド 28



感染材料（HBV のジェノタイプ A、HBV DNA 量 2.6×10^6 コピー/ml、HBc 抗体陰性）を 37℃温浴にて穏やかに融解し、あらかじめ採血し、保存しておいた、実験に用いるチンパンジーの自己血清を用いて 10^N 倍に段階稀釈します。稀釈後、各３本に分注した後に液体窒素にて瞬間凍結し、-80℃のディープフリーザー中に保存します。

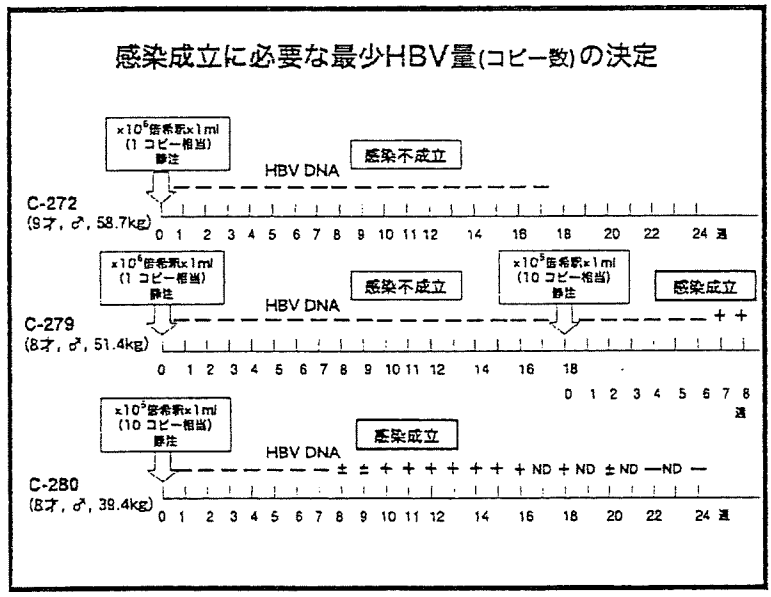
各３本のうちの１本を用いて、HBV DNA 量を定量し、10^2 コピー/ml 相当（10^4 倍稀釈）まで、正しく稀釈されていることを確かめた上で、10^5 倍稀釈のもの（10 コピー/ml 相当）、10^6 倍稀釈のもの（1 コピー/ml 相当）を接種材料として選び、各３本のうちの２本目を 37℃温浴にて穏やかに融解し、それぞれを、図に示した順序に従って３頭のチンパンジーの静脈内に、正確に 1ml ずつ注射して経過を観察しました。

スライド29



その結果、HBV DNA量に換算して1コピー相当のHBVを接種した2頭のチンパンジーでは感染は成立しなかった（C-272、およびC-279、1回目の接種）のに対して、10コピー相当のHBVを接種した2頭のチンパンジー（C-280、C-279、2回目の接種）では接種後7～8週目に末梢血中にHBV DNAが出現し感染の成立が確認されました。

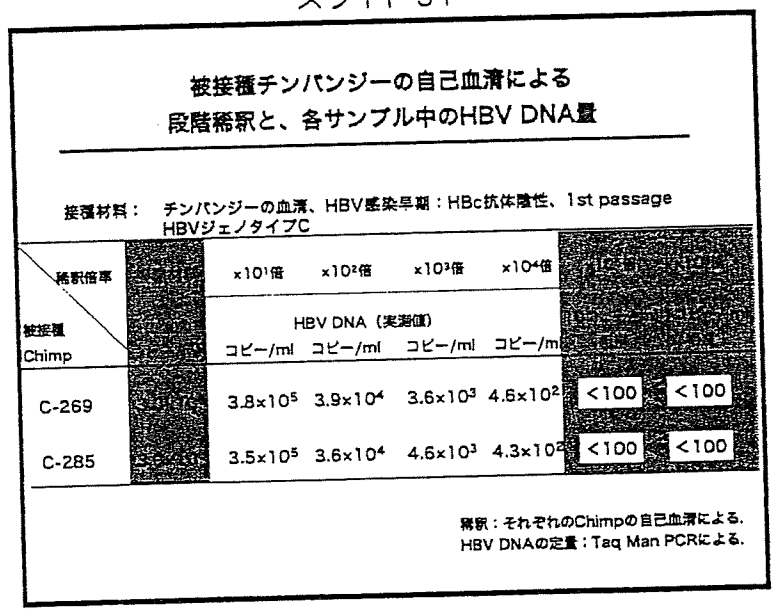
スライド30



また、次の感染実験では、同様に準備したジェノタイプCの感染材料（HBV感染初期のヒト新鮮凍結血漿接種後29日目のチンパンジー由来の血清、HBV DNA量3.0×10^6コピー/ml、HBc抗体陰性）を同様に37℃の温浴にて融解後、それぞれのチンパンジーの自己血清で10^N倍に段階稀釈し、稀釈した3本のうちの1本を用いてHBV DNA量を定量しました。

3本のうちの2本目を37℃温浴にて穏やかに融解し、今度は、2頭のチンパンジーを用いて、図に示した順序に従って、それぞれを正確に1mlずつ静脈内に注射して経過を観察しました。

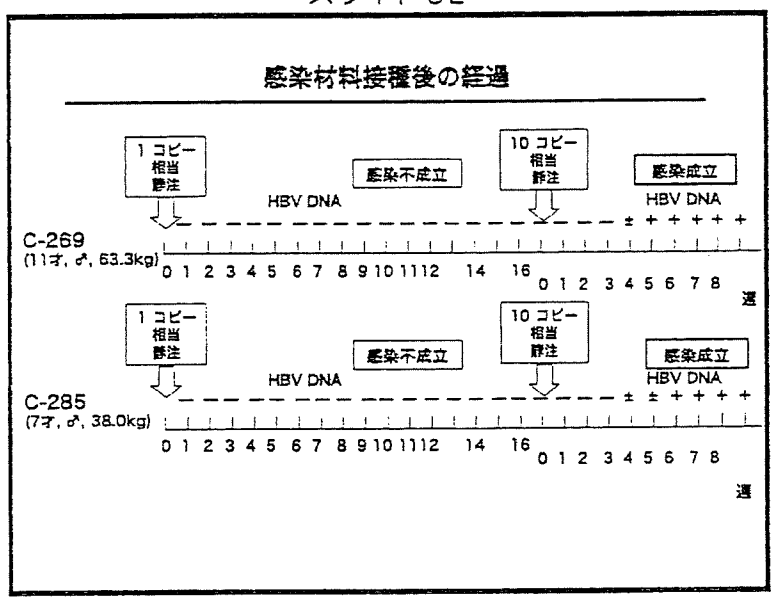
スライド31



その結果、1コピー相当のHBVを接種した1回目の実験では、2頭とも感染は成立せず、10コピー相当のHBVを接種した2回目の実験で、接種後5週～6週目に、末梢血中にHBV DNAが出現し、2頭とも感染の成立が確認されました。

ここまでの実験で、感受性の減弱を最少限に抑えた「HBV感染早期の血清」を用いた場合、感染成立に必要なHBV DNA量に換算したウイルス量は、HBVのジェノタイプA、もしくはCにかかわりなく10コピー相当であることが明らかとなりました。

スライド32

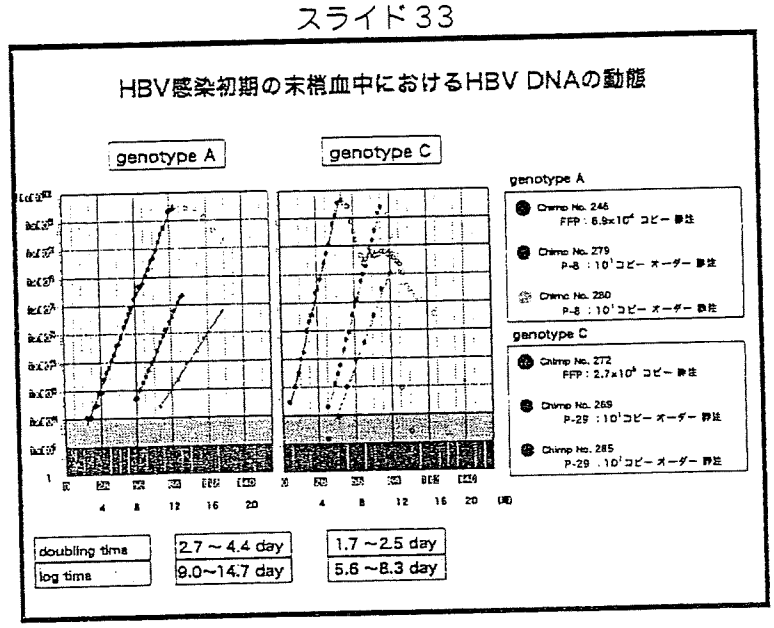


感染成立後の末梢血中における HBV DNA が 2 倍量に増えるために必要な時間（doubling time）を求めるとジェノタイプ A の HBV の場合は 2.7 日～4.4 日、ジェノタイプ C の HBV の場合は 1.7 日～2.5 日となりました。同様に 10 倍量に増えるために必要な時間（log time）を求めると、ジェノタイプ A の HBV の場合は 9.0 日～14.7 日、ジェノタイプ C の HBV の場合は 5.6 日～8.3 日となることがわかりました。

また、感染成立に必要な最少 HBV 量（10 コピー相当）を接種した場合の核酸増幅検査（NAT）のウインドウ期間（末梢血中の HBV 量が 10^2 コピー/ml に達するまでの期間）は、ジェノタイプ A の HBV の場合は 8 週～11 週、ジェノタイプ C の HBV の場合は 5 週～7 週であることが明らかとなりました。

一方、検出感度の高い酵素抗体法（EIA 法）で検出した場合の HBs 抗原のウインドウ期間は、ジェノタイプ A の HBV の場合は 10 週～14 週、ジェノタイプ C の HBV の場合は 7 週～9 週であることが明らかとなりました。

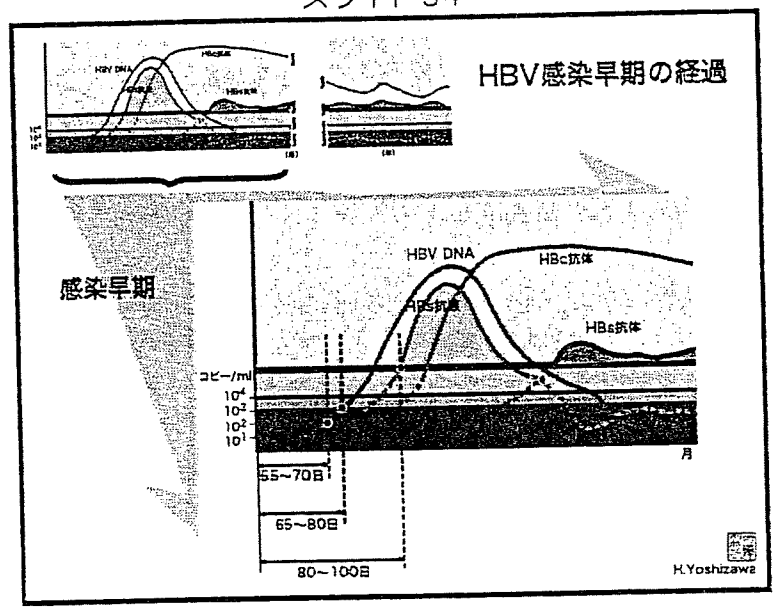
スライド33



感染実験によって得られた結果をもとに、「HBV感染早期」の経過をまとめるにあたっては、安全を見込むために増殖速度が遅いジェノタイプAのHBVを感染させて得られたデータをもとに作図することが望ましいと考えられます。

そうしますと、HBVに感染してから、日赤血液センターにおいて日常検査として行われている核酸増幅検査（ミニプールNAT）によるHBV DNAのウインドウ期間は65日～80日を、最も検出感度が高いEIA法によるHBs抗原のウインドウ期間は80日～100日を見込む必要があるということになります。

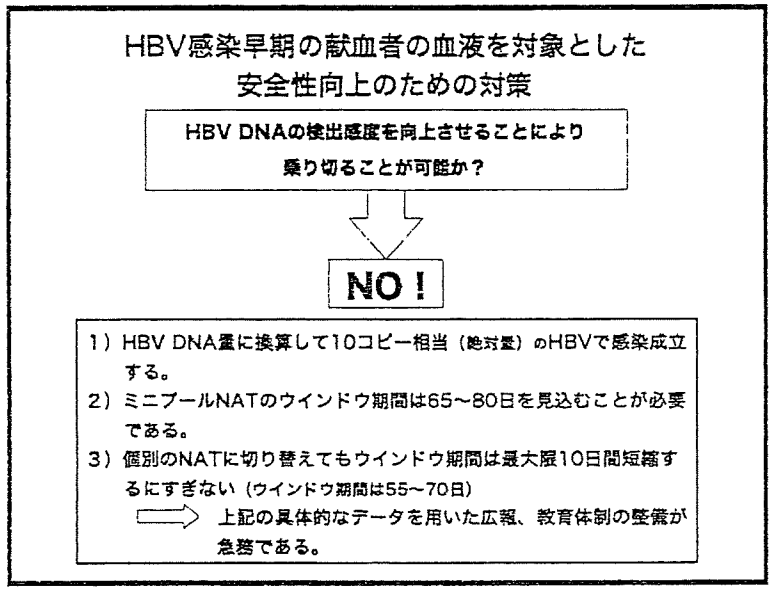
スライド34



つまり、結論はこういうことになります。すなわち、「HBV感染早期」の血液を対象とした場合、核酸増幅検査（NAT）によるHBV DNA検査も含めて、検査によって血液の完璧な安全性を確保することは、そもそも不可能であるということです。

従って、残された方法は、感染実験によって得られたデータも含めて、これまでに話した具体的なデータを用いた広報と教育を徹底して行うことに尽きると言うことになります。

スライド35



これまでの話をまとめるとこうなります。

日赤血液センターが自主的に行った調査から、以下のようなことがわかりました。すなわち、わが国では年間約100万人が輸血を受け、このうちの約50万人が輸血後1年以上にわたって生存することができている。この約50万人の中で、最大限に見積もっても年平均20人前後の輸血後B型肝炎が発生しているにすぎず、その感染源のほとんどは「HBV感染早期」に献血された血液であるということです。

このわずかに残っている輸血後肝炎の発生数を1例でも減らすためには、3つのHBV感染の態様を十分に理解した上でそれぞれの態様に則した合理的な対策を立て、実施に移す必要があるということになると思います。

そして、検査によってはどうしても捉えることができないHBV感染早期の献血者の血液に対する対応としては、HBV感染早期の自然経過を基とした具体的なデータを用いた広報、教育を徹底して行う以外に方法は無いということになると思います。

スライド36

まとめ

1）輸血後B型肝炎の現状

HBs抗原検査、HBc抗体検査、NATによるHBV DNA検査を組み合わせたスクリーニングの導入により、わが国における輸血後B型肝炎の発生数は最大限に見積もっても年間20例前後にまで減少したと推定される。

2）輸血後B型肝炎の更なる減少のために

HBV感染の特性に基づいた合理的な対策の樹立

（1）（定型的な）HBV持続感染者（HBVキャリア）

現行のHBs抗原検査のみで充分対応が可能

（2）（非定型的な）HBV持続感染者（HBV感染既往/感染晩期）

HBc抗体価の測定とHBV DNA検査（NAT）を合理的に組み合わせたスクリーニングの実施

（3）HBV感染の新規発生（HBV感染の早期）

現行のHBs抗原とHBV DNA検査（NAT）を組み合わせたスクリーニングの実施

献血者を対象とした安全教育の実施

・HBV感染の回避、検査を目的とした献血の停止など

3）今後の目標

輸血後B型肝炎の発生ゼロは関係者の共通の到達目標である。

最後に、今回用いたデータは、スライドに示す３つの研究グループに所属する人々を始めとする多くの共同研究者の10年以上にわたる献身的な努力によって得られたものであることを付け加えておきたいと思います。

長時間にわたり、御清聴ありがとうございました。

スライド37

謝　辞

本研究を共に推進した下記の方々に深く感謝いたします。

1．日本赤十字社　NAT研究グループ

Yuji Hoshi BS	JRC Tokyo NAT center
Hiroyuki Emura BS	JRC Hokkaido NAT center
Eiko Mine PhD	JRC Kyoto NAT center
Hiroyuki Murokawa BS	JRC Tokyo NAT center
Hisao Yugi PhD	JRC Tokyo NAT center : chief

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Shigeru Tamatsukuri PhD	Roche Diag. KK. Japan
Tohru Yamada PhD	Dinabott Co. Ltd. Japan
Hidetaka Minagewa PhD	Fujirebio Inc. Japan
Masaaki Mizui BS	JRC Hiroshima Blood Center
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Hiroshi Yoshizawa MD	Hiroshima University : chairman

3．厚生労働省　ウイルス肝炎の疫学研究班

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Keiko Katayama MD	Hiroshima University
Yutaka Komiya MD	Hiroshima University
Junko Tanaka PhD	Hiroshima University
Hiroshi Yoshizawa MD	Hiroshima University : chairman

【飯野】どうもありがとうございました。会場のほうから，どなたかご質問はありませんか。

実際，以前は健康保険の制約がいろいろあって，輸血後 virus marker の検査ができないということがありましたが，実際的にはＢ型肝炎というのは，感染して，トランスアミラーゼが 1,000 程度上がっても，自覚症状がない感染者，そしてそのまま治っていく。あるいは最近の例ですと，ジェノタイプＡだとそのままキャリア化するのが現実にはいるので，輸血後で発生するＢ型肝炎は，つかまらないのがもう少し多いのかもしれませんね。

ですから，保険のほうで制約があったから，輸血後でも臨床の先生は，そうでなくても検査しないのですが，それを徹底するようなことも，輸血後肝炎の実態をはっきり知るためには必要ですね。

【吉澤】その通りだと思います。

輸血前、後のウイルスマーカーの検査については、平成 17 年（2005 年）3 月に厚生労働省から「血液製剤等に係る遡及調査ガイドライン」が出され、HBV、HCV、HIV の検査については、（原則として）保険で認められることになりました。

ただ、モデル地区を選んで、pilot 的に全数調査をやってみると、受血者の 2.2%（22/1,021）は輸血前から HBV DNA が陽性、すなわちキャリアであること、また、8.6%（86/1,000）は輸血前から HCV RNA が陽性、すなわち HCV キャリアであることがわかりました。

ですから、受血者の検査をする際には、必ず輸血前の血清を保存しておくことが必要です。ちなみに、輸血後に HBV が陽転したのは 9 例（0.9%）、HCV が陽転したのは 2 例（0.2%）でしたが、このうち輸血に用いた血液が感染源になったことが立証されたのは HBV が陽転した 1 例だけでした。

輸血が原因で、HBV、HCV 又は HIV に感染したとの因果関係が立証された場合には、救済制度が設けられています。

【飯野】キャリアというか，非常に幅の広い意味のキャリアですね。既感染者のウイルスの動態は，常に増えたり減ったりしているわけですね。免疫状態，ＨＢｓ抗体の状態と思いますが，抗体が減ってくればウイルスが表に出てくるという状態は，ある意味では始終起こっている現象かもしれませんね。

ですから，そこのところの問題ですが，先生がちょっとおっしゃいましたが，日赤が 50 本プールから 25 本プールにしましたね。個別ＮＡＴでも無理な話で，それはお金が倍かかっているわけで，そこで得られるメリットは微々たるものですね。

【吉澤】メリットと言える程のものは得られないと思います。

HBV DNA の検出は桁の問題ですので…。

【飯野】「微々たる」と言ったのは，吉澤先生と同じ意味ですが。

【吉澤】はい。

【飯野】そのお金の分で，もう少し有効に使うべきで，50 本に戻したほうが……。500 本から 50 本というのは，そこの領域は意味があるのだけれども，そのあとは 2 倍しか上がらないので，ほとんどナンセンスですよね。そこに属する集団は，ほとんどない

わけですから。50本に戻そうという話はないのですか。こんなばかばかしいことはやめようと。

【吉澤】今のところはないようです。

今日のような話を聞いていただいて、多くの方々に理解してもらうしか方法はないのかも知れません。そんなことを考えて、今日、この時間をいただいたわけです。

【飯野】会場のほうからどなたかありませんか。いろいろな方には，感覚的にわかっても，すべてクリアにはなかなかわかってもらえない。特に行政の方にはそうだろうと思いますが，どなたかいかがでしょうか。

【会場】HBc抗体陽性（HBs抗原は陰性）の血液の輸血によりHBVに感染した場合、受血者が劇症肝炎になる頻度は高いのでしょうか。

【吉澤】日赤血液センターが行なった遡及調査により見出された輸血後B型肝炎例でみる限り、必ずしも全ての例が劇症化するとは限らないようです。

一方、HBc抗体測定によるスクリーニングが導入された1989年11月以前にB型劇症肝炎と診断された患者では、輸血が原因となっていたことを推定させる例が比較的多くみられたと言われています。

ですから、ご質問の件については、まだはっきりした答は得られていないというのが実際だろうと思います。

【飯野】ＨＢｃ抗体の200倍というのは頭から一切除いて，ＨＢｃ抗体というものをもう少し広く測定をして，ＨＢｃ抗体陽性者については，特にステロイドホルモンを含む免疫抑制剤を使う。これは極端な場合だけはいま注目されていますが，悪性腫瘍などで免疫抑制剤，抗腫瘍剤を使うとき，一般的な他の人たちでも結局同じことですよね。

【吉澤】そうです。

【飯野】そういうときに，ウイルスのreactivationを引き起こしているということを，みんなが認識しなければいけない時代だと思うのですが，どうでしょうか。

【吉澤】その通りだと思います。

occult infectionとか、低濃度キャリアとか、非定型的なキャリア状態とか、これまで様々な名前で呼ばれてきましたが、ウイルス学的には「HBV感染晩期」の状態という名前で1つにくくることができるのではないかと思います。この状態にある人は、HBc抗体の力価にかかわらず、肝細胞の中にHBVが存在し続けているわけですから、どのような原因であれ、生体側の免疫が低下すればreactivationを引きおこすということを認識した上で経過を見ていただくことが大事だと思います。結核の石灰化巣とreactivationとの関係を思いうかべるとわかりやすいのではないでしょうか。

ただし、一般には急性B型肝炎あるいは定型的なキャリア状態から離脱して臨床的に治癒した後は、ほとんどの人はそのまま健康な状態で生涯を全するわけですから、いたずらに不安をあおることは必要のないことだろうと思います。

【飯野】吉澤先生，長時間ありがとうございました。

【雑　　誌】

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National Prevention of Hepatocellular Carcinoma in Japan Based on Epidemiology of Hepatitis C Virus Infection in the General Population

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Key Words
Chronic hepatitis · Cirrhosis · Epidemiology · Hepatitis C virus · Hepatocellular carcinoma · Prevention · Transfusion

Abstract
During the past 30 years, hepatocellular carcinoma (HCC) in Japan has kept linearly increasing from 10 to 30 per 100,000 population per year and is expected to grow further. The increment is attributed to infection with hepatitis C virus (HCV). Hence, there is a pressing need to find subjects with persistent HCV infection in the general population of Japan and take necessary measures to prevent HCC developing in them. As a first approach toward this goal, the sex- and age-specific prevalence of ongoing HCV infection was surveyed in 3,485,648 first-time blood donors during 1995–2000. Taking into account the size of subpopulations with different sex and age in Japan registered at the Census 2000, there are an estimated 884,954 HCV carriers aged from 16 to 69 years, and 759,316 (86%) of them are older than 40 years, with an increased risk for HCC; they are hidden in the society, without overt liver disease. The national 5-year project searching for HCV carriers in the general population was started in April 2002. Subjects are examinees of health check-ups, which they receive every 5 years when reaching the age of 40, as well as those at increased risk for HCV infection. The project detected HCV RNA in 14,672 of the 1,298,746 (1.1%) health check examinees and in 16,721 of the 624,734 (2.7%) high-risk individuals during the first fiscal year. Subjects found with HCV RNA have been referred to clinics and hospitals with expert hepatologists. Hopefully, this project will decrease HCC development in HCV carriers in Japan and be considered in other countries where increases in HCC are predicted from the current age-specific prevalence of anti-HCV.

Introduction

Hepatocellular carcinoma (HCC) ranks as the fourth most frequent cause of death due to malignancy in the world. HCC in men is the third most frequent malignancy in Japan, only next to lung and stomach cancers, while it is the fourth in women, following stomach, colon and lung cancers. The vast majority of patients with HCC are persistently infected with hepatitis B virus (HBV) or hepatitis C virus (HCV). The roles of HBV and HCV in the development of HCC vary widely in different countries and have changed with time. On the global scale, HBV

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induces HCC much more frequently than HCV. There are an estimated 350 million HBV carriers (corresponding to 6% of the world population) [1], which is twice as many as 190 million who are presumed with persistent HCV infection [2].

However, several lines of epidemiological and clinical evidence predict that the role of HCV will increase and exceed that of HBV in the future. First and foremost, there is no means of preventing HCV infection with vaccines, unlike the infection with HBV that has been prevented since 1980 by hepatitis B vaccine [3]; it is proven to have a long-term effect in suppressing HCC associated with HBV infection [4]. Secondly, HCV infection can persist in 80% of adults parenterally exposed to it [5], in contrast to only a few percent of HBV infection in the adulthood that becomes chronic [6]. Thirdly, HCV infection prevails globally and keeps spreading, in remarkable contrast to HBV infection that is restricted to Asia and Africa. Finally, new HBV infections have been prevented by the global mass vaccination campaign advocated by the World Health Organization.

Hence, there is every reason to believe that HCV will have an ever increasing role in the development of HCC anywhere in the world where HCV prevails. However, it is not easy to foresee with a reasonable precision when and how often HCC develops in persistently infected individuals. The natural history of HCV infection is poorly defined [7, 8]. It varies widely according to the influence of diverse host and virus factors, of which the time factor is most important. It is presumed that 30 years elapse before HCC develops in the recipients of transfusion contaminated with HCV [9, 10]. However, the incubation time for HCC may not be constant in view of the velocity of fibrosis that differs widely by many factors, including the age at infection and gender [11–13].

Despite these differences in the development of HCC after HCV infection, a tendency would emerge if many HCV carriers are observed macroscopically en masse, in a national scale for instance. Furthermore, observing trends in HCV infection and associated HCC would help predict what will happen in the future and allow taking measures to prevent HCC arising in HCV carriers. Considering the Japanese as a whole, there has been a unique tendency for HCC during the past 50 years. Remarkably, the yearly incidence of HCC started to increase abruptly in 1975, and the role of HCV has kept increasing; now, it surpasses that of HBV by a margin of fourfold [14].

Driven by the need to comprehend how many people are at risk of developing HCC, we surveyed persistent infections with HBV and HCC in 3,485,648 first-time blood donors during 1995–2000 in the 8 jurisdictions of the Japanese Red Cross Blood Centers [15]. Based on the sex- and age-specific prevalence of infection, there are an estimated 759,316 HCV carriers in Japan who are older than 40 years and at increased risk of developing HCC. These trains of evidence have strongly indicated that HCC deaths in Japan will keep increasing at least until 2010, then flatten out and decline. This instigated doctors, as well as the personnel in the Ministry of Health, Labour and Welfare of Japan, to launch a 5-year project (which started in April 2002) to spot HCV carriers older than 40 years in Japan, urging them to take medical examination and receive treatment as required.

Geographical Distribution of Deaths due to HCC in Japan Shifting from 1975–1980 through 1991–1995 toward 1996–2000

Figure 1 pictures the distribution of deaths due to HCC examined over three 5-year ranges. Japan consists of four major islands, with Hokkaido situated up in the north, the mainland lying from northeast to southwest, accompanied by Shikoku down in the south and Kyushu in the west. There are 48 jurisdictions which are further broken down into many cities and villages. The standardized mortality rate (SMR) due to HCC in each of the 3,212 municipalities was determined by the Bay's method. The mortality rate of each municipality was classified as follows: highest (SMR >140, red), higher (SMR 140–120, orange), medium (SMR 120–80, yellow), lower (SMR 80–60, green) and lowest (SMR <60, blue). During 1971–1975, the annual incidence of HCC was pretty much the same all over Japan with most municipalities colored in yellow with the average frequency, although some areas in the southern Kyushu were in red with the highest incidence. During 1991–1995, the coloring became uneven, with areas in red or orange increasing all over Japan. The trend for this uneven distribution is further intensified on the last survey during 1996–2000. Although municipalities in green or blue increased in number, this by no means represents the incidence of decreased HCC. On the contrary, yearly incidence of HCC as well as the average total deaths due to HCC more than tripled during the three surveys covering 30 years from 1971 to 2000. Overall, the coloring highlights the incidence of HCC in Japan that decreases from Kyushu in the southwest, along the axis of Honshu toward Hokkaido in the northeast.

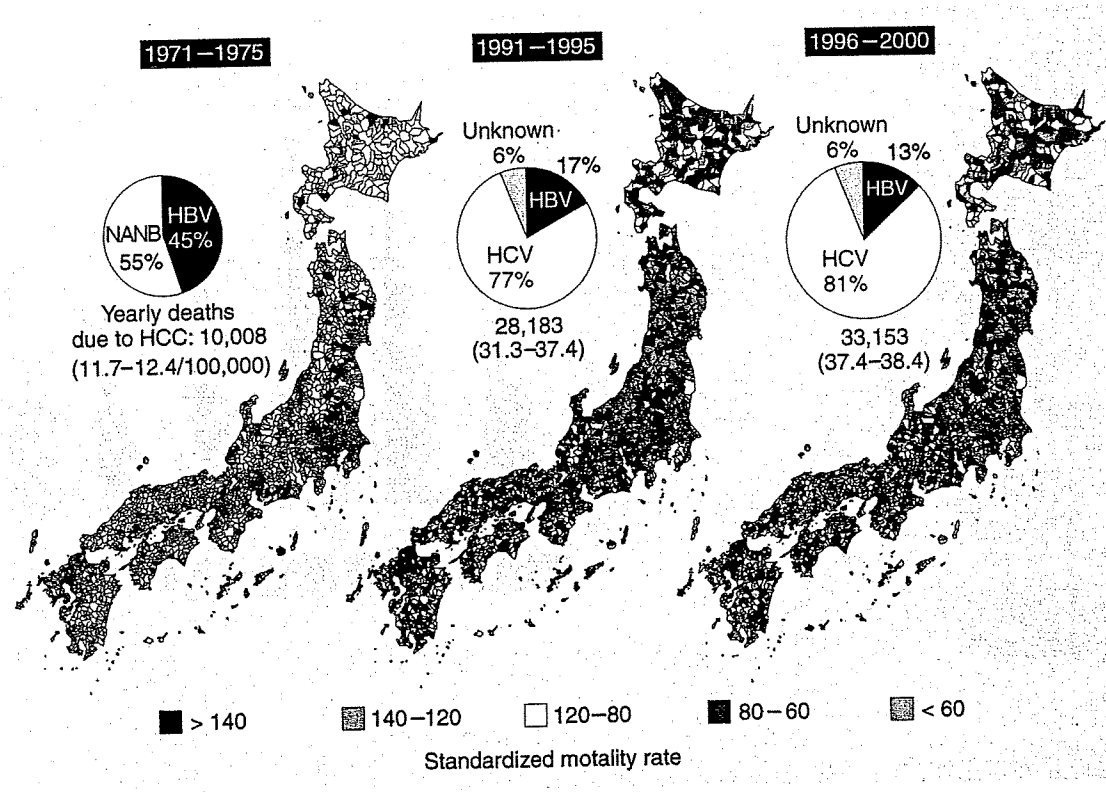

Fig. 1. Changes in the geographical distribution of deaths due to HCC in men during the past 30 years in 3,212 municipalities in Japan. Etiology of HCC is shown in a pie graph in size reflecting the yearly HCC during the survey period, with the average yearly deaths during the 5 years below and deaths due to HCC in 100,000 population per year in parentheses. Maps were produced by Dr. Yoshihiko Mirua of Saitama Prefectural University.

The center of Honshu over the skirt of Mount Fuji is an exception to this gradient. The incidence of HCC in Yamanashi prefecture stands out in red. This is due to the endemic *Schistosoma japonicum* along the Fuji River running through Yamanashi. Intravenous injection with antimony sodium tartar, performed during 1923–1980, spread HCV among patients infected with this parasite through repeated intravenous injections with insufficiently sterilized needles and syringes [16]. Similar endemic *S. japonicum* and iatrogenic diffusion of HCV happened in Saga and Fukuoka (both in northern Kyushu) and Hiroshima (in the western tail of Honshu) [17]. This was also the case along the riverside of the Nile in Egypt where *Schistosoma mansoni* once prevailed [18]. In Japan, parenteral antischistosomal treatment started to locally create small cores of HCV infection since the 1920s, and a more robust outbreak of HCV infection occurred since the end of World War II in 1945, through the 1960s to the 1970s [14].

Uneven geographical distribution of HCC in Japan, increasing with time (fig. 1), reflects the prevalence of HCV infection that differs regionally. Age-specific prevalence of HCV infection in the 3,485,648 first-time blood donors during 1995–2000 is shown in figure 2, individually for the 8 jurisdictions of the Japanese Red Cross Blood Center [15]. The frequency of HCV infection is higher in the four jurisdictions in the southwest than the other four in the northeast; there is a trend in the frequency of HCV infection, showing a decreasing gradient through the axis of Honshu from Hiroshima to Tohoku. The pattern matches the distribution of HCC in Japan at the last survey during 1995–2000, depicted in figure 1.

There are marked differences in the age-specific prevalence of HCV infection in every district (fig. 2). HCV is least prevalent in the age group of 16–19 years, gradually increases in the age group of 50–59 years and is highest in the 60–69-year-olds. Looked at more closely, in blood donors divided by a 1-year notch, the increase of HCV infection with age is even more conspicuous and reaches

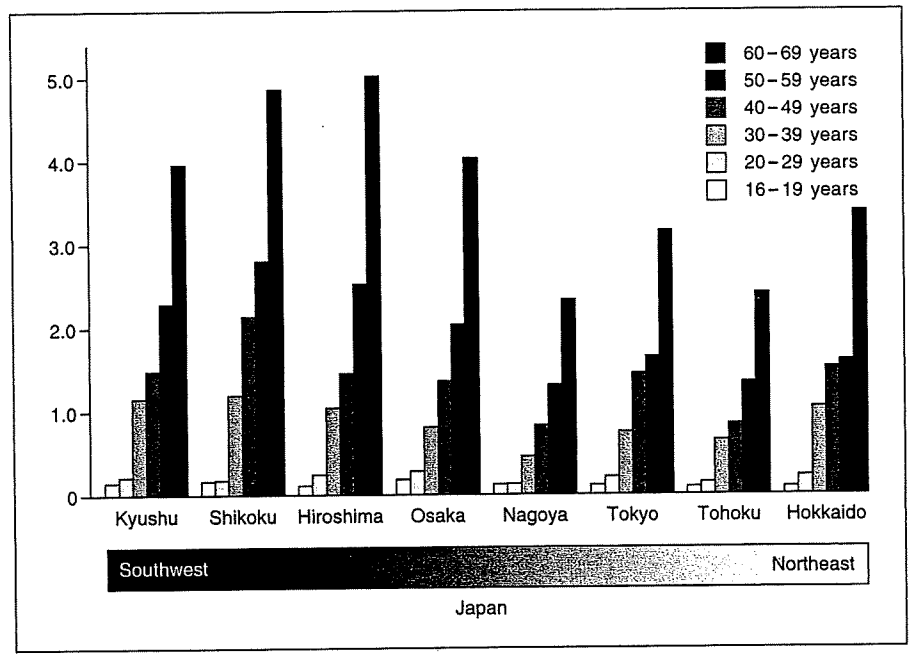

Fig. 2. Age-specific prevalence of anti-HCV in a large-scale survey on the first-time blood donors in eight districts of Japan in the order from southwest to northeast.

11% in those aged 70 years, extrapolated to the year 2000 (fig. 3). In Japan, in the year 2000, the number of total HCV carriers aged from 16 to 64 years who were eligible for blood donation was estimated at 884,954, 95% confidence interval (95% CI) 725,082–1,044,826, among 93,325,570 (0.95%) individuals aged 15–69 years [15]. They comprised 464,363 (95% CI 377,927–550,799) of the 46,638,636 (1.00%) men and 420,591 (95% CI 347,156–494,027) of the 46,686,934 (0.95%) women of the same ages. They were calculated as the sum of sex- and age-specific prevalence of HCV infection multiplied by the subpopulation with the corresponding sex and age. Thus, there are at least 759,316 HCV carriers older than 40 years who would be at increased risk of developing HCC.

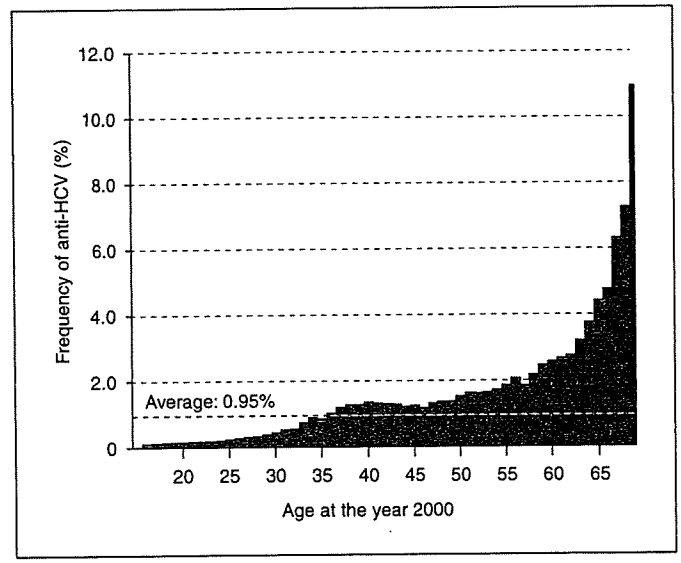

Fig. 3. Age-specific distributions in the 884,954 first-time blood donors with anti-HCV in Japan [modified from ref. 15].

Liver Disease in Blood Donors Found with Ongoing HCV Infection at the Screening

Liver disease induced by persistent HCV infection is insidious and progresses slowly at the speed differing according to various factors. Therefore, patients with HCV-associated liver disease are not aware of it before they develop signs and symptoms of cirrhosis or HCC, which even then can be missed for years due to ample reserve in the life-maintaining capacity of the liver. Hence, most HCV carriers are asymptomatic, as those typically identified among blood donors. During 10 years, from 1991 to 2000, 912 apparently healthy individuals were found to be persistently infected with HCV at a screening for blood donation at the Japanese Red Cross Blood Center in Hiroshima. They consulted doctors and were clinically examined [19]. It does strike us as a surprise that chronic

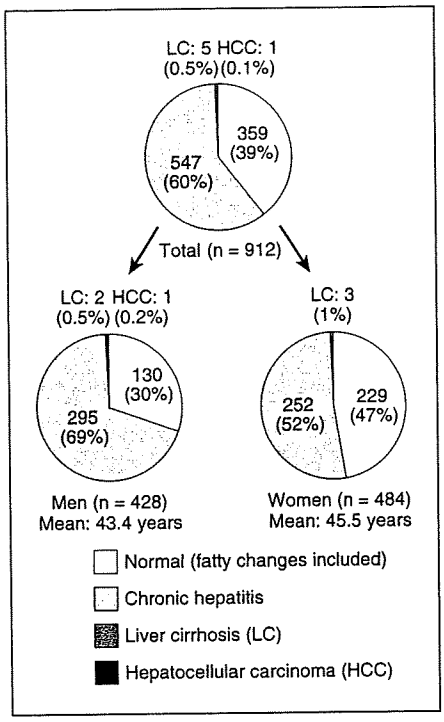

Fig. 4. Distribution of liver disease in 912 blood donors in Hiroshima who were found with HCV infection.

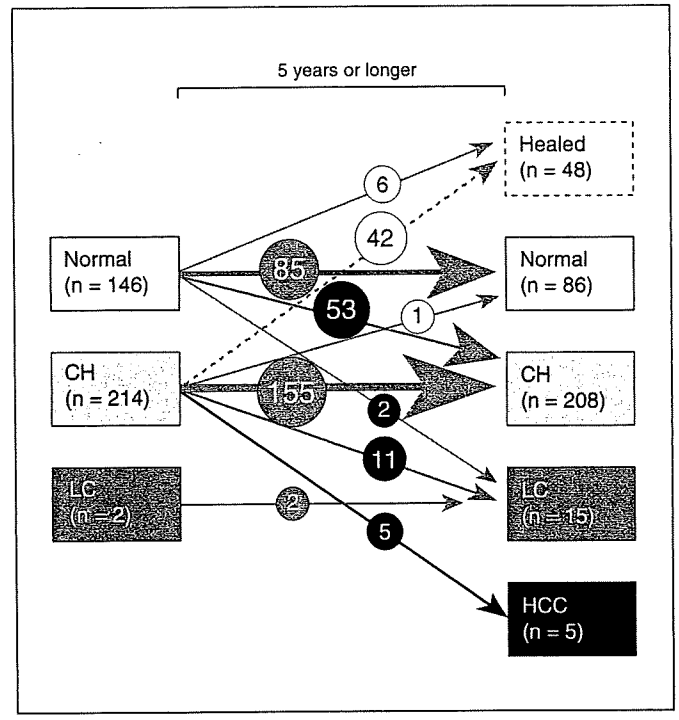

Fig. 5. Evolution of liver disease in 362 blood donors with anti-HCV in Hiroshima during follow-ups for 5 years or longer. CH = Chronic hepatitis; LC = liver cirrhosis.

hepatitis C with elevated serum levels of alanine aminotransferase was diagnosed in 60% of the subjects who had felt healthy enough to donate blood (fig. 4). Furthermore, cirrhosis had developed in 5 (0.5%) patients and HCC already in 1 (0.1%). Men had chronic hepatitis more frequently than women – 252/428 (69%) versus 252/486 (52%), $p < 0.01$ – despite the mean age which was comparable between them.

At least 5 years later (average 8.2 years, range 5.0–10.3 years), 362 (39.7%) subjects again received medical examination [19]. Clinical diagnoses remained unchanged in the majority of asymptomatic carriers and in those found with chronic hepatitis at the time of blood donation (fig. 5). However, 5 patients with chronic hepatitis had developed HCC, including 4 men at the ages of 53, 62, 64 and 68 years and 1 woman at the age of 67 years. In addition, liver cirrhosis had elicited in 11 of the 214 (5.1%) blood donors who were found with chronic hepatitis and in 2 of the 146 (1.4%) who were asymptomatic carriers at the first examination. As a positive result, HCV infection had resolved by interferon (IFN) therapy in 42 blood donors with chronic hepatitis C; they would never have received IFN, if the HCV infection had not been found at the time when they wished to donate blood. In addition, 6 of the 146 (4%) asymptomatic carriers cleared HCV infection during 5 years or longer. Five subjects resolved the infection by IFN, and the remaining one spontaneously; he might have been acutely infected with HCV and clearing it when he visited the blood center for donation.

Simulating the Natural History of HCV Infection in Asymptomatic Carriers by the Markov Model

The prospective study on blood donors who were found with HCV has unfolded liver disease advancing in some of them [19]. In order to plan the strategy to deal with inapparent HCV infection, it is necessary to foresee when and what will happen how frequently in asymptomatic carriers. However, the natural history of HCV infection is hard to define. As is evident in their wish to donate blood, subjects found with HCV infection were utterly unaware of it. The time when HCV infection was contracted is unknown in most carriers, except in those with

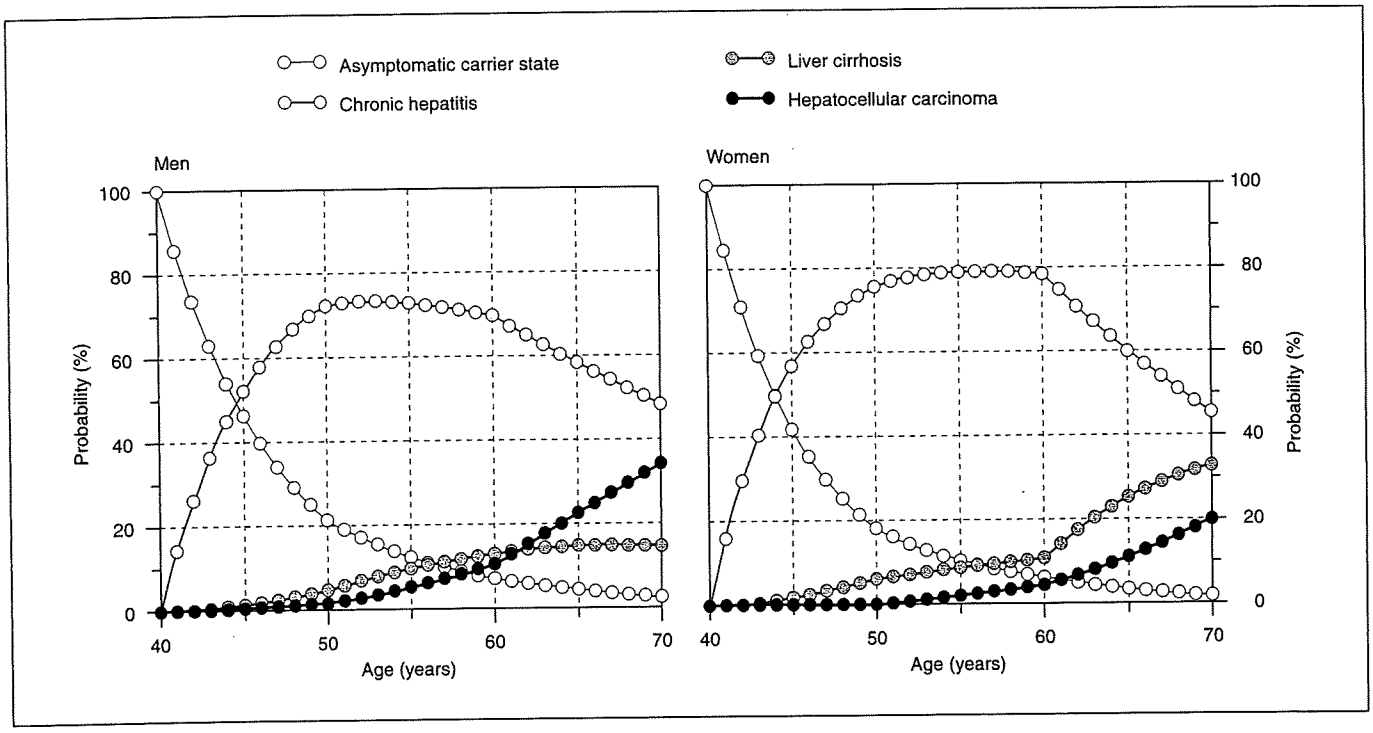

Fig. 6. Simulation of clinical courses of imaginary cohorts of 40-year-old male and female asymptomatic HCV carriers during 30 years by the Markov model [modified from ref. 21].

a history of transfusion or blood products by which they may have been infected. Since it takes decades until cirrhosis and HCC develop after HCV infection, doctors can hardly see through the entire history of their patients. Furthermore, it is unethical and not permitted to observe patients without any treatment at present.

The Markov model is powerful for simulating the natural history of chronic disease [20]. First, probabilities of transitions between any two clinical states within a cycle (usually a year) are assessed by the observation of a limited number of patients who have been followed regularly and rigorously for only a few to several years. Then, the natural history through many years is simulated in imaginary cohorts by integrating all probabilities for each transition per year that are multiplied successively for years elapsed. We have simulated the natural history of HCV infection in men and women by the Markov model [21]. Probabilities for transition per year between any of four clinical states (the asymptomatic carrier state, chronic hepatitis, cirrhosis and HCC) were calculated on 2,251 person-years from 942 patients with HCV infection who had been rigorously examined at least every year. HCC or death was defined as the absorbing state from where no transitions occur.

Based on the transition matrix constructed, the cumulative probability for developing chronic hepatitis, cirrhosis and HCC during 30 years was simulated on a hypothetical cohort of men and women who are found to be asymptomatic HCV carriers at the age of 40 (fig. 6). Of male asymptomatic carriers who enter their fifties, chronic hepatitis is estimated to develop in 72.4%, cirrhosis in 4.6% and HCC in 1.5%; 10.6% are expected to have HCC when they become 60 years old. In contrast, of female asymptomatic carriers who enter their fifties, chronic hepatitis is estimated to develop in 75.9% and liver cirrhosis in 6.2%, but HCC in none. HCC is expected to elicit only in 4.5% of female asymptomatic carriers at the age of 60, with a probability much less than 10.6% in male asymptomatic carriers. There is a steep rise in the cumulative probability for developing HCC in males in their sixties, and it reaches 34.4% at the age of 70, which is higher than that for liver cirrhosis at 14.6%. Although the cumulative probability for developing HCC increases in females reaching 70 years of age, it remains 20.0% and is much lower than that for liver cirrhosis at 32.8%.

The ability of this Markov model in predicting the clinical outcome of HCV infection was evaluated by comparing the results of 5-year follow-ups in 70 men and 83

women with those simulated by it [21]. The results were in good agreement, thereby attesting to the reliability of this model in predicting the natural history of HCV infection in asymptomatic carriers and patients with chronic hepatitis.

Efficient Screening of Individuals with Persistent HCV Infection in the General Population

There are two groups of individuals who test positive for anti-HCV. Subjects in the first group have ongoing infection with HCV RNA in serum, while those in the second group already have cleared infection without serum HCV RNA. Hence, nucleic acids need to be extracted from sera of subjects with anti-HCV, amplified by polymerase chain reaction, also known as nucleic acid amplification test (NAT), with primers deduced from the nucleotide sequence commonly expressed by HCV isolates of any genotypes, and examined for amplification products. However, this is by no means easy, demanding high cost, labor and equipment. Hence, polymerase chain reaction for detecting HCV RNA is not suitable for mass screening of ongoing HCV infection on a national scale.

We have invented a method to efficiently and sensitively screen ongoing HCV infections at low costs. The method takes advantage of the titer of anti-HCV that is higher in individuals with ongoing infection than in those who have cleared HCV infection. The bottom of anti-HCV titers over which HCV RNA can coexist and the ceiling below which HCV RNA is absent can be determined on the panel of anti-HCV-positive sera with or without HCV RNA; however, there is an inevitable grey zone in between. The flow chart depicted in figure 7 retains the benefit of this method, compensates for uncertainties given rise to by the grey zone and avoids missing rare sera with low-titered anti-HCV accompanied by HCV RNA.

First, sera of examinees are tested for anti-HCV, and those which are negative are regarded without HCV RNA and not tested further. The anti-HCV immunoassay has been calibrated for three categories of positive results: (1) high titers for the presence of HCV RNA, (2) low titers that can be very rarely accompanied by HCV RNA and (3) medium titers in the grey zone that may or may not co-occur with HCV RNA. Sera with high-titered anti-HCV are deemed to contain HCV RNA and are not examined any further. Although extremely exceptional sera with high-titered anti-HCV do not contain HCV RNA,

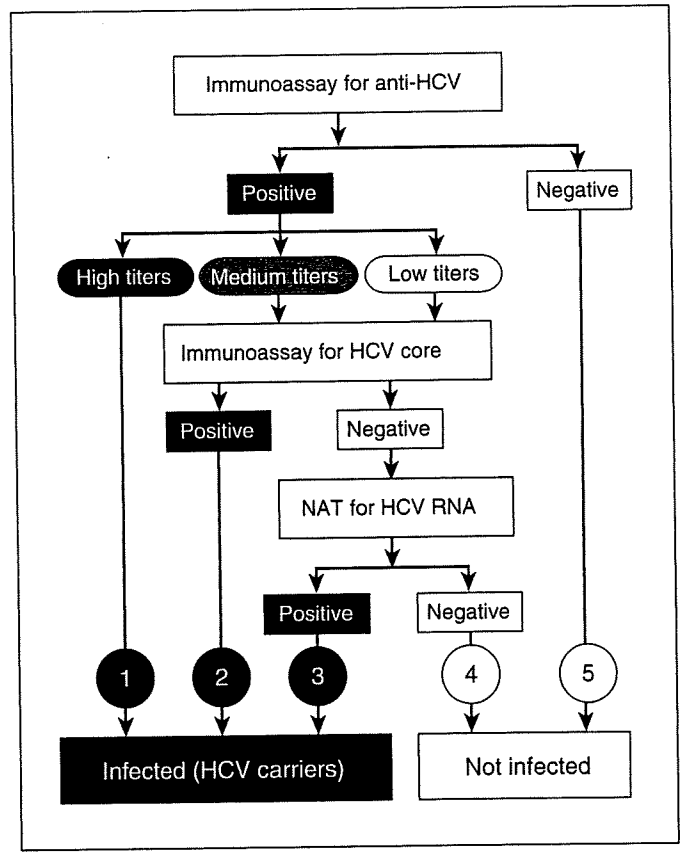

Fig. 7. Strategy for the national screening for persistent HCV infection in Japan with immunoassays for anti-HCV as well as HCV core protein combined with NAT.

we consider false-positive results far more acceptable than false-negative results in judging HCV infection and staying on the safe side. Sera with medium-titered or low-titered anti-HCV are then screened by immunoassay for HCV core antigen; it is easier and less costly than NAT for HCV RNA. If HCV core antigen is detected in the test serum, it indicates ongoing HCV infection, which is theoretically justifiable, and is not tested by NAT. Finally, NAT is performed on all sera with low- and medium-titered anti-HCV, including those that are negative for HCV core antigen. All sera fall into one of the five categories at the bottom of figure 7, of which three (1–3) indicate the presence of ongoing HCV infection and the remaining two (4 and 5) the lack of it. Examinees receive this chart with a mark on one of the categories 1–5 to let them know where they stand.

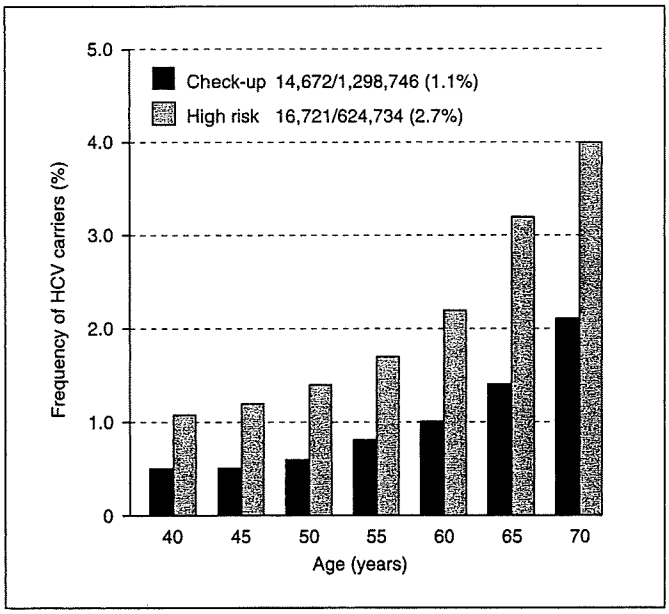

Fig. 8. Age-specific distribution of persistent HCV infection in Japan determined by the national screening during the first fiscal year (April 2002 to March 2003). HCV carriers identified among examinees at regular health check-ups and individuals at high risk, who were stratified by age, are shown separately.

A Nation-Wide Project to Spot HCV Carriers Older than 40 Years in Japan

A 5-year project for screening the Japanese for ongoing HCV and HBV infections on a national scale was launched in April 2002 [19]. It has been performed on two distinct populations. Health check-ups are offered to the Japanese every 5 years when they become 40 years old until they reach 70 years of age. Therefore, by screening health check examinees for 5 years, all carriers of hepatitis viruses who are aged 40 years or older can be identified by March 2007. Thereafter, each year, only individuals who reach 40 years of age need to be screened. This project is based on an extremely low incidence of de novo infection with HCV or HBV in Japan [14], which makes it sufficient to screen for these hepatitis viruses only once in the lifetime.

Subjects who cannot wait for the screening for HCV infection at regular health check-ups, because of an urgent requirement for it, are provided with a chance to receive it at least once. They include individuals at high risk for HCV infection: (1) those who were found with abnormal liver function in the past; (2) those who may have received transfusion with blood or its products before 1992 at a major operation or because of massive hemorrhage at delivery, which had not been screened for anti-HCV by the second-generation immunoassay, and who are not regularly monitored for their liver function; and (3) those in whom liver function tests gave abnormal results at regular examination which they receive in clinics of their companies and institutions. Individuals older than 70 years and off regular health check-ups are tested for virus markers if they wish.

The Ministry of Health, Labour and Welfare of Japan has reported results of the national screening project in the first fiscal year from April 2002 to March 2003. Of the 3,212 municipalities in Japan, 2,997 (93.3%) started this project with a good compliance. The Department of Welfare for the Aged compiled results in the first fiscal year and registered 1,923,480 examinees, of whom 31,393 (1.6%) were found with ongoing HCV infection. There were 1,298,746 examinees at regular health check-ups and 14,672 (1.1%) were found to be HCV carriers. In comparison, of 624,734 high-risk subjects who received tests on other occasions, 16,721 (2.7%) were found with ongoing HCV infection, at a frequency twice as high as that in health check examinees. The results are shown for both groups, stratified by age (fig. 8). HCV carriers were found in 3.2 and 1.3% of the individuals aged 65 at high risk and those examined at check-ups and more often in those aged 70 years, at 4.0 and 2.1%, respectively.

Figure 9 illustrates the results of persistent HCV infection in examinees of regular health check-ups and individuals at high risk from the 48 jurisdictions in Japan. Without exception, the prevalence of persistent HCV infection is higher in individuals at high risk than in health check examinees. Additionally, there is an apparent gradient in the persistent HCV infection from the southwest to the northeast of Japan; the gradient mirrors that of blood donors in the 8 jurisdictions of the Japanese Red Cross Blood Center (fig. 2).

Projection of HCC in Japan to Other Countries toward the Future

The age-specific prevalence of HCV infection in the year 2000 is compared among Japan, Ukraine and the United States (fig. 10a). It is based on 275,868 blood donors in Hiroshima for Japan, 41,021 blood donors in Kiev for Ukraine and data reported by the Center for Disease Control and Prevention for the United States. The age with the highest prevalence of anti-HCV is markedly different among the three countries. The prevalence