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1-2 October, 1990

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Proceedings of a Symposium on HEPATITIS C VIRUS Toronto, Ontario 1-2 October, 1990

Organized by

the Laboratory Centre for Disease Control
Health Protection Branch
Department of National Health and Welfare Canada

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Introduction and Welcome

DR. J. LOSOS (Director General, Laboratory Centre for Disease Control, Health and Welfare Canada)

On behalf of the Laboratory Centre for Disease Control, Health and Welfare Canada, I would like to welcome you to this symposium on hepatitis C, the latest hot topic in hepatitis.

Particular thanks go to the organizing committee. If you look at the program, you realize the amount of effort and work that it took to get this group together. Specific commendations to Dr. Bishai, Ministry of Health, Ontario, Dr. Chaudhary from LCDC, Dr. Spencer Lee from Halifax, Dr. Marusyk from Alberta, Dr. Montplaisir, University of Montreal, and Dr. Rozee.

Also thanks to the private industries, Abbott Laboratories and Ortho Diagnostics for their participation.

We have an impressive list of speakers and look forward to an intensive review of the topic, and also to the recommendations and ideas for the next steps against this virus.

Thank you for your participation.

Prologue

DR. F.R. BISHAI (Ontario Ministry of Health, Toronto)

As we all know, the identification of hepatitis A virus and the hepatitis B virus and the development of serological tests for diagnosing hepatitis A and B have revealed that many episodes of acute hepatitis are caused by neither hepatitis A nor by hepatitis B viruses.

Neither are they caused by cytomegalovirus, nor by Epstein-Barr virus, for which serological assays are available.

After excluding these and other possible known causes of acute hepatitis, such as drugs, alcohol abuse, metabolic liver diseases and auto-immune hepatitis, the remaining acute hepatitis episodes have been called non-A, non-B (NANB) hepatitis.

Since the early 1970s the search for NANB hepatitis virus has been a challenge. Since that time it has been realized that more than one virus can cause NANB hepatitis. One of them is called hepatitis C virus. Despite various reports describing hepatitis C more fully, there is no confirmed visualization of virion particles.

Since 1985 a research group at Chiron Corporation in California under the leadership of Dr. Overby - whose presence today in this symposium is much appreciated - has applied a variety of molecular, biological and immunological methods to the diseases of NANB hepatitis in an effort to identify the causative agent. In the late 1980s they succeeded in cloning the hepatitis C virus genome.

In the spring and summer of 1990 the Food and Drug Administration in the United States approved two new immunoassays designed for the detection of antibody to hepatitis C virus. With this direct serologic test for NANB hepatitis infection, we can expect not only a substantial improvement in the safety of the blood supply, but we can also anticipate a tremendous surge of new information concerning epidemiology, clinical course, prevention and control of NANB hepatitis infection.

The Laboratory Centre for Disease Control, in Ottawa, Canada, which is represented by the Director General, Dr. Losos, and the Director of the Bureau of Microbiology, Dr. Rozee, have recognized the importance of this subject, which is why they decided to sponsor this symposium. We are grateful to them for so much effort on our behalf, and grateful to the speakers who will share their expertise with us, and have come from near and far to do so.

The members of the organizing committee put forward the objectives of this symposium which are:

- to provide the latest information on the pathogenesis and epidemiology of hepatitis C;
- to provide an analysis of the usefulness of currently available diagnostic tests and commercial kits;
- to present recommendations on the laboratory's place in the diagnosis and control of hepatitis C virus;
- and to publish the proceedings in international journals.

On such an occasion when we are accompanied by the most prestigious and distinguished scientists in this field, it would be improper not to ask all questions we have in mind in order to obtain the right answers. Therefore, I will bring my questions forward.

First, relating to the epidemiological features of HCV:

- What proportion of diagnosed NANB hepatitis truly represents hepatitis due to specific hepatotrophic virus?
- What is the distribution pattern of hepatitis C virus infection in different parts of the world and among different populations?
- The third question here is what proportion of anti-hepatitis C virus negative patients is caused by hepatitis C virus?
- How often would an anti- hepatitis C virus positive donor transmit hepatitis C virus infection?
- Is hepatitis C virus transmitted sexually?
- Is hepatitis C virus transmitted in utero or parenterally?
- Since hepatitis C virus is flavivirus-like, is it transmitted by arthropod vectors?

The following questions relate to immunodiagnosis:

- In what way is hepatitis C virus similar to flavivirus?
- Are there different strains of hepatitis C virus?
- How specific and reproducible is the test for hepatitis C antibodies?
- What do reactives or non-reactive tests indicate?
- Are existing tests helpful in identifying the silent hepatitis C virus carrier?
- Would other epitopes of hepatitis C virus distinguish between acute versus convalescent cases?
- Would other epitopes of hepatitis C virus result in more sensitive and specific tests?
- Should a donor's screen show a repeatedly seronegative, how long should the new hepatitis C virus test be continued?

Relating to the clinical features of HCV infection:

- Is the chronicity rate in intravenous drug addicts and in patients with sporadic form similar to that in transfusion hepatitis patients?
- Does hepatitis C virus play a role in hepatocellular carcinoma? If so, is it directly oncogenic or secondarily by enhancing mitotic activity?
- Are persons with silent hepatitis C virus infection at risk for hepatocellular carcinoma?
- Is there a recommended treatment for hepatitis C patients?

Last, related to the prevention and control of HCV:

- Who should be tested for hepatitis C?

- What advice and/or information should anti-hepatitis C virus reactive patients receive?
- Does standard gamma globulin containing anti-hepatitis C virus transmit hepatitis C? If so, should all gamma globulin preparations be tested before being released?
- Is passive and/or active immunization a hope or an illusion?
- By the end of this symposium, I am sure that many, if not all, of these questions will be answered.

SESSION I

EPIDEMIOLOGIC FEATURES OF HCV

Chairman: DR. L. SPENCE (University of Toronto)

Historical Background of Viral Hepatitis

DR. S. KRUGMAN (New York University Medical Center, New York)

It is a pleasure for me to join you this morning to review historical aspects of viral hepatitis, a disease that has an ancient history.

Reports of epidemic jaundice were described in Greece during the fifth century B.C. and many outbreaks occurred during the 19th and 20th centuries.

Epidemics of so-called "campaign jaundice" were prevalent during various wars. For example, more than 70,000 cases of hepatitis occurred in Union troops during the Civil War in the United States, and many hundreds of thousands of cases occurred among American, British and French troops during the Second World War. In retrospect, it is likely that most of these outbreaks were caused by hepatitis A.

The first recognized outbreak of hepatitis B occurred in Bremen, Germany, in 1883 during a smallpox immunization program[1]. At that time there was an outbreak of smallpox and many thousands of persons were inoculated with vaccine prepared from glycerated lymph of human origin. Of approximately 1,200 persons working in the shipyard, 15 per cent developed jaundice several weeks to about eight months after they were inoculated with the smallpox vaccine. In striking contrast, jaundice was not observed among 700 uninoculated employees at this particular shipyard. In retrospect, it is likely that the source of the human lymph was a hepatitis B carrier.

During the first half of the twentieth century, outbreaks of so-called "long incubation-period" hepatitis were observed in many countries of the world and amongst various groups, such as patients who attended venereal disease, diabetic and tuberculosis clinics, persons who received blood transfusions, persons who were inoculated with mumps or measles convalescent serum of human origin, and in military personnel who received yellow fever vaccine.

These infections were caused by the use of hepatitis B contaminated needles, syringes, blood and blood products. The yellow fever vaccine at that time contained human serum that was obtained from an unrecognized hepatitis B carrier.

Initially, knowledge of the pathogenesis of the disease was based on clinical and pathologic observations. The concept proposed by Virchow in 1865 was accepted for many years[2]. He proposed that so-called "catarrhal jaundice" was caused by a plug of mucous in an ampulla of vater. However, the presence of diffuse hepatic necrosis demonstrated by Eppinger in 1922 and Rich in 1930 indicated that an infectious agent was the cause of the disease[3,4].

It was during the late 1930s and early 1940s that studies in human volunteers by various investigators in Europe and in the United States provided convincing evidence of a viral etiology. Their findings indicated that two viral agents were responsible for the epidemics of jaundice in military personnel during the Second World War.

At that time McCallum proposed the terminology of hepatitis A virus (HAV) and hepatitis B virus (HBV). The evidence for two types of viral hepatitis was based on differences in the incubation period and on presumed differences in the mode of transmission[5].

Human volunteer studies during the 1940s at the time of the Second World War revealed that feces or serum obtained during the acute phase of infectious hepatitis (hepatitis A) induced the disease after an incubation period of about 15 to 30 days.

In contrast, acute phase serum obtained from patients with homologous serum jaundice (hepatitis B) induced disease after a much longer incubation period, 50 to 160 days[6,8].

Both Havens and his colleagues and Neefe and his colleagues failed to transmit hepatitis B by oral inoculation of infectious serum, the same serum that produced disease regularly when administered parenterally. On the basis of these findings, it was assumed at that time that parenteral inoculation was the only mode of transmission of hepatitis B.

The observations of the epidemiology, natural history and prevention of hepatitis A and hepatitis B during the 1940s were confirmed and extended during our Willowbrook studies in the 1950s and the 1960s[9]. The development of serum enzyme assays in 1955 provided us with sensitive markers of hepatitis B infection with and without jaundice. The ability to detect anicteric hepatitis enabled us to clarify further the epidemiology of the two diseases.

During the 1960s we identified two types of viral hepatitis; each type had distinctive epidemiological, clinical and immunological features[10]. One type that we designated MS-1 at that time resembled hepatitis A and the other designated MS-2 resembled hepatitis B.

The MS-2 strain of hepatitis B was infectious orally as well as parenterally and, therefore, contrary to the prevailing concepts at that time, the findings indicated that hepatitis B could be transmitted from person to person by intimate physical contact.

These new epidemiological findings were confirmed during the late 1960s when the discovery of Australia antigen by Dr. Blumberg and his colleagues and its association with hepatitis B led to the development of specific tests for the identification of hepatitis B infection[11-13].

The Willowbrook studies confirmed the existence of homologous immunity following hepatitis A and hepatitis B infections. On the other hand, one hepatitis agent did not confer immunity against the other. There was no evidence of heterologous immunity.

The period between the mid 1960s and the mid 1980s proved to be a golden era in the history of hepatitis and hepatitis A and hepatitis B research. Other types of hepatitis identified during that period included hepatitis D, parenterally transmitted non-A, non-B (NANB), and enterically transmitted NANB.

Hepatitis A

It was in 1973 that Drs. Feinstone, Kapikian and Purcell detected hepatitis A virus particles in stools from patients who were infected with the MS-1 strain of the virus[14].

The identification of hepatitis A antigen in stool specimens and in the liver of marmoset monkeys provided a source of hepatitis A antigen. By the mid 1970s highly sensitive immunoassays were developed for the detection of HAV and its antibody and the development of IGM specific anti-HAV serologic tests enabled physicians to distinguish a recent HAV infection from a past HAV infection.

The first successful cultivation of hepatitis A virus was reported by Provost and Helleman in 1979[15]. This important contribution was confirmed by other investigators. It provided the technology needed for the development of hepatitis A vaccine. Studies with live attenuated and formalin-activated hepatitis A vaccines are currently in progress.

The RNA genome of hepatitis A virus, a picornavirus, was molecularly cloned and partially sequenced during the 1980s. These clones can be used as sensitive probes for the detection of viral RNA in clinical samples and cell cultures. These new developments make it possible to prepare recombinant hepatitis A vaccines.

Hepatitis B

The sequence of events that led to the identification of HBV began, as I indicated earlier, in 1975 when Blumberg, Alter and Visnich identified an antigen in serum obtained from an Australian aborigine[11].

The subsequent association of Australia antigen with hepatitis B was confirmed by many investigators[12,13]. Extensive studies during the 1960s and 1970s revealed the distribution of this antigen in various population groups and in patients whose diseases appeared to be unrelated to hepatitis B. Seroepidemiologic surveys found Australia antigen to be present in the blood of a very small percentage of blood donors, somewhere in the neighbourhood of 0.1 to 0.3 per cent.

In contrast, the antigen was detected in 10 to 20 per cent of persons living in various African and Asian countries, in 10 to 15 per cent of patients with leukemia or Hodgkin's disease, in 20 to 30 per cent of institutionalized patients with Downs syndrome, and in about 20 per cent of patients with viral hepatitis.

The convincing data to support the conclusion that Australia antigen was indeed a hepatitis B antigen were reported by Prince and Giles and her colleagues[12,13]. Thus, it was obvious that the prevalence of Australia antigen in Africa and in Asia and in persons with leukemia and Downs syndrome indicated that these high-risk groups were likely to contract chronic hepatitis B infection.

Electron microscopic studies by Dane and his colleagues in 1970 demonstrated the presence of a 42-nanometer particle in the blood of patients with hepatitis B[16]. The surface component of this particle was shown to be immunologically distinct from the core component and the accumulated evidence indicated that the Dane particle was indeed the hepatitis B virion, and its surface component was designated hepatitis B surface antigen (HBsAg).

The core component contained endogenous DNA and hepatitis B core antigen (HBcAg). A third antigen related to infectivity, the hepatitis B e antigen (HBeAg), was subsequently described by a Magnius and Espmark in 1972[17].

The development and the use of sensitive specific markers of hepatitis B infection enabled many investigators to clarify the natural history of hepatitis B infection. During the 1970s it was observed that chronic hepatitis B infection was a cause of cirrhosis of the liver and primary hepatocellular carcinoma.

The development of tests to measure hepatitis B antibody provided the technology to identify units of blood that contained anti-HBs. These antibody positive units were used for the preparation of hepatitis B immune globulin (HBIG). Our preliminary studies indicated the HBIG was effective for the prevention or modification of hepatitis B[18].

During the course of our studies on the natural history of hepatitis B, in 1970, we developed by serendipity a crude inactivated hepatitis B vaccine. This development was the result of an investigation that was designed to determine the effect of heat (boiling MS-2 serum in distilled water for one minute) on the infectivity of 1 to 10 dilution of MS-2 serum. A previous study had revealed the presence of both HBV and HBsAg in MS-2. The one-minute boil, to summarize briefly, destroyed the infectivity of HBV, but the heat-inactivated MS-2 serum proved to be antigenic[19]. Later it was shown that the inactivated MS-2 serum was indeed immunogenic and partially protective; it possessed the characteristics of an inactivated hepatitis B vaccine[20].

By 1975 Purcell and Gerin and Hillemann and his colleagues had developed plasma-derived subunit hepatitis B vaccines[21,22]. Later, the successful cloning of HBsAg by DNA recombinant technology led to the development and licensure of a yeast recombinant hepatitis B vaccine in 1987[23].

Before discussing NANB hepatitis, it is appropriate to comment briefly about hepatitis D virus (HDV). It was in 1977 that Rizetto detected by immunofluorescence an antigen in liver cell nuclei and in the serum of HBsAg carriers who had chronic liver disease. This unique antigen, HDAg, was distinct from HBcAg[24]. Studies in chimpanzees have revealed that it is a transmissible agent associated with, but distinct from, HBV.

It is a 35-nanometer particle containing an RNA core and HBsAg as its surface component. Replication of this agent is initiated and maintained by the helper function provided by HBV infection. Delta infection of a chronic hepatitis B carrier may increase the risk of complicating hepatitis.

NANB hepatitis

Before the 1970s HAV was recognized as the most common cause of enterically transmitted viral hepatitis and HBV was thought to be the most common cause of parenterally-transmitted viral hepatitis. In spite of the initiation of routine screening of blood for HBsAg and the recruitment of voluntary blood donors, the problem of post-transfusion hepatitis was not solved.

It soon became apparent that NANB agents were present in the blood of certain donors. In addition, by the 1980s an enterically-transmitted NANB agent was recognized as the cause of certain waterborne epidemics of hepatitis. This agent has been designated as a hepatitis E virus, HEV.

One year ago in 1989, two articles in the journal Science provided

convincing evidence that a parenterally-transmitted NANB agent named hepatitis C virus (HCV) was identified by molecular cloning and characterization of its RNA genome. In addition, a specific serologic assay was developed for the detection of antibody, anti-HCV, to an epitome of this virus.

This afternoon, Dr. Lacy Overby, one of the co-authors of one of the articles in Science, will describe the discovery and molecular organization of HCV. The use of this new technology by many investigators has shed and will continue to shed new light on the natural history of HCV infection.

The four sessions of this symposium, as you have heard from Dr. Bishai, will include a review of the present status of epidemiologic features, immunodiagnosis, clinical features, prevention and control of hepatitis C infections.

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Post-transfusion Hepatitis

DR. J. MOSLEY (University of Southern California, Los Angeles)

I would like to start with a point mentioned by Dr. Krugman this morning, about the simpler times of the 1940s when experiments with human subjects and epidemiologic observations suggested that there were only two different agents of what was called viral hepatitis. One of the characteristics distinguishing between these two, as Dr. Krugman mentioned, was the incubation period.

In fact, at that time, it was generally accepted that if one knew the incubation period of a case of hepatitis, its etiology was easily inferred. For hepatitis A virus, the longest experimentally or epidemiologically established interval was about 45 days. For hepatitis B virus, the shortest interval generally accepted on the same basis was about 50 days.

Earlier data concerning incubation periods

With regard to hepatitis B, I would like to show you two sets of observations from the 1940s. These are pieces of information that helped form our impression of the incubation period of hepatitis B and reinforced data derived from volunteer studies.

This curve (Figure 1) shows intervals from battle injury to onset of jaundice in a group of 491 cases of post-transfusion hepatitis among military casualties during World War II[1]. Most had received highly icterogenic pool plasma on the battlefield instead of blood.

Figure 2 represents incubation periods in one of the epidemics of yellow fever vaccine-induced hepatitis due to contamination with the hepatitis B virus[2]. You see that the distributions of incubation period in the two series of cases were very similar and corresponded to what was expected from inoculation of human subjects at that time.

We were all very comfortable with this image of the typical range of incubation periods for "serum" hepatitis until 1962. That year, Allen and Sayman published a paper that I regard as a classic in terms of a great deal of new information about post-transfusion hepatitis as it was occurring in the civilian population[3] There was, however, one thing that was immediately disturbing, and that was the distribution of incubation periods (Figure 3).

Of the 113 cases that Allen and Sayman called post-transfusion "serum" hepatitis, 39 per cent had onset of jaundice of less than 50 days after transfusion. More important, the modal incubation period was not between 75 and 100 days, as had been seen in cases associated with yellow fever vaccine and pooled plasma given to battle casualties. The peak was from 40 to 59 days. It may be difficult for our younger colleagues to appreciate how unsettling these data were 28 years ago. If Allen and Sayman's cases were a composite due to both hepatitis A and hepatitis B, one would have expected a bimodal distribution with a low point in exactly the area in which the unimodal peak occurs.

I would also like to call your attention to the fact that even though a report of the cases was not published until 1962, they occurred from 1946 through 1956. This pattern of incubation periods has been occurring for a long time.

As an epidemiologist at the Center for Disease Control at that time, I was intrigued by this observation. From the tens of thousands of surveillance reports available, I was able to cull 718 cases of persons transfused on a single day for whom the date of onset of jaundice was known[4]. Figure 4 shows that I obtained essentially the same distribution as Allen and Sayman. At first, I considered two explanations that did as little violence as possible to established ideas. One was to assume that hepatitis A virus (HAV) was causing far more cases of parenterally transmitted disease than suspected, and that its modal incubation period was delayed from its usual peak of about 28 to 30 days because of partial neutralization by antibodies to HAV in other, concomitantly administered blood units. The second possibility I considered was that the incubation period of hepatitis B was shortened, perhaps because of the large inoculum in a unit of contaminated blood.

Figure 1
Sartwell, 1947: Post-transfusion hepatitis observed during World War II.

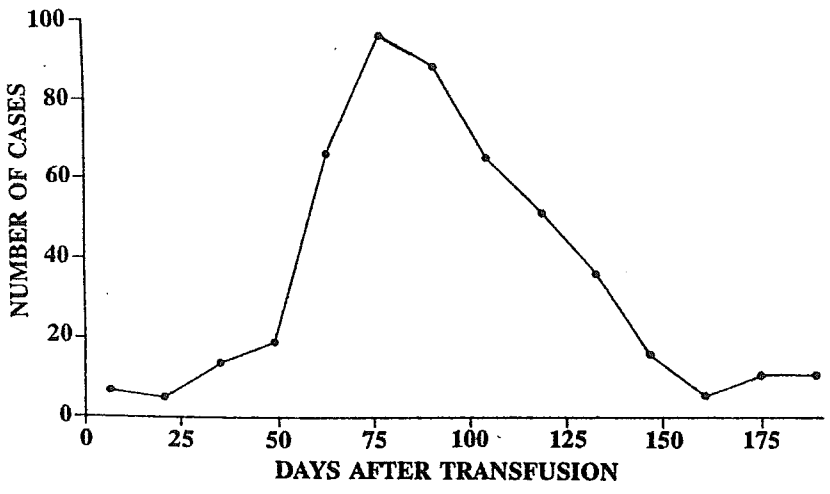


Figure 2
Freeman, 1946: Yellow fever vaccine-induced hepatitis due to contamination with hepatitis B virus

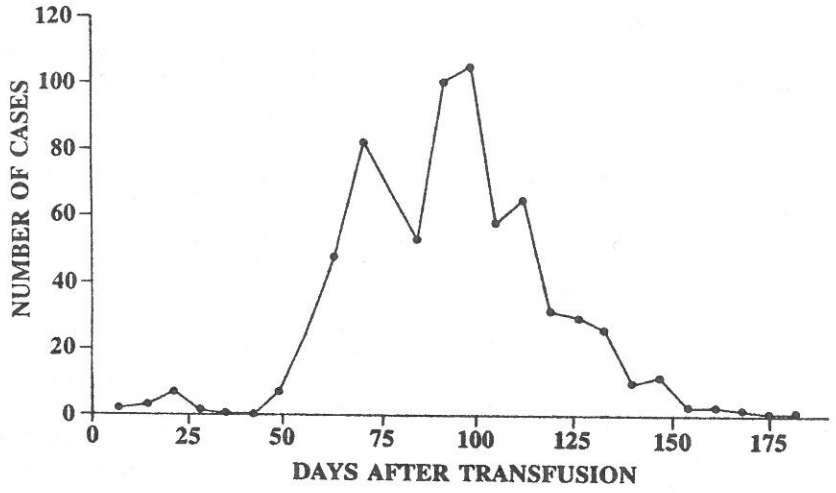
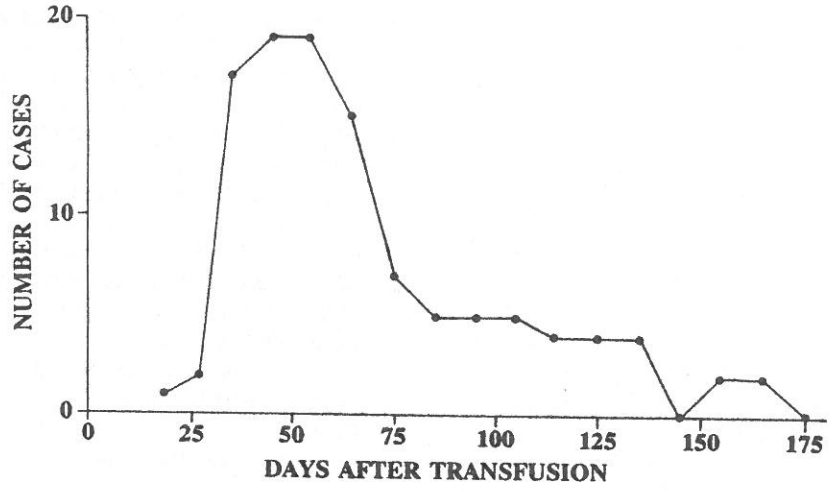


Table 1
Age distribution by incubation period of transfusion-associated hepatitis

Incubation period (days)	Age group in years No. and (%)					
	0-19		20-39		≥40	
<40	5	(3.4%)	63	(42.9%)	79	(53.7%)
40-59	2	(0.1%)	73	(42.9%)	102	(57.6%)
≥ 60	14	(3.6%)	155	(40.3%)	216	(56.1%)

Figure 3
Allen and Sayman, 1962: Post-transfusion hepatitis, 1946-1956



One way of testing this hypothesis was to examine the age distribution of the transfusion-related cases in relation to the incubation period. In the early to mid-1960s, HAV was still causing cyclic epidemics in the United States[5], and most adults, particularly older adults, were immune. One would expect, therefore, that is any large number of transfusion-associated cases were caused by HAV, they would have a younger age distribution. Table 1 shows that this is not the case. There were no statistically significant differences in age-specific proportion by incubation period. Other characteristics not compatible with a hepatitis A etiology were a fatality rate in the range of 4.1 per cent for short incubation period disease, and the lack of any effect in instances in which there was immunoglobulin administration around the time of transfusion[6].

No epidemiological approach seemed feasible at that time to the alternative hypothesis, that the incubation period of hepatitis B was often shortened by the large inoculum. There were, however, some experimental data of pertinence, although in the absence of specific serologic tests they could be interpreted only to a limited extent.

Neefe, Murray and colleagues[7,8] studied 14 recipients who developed post-transfusion hepatitis from 32 to 111 days after blood administration. Five donors were re-bled and 1.0 mL of their serum was inoculated into various numbers of volunteers (Table 2). In general, the incubation periods for the volunteers were very similar to those in the original recipients of blood.

I will anticipate future information by pointing out that in 1972 Barker and Murray[9] found that two of the five volunteers were HBsAg positive, as were their recipients. The unexpected early peak in incubation periods in Figure 3 and 4, therefore, could be explained by hepatitis B cases. For example, if we look at data for recipient 3 who had known HBV infection, we find that his incubation period for HBV was 44 days, and the range in subjects inoculated with this serum was 32 to 72 days. I will anticipate what I will say shortly, however, by pointing out that I have been unable to find information concerning the serologic status of all the subjects in Table 2. To my knowledge, the others have not been tested.

The advent of serologic tests for hepatitis B virus at the end of the 1960s opened the way to establish the cases associated with the agent and the distribution of their incubation periods. At the beginning of the 1970s, the optimism of the HBV discovery led many to feel transfusion-transmitted hepatitis cases would disappear regardless of their incubation periods.

By 1973, however, it was clear that many cases of post-transfusion hepatitis were still occurring. More important, most of the cases during that period could not be serologically attributed to hepatitis B virus by the assays then available. The initial assumption was that more sensitive tests were needed, both for donor screening and for diagnosis of cases. I have been unable to find, however, any examination of the serologically undiagnosable cases in terms of incubation period to see if a disproportionate number occurred in the range of under 60 days. We may use data from the Transfusion-Transmitted Viruses Study (TTVS), discussed below, to illustrate the point. In TTVS, however, most cases were asymptomatic rather than icteric. Accordingly, Figure 5 shows the intervals from the day of transfusion to the day the patient's ALT was first observed to be 90 U/L or greater. The distribution of latent intervals for the 15 HBV infections ranged from 28 to 150 days, with a median of 108.

Comparing the pattern in Figure 5 with those reported before HBsAg screening, one sees that there were fewer cases in the left limb of the skewed distribution. In Table 3, I have compared the proportion of cases with intervals of 60 days or more in the earlier series, with that in TTVS. Keeping in mind the difference in definition of incubation periods, it is nevertheless obvious that HBsAg screening of donors did have an impact on the proportion of cases with longer incubation periods, whatever the data in Table 4.

Table 2
Incubation periods in cases of post-transfusion hepatitis among subjects experimentally injected with their blood[7,8]

Recipient ID	Incubation periods in days	
	Original transfusion	Experimentally inoculated subjects (1.0 mL of serum)
1*	32	(50), 57, 67
2	40	48
3*	44	32, 43, 49, 50, (50), 56,56, 57, 63, 7
4	48	(18), 30,(43), 45,46,(46), 56
5	70	84

*later demonstrated to be HBsAg + (Reference 8)

Table 3
Proportion of cases with incubation periods ≥ 60 days in various reported series

Investigator	Text Reference	Years of Study	% of cases ≥ 60 days
Allen & Sayman	3	1946-56	47%
Grady et al	17	1952-62	53%
Mosley	4	1961-65	55%
TTVS	13	1974-79	25%

Early studies in the TTVS

The plan for the TTVS emerged in 1973 from this problem of post-transfusion cases undiagnosable as being due to HBV, and it was intended to serve several purposes:

1. to determine the incidence of clinical and subclinical transfusion-associated hepatitis following HBsAg screening;
2. to apply available serologic procedures to try to explain the etiology of HBV serologically negative cases; and
3. most important, to store serum samples for evaluation later of subsequently developed serologic tests.

Cases of transfusion-transmitted HAV and HBV infection were defined on the bases of specific serologic tests. Cases not serologically definable as due to HBV were obviously more of a problem. We wanted to identify all possible such cases, and we knew that many of them would not only be subclinical but they would also, perhaps, have relatively mild hepatic abnormalities. Accordingly, we defined non-A, non-B (NANB) hepatitis as the occurrence of at least one serum alanine aminotransferase (ALT) activity of 90 U/L or greater, and a second of 45 U/L or greater three to 17 days before or after, during the interval from 15 through 160 days after the reference event (for recipients, this was the day of first transfusion and for most controls, the day of surgery). Enrolled transfusion recipients and controls meeting these criteria were evaluated by both the investigators and an independent expert committee unaware of whether the patient had been transfused.

Between July 1974 and October 1979, TTVS observed 1,533 recipients of one to 15 units of homologous blood, and 1,588 hospitalized patients cross-matched for similar surgical or medical indications who did not require blood components. The incidence among recipients of HBV infection was 1.0 percent (15 cases), and of NANB hepatitis, about 10.2 percent (156 cases) (Figure 5). There was almost no background rate for HBV, but one of a moderate level (2.9 per cent) existed for NANB hepatitis by the criteria used[10].

Recent studies of TTVS specimens

Following completion of the TTVS/NHLBI Repository in late 1979, panels of selected samples were provided to a total of 26 investigators who offered evidence of a potentially specific test for NANB hepatitis. All 26 failed. In 1988, a similar panel was provided to Houghton and his colleagues at Chiron for evaluation of the cloned peptide from NANB material. All specimens were appropriately

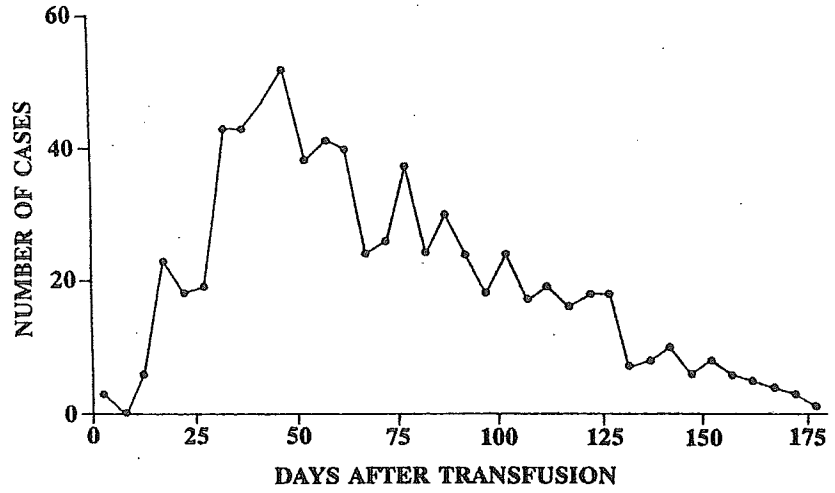
Table 4
Hepatitis B virus, hepatitis C virus, and presently unexplained NANB hepatitis in the Transfusion-Transmitted Viruses Study

Category of subjects	No. of subjects	HBV	NANB		
		No/%	Total No/%	HCV No/%	Other No/%
Recipients	1,533	15/1.0	156/10.2	51/3.3	105/6.8
Controls	1,588	1/0.1	46/2.9	1/0.1	45/2.8

Table 5
Donor anti-HCV and its surrogates in cases of post-transfusion hepatitis: Transfusion-Transmitted Viruses Study

Status of donors	Recipient seroconversion (No/%)			
	YES (n=48)		NO (n=53)	
Anti-HCV +	43	(90%)	9	(17%)
only	12	(12%)	0	(0%)
anti-HBc+ also	11	(26%)	3	(6%)
ALT + also	13	(30%)	2	(4%)
anti-HBc+ and ALT+	7	(16%)	5	(9%)
Anti-HCV -, surrogate +	3	(6%)	11	(21%)
anti-HBc + only	0	(0%)	5	(9%)
ALT + only	2	(4%)	3	(6%)
anti-HBc + and ALT +	1	(2%)	3	(6%)
All 3 tests -	2	(4%)	33	(62%)

Figure 4
Mosley, 1968: Post-transfusion hepatitis, 1961-1966



coded[11] as in other evaluations by the Chiron group[12], and hepatitis C virus (HCV) was identified as another etiologic agent of human hepatitis.

Subsequently, Dr. Cladd E. Stevens of the TTVS group tested for anti-HCV with enzyme-linked immunoassay representive specimens from 132 (85 per cent of the total) recipients and 35 (76 per cent of the total) non-transfused, hospitalized controls classified as having had NANB hepatitis.

Initially, positive specimens were retested in duplicate. In addition, specimens key to the interpretation of the pattern of serologic status were selected for retesting in duplicate to verify the results. We have presently identifed 51 recipients and one control with NANB hepatitis who had anti-HCV seroconversion. For the recipients, this represents an incidence of 3.3 per cent (Table 5).

In all these instances, the pre-transfusion sample was negative, and for recipients, the values for sample/cut-off ratios had a pattern consistent with active infection rather than, or in addition to, passive acquisition of antibody from transfusion. For controls, the incidence was identical to that of HBV infections, 0.1 per cent[13].

Forty-four (86 per cent) of the 51 cases in recipients with seroconversion were associated with a known positive donor. Only five, or 10 per cent, of all seroconverting recipients did not have a positive donor identified.

Of the recipients with seroconversion, 67 per cent had a peak ALT of 450 U/L or higher, and 59 per cent had abnormalities that were observed to persist for six months or more. In contrast, 69 per cent of the seronegative recipients with seronegative donors had peak ALT abnormalities under 200 U/L, and in 85 per cent all elevations had returned to normal within six months. It is obvious that the seronegative recipients with a positive donor closely resemble the recipients who seroconverted, and one might suspect that for most, the NANB hepatitis was attributable to HCV transmission.

The seronegative recipients with seronegative donors resemble the seronegative control subjects with what was diagnosed as NANB hepatitis. This finding, as well as the fact that NANB hepatitis was only about twice as

Table 6
Anti-HCV by ELISA in autoimmune chronic active hepatitis

COUNTRY	AI-CAH	NO. ANTI-HCV+	%	REFERENCE
Italy (Palermo)	22	13	59	Hepatology 1990;12:424
Japan	18	9	50	Hepatology 1990;12:430
Italy & France	38	23	60	Lancet 1990;5:1160-1161
*after treatment England	53	21	40	Lancet 1990;335:754-757
*after treatment Canada (Toronto)	19	5(3)	26	

Note the marked drop in anti-HCV frequency in some patients after treatment

frequent among seronegative recipients of seronegative donors as in seronegative controls brings the nature of the NANB diagnosis into question.

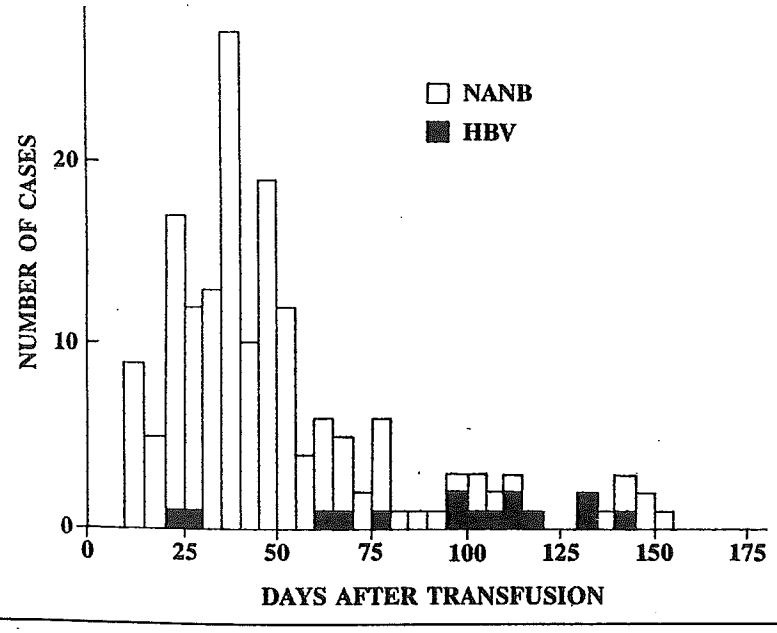
If we return to the question of incubation periods, we find that those for recipients with HCV seroconversion were very symmetrically distributed, with a media of 42 days and a range of 17 to 85 days. Seronegative recipients of seronegative donors and seronegative control subjects had slightly shorter incubation periods than seroconverting recipients, but their distributions did not differ significantly from the seroconverting recipients. HCV cases in recipients, therefore, differ in severity and outcome, but do not clearly differ in incubation period.

Perhaps of the greatest interest are the instances in which both recipients and control subjects who were diagnosed as having acute NANB hepatitis were anti-HCV positive as the time of hospital entry. There was a total of nine such cases, five among recipients and four among controls. The characteristics of their NANB episode differ widely, and they may represent a group with a heterogeneous etiology.

Table 6 is a summary of most of our findings. Our first analysis of the NANB cases in the TTVS shows that about half of the cases diagnosed by criteria considered acceptable in the 1970s are attributable to HCV, and about half cannot be so attributed at the present time.

Furthermore, it seems unlikely that most of the anti-HCV negative cases will become attributed to HCV by future testing because their characteristics, including the frequency of similar cases among untransfused controls, are different. Whether they are attributable to a nosocomially transmitted or re-activated virus, remains to be seen.

Figure 5
Transfusion-Transmitted Viruses Study: Hepatitis cases



Surrogate tests for NANB hepatitis

Finally, we are now in a position to assess the frequently controversial impact of surrogate tests [10,14] in the United States [15, 16]. For HCV prevention, serum ALT and anti-HBc testing have been, in fact, good surrogates. For the non-B, non-C cases, they were ineffective but it is not yet clear to what extent we need to prevent these occurrences, whatever their etiology. Fortunately, the HCV cases appear to have been the more serious, and the use of surrogate tests in the United States probably made at least some contribution to the public health.

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SPEAKER : I wonder if Dr. Mosley has tested the non-B, non-C hospital-associated cases with so-called second generation tests for HCV.

DR. MOSLEY: No, we have not. Unfortunately, our experience with the second generation tests has suggested that they have been less sensitive than the EIA so that we have been waiting for, perhaps, further development before using our sera which are very limited in amount for that purpose.

Mode of Transmission and High-Risk Groups

DR. M. ALTER (Centers for Disease Control, Atlanta)

As you heard this morning, non-A,non-B (NANB) hepatitis is traditionally considered a transfusion associated disease and most of the studies of NANB hepatitis have been conducted in that setting.

However, over the last decade it has become clear that NANB hepatitis occurs in patients with histories of other risk factors as well as those with no known source for infection.

In the United States, NANB hepatitis is a very poorly reported disease and only about 5 per cent of the acute viral hepatitis reported to CDC can be classified as NANB hepatitis with an incidence of one case per 100,000 population.

Because of this poor reporting, we started a more intensive surveillance system in the late 1970s in four sentinel counties with complete serologic testing as well as the exclusion of other causes.

In these four counties, located in Florida, Alabama, Colorado and Washington State, NANB hepatitis represents an average of 25 per cent of the acute viral hepatitis. The overall incidence of the disease has remained relatively stable over the last seven years, approximately seven cases per 100,000 with considerable variation between the counties as is typical of the United States as a whole.

The clinical features of the NANB patients with risk factors other than transfusions are similar to patients with transfusion-associated NANB hepatitis. About two-thirds of the patients have peak ALT levels during their acute illness greater than 15 times the upper limit of normal. About one-quarter have ALT levels six to 15 times the upper level of normal, and only 10 per cent of patients have minimally elevated ALT levels.

The majority of patients are between the ages of 15 and 39. However, over the course of the last seven years, we found there have been some slight declines in the youngest and the oldest age groups while there have been some moderate increases in patients 30 to 39 years of age. This reflects changes in the risk factors for acquisition of disease.

The male to female ratio is about 1:1 and has remained that over the study period.

The highest incidence rate by ethnic group are in Hispanics (26 per 100,000). The incidence rates in other whites and blacks are relatively similar (6 per 100,000). In one report of blood donors from the New York Blood Center by Cladd Stevens and colleagues, the highest prevalence of anti-HCV was found among Hispanic blood donors.

Although the incidence of the disease has remained stable over time, there have been significant changes in disease transmission patterns. Between 1982 and 1985 an average of 17 per cent of patients reported a blood transfusion in the six months preceding the onset of their illness. This percentage declined significantly to 8 per cent in 1986 and to 6 per cent in 1988. The majority of this decline occurred before surrogate testing of blood donors was instituted and was temporally associated with changes in the donor population related to the prevention of HIV infection.

In contrast, the percentage of patients reporting a history of drug use was 20 per cent between 1982 and 1985 and dramatically increased to an average of 42 per cent between 1986 and 1988.

A consistent 10 per cent of patients reported heterosexual contact as their exposure for infection, defined as exposure to a household or sexual contact who had hepatitis or exposure to multiple partners.

We have recently shown in a case control study which I will describe a little later in this presentation that such factors are significantly associated with acquiring NANB hepatitis.

Only about 2 per cent of the cases are associated with occupational exposure to blood. Forty per cent have no known source for their infection.

One of the most important features of NANB hepatitis is the frequency with which patients progress to chronic liver disease.

The majority of studies on chronic NANB hepatitis have been in the transfusion setting.

In the sentinel counties we conducted a study to determine the frequency and severity of chronic hepatitis in patients with NANB hepatitis as a result of all types of exposures. These patients were followed from the onset of acute disease every three months for up to four years with serologic markers, biochemical tests, history and physical and biopsy when possible.

Of the 131 patients successfully followed for six months, 50 per cent had an abnormal ALT at that time. Of the 121 patients followed for at least nine and up to 48 months after onset of illness, 65 (54 per cent) had persistently abnormal ALTs.

Abnormal ALT levels persisted independent of the source for infection. Abnormal ALT levels persisted in 58 per cent of patients with transfusion associated NANB hepatitis, 55 per cent of patients with a history of drug abuse, 40 per cent of health care workers with occupational exposure to blood, 63 per cent of those who acquired their infection as a result of heterosexual activity or household exposure to a contact who had had hepatitis and 48 per cent of those who had no known source for their infection.

We tested the patients followed in this study for antibody to the hepatitis C virus by the currently licensed enzyme immunoassay. Of patients followed for at least six months, a total of 68 per cent are positive for anti-HCV. Forty-five per cent of these patients were positive within six weeks of the onset of their illness, 99 per cent were positive by six months after onset of illness, and one patient did not seroconvert until nine months after onset of illness.

Patients with no history of blood transfusion were just as likely to be anti-HCV positive after prolonged follow-up as were patients with transfusion associated NANB hepatitis.

However, patients who were anti-HCV positive were more likely to

have persistently abnormal ALT levels (60%) than were patients who were anti-HCV negative (40%), even after prolonged follow-up.

Thirty patients have had biopsies evaluated to date. Of these, about 70 per cent have chronic lobular or chronic persistent hepatitis and about 30 per cent have chronic active hepatitis.

Three studies have evaluated the persistence of anti-HCV in relationship to the outcome of disease.

The first study is of a group of patients from the CDC Sentinel Counties Study. The second is the group of patients from NIH published by Harvey Alter and colleagues in the November 1989 New England Journal of Medicine, and the third is a small study published in Lancet from Italy by Bortolotti and colleagues.

In the study from CDC, of the 88 patients with hepatitis C who were followed for four years after onset of the disease, anti-HCV became undetectable in only three, all of whom appeared to have resolved their hepatitis. One of these three patients, however, regained the antibody a year later.

In the series of 18 patients with hepatitis C from the NIH, anti-HCV became undetectable in four, three of whom resolved their hepatitis.

In the small study from Italy, both patients with hepatitis C resolved their infection and in both, anti-HCV became undetectable.

The prevalence of antibody to hepatitis C varies depending on the population tested.

Among blood donors in the U.S. who were tested during the original Ortho Diagnostic Systems Clinical Trial, it was found that about 0.6 per cent were repeatedly reactive for anti-HCV with some variation depending on the part of the country in which the testing was done.

As you have seen in previous presentations, the anti-HCV rate in those donors with one or both surrogate markers. In blood donors with the highest ALT levels who are also positive for anti-HBc, 56 per cent were found in the New York Blood Centre Donor Study to be positive for anti-HCV.

Anti-HCV positivity among drug users was found to be extraordinarily high (60 to 90 per cent) in the original clinical trials, as were the rates among haemophilia patients. The available data on sensitivity and specificity of the licenced enzyme immunoassays are very limited. Harvey Alter from the NIH has reported that 20 per cent of donors who were anti-HCV negative transmitted disease to recipients suggesting that the sensitivity of the currently licenced test is not as high as we would like. In addition, the specificity varies, depending on the prevalence of the infection in the population tested.

There are two supplemental tests for specificity available on an investigational basis. One of these is an immunoblot type assay and the other is a neutralization or blocking assay type. At CDC we have tested several groups with the neutralization or blocking assay and found that EIA-positive samples from patients with the highest likelihood of having hepatitis C - chronic hepatitis C or IV drug users - 90 per cent tested positive on the supplemental assay.

Tom Zuck tested previous transfusion recipients and found that of the 76 EIA positives, 84 per cent tested positive on the immuno blot supplemental assay.

In Harvey Alter's study, of the 28 anti-HCV positive donors implicated in HCV transmission, 89 per cent tested positive on the immunoblot supplemental assay, whereas, of the 21 non implicated donors who tested positive by anti-HCV only 33 per cent tested positive on the supplemental assay.

Finally, of randomly selected volunteer blood donors tested by Jay Menitove and reported in Lancet in July of 1990, of 76 EIA positive blood donors, only 20 per cent tested positive on the supplemental assay.

I want to discuss the evidence for sexual transmission.

As I mentioned earlier, we conducted a case-control study published in JAMA in September 1989, which suggested that heterosexual activity played a role in the transmission of NANB hepatitis or hepatitis C.

We selected cases of acute NANB hepatitis from our sentinel counties who had no history of either drug use or transfusions and matched them to healthy adults selected at random from the general population who had normal ALT levels and no history of parenteral exposures.

We found three characteristics to be associated with the cases compared with the healthy adults from the general population: fewer years of education, multiple sex partners, and a history of hepatitis in a household of sexual contact.

Fewer years of education was associated with a threefold greater risk of acquiring disease; multiple sex partners in the six months preceding illness was associated with an elevenfold greater risk of acquiring disease; and having a household or sexual contact who had had hepatitis was associated with the sixfold greater risk of acquiring disease. This latter factor was found in another case control study published in 1981 to be associated with acquiring NANB hepatitis.

Interestingly, neither of these case control studies have found homosexual activity to be associated with acquiring NANB hepatitis or hepatitis C. Since bloodborne sexually transmitted diseases are generally more efficiently transmitted between homosexual men than between heterosexual men and women, it is unclear why heterosexual activity may be associated with acquiring hepatitis C when homosexual activity is not.

We found no evidence of an association between infection and occupational exposure, being a dialysis patient, being hospitalized in the six months preceding onset, having surgery, having any type of dental work, having any other medically related injections, having percutaneous exposure such as tattooing or acupuncture or ear piercing, or being in prison or jail. Neither was there evidence for homosexual activity, ingesting or shucking raw shellfish, or international travel.

Another way to examine sexual transmission of HCV is to look at the prevalence of infection in populations with different sexual behaviours.

In a study conducted at CDC in individuals without evidence of NANB hepatitis, we found that 16 per cent of heterosexuals with more than two partners in the preceding four to six months were anti-HCV positive compared with 3 per cent of individuals with zero to two partners.

In a study from West Germany, Hess and colleagues found that heterosexuals attending an STD clinic had a rate of anti-HCV of 5 per cent compared with 0.5 per cent in blood donors.

Esteban and colleagues in Spain have found increased rates among

homosexual men compared with blood donors and pregnant women, but not a rate you would expect for a sexually transmitted disease when you compare the rate of anti-HCV with that of HBV and HIV in the same population. In a study by Mortimer from the U.K. anti-HCV was found in 15 per cent of homosexual men.

Finally, there are several studies of contacts of anti-HCV patients. In one study, anti-HCV was found in 20 per cent of the non-drug using female sex partners of 25 anti-HCV positive drug users, compared with no anti-HCV in female sex partners of 20 HCV-negative drug users.

Esteban reported no anti-HCV positive partners of five HCV positive drug users in Spain and of 13 drug users not tested but presumed to have a high rate of hepatitis C, one sex partner was positive.

Of 13 patients with chronic hepatitis C reported by Kamitsukasa in Japan, 34 household and sexual partners were tested and three (9 per cent) were positive, two of whom had no other risk factors for hepatitis C.

Of 88 family members of 34 patients with chronic hepatitis C published by Ideo and colleagues in Lancet, seven (8 per cent) were positive. These individuals also had no other risk factors for hepatitis C and included two spouses, three parents, a child and a sibling. The study also included 26 contacts of eight individuals with chronic hepatitis who were anti-HCV negative, and none of those contacts were positive for anti-HCV. Finally, of 40 patients with chronic hepatitis C from a study published by Everhart and colleagues, none of 54 household and sexual contacts, were found to be positive.

The majority of the studies just described found some evidence of sexual or household transmission of hepatitis C, but the differences observed between studies are not unlike those observed between the studies that were originally conducted for hepatitis B.

We now have the benefit of knowing why the studies for hepatitis B were so different. That is because we now know about hepatitis B e antigen (HBeAg), a marker of infectivity. We know that people who are HBeAg positive are much more likely to transmit infection than those who are HBeAg negative. We do not have the benefit of that knowledge for hepatitis C.

We estimate that in the United States there are 170,000 infections with hepatitis C annually and, although only one quarter of these may be symptomatic and very few die of fulminant disease, 85,000 develop chronic infections. Of these, 20 per cent or more go on to develop chronic active hepatitis or cirrhosis.

There are many unresolved issues with respect to hepatitis C. Why do some patients with NANB hepatitis remain anti-HCV negative even after prolonged follow-up? They may be infected with a second NANB agent. They may have been misdiagnosed and not have viral hepatitis. Or, they may lack an immune response detectable by the current assay.

What is the meaning of an anti-HCV positive test particularly in an asymptomatic blood donor? The current test does not distinguish between those who are chronically infected and those who have resolved their infection. I think for the time being we have to assume that anyone who tests positive for anti-HCV is potentially infectious, assuming that the test is a true positive.

What are the long-term consequences of HCV infection?

We still have much to learn about the natural history of infection, particularly in the asymptomatic individual.

What is the role of preventive and therapeutic measures? You are going to hear more about interferon therapy from Dr. Schiff, but as far as prophylaxis is concerned, we have nothing to offer at this time.

Finally, although we have waited a long time to screen out hepatitis C in our blood supplies, the ability to do that will prevent only a very small percentage of infections. To have a significant impact on the overall disease burden, we need to better define the role of sexual, perinatal, and other routes of transmission for hepatitis C so that prevention measures outside the transfusion setting can be developed.

DR. LEE: Dr. Alter, in your supplemental testing study, can you draw any relationship between the false positivity based on the neutralization or blocking test and the OD reading on the EIA result?

DR. ALTER: Individuals who tended not to test positive on the supplemental test had OD values below 1.5.

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Blood Donors in Canada and HCV

DR. P. GILL (Canadian Red Cross Society, Ottawa)

What I want to give you today is an update on what we have found in blood donor testing in the Red Cross since we initiated testing, a little bit of our experience in evaluating the test, and the results since it became a test of record as of June 30th this year, which will include some experiences with supplemental testing.

So, this morning I want to focus on the actual data arising out of blood donor testing in Canada of our volunteer blood donors.

As you will see, the Red Cross Centres across Canada cluster in the southern region of the country near the border with the United States.

All Canadian blood donor collections are from volunteer donors to the Canadian Red Cross Society, which has the monopoly on the distribution of blood products. The difference between the United States and ourselves in terms of this volunteer population is that we do not do surrogate tests for non-A, non-B (NANB) hepatitis. We do not test for ALT and we do not test for anti-hepatitis B core antibody. The questions asked on our donor questionnaire are: "Have you ever had yellow jaundice (other than at birth), hepatitis or liver disease?" and, "To your knowledge, in the last six months have you come in close (intimate) contact with someone who has had yellow jaundice or hepatitis?"

This questionnaire is the mechanism for excluding donors who may be at risk, without specific testing. However, supplemental to this is the recent history of the screening for risk activities associated with HIV infection; this certainly has caused a decrease in the transfusion-associated NANB hepatitis in the country.

So, there is a parallel to what you have heard from Dr. Miriam Alter regarding the effect of HIV screening in terms of questionnaires and the increased sensitivity of the donor population to risk activities.

The data that I am going to report on were compiled mainly through activity in the National Reference Laboratory in terms of the supplemental testing along with information that was gathered by the Department of Laboratory Services in our national office. Obviously, we are thankful for the actual real testing, that is, the screen testing done by the centre staff, and their collaboration in providing data to us from the centres across the country.

The test that we selected in the Canadian Red Cross Society was the Ortho HCV antibody test, an EIA test using the recombinant C100-3 peptide sequence of the hepatitis C virus.

The antigen (as shown in Figure 1) is this C100-3 peptide which is linked to a superoxide dismutase region and this is the antigen that is used to coat the wells. It is an EIA test which is dependent on the OD reading of the enzyme reactivity done through a normal EIA procedure using antigen-coated wells and donor serum and plasma for the detection of antibody.

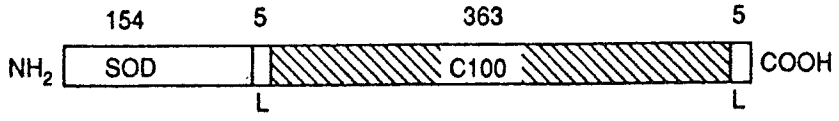
The cut-off in this particular test is an OD of 0.4 plus the negative control value, usually between 0.05 and 0.15. So, you are looking at an OD value of around 0.5 as your cut-off value.

You have a very low OD value in the normal blood donor population distributing upward through a limited number, obviously, who are reactive. The distribution around the cut-off is shown in Figure 2, which are not our data, but a cumulative assessment of blood donors. Anything above the cut-off value would have an S/C or sample (or signal) to cut-off ratio of 1 or greater.

We raise several concerns with using this test to screen blood donors. First, we recognize that it is a recombinant peptide; it is a not a viral lysate. We expect it to be cleaner than a lysate in terms of being able to differentiate false positives and false negatives. Second, however, it is a non-structural region of the gene and, therefore, the antibody response is not to classic viral structures of envelope and surface antigens.

We were concerned, obviously, about the specificity of this response and the titre of the immune response. In terms of the blood donor population, this would indicate whether an individual is going to be classified as a reactive or non-reactive in a screen test.

Figure 1
Composition of the yeast recombinant HCV antigen* (C100-3) is used to capture circulating antibodies.



* The antigen comprises 154 amino acids of human superoxide dismutase (SOD) and 363 amino acids from the non-structural part of the HCV polyprotein (C100). Due to the cloning procedures, there are an extra 10 amino acids derived from adjoining linker (L) DNA.

Figure 2
Frequency distribution of blood donors

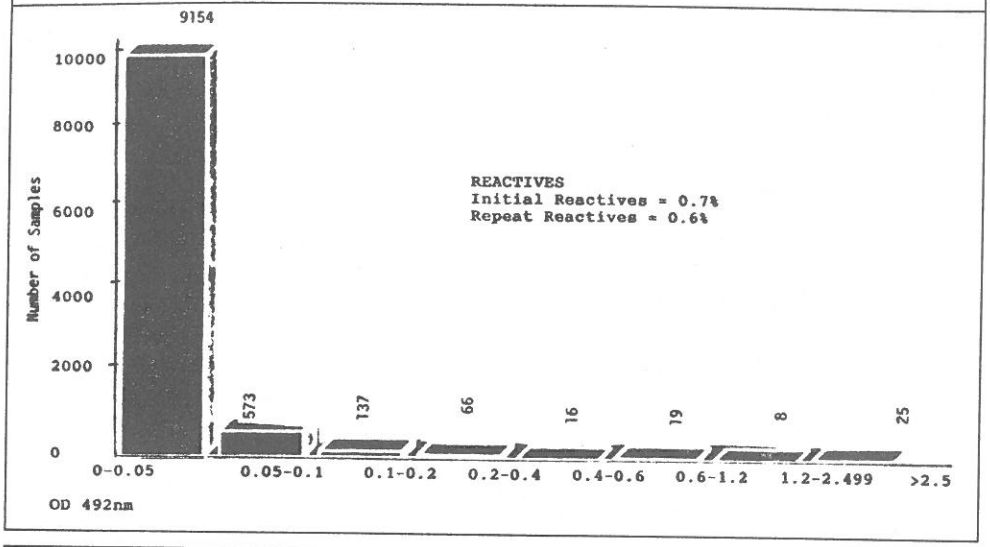


Figure 3
Time of Infection

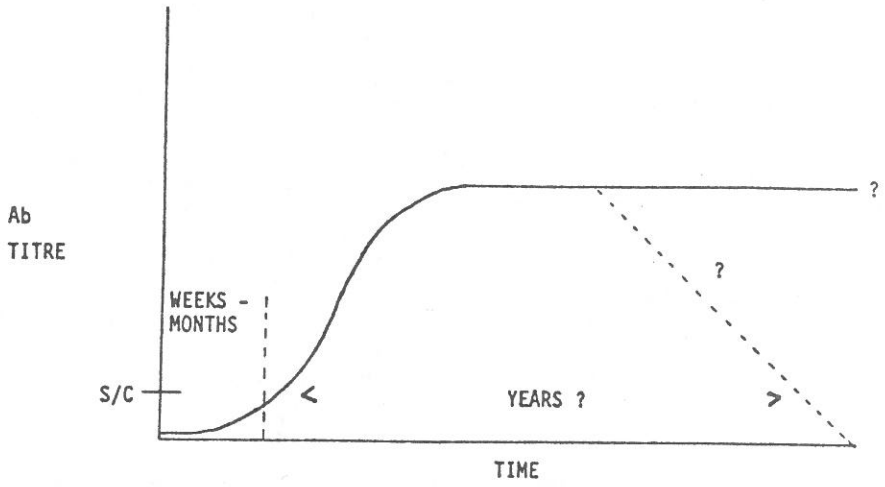
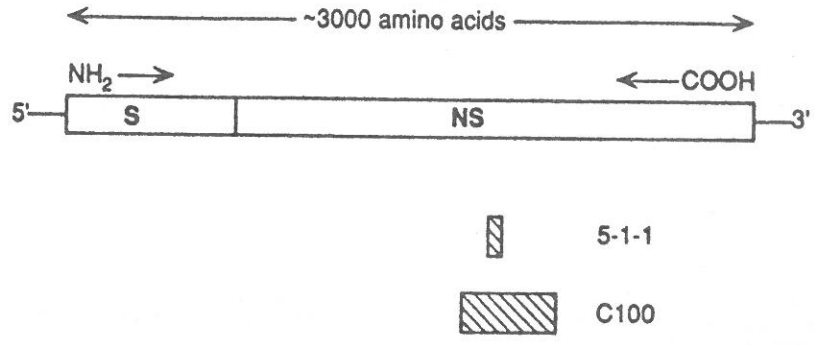


Figure 4
Basic organization of the HVC polyprotein. Virion structural proteins (S) are situated at the N-terminus while the majority of the polyprotein is responsible for the synthesis of a large variety of non-structural (NS) proteins. The approximate positions of the polypeptides encoded by clones 5-1-1 and C100 are indicated.



As can be seen in Figure 3, concerns arise in relation to time of infection, antibody levels to seroconversion, and whether antibody is reflecting a declining response in resolved infection, or a persistent infection.

It is important to remember that the difference that we are looking at in terms of chronicity is in hepatitis B virus-infected blood donors compared to hepatitis C. In terms of the natural HVB infection we are looking at only 6 to 10 per cent becoming chronic, whereas in non-B and particularly hepatitis C, we are looking at a much higher number of individuals being chronically infected, with perhaps up to 75 per cent of those being asymptomatic.

Who is going to be reactive in the test? From looking at our own data and what is being published, this non-structural antigen is really a marker for persistent infection. Thus, those that we are detecting, we assume, will be individuals who are asymptomatically persistently infected who are donating blood. The purpose of the test is to screen out those individuals.

However, we do recognize that we are uncertain about the duration and the antibody response in individuals so that in our donor population we may be getting people who have resolved an infection, but who are antibody positive. These individuals are not persistently infected and will not transmit the virus. Eventually we expect to be able with novel tests to sort out the difference between those who are persistently infected and those who are not but are antibody positive.

In terms of the accumulated data that we have obtained, using this Ortho EIA screen test, we began in April of 1990 and by June 30th it became the test of record for all blood donations.

The data are presented in Table 1. The initial reactive rate reflects the reactivity with a sample cut-off ratio of 1 on the initial testing of a donor's sample of serum or plasma.

The repeat reactive rate means that when an initially reactive donation is retested in duplicate, one of those test results has to be reactive with the sample cut-off ratio of 1 or greater to designate that donation as a repeat reactive donation. Any such donor having that reactivity is excluded from further donation.

In terms of the change in repeat reactive rates with time, this is accounted for partly by the learning curve of the centre technical staff becoming more familiar with the procedure until it became a test of record. As you can see in Table 2, it is fairly stable now, running at about 0.52 per cent in our donor population, but probably just under 0.5 per cent because these are cumulative data including the earlier results.

I am still a little surprised that we are finding that the initial reactive rate is almost double the repeat reactive rate for an EIA screen test using a recombinant antigen. Thus, out of the 353,000 donations we have looked at, roughly half the initial reactives proved to be not repeat reactive on duplicate testing.

The distribution of these repeat reactives across the country is shown in Table 3. Low repeat reactive rates show in St. John's, Newfoundland; Saint John, New Brunswick; and Halifax, Nova Scotia. The repeat reactive rate amongst our donors in the Maritimes is roughly 0.3 per cent. Vancouver Centre has a reactive rate of 0.6 per cent and includes, obviously, the cosmopolitan population of Vancouver. In the western provinces, in the Calgary, Edmonton, Saskatoon, Regina, and Winnipeg centres, we have a range of about 0.39 to 0.74 in the repeat reactive rate of the blood donors. In Central Canada we are running 0.48, 0.62, 0.57 in Ontario, with Toronto (0.64) slightly higher than Ottawa (0.50).

In Quebec City and Montreal the rates are running around 0.60 and 0.57 per cent, respectively.

The rates in the Maritimes presently appear somewhat lower than the rest of the country, but we will with time, over another six months or so of testing, get firmer figures on these. I do not have the initial numbers of donors to give you the exact variation from centre to centre.

Given the fact that we were looking at this EIA as a screen test, we felt the need not only to find out more about the meaning of this reactivity in the blood donors, but also, for investigation of post-transfusion cases, to take a close look at the recombinant immunoblot assay (RIBA) supplemental test and apply that to our data.

This involves using the recombinant C100 antigen on a test strip along with another smaller 5-1-1 region of the same C100 region. Instead of being produced in yeast as a recombinant peptide, 5-1-1 is produced in *E. coli*.

As shown in Figure 4, you have two antigens produced in two different systems from the same non-structural region of the virus. This RIBA is used as the current supplemental test to get a better specificity out of the blood donor screen data.

The response to this particular blot strip requires for it to be positive to have a reactivity to the *E. coli*-produced 5-1-1 antigen, and reactivity to the yeast-produced C100 antigen, and no reactivity to the human superoxide dismutase gene product, which is present on the strip. The intensity is measured against two levels of globulin intensity to determine whether the donor serum is positive or not on reactivity with the individual peptides. We were convinced of the value of the RIBA test when we examined several post-transfusion hepatitis cases. Data is presented in Table 4. Using this RIBA supplemental test, 86 per cent, or 24 out of 28, were positive on RIBA -- and this is from a single serum sample we had in our serum bank from post-transfusion

Table 1
Canadian blood donors anti-HCV EIA reactivity

	TOTAL	IR	RR
April 1990	42,818	1.62%	0.80%
June 1990	162,044	1.23%	0.62%
August 17, 1990	303,093	1.07%	0.53%
August 23, 1990	353,228	1.04%	0.52%

Table 2
Anti-HCV initial and repeat reactive rates (lot by lot)

Master Lot	# Tested	% IR	% IR
119	12,662	1.45	0.58
128	39,033	1.20	0.58
137	42,349	1.04	0.53
152	58,549	0.98	0.49
147	48,451	0.93	0.47
157	81,459	1.01	0.50
162	36,722	0.94	0.55
316	34,088	1.13	0.53
TOTAL	353,228	1.04	0.52
	Range	0.93-1.45	0.47-0.58

Table 3
Anti-HCV repeat reactive rates of 353,228 blood donors

	RR%
Saint John	0.31
St. John's	0.32
Halifax	0.33
Vancouver	0.60
Calgary	0.39
Edmonton	0.48
Saskatoon	0.49
Regina	0.61
Winnipeg	0.74
Sudbury	0.48
London	0.62
Hamilton	0.57
Toronto	0.64
Ottawa	0.50
Quebec City	0.60
Montreal	0.57

Table 4
RIBA confirmatory test results

	EIA	RIBA Interpretation			
	RR	+ve	-ve	Ind.	N.S.
Post-Transfusion Hepatitis	28	24 (86)	0	4(14)	0
Implicated Donors	14	13 (93)	1 (7)	0	0

( ) = %

Table 5
S/C ratios of RR donors (78) correlated with RIBA results

	S/C Ratios			
	RIBA + ve	RIBA - ve	RIBA Ind.	RIBA n.s.
	(22)	(20)	(31)	(5)
	6.88	1.06	2.16	2.95
	6.13	1.94	2.33	3.86
	5.79	2.06	2.08	4.29
	6.09	3.09	4.83	5.82
	6.50	1.07	6.45	1.27
	6.37	1.48	2.03	
	6.51	1.44	1.22	
	6.16	2.90	2.44	
	6.27	1.02	1.19	
	5.58	3.69	1.79	
	5.67	3.30	2.12	
	6.36	1.20	1.15	
	5.90	1.38	2.21	
	6.91	1.27	1.28	
	6.71	1.31	1.17	
	4.20	1.63	2.76	
	6.55	1.78	1.45	
	6.03	1.12	5.49	
	5.89	1.28	4.40	
	5.94	1.64	1.15	
	5.87		2.22	
	6.04		1.59	
			1.11	
			1.34	
			1.21	
			1.48	
			6.95	
			2.15	
			2.10	
			1.21	
AV	6.10 (4.2-6.91)	1.78 (1.02-3.69)	2.32 (1.11-6.95)	3.64

cases. In the implicated donors from the donor pools going into those, we identified 13 out of 14, or 93 per cent on the supplemental test as being positive, which gave us a reason to consider the supplemental test as a reasonable tool to find out what the true positivity was in our blood donor population.

As regards the RIBA interpretation in donors, the question was raised about the S over C ratio in terms of the signal that you get in donors who are screen positive compared to negative status on RIBA. In the blood donor population that we looked at, some 78 in a pilot study, you can see (Table 5) that the signal in RIBA positives is significantly different from that in RIBA negatives. Looking at the supplemental test results on our blood donors, at least in this particular study, we found that its application was quite significant.

We have done further testing. We have broken it down to the actual reactivity on the supplemental test and we have looked at 2,974 EIA reactive samples from blood donors. Of these, 54 per cent are negative and 27.6 per cent are positive by RIBA, and of the indeterminates you can see a distribution in Table 6 of the reactivity to the given peptide sequence on the blot strip. The C-100, which is the same antigen used in the multi-well plate for the screen test, results in, not surprisingly, a high percentage of indeterminate results.

We then requested from our centres more data on the RIBA-positive donors. The limited data are whether the donor is male or female, what age and whether the donor comes from a rural or urban community. (We defined "urban" as having a population of 30,000 or greater.) We found that for male/female RIBA positives, the distribution is very similar between male and female and between the urban and rural populations.

In terms of age distribution (Table 7), and again looking at male and female, at least of the total number reported so far, the ratio of male to female in the donor population which should be a ratio of 60:40, actually was 76:24. So, it seems skewed in favour of the male donor being more positive than the female donor in terms of the percentage of the populations of males and females of the donor population. All these data are based on "confirmed" positive donors by supplemental testing following the screen test.

Related to the age groups, of all the reactive donors something like 70 per cent of males and 70 per cent of females are under the age of 40. Thus, the numbers of positive male and female donors in that age group are very similar. This is as far as we have got in terms of accumulating data. Obviously we need the denominators to go with these figures in terms of how many donors were actually tested in which centres. The numbers coming in from some centres are rather small at present so I have not attempted to break it down any further.

Within a couple of months we will have accumulated more data on supplemental testing which will give us a better idea of what the real distribution is and whether we can start to see any patterns and ask questions about the distribution of hepatitis C in the blood donor population.

Given that these are essentially healthy individuals who have not had any clinical evidence of hepatitis infection, this makes for interesting epidemiological studies.

DR. BISHAI: Dr. Gill, have you tried to confirm each positive result, repeated positive, by RIBA or just a few cases, every positive, repeated positive in blood donors? Have you confirmed them by RIBA before you send out the letters to the donors?

Table 6
CHIRON® - RIBA test system results for Ortho HCV EIA repeat reactive (RR) donor samples collected from July 24 - September 21, 1990

No. of Ortho HCV EIA RR Samples Tested	CHIRON® RIBA Test System				
	Neg (%)	Reactive (%)	Indeterminate		
			C100-3 (%)	5-1-1 (%)	SOD (%)
2,974	1,618 (54.4)	820 (27.6)	433 (14.6)	86 (2.9)	17 (0.6)

Table 7
Canadian volunteer blood donors RIBA supplemental test "confirmed" vs age

Age	# Male	%	# Female	%
<20	3	0.67	4	2.90
20-29	48	10.70	25	18.00
30-39	263	58.70	70	50.30
40-49	103	23.00	24	17.30
50-59	23	5.10	12	8.60
60-69	8	1.80	4	2.90
Total	448	100.00	139	100.00

Table 8
Prevalence of anti-HCV correlation with ALT and anti-HBc

ALT	Anti-HBc		N = 1544 Total tested in ALT/anti-HBc	N = Number of samples anti-HCV reactive	Rate %
NOR.	-	1508	1508	** 6	0.40%
ELV*	-	11	11	0	0
NOR.	+	24	24	0	0
ELV.	+	1	1	0	0

Precise ALT normal level in international units (male or female)
* ELV = ALT level > 2 normal levels
** :n = Number of blood units detected as HCV + and not eliminated through ALT and anti-HBc

DR. GILL: No. The initial strategy was that we would do screen testing and advise all reactives on screen testing that they had a reactivity. The purpose was to be, as usual, conservative; that is, eliminating risk to the blood supply was our main concern, not the actual definition of the status of an individual.

So, we are now testing by RIBA after the fact, after having notified donors that they are screen positive. The information which we could forward to the donors' physicians would be whether they are supplemental test-positive or supplemental test-negative or indeterminate.

I think our position would be to raise concern about doing liver function tests, et cetera, on individuals who are RIBA positive or RIBA indeterminate as opposed to the RIBA negatives.

In one instance it would be important to determine whether these people were chronic and persistently asymptomatically infected and consider what might be done to further evaluate them or treat them. On the other hand, it might be appropriate to be a little bit more laid back and not be alarmist, and allow a physician to make the judgment whether he or she would do further tests on an individual, or just monitor them in routine annual check-ups.

That is the position we are in now. So, physicians will be getting to know the RIBA supplemental test results. So far, of those repeat reactives we have tested, I indicated almost 3,000 which were repeat reactives and have been analyzed by the RIBA supplemental test. But, that is to be ongoing. We anticipate by November this year that the second generation RIBA test will be available with more antigens on it and with a better sensitivity.

We have some data suggesting that the present RIBA test is not as sensitive as it should be. We look forward to switching as soon as it is made available to us and to continuing our evaluation with RIBA second generation.

DR. ROZEE : The rate for confirmed positives for the Maritimes was about 0.3 and for the city with the longest winter in Canada - Winnipeg - about 0.7. These were all done in the same lab?

DR. GILL: Yes.

DR. ROZEE: They were not done in regional labs?

DR. GILL: No, no. All the confirmatory testing was done by our own staff in the National Reference Laboratory.

DR. SPENCE : Peter, I just want to ask you, do you still exclude donors who have a history of jaundice in childhood?

DR. GILL: We do not exclude. We do not solicit them.

Epidemiologic Features of HCV Infection in Northern Alberta

DR. B. LARKE (Canadian Red Cross Society, Edmonton)

Dr. Peter Gill, Director of the National Reference Laboratory of the Canadian Red Cross Blood Transfusion Service in Ottawa, has presented the aggregate data from blood donor screening for anti-HCV as complied by the various Blood Centres coast to coast. The first part of my presentation will provide some of the more detailed results from anti-HCV screening in Edmonton, Alberta.

Edmonton is the northernmost of the Blood Transfusion Centres in Canada and we provide blood and blood products to the northern half of the Province of Alberta, including the City of Red Deer. We actually extend beyond the Arctic Circle to Inuvik, and serve all of the western part of the Northwest Territories as well.

Edmonton was one of the cities selected as a pilot centre for anti-HCV testing. The data I will present are mainly from the early part of 1990, although we checked back on blood products we had in inventory and some of the results are from the end of 1989 year as well.

I want to acknowledge my thanks to Dr. Robert Turner, Medical Director of the Red Cross Blood Transfusion Service in Edmonton, and to Jean McLellan, who is the Laboratory Manager and her staff, who were very helpful in pulling together these results.

As Dr. Gill outlined, all of the initial laboratory testing of blood donors by Canadian Red Cross Centres was carried out using the Ortho Diagnostics anti-HCV testing ELISA. Of 37, 946 donor units assayed between November 1989 and May 1990, 225 (0.59 per cent) were repeatedly reactive for anti-HCV. We have had experience with more than twice that number of donor units through the end of September, 1990, and the overall proportion of anti-HCV reactive units has been remarkably consistent over time, as Dr. Gill mentioned from the national perspective.

Every time a person donates blood, he or she is asked a series of questions designed to eliminate donors who may be unsuitable for a variety of health reasons. One might assume that with this regular questioning, there would be less likelihood of a repeat donor being anti-HCV reactive in comparison with a first-time donor. With this possibility in mind, we compared 3,806 first-time donor units with 20,609 repeat donor units collected furing the first four months of 1990. The difference, however, between the proportions of repeat reactive tests (0.71 per cent and 0.65 per cent, respectively) was not statistically significant.

Our apheresis donors may give frequently. The anti-HCV rates among 2,033 apheresis donors was exactly the same (0.69 per cent) as among 12,047 units collected from urban donors of blood.

We have also looked at the male/female situation: males comprise about 58 per cent of the donors in the northern half of Alberta. A slightly greater proportion (0.87 per cent) of the 14,081 male donor units were repeatedly reactive for anti-HCV compared to 0.37 per cent of 10,314 female donor units ($p < 0.001$). When the donor groups were further analyzed by age, it was evident that anti-HCV seropositivity rates increased as donor age increased and this was more striking among females (Table 1). Highest rates of anti-HCV reactivity (1.19 per cent) were among male donors between 30 and 39 years of age.

In searching for possible clues as to the epidemiology of hepatitis C virus infection among northern Alberta blood donors, we compared seropositivity rates of persons attending urban and rural clinics during the first four months of 1990. There were 145 repeatedly reactive units collected from 20,922 urban donors (0.69 per cent) and 16 collected from 3,493 rural donors (0.46 per cent); the proportions do not differ significantly upon statisical analysis.

We took a closer look at 35 repeat donors who were screened for anti-HCV on a number of occasions between January 1 and May 17, 1990. There were 28 whole blood donors who had one or two subsequent donations, and seven apheresis donors with one to nine subsequent donations. Of the total number, 32 were repeatedly anti-HCV reactive on all subsequent donations. One donor was repeatedly anti-HCV reactive on two subsequent occasions, and another donor was non-reactive on one subsequent donation. One apheresis donor, however, was repeatedly reactive on January 12 and on four subsequent occasions (January 22, January 31,

Table 1
Anti-HIV Seropositivity Rates by Age and Sex

Sex	Age	Anti-HCV		
		No. repeat reactive/ no. screened	% +	P
Female	17-29	9/4,927	0.18	
	30-39	13/2,833	0.46	<0.01
	≥40	16/2,554	0.63	
Male	17-29	28/5,212	0.54	
	30-39	48/4,046	1.19	<0.005
	≥40	123/14,081	0.87	

March 14, and April 5) but non-reactive on February 20 and April 26.

Because Edmonton was in the pilot screening project, donors who tested repeatedly reactive for anti-HCV during this time were not advised as to their status. Our experience with donors whose anti-HCV tests fluctuated from reactive to non-reactive and the general uncertainty of factors that may contribute to false positive reactions emphasizes the pressing need for improved supplementary tests or solid confirmatory assays for HCV infection.

All the donor blood samples that we found repeatedly reactive for anti-HCV by the Ortho Diagnostics screening ELISA have been sent to the National Reference Laboratory of the Canadian Red Cross Society in Ottawa for supplementary testing by RIBA, as described by Dr. Gill.

In summary, the pilot blood donor screening program for anti-HCV on units collected between November 1989 and May 1990 yielded the following observations:

1. the repeat reactive rate for anti-HCV was 6 per 1,000 donations and was remarkably stable month to month;
2. male donors were more likely than females to be seropositive for anti-HCV;
3. seropositivity rates increased with age (17 to 70 years) in males and females. The 30 to 39-year-old cohort of male donors had the highest rate;
4. apheresis donors did not have a lower rate of seropositivity compared to urban donors of whole blood;
5. urban rather than rural place of residence in northern Alberta did not significantly affect rates of seropositivity.

I would like to change focus and present some preliminary results from our screening of prenatal women for evidence of HCV infection. Among the unanswered questions regarding the epidemiology of HCV is whether the virus may be transmitted from mother to her infant *in utero*, during passage through the birth canal where they may be exposure to infectious cervical secretions or maternal blood, or post-natally through breast milk.

Dr. Miriam Alter has reviewed some of the data on this subject. Except where the mother is also positive with the human immunodeficiency virus (HIV), transmission of HCV from mother to infant appears to be infrequent[1].

However, simply in terms of looking at the distribution of HCV infection throughout the population, there are a number of advantages in screening pregnant women for anti-HCV. These women are drawn from a wide geographic area. Since Edmonton Centre serves much of the western portion of the Northwest Territories, as well as northern Alberta, we include some Dene and Inuit women along with the prenatal sampling. Apart from the fact they are pregnant, women are generally in good health and are not seeking medical care or blood testing for other unrelated reasons. By definition, all of the pregnant women are sexually active. They also represent all races and come from the full spectrum of socioeconomic circumstances, educational backgrounds, and lifestyles.

Alberta has had a highly successful province-wide prenatal hepatitis B virus screening program since August, 1985. All prenatal sera are sent to the Edmonton or Calgary Red Cross Blood Transfusion Service Centre where they are screened for hepatitis B surface antigen (HBsAg) in addition to routine testing for anti-erythrocyte antibodies and other factors. We have excellent follow-up on infants born to HBsAg-positive mothers and have completed hepatitis B vaccination on more than 90 per cent of at-risk infants throughout the province[2].

The existence of this highly efficient prenatal screening program provided an excellent opportunity to determine the prevalence of anti-HCV among pregnant women in northern Alberta. We had two objectives. Depending on the rates of HCV infection, such information might suggest whether we should consider a future study of perinatal HCV transmission, with prospective follow-up of infants for liver disease and possible therapeutic interventions. Secondly, an examination of the demographics of anti-HCV reactive persons among a poulation of pregnant women might identify possible risk factors for infection that should be added to the list of routine questions asked in our selection of healthy blood donors.

During August and September, 1989, we selected at random a number of serum samples submitted to the Edmonton Centre for routine prenatal HBsAg screening. Name identifiers were removed and residual sera were screened for anti-HCV, following similar protocols approved for anonymous, unlinked screening of pregnant women for anti-HCV[3]. All of the selected prenatal sera were screened for anti-HCV using Abbott Laboratories enzyme immunoassay, following testing procedures specified by the manufacturer.

To date, we have screened 1,191 prenatal sera for anti-HCV. The age distribution of women included in our serologic survey was representative of the pregnant population served by the Edmonton Centre. The majority (55 per cent) were aged 20 to 29 years, 33 per cent were 30 to 39 years, and only 1 per cent were 40 years or older. The number of pregnant women under 20 years of age appears to be declining and is at present about 11 per cent of our study population.

Of the 1,191 serum samples screened, four women were confirmed positive for HBsAg, as determined through our routine prenatal testing program, and six were positive for anti-HCV. None of the women with HBsAg was positive for anti-HCV. Due to some similarities in transmission patterns of HBV and HCV, other studies have indicated concurrent seropositivity for the two viruses. As Dr. Miriam Alter pointed out, among injection drug users, the presence of serologic markers for

Table 2
Characteristics of women seropositive for anti-HCV among 1,191 prenatal women tested in Edmonton, August and September, 1990

Age (years)	Pregnancy history	Place of residence
23	P1 G2	western rural
25*	P0 G1	Edmonton
26	P0 G1	northern rural
26	P2 G3	northern rural
27	P1 G2	Edmonton
40	P3 G4	suburban

* history of hepatitis in 1986

HBV and HCV in the same individual is relatively common. The four pregnant women who were positive for HBsAg were entirely representative of other women identified in the past five years of our hepatitis B screening program in Edmonton[2]: three women were of Oriental birth (including one health care worker), and one was from a community in the Northwest Territories previously known to have a high incidence of HBV infection.

Table 2 indicates the information available on the six women who tested positive for anti-HCV. Keep in mind that the sera were screened on an anonymous unlinked basis only after HBsAg testing and other routine prenatal serology had been completed. Information on the place of residence was limited to the first three characters of the postal code; the ethnic background and place of birth was unknown. The ages of the women positive for anti-HCV and the pregnancy history are generally representative of the population of pregnant women screened at the Edmonton Centre.

To summarize, we have conducted a pilot program of screening pregnant women for anti-HCV. Among the nearly 1,200 prenatal sera tested to date using the Abbott Laboratories enzyme immunoassay, the repeat positivity rate of 5 per 1,000, or 0.5 per cent, was slightly below the .59 per cent overall rate among male and female blood donors tested, but higher than the rate of .37 per cent among the female donors. We realize that since we have not done supplemental RIBA or confirmatory testing on the six prenatal sera positive for anti-HCV, as many as a half to two-thirds may be falsely reactive. A note written on the requisition of one of the women who tested positive for anti-HCV indicated she had a history of hepatitis in 1986.

Any decision to implement anti-HCV screening into Alberta's routine prenatal program in the future will depend to a large extent on the outcome of additional ongoing prospective studies that should establish the frequency of mother to infant transmission of HCV in various populations. Information that will be presented later in the conference regarding the prospects for passive or active immunoprophylaxis against HCV infection will also influence our decisions about possible routine anti-HCV screening of pregnant women to identify their at-risk infants. Even if the virus carrier state cannot be prevented in early childhood, new therapeutic agents may become available for those who progress to chronic liver disease caused by HCV.

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DISCUSSION

MS TISCHLER: I would like to find out if anybody tested patients with HIV positive sera against HCV to see if there is any increase or any comparable incidence to the hepatitis B and HIV?

In other words, a comparison, HIV HCV versus HIV HBV.

SPEAKER: Not quite like that. What we did was receive a set of sera, including serological bleeds, from patients who had a history of transaminitis who were haemophiliacs and we tested them for anti-hepatitis C.

What we found, including the supplemental test, was that we got some indeterminates or positives going to indeterminate amongst that subset. When we broke the code, we found that all were HIV positive and that the titre seems to sort of be unstable in terms of anti-HCV in these HIV positives.

Not only were they HIV positive, they were in the final stages of disease, many of them. So, there is some perturbation, maybe, of the HCV tests in HIV-positive individuals in terms of both screen and supplemental testing.

That is all I can say.

SPEAKER: We have studied a large number of haemophiliacs who obviously have a high prevalence of both infections and we have not been able to see any interaction in terms of one having an adverse effect on the other at least in the asymptomatic. Although, so far as the antibody is concerned, in the terminal stages there may well be a difference.

DR. SAKLOV: I have two questions. One is the role of anti-IGM antibodies in sero-converters and the second one is, what type of pattern has been used to define the specificity of the tests?

SPEAKER: I can comment on IGM. We have been looking at that and we have had some 30 sero-conversions among haemophiliacs being treated various ways and we do frequently find IGM for a period of about six months.

I am not sure I have any information on the second question.

DR. FREEDLE: Is surrogate testing with anti-core and ALT still being done at the present time?

DR. ALTER: Yes, and it will continue to be done. The three blood banking organizations as well as the FDA are recommending that both ALT and anti-core continue to be done.

The Food and Drug Administration has basically stayed out of saying anything about surrogate tests since they first were recommended by the blood banks, but I understand that they are now going to recommend, in addition to anti-HCV testing, anti core testing, so that the surrogate markers will remain.

DR. FREEDLE: Do you have any idea how many more donors are being eliminated with the surrogate markers compared to just anti-HCV alone?

DR. ALTER: I am not sure that I can answer your question exactly. I know that about 40 per cent of individuals with surrogate markers are not anti-HCV positive, but how much of the donor population it is actually, I do not know.

DR. ENGLAND: I would like to ask the panel about the merits of retrospective testing of transfusion recipients.

DR. ALTER: From a public health perspective, not from the blood banker's perspective, we would not recommend either directed or universal look back of previous transfusion recipients for a variety of reasons.

One, the meaning of the test is not clear so if you do not know whether an individual who is anti-HCV positive has resolved their infection or is chronically infected, therefore, what are you going to tell the person when you detect the antibody?

Two, the rate of false positivity in low prevalence disease populations is high and no confirmatory test is available to distinguish true from false positives.

The effort involved in such a screening or media campaign whatever would be very large in comparison to what you get in return which is probably a very small number of people being reached. And, because transfusion recipients represent a very small proportion of the disease, we do not feel that is where the resources ought to go. If you are going to recommend that that group be screened, perhaps you should recommend that anyone who has a risk factor for hepatitis C be screened as impractical as that may be. But, from a public health point of view you cannot discriminate between risk groups.

You would be finding a lot of people who were anti-HCV positive and what would you tell them? So, at this time we do not recommend it.

SPEAKER: May I just add to that as another non-blood banker that I agree very emphatically. I think for the blood bankers it is legally driven and it is not a good use of public health resources.

DR. BARKER: I could just about say ditto and save a lot of time tomorrow by referring to the points that Peter Gill and Bryce Larke made so nicely on the findings of blood donors.

I do have one comment and then a question for perhaps several members of the panel. We do have some data, incidentally, on the correlation with the surrogate markers which I will present tomorrow.

I think it may be a little early to see a difference between first time and repeat donors. Our experience with every test we have introduced over the past 20 years or so has been that there is a point at which considerable difference appears.

That is because you start out with the repeat and first time donors all being tested for the first time, but we will get to the point where the repeat donors are a fully at least once tested population, at least those who donate at intervals of roughly a few months to a year so they will all have been tested. And, then we see incident or new cases for the most part in those repeat donors whereas we see both new and established or chronic cases in the first time donors. I expect that a bigger difference will turn up in maybe a year or two, Dr. Larke, in what you have seen so far. We are in the same situation as you are in this early stage.

My question for the group is, while I think it is obviously desirable to have a handle on specificity of this test like all of the viral tests that we do, I am not so convinced at this point that it is as important to have a handle on infectivity and I say that because there is so much

interaction, I guess, is the right term for the risk behaviours.

Basically, I do not think we want these people in the donor population; that is to say, people who have even a history of exposure with perhaps full recovery, not a chronic viral infection, but antibody markers only.

I am wondering how Dr. Alter or Mosley or those of you in the Canadian blood transfusion services feel about that. My view at this point would be that if we have a specific marker for infection with HB virus or HC virus or one of the retro viruses, that probably ought to be enough to say, thank you very much, we would rather you not be a blood donor.

SPEAKER: I think, as I interpret this comment, that specificity is not so important at the present time. The donor could simply be excluded.

I think the real difficulty with that is while I do not feel that looking back to identify infected recipients is worthwhile, I think blood banks would be in a very poor position if they did not advise the donor of possible infection, a possibly chronic infection, that could be transmitted in a variety of ways.

I think it is really in terms of informing the donor that we need to have as good an answer concerning specificity as possible.

DR. ALTER: The FDA is not going to allow any blood banks to use repeatedly reactive anti-HCV donations no matter what the specificity of the test is. But, on the other hand, the terms of notification of donors, given the rate of false positivity with this test or at least what we understand to be the rate of false positivity based on the supplemental testing, I do not think it is fair to the donors when they have such a high probability of being false positive that we tell them, but then we do not let them know about the availability of supplemental testing. Optimally all positive donors should be tested with a supplemental test prior to their notifications so that you would have a better idea, or the blood banking group would have a better idea of what to tell the donor as to the possibility that the test represents a true or false positive.

I think that is only fair to the donor.

Let's assume that you have a donor who has a normal ALT level, no surrogate marker - and I realize in Canada they do not test with surrogate markers anyway - and the test is positive for anti-HCV, you can tell the donor that this is very likely a false positive if his/her ALT is also normal and yet, the donor can no longer donate given the state of the art of the test at the moment.

DR. BARKER: I can tell that my question was not clear. I was distinguishing between two kinds of specificity. My view is that specificity for history of active viral infection is very important, desirable and useful from a practical standpoint.

My question was, should we worry so much these days about whether the individual is in fact a carrier or is a previously infected person? I absolutely agree that specificity of the marker for the virus having been there at some point is important and desirable, so I did not make that point clear, evidently.

My question is should we worry much about whether or not the individual is chronically infected since these viruses do travel around together quite a bit?

DR. LARKE: I just want to make a comment to Dr. Barker's comment about repeat versus first time donors.

For the first seven months before the National Red Cross uniformly introduced hepatitis C antibody testing, we were doing that in Edmonton, but we were not notifying the donors who were anti-HCV positive.

So, while we clearly did not use their blood, they were still coming in to donate so they remained in the pool and I agree over time as they are withdrawn with the current system, they would clearly diminish.

I wonder if I could ask a question of Dr. Alter (about advising donors of their status). I am just wondering what Dr. Alter, in view of the data that she has presented with respect to the possible or probable transmission of this virus, at least in some individuals, through sexual activities, what she would recommend we tell our blood donors at this time.

DR. ALTER: I always get asked this question.

First of all, I think it is incumbent on those of us doing testing that before we start counselling individuals about changing their lifestyles that we do supplemental testing and make sure that to the best of our current ability to tell that this is a true positive. So, that is number one.

So, assuming that you do have, to the best of our ability to tell a true positive, I think you have to let someone know that there is some evidence that this disease can be sexually transmitted.

If they have a monogamous relationship and they have had this one partner for a very long time, chances are there may be no reason to change their behaviour. You could test the sexual partner and determine whether transmission has already taken place. If it has, then obviously they may not need to change their behaviour.

On the other hand if it has not, don't you think that they have a right to know that it is a possibility?

First, any individuals who have multiple partners should be using safe sexual practices anyway and they do need to consider the number of partners that they are having sex with; second, they should be informing their partners that they have a potentially infectious disease and; third, they should be taking precautions to prevent the transmission of all sexually transmitted diseases, not just hepatitis.

DR. ROZEE: May I just comment. One of the very few things that I have to disagree with Miriam about are the data on sexual transmission, at least so far as our own data are concerned.

We have looked at sexual and household contacts of haemophiliacs who have an infection rate between 60 and 70 per cent and many of whom have chronic ALT elevations suggesting persistent hepatitis. The rate of hepatitis B positivity ranges from about 10 per cent in all household contacts to 20 to 30 per cent for sexual contacts and for mothers of children. Obviously the mothers are providing care.

In contrast to that, we have a frequency of anti C of about 0.5 to 2.0 per cent which is not really different from the background rate for a donor population.

I find it difficult to think there is much sexual transmission in view of these figures. I think there is another study with similar data.

SPEAKER: There was a suggestion earlier that in some instances patients are referred to their own physician.

It would seem that before doing that one would have to be sure that the

patient's own physician at least had the opportunity, as the members of this audience have had, to know something about the natural history of this disease.

Most physicians are really not in a position to advise their patients at the present time. Now, perhaps a year or two or three from now, with education, they may be. If I were the usual private physician who had not had an opportunity to read or to understand much of what has been going on this past year it would be very, very difficult.

SPEAKER: I agree. We certainly began to send the notices out with great trepidation. The first letters were just a generic letter that said there is a problem with one of your tests and then people were coming in after sweaty weekends thinking they had HIV infection or leukemia or some other dreadful situation.

However, we have to send out some kind of a letter as a first thing to get the physician's name because this is not routinely collected as a donor item. Once we have the name of the physician, then we send them out an educational package.

But you are quite right, most physicians have never even heard of hepatitis C. They may have heard of non-A, non-B, but not under hepatitis C as a name and many of them refer their patients right back to me anyway.

DR. BISHAI: I have just one comment and then a question after that which has been partly answered so far, but I would like to hear the answer more clearly.

First, Dr. Bryce Larke identified the sexually active woman. We had one study in which we screened more than 100 sexually active women and the ages ranged from 19 to 65, so I would point out that sexually active women are not restricted to those women under 40.

The second point is that we found only five confirmed positives for hepatitis C among the 100. However, all five were drug abusers.

The second comment I would like on the panel here is, what recommendation do you give a healthy person who donated his blood and he was HCV positive but he has no other symptoms, neither elevation of ALT or any other symptom. Are you going to put the same restriction on that person?

DR. ALTER: Was he tested by a supplemental test? Do you have supplemental test results available?

DR. BISHAI: Yes, assuming that we have a variety of tests, we have some patients which I am going to project were positive by skin test, negative by RIBA, positive by ELISA, Abbott and positive by peptide, sensitive to peptide C-100.

DR. ALTER: For a patient who is anti-HCV positive on EIA who is also supplemental test positive, I have to assume that that person truly has hepatitis C.

I think that even Dr. Schiff and Dr. Gitnick would agree with me in this case that individuals who develop chronic liver disease with hepatitis C are very often asymptomatic and, in fact, even individuals with cirrhosis on biopsy may experience no symptoms.

So, I am not sure if the fact that a person feels healthy is a good indication of infection. On the other hand, a person who feels healthy and has no signs or symptoms of hepatitis, who is anti-HCV positive on EIA, but without supplemental testing, I would really have to suggest that that very well may be a false positive.

DR. CHERNESKY: I would like to ask the panel what is happening as far as testing in transplantation programs? Is this a marker that we should now be screening as well as HIV and hepatitis B and CMV?

DR. LARKE: Certainly in Edmonton and I imagine it is true everywhere, most of the provincial laboratories and hospital laboratories can now perform this assay. Since the beginning of this year, we have screened all of our transplant candidates and the donors of those organs for antibody to hepatitis C as well as HIV, hepatitis B, et cetera.

DR. ALTER: What would you do with a relatively rare donor tissue in which the donor tested anti-HCV positive without benefit of supplemental testing? What would you do with that tissue?

DR. LARKE: If I had the option of getting that, I would certainly opt for it. We make that very clear to the transplant surgeons, the status of this assay, but for medical and legal reasons we have the test available and we are performing that. They have the option whether they will go ahead and use that tissue.

DR. ALTER: My other question is, what do you think ought to be done with inventory when donor serum is not available for anti-HCV testing? Do you think that inventory should be used, particularly with respect to semen, or do you think we should discard our inventory if we cannot test the donor?

SPEAKER: I think you would have to decide what tissues you are looking at. If it was a bone donor, I would say, no, there are other options. Semen is retained, and most places have the ability to bring that person back after six months so it is not used anyway because of anti-HIV testing.

We just do not have enough information, I think, about the epidemiology and natural history of this disease to answer some of those questions.

I think the lawyers would tell us that if anybody is anti-HCV positive, even without supplemental testing, it is better to steer clear.

SESSION II

IMMUNODIAGNOSIS

Chairman: Dr. R. PURCELL (NIH, Bethesda)

Discovery and Molecular Organization of HCV

DR. L. OVERBY (Biotechnology and Medical Diagnostics, Alamo)

Most of us became acquainted with non-B hepatitis in 1972 by confirming that there was some transfusion-associated hepatitis that did not induce hepatitis B serological markers. By 1974, when hepatitis A virus was discovered, this post-transfusion hepatitis was also not shown to be the result of hepatitis A virus infection, and was designated as non-A, non-B (NANB) hepatitis.

Why did it take almost 15 years to discover this virus? I do not know the answer, but it was discovered through molecular cloning by a research team at Chiron Corporation in collaboration with scientists at the Centers for Disease Control in 1987[1,2]. Since then, there has been only one other completely independent isolate of the virus. Clearly, many other isolates will be needed before we know the whole story of hepatitis C virus (HCV).

I will review the background of how HCV was discovered at Chiron, summarize current understanding of the molecular organization of the viral genome, indicate the diagnostically significant viral proteins, and identify some major challenges and opportunities for the future study of hepatitis C, using the molecular and immunological tools now in hand. We can now expect rapid progress through combinations of clinical, virologic and molecular studies of this interesting new hepatropic virus.

HCV discovery

HCV represents the first example of successful cloning of a viral genome in the absence of any prior growth in cell culture, nucleic acid or protein sequence information, or identificaton of virus-specific antigens or antibodies during infection in animals or humans. Was the successful cloning of HCV from titered chimpanzee plasma:

- easy application of cloning grafts to a plasma source containing 1 million chimpanzee infectious doses per millilitre?
- just a lucky hit in the cloning lottery?
- or the application of innovative techniques and skills developed to discover an extremely rare nucleic acid in a universe of adventitious DNA or RNA?

Succesful cloning of the HCV genome was neither lucky nor easy. It was the result of persistent application of improved molecular cloning and selection techniques that enhanced probabilities of cloning and identifying a viral nucleic acid present at only one part in 10 million of unrelated nucleic acids. A key to developing the methodologies was the cloning and sequencing of the hepatitis delta virus (HDV) in 1985[3]. Specific procedures of concentration, reverse transcription, library preparation, and immuno-screening were validated to be successful with HDV at a level of one to 10 million delta RNA genomes per milliliter of chimpanzee plasma. The exact techniques were then applied to chimpanzee plasma containing known levels of NANB infectivity.

Initial studies

There have been hundreds of publications about NANB hepatitis since 1972. Many patents have been issued claiming hepatitis C virus and methods for identifying antigens and antibodies. Most of the patents and many of the publications were not helpful teachings to guide molecular approaches to identifying the virus through molecular cloning. Many studies clearly established serious long-term consequences of transfusion-associated infections, and the probable virus nature of the infectious agent. Our knowledge and accomplishments up to 1987 are as follows:

- a high risk of infection (up to 10 per cent) from transfused blood
- NANB hepatitis frequent in sporadic liver disease
- chronic asymptomatic viremia in healthy blood donors
- high risk of serious long-term chronic hepatitis after transfusion infections
- NANB hepatitis associated with hepatocellular carcinoma
- infectious agent has biophysical properties of a small chloroform sensitive virus
- disease transmitted to chimpanzees and accurate diagnosis from ultrastructural changes to hepatocytes
- infectious titre of plasma and liver extracts determined in chimpanzees
- worldwide epidemiology similar to hepatitis B.

Many other studies were disappointments and would be misleading for designing molecular strategies for cloning and identifying the virus genomes:

- serological antigen/antibody systems, complicated by autoimmune systems during disease process
- two or more bloodborne agents without cross protection
- identification of virus-like particles by electron microscopy
- NANB hepatitis as a form of serologically silent hepatitis B
- NANB hepatitis with serologic retrovirus markers
- isolation of viruses in hepatocyte tissue culture.

Some of these studies and reports, although disappointments in guiding molecular strategies, may eventually be important in understanding the disease processes when the hepatitis C virus life cycle and replication strategies are better understood.

Cloning strategies

In 1987, there were formidable and discouraging unknowns about the transfusion-associated NANB hepatitis virus. Cloning strategies were developed at Chiron to deal with the following options:

- DNA or RNA genome
- double or single-stranded genome
- single, segmented or multiple genomes
- positive (sense) or negative (antisense) polarity
- no nucleic acid or protein sequences to construct probes

Figure 1
Recombinant clones of HCV: Preparation

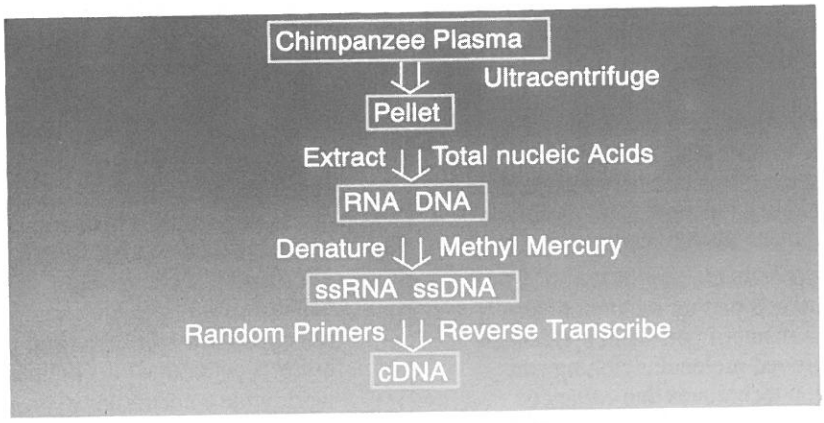


Figure 2
Recombinant clones of HCV: Isolation

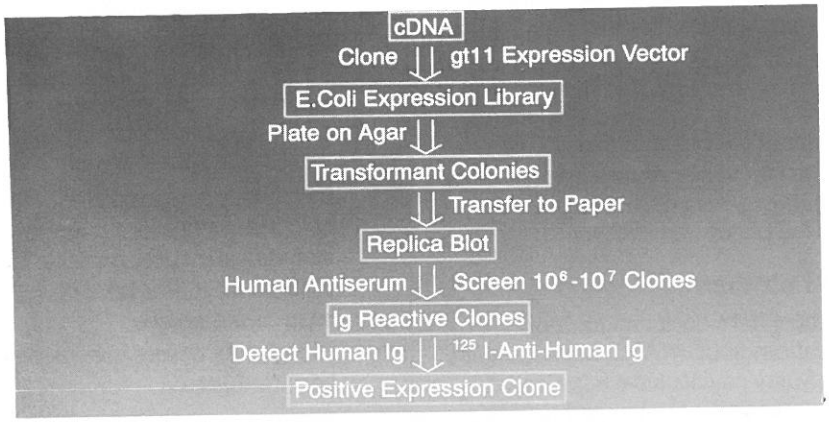
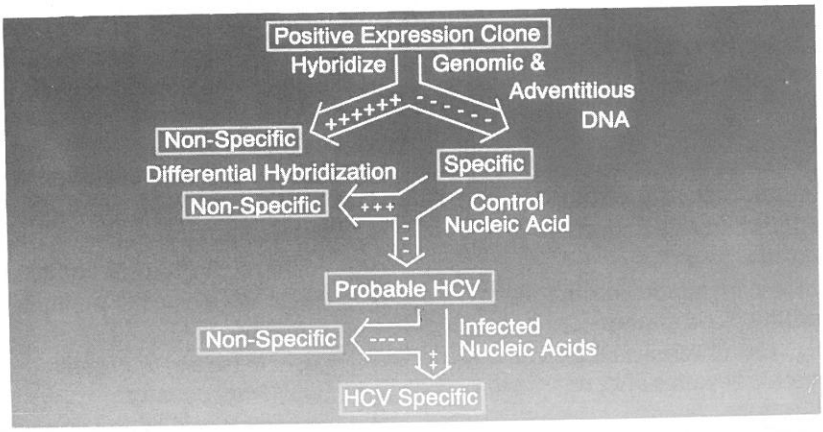


Figure 3
Recombinant clones of HCV: Identification



- no validated sources of antibody positive serum for immunoscreening.

Several years investigating RNA extracted from normal and infected human and chimpanzee livers led to no specific clones of cDNA associated with infection. This work did lead to improved molecular techniques that were applied to centrifugal concentrates of chimpanzee plasma containing one million infectious doses per milliliter.

Expression libraries were screened with serum from human patients with well diagnosed chronic NANB hepatitis. Notable, immunoscreening of expression libraries with human antiserum detected hundreds of genomic clones that produced host proteins associated with autoimmunity during chronic NANB hepatitis. An essential for success was to rapidly recognize and eliminate such recombinant clones as candidate virus specific clones.

Figures 1, 2 and 3 summarize the sequential steps in constructing expression libraries and eliminating immunopositive clones of host and adventitious cDNA. These procedures were rigorously followed in preparing and screening hundreds of cDNA libraries.

Virus specific clone

Eventually, after screening about 20 million recombinant clones derived from liver or plasma nucleic acids, a clone designated 5-1-1 was identified that met all criteria for being virus specific by both its hybridization properties and seroconversion to the expressed protein in both humans and experimentally infected chimpanzees. In addition, a high percentage of patients with chronic NANB hepatitis were positive for the antibody, and most healthy people were negative.

The 93 nucleotide sequence of 5-1-1 was then used as a hybridization probe to identify larger overlapping clones in the same and subsequent libraries. The overlapping clones were shown to be derived from a high molecular weight (10,000 kb) positive stranded RNA found only in plasma or liver during NANB hepatitis infection[4]. The agent was thus designated hepatitis C virus (HCV).

Figure 4
Flavivirus organization

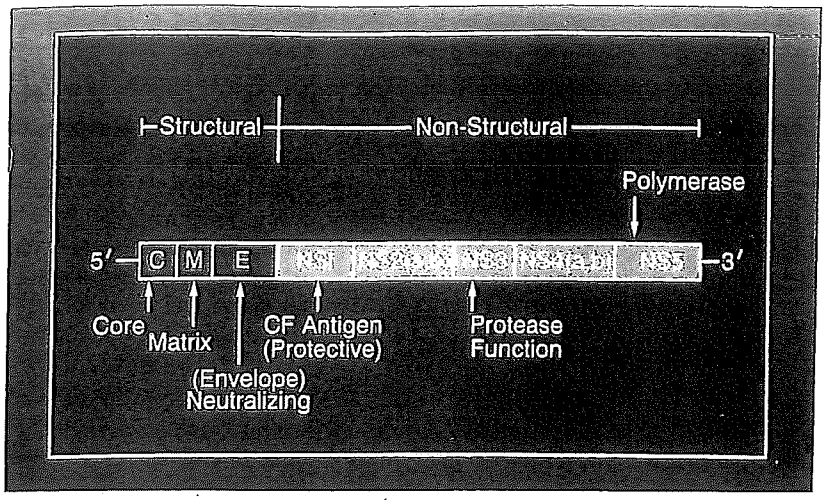


Figure 5
Flaviviridiæ

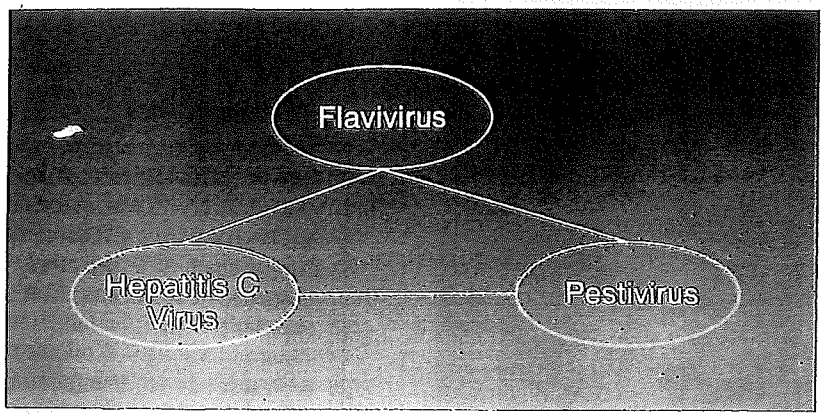
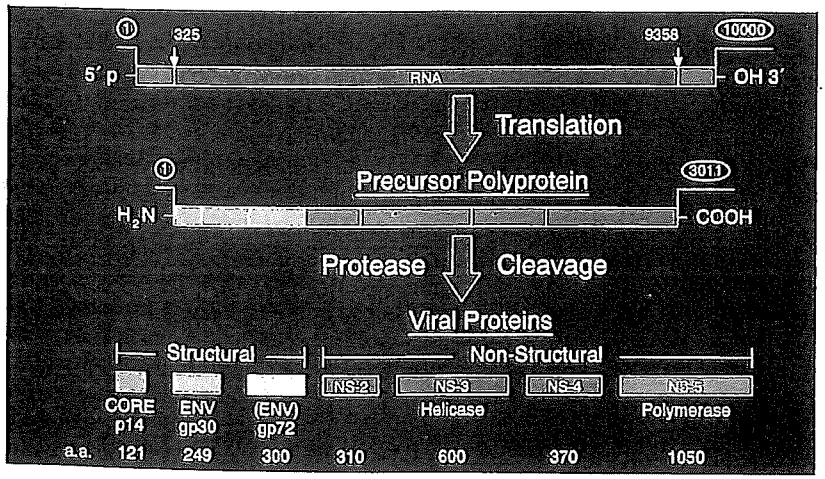


Figure 6
HCV genomic organization



The HCV genome

The nucleotide sequence of the HCV genomic RNA was shown to be a continuous translational open reading frame (ORF) that encoded a polyprotein of about 3,000 amino acids. Comparison with other known viral sequences showed some co-linear homologies with the flaviviruses. This suggested similar genomic organization for HCV. Figure 4 shows the typical genomic organization of the flavivuris, yellow fever virus (YFV). The genomic RNA codes for a polyprotein with structural proteins at the 5'end (N-terminal), followed by non-structural proteins at the 3' end (C-terminal).

Examination of the amino acid sequence of the encode polyprotein of HCV for hydrophobicity and hydrophilicity revealed exceptional correspondence with the yellow fever virus and also with pestivirus, bovine viral diarrhea virus (BVDV). These similarities suggest tentative classification of HCV in the flaviviradae, wherein HCV shares a distant relationship to the vectorborne flavivirus and the animal pestivirus families (Figure 5). However, there are notable differences for HCV:

- the structural regions differ substantially from flaviviruses and pestiviruses;
- the viral RNAs have different binding properties for oligo-dT, suggesting differences in 3' terminal poly-A;
- HCV has a significantly lower buoyant density in sucrose.

The entire nucleotide sequence for HCV is not yet available. In particular, the 5' and 3' terminal regions are incomplete. The current proposal for organization of HCV genomic RNA is shown in Figure 6, indicating the following characteristics:

- a minimum 340 nucleotide 5' untranslated region;
- 9,033 nucleotides coding for a 3,011 amino acide precursor polyprotein;
- a minimum 30 nucleotide 3' untranslated region;
- 3'-poly-A has not been clearly established;
- functionality for the proposed viral proteins has not been determined.

HCV antigens

The original 5-1-1 cloned identified a highly immunodominant

Figure 7
Dominant epitope region

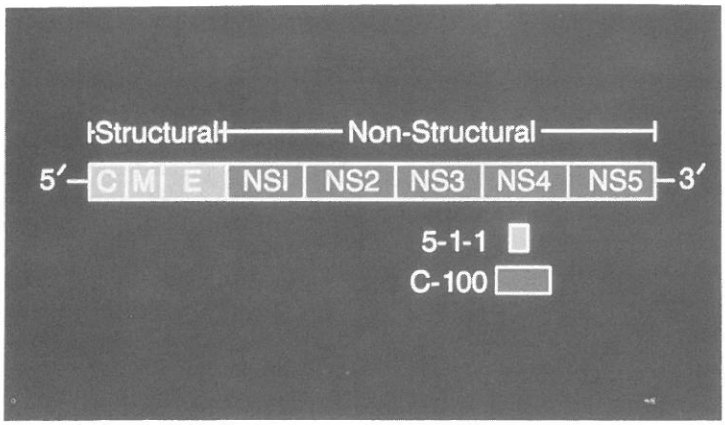


Figure 8
HCV viral proteins

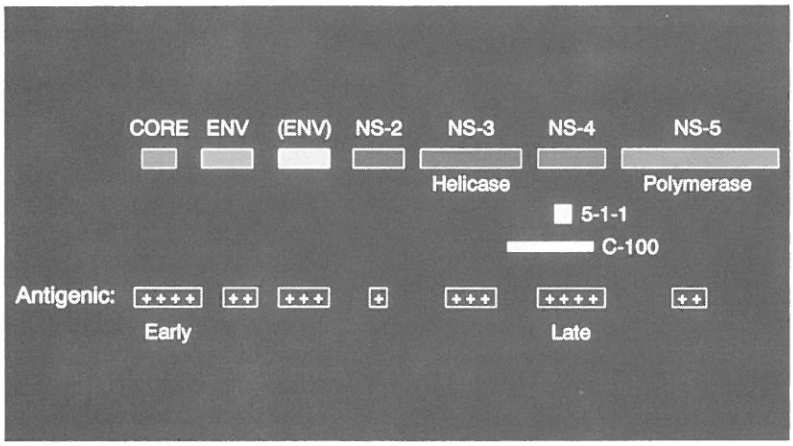
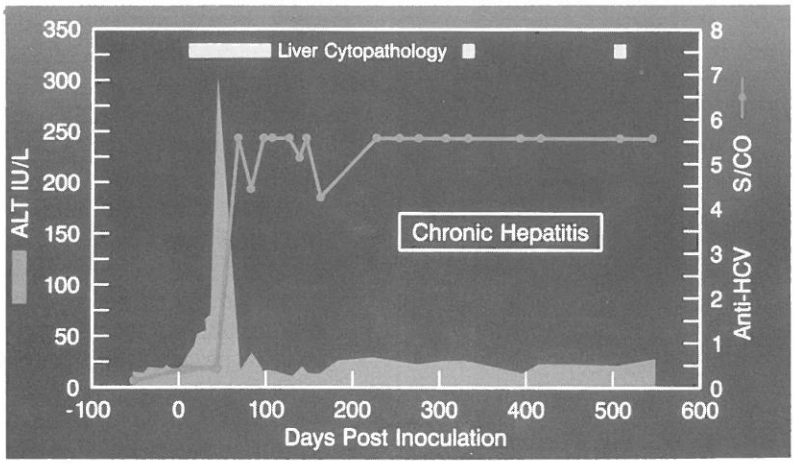


Figure 9
Chimpanzee HCV infection



non-structural region, correlating with the NS-4 region in flavivirus nomenclature. For test designed for blood screening a recombinant protein embracing the NS-4 region and part of the NS-3 region (designated C100-3) was used (Figure 7). The protein was produced in recombinant yeast cultures, and configured into solid phase ELISA assays for serum antibodies. Proteins representing the entire precursor polyprotein have now been produced in recombinant bacteria, yeast, or higher cells and tested for antigenicity in human and chimpanzee infections. Relative antigenicities are illustrated in Figure 8. The nucleocapsid (core) protein is highly antigenic and the antibody may become detectable either early or late in infection. Likewise, the NS-3 non-structural protein is highly antigenic, and the antibody is also detectable both early and late in infection.

Second generation tests are being developed using combinations of the core, NS-3, and NS-4 recombinant HCV proteins.

HCV diagnosis

Most studies to date have used tests with only the C100-3 recombinant protein to detect seroconversions. This has been a very useful and accurate diagnosis of HCV infection. The time sequence of seroconversion in an experimentally infected chimpanzee is shown in Figure 9. This serology is typical for an HCV infection, but there are no absolute timings of appearance and persistence of the HCV C100-3 antibody in humans or chimpanzees. Generally, relatively late seroconversion and long persistence of this antibody is found. There is high association with persisting viremia. However, samples of early seroconversion with and without long persistence are observed. Retrospective analyses in transfusion-associated hepatitis studies have confirmed 70 to 80 per cent of patients who receive an anti-HCV positive blood unit will become infected. The current screening test and improved future tests will therefore identify the majority of healthy blood donors who are persistent carriers of HCV. This will have a major positive impact on the long term consequences of HCV infection acquired through transfusion. A high proportion of these infections lead eventually to chronic hepatitis, cirrhosis, and hepatocellular carcinoma.

Challenges and opportunities

Our knowledge about NANB hepatitis has expandly vastly since the molecular and serologic characterization of HCV, beginning in 1987. There is still much to learn.

We still have knowledge gaps, and these will be challenges. The tools are now available, however, for attacking these problems. Multidiscipline groups composed of clinical, biological, and molecular teams can now attack some of the following, high-priority problems:

- to make new independent isolates of HCV, not biased by existing sequences;
- is there a chloroform-resistant, blood borne agent different from HCV?
- what is the molecular or immunologic basis for persistent infection?
- what is the basis for the association of NANB infection with hepatocellular carcinoma? and
- develop therapy for NANB associated chronic liver disease.

The opportunities are even more numerous. There are no serious knowledge gaps or missing molecular techniques that prevent immediate attacks on the following questions by most laboratories:

- the presence of signatures of virus replication in extrahepatic tissues, cells and organs;
- a profile of serological antigen and antibody markers to stage the disease and virus life cycle;
- grow HCV in hepatocyte and other cll cultures;
- identify and determine the significance, if any, of envelope subtypes;
- is there a basis for either active or passive immunity?
- the transmission route for sporadic infection with no known epidemiologic risk.

Solutions to these challenges and opportunites will not end our common interests in the hepatotropic viruses. They will no doubt represent just another beginning of another search for answers. And here, too, as with NANB hepatitis, it was not the discovery of HCV, it was the journey that was the reward.

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Recent Progress on Anti-HCV Screening

DR. I. K. MUSHAHWAR (Abbott Laboratories, Chicago)

The C100-3 antigen currently used in the First Generation Anti-HCV assay (FGA) represents only 12 per cent of the encoding capacity of the virus[1] and, though it is an adequate marker for chronic viremia, it obviously has its limitations[2].

First, it is not an adequate assay for detecting all stages of hepatitis C virus (HCV) infection. Second, the test's inability to flag all infectious blood units hints at its poor sensitivity, especially among acutely infected individuals. Third, delay in anti-HCV appearance suggests that some blood donors capable of transmitting HCV infection will not be detected by this assay. For these reasons, recombinant antigens from different regions of the HCV genome have been expressed in *E. coli* by Abbott scientists, purified and utilized to develop a Second Generation Assay (SGA).

The antigens utilized include a structural antigen encoded by sequences at the 5' end of HCV (core region) and non-structural antigens encoded by the NS-3 and NS-4 regions of HCV using epitopes of 33c and C100. These antigens (representing approximately 27 per cent of the encoding capacity of HCV) were bound to polystyrene beads by absorption. The serum to be tested for HCV was incubated with the antigen-coated bead giving a complex of anti-HCV in the solid phase. The horseradish peroxidase-labeled probe used in a second step was highly purified anti-human IgG. The resulting absorbency at 492 nm of the multiple layered bead after incubation with an appropriate enzyme-substrate was then in direct proportion to anti-HCV in the serum sample.

We have evaluated the SGA assay and compared it with the First Generation Assay as to its diagnostic and screening utility. Sera tested included volunteer blood donors, sequential bleeds from verified non-A, non-B patients, and experimentally infected chimpanzees with HCV, sera from the Transfusion-Transmitted Viruses Study (TTVS) Group of the National Heart Lung and Blood Institute (NHLBI), and a variety of sera from acute and chronic patients. All repeat reactive specimens were confirmed by alternate antibody tests that utilized different antigen sources and different assay configuration[3].

Table1
Comparison of the sensitivity of first and second anti-HCV assays in volunteer blood donors

Assay	Total	No. Repeat Reactive(%)	No. Confirmed (%)
First Generation	2010	17 (0.84)	8 (47.1)
Second Generation	2010	18 (0.89)	13 (72.2)

Volunteer blood donors

The detectability of anti-HCV in 2,010 blood donors was determined by FGA and SGA. The results are shown in Table 1. Both assays showed equivalent detectability of anti-HCV; however, only 47.1 per cent of the FGA repeat reactives were confirmed positive in comparison to 72.2 per cent of the SGA.

Sequential serum samples

Sequential serum samples from several patients diagnosed with NANB hepatitis were tested for anti-HCV utilizing FGA, SGA and Ortho assays. Without exception, the SGA always demonstrated seroconversion to anti-HCV before the FGA or the Ortho assay, as illustrated in Figure 1. In this patient, the SGA detects anti-HCV 14 weeks earlier than either of the other assays.

Chimpanzee studies

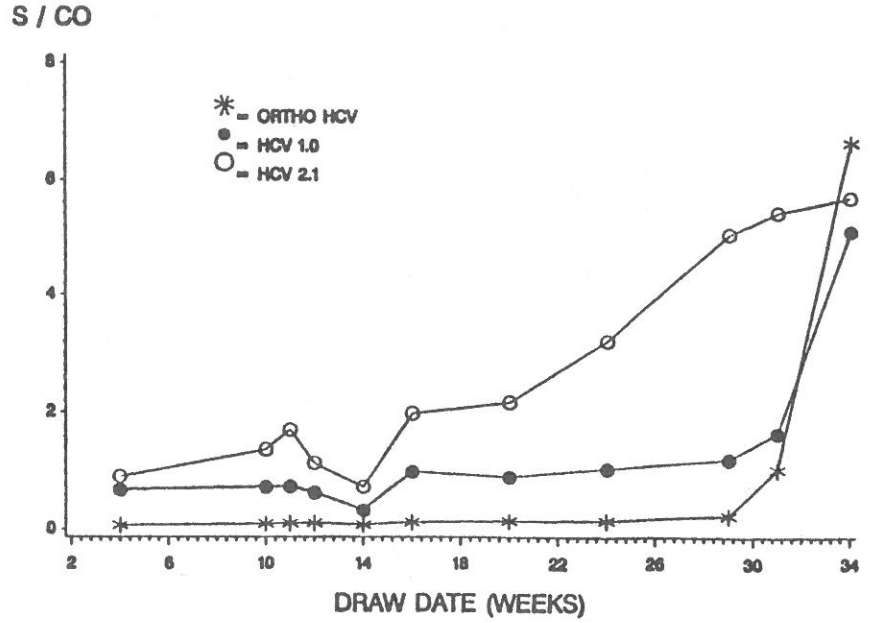
Serial bleeds from chimpanzees experimentally infected with the Hutchinson's strain of HCV were tested for anti-HCV by both the FGA and the SGA. Anti-HCV was detected earlier with SGA in five of six chimpanzees. In most cases, anti-HCV was detected at least three weeks earlier as shown in Table 2.

Table 2
Serologic profile of chimpanzees inoculated with hepatitis C virus as revealed by first and second anti-HCV assays

	ALT Evaluation*					Detection of Serocoversion to HCV proteins		
ID#	Pre** ALT (mIU/mL) (Range)	First (DPI)	Peak (DPI)	Duration (Days)	Maximum ALT Value (mIU/mL)	1st Gen (DPI)	2nd Gen (DPI)	Days Different
CH 427	29-53	56	75	24	280	77	56	21
CH 479	15-20	91	91	7	156	133	98	35
CH 477	17-31	30	35	12	107	70	70	0
CH 335	16-20	38	46	21	295	59	38	21
CH 21	12-30	68	75	14	190	82	66	16
CH 379	19-27	49	68	28	250	119	91	28

* Twice the upper limit of normal values
** Eleven preinoculation samples per animal

Figure 1
Anti-HCV profiles as measured by three assays in a patient who developed a post-transfusion NANB hepatitis



S/CO = sample/cutoff

Table 3
Performance of Abbott HCV EIA assays on transfused recipients (TTVS)*

	Seroconversion of			
	Control Patients (no hepatitis)		PT-NANBH** Patients[a]	
Anti-HCV Assay	No. Tested	No. Pos.	No. Tested	No. Pos.
First Generation	28	0	100	45
Second Generation	28	0	100	58

* TTVS: Transfusion-Transmitted Viruses Study
** NANBH = non-A, non-B hepatitis
[a] 3 of these patients were reactive in both tests before being transfused - yet developed acute PT-NANBH in spite of anti-HCV antibodies being present.

Table 4
Comparison of Abbott and Ortho HCV EIA assays on donors associated with PT-NANBH (TTVS)

Anti-HCV Assay	No. Donors Tested	Anti-HCV Positive[a]	
		No.	%
Ortho HCV EIA	410	56	13.7
First Generation	410	56	13.7
Second Generation	410	68	16.6

[a]All positive results have been confirmed
TTVS: Transfusion-Transmitted Viruses Study

Table 5
Comparison of first and second generation anti-HCV assays on sera from patients with acute and chronic post-transfusion NANBH

NANBH Category	No. Specimens	First Generation				Second Generation			
		No. Repeat Reactive	% Repeat Reactive	No. Confirmed	% Confirmed	No. Repeat Reactive	% Repeat Reactive	No. Confirmed	% Confirmed
Acute	32	4	12.5	4	100.0	14[a]	43.8	11/12[b]	91.7
Chronic	164	138	84.1	137	99.3	155	94.5	155	100.0

[a]One specimen FGA positive is just under cutoff in SGA
[b]Two samples were unavailable for confirmation

TTVS

Specimens collected between 1974 and 1979 by the TTVS Group of NHLBI were tested under code. Repeatably reactive specimens were confirmed by a blocking assay and/or synthetic HCV peptide assays. In 100 post-transfusion NANB hepatitis patients, FGA revealed seroconversion in 45 per cent, while SGA detected 58 per cent (Table 3). In 410 donor units (available for testing), given to these patients, both the Ortho and the Abbott FGA detected 13.7 per cent, while the Abbott SGA detected 16.6 per cent anti-HCV positive donors (Table 4). In 42 post-transfusion NANB hepatitis patients who did not receive an anti-HCV positive unit the FGA detected seroconversion in 4.8 per cent while SGA detected 14.3 per cent.

In summary, we found that the SGA has an increased detection rate in both donors (13.7 vs 16.6 per cent) and seroconversion of recipients (44.3 per cent vs 58.8 per cent).

Acute and chronic NANB hepatitis

The sensitivity and confirmability of SGA was compared to FGA in a collection of specimens from patients diagnosed with acute or chronic NANB hepatitis infection. As shown in Table 5, in acute cases, FGA detected 4 of 32 specimens (12.5 per cent) as repeatable reactive, compared to 14 of 32 (43.8 per cent) for SGA. All repeatably reactive specimens by both assays were confirmed by our confirmatory procedures[3].

Summary

As I outlined this morning, SGA is a highly senitive and specific ELISA for the detection of anti-HCV. The assay's ability to detect seroconversion to HCV three to 14 weeks prior to detection by FGA, together with its improved sensitivity and specificity in volunteer blood donors, acute and chronic NANB hepatitis patients, would contribute toward further decreasing post-transfusion hepatitis.

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SPEAKER: Having been through the specificity, I think you will agree with me that it is still something less than ideal.

As more is being learned about the hepatitis C virus genome, is there anyone working on a Western blot assay in order to improve the specificity?

DR. MUSHAHWAR: We have been working on a Western blot assay, but as you know, it still needs some refining. It is very, very hard if you do not have the proper antibodies to follow that up.

DR. MONTPLAISIR: Could the mode of conservation of the serum sample in terms of freezing or thawing or being kept frozen for many years influence the sensitivity of the tests?

DR. MUSHAHWAR: Yes. We really prefer freshly frozen, recently drawn samples. Very old samples without centrifugation cause some problems and, like the HIV test, also heated samples; all should be handled very carefully. We do not recommend old samples; they do not give you false positives that can be confirmed.

DR. LARKE: Talking about what may give you false positive reactions, is there any evidence that people who are sensitive to the various yeasts that you have used to generate these antigens cross-react?

DR. MUSHAHWAR: We have been in this business now for at least 20 years and we know the trick to making an immunoassay is the nature of the diluents for your conjugate and the specimen and those, obviously, should have SODs and yeast specimens and so on to cover the titre range of anti- *E. coli* or anti-yeast you have. So, those are well taken care of.

Now, I do not want to knock the Ortho test. I do not think their monitoring in that area is as good as ours. This is why you will find the repeat non-confirmed results are a little higher. The nature of the SOD, how pure it is in your diluents, those are techniques well known to the ELISA people.

Experiences with HCV Serologic Tests

DR. M. CHERNESKY (St. Joseph's Hospital, Hamilton)

This is going to be a short presentation on some of the studies that we are doing in Hamilton.

Table 1 shows unconfirmed anti-HCV positivity rates from the literature for risk groups. In Hamilton we offer a diagnostic service for anti-HCV, but we found that because of the lack of a confirmatory test, it is necessary to interpret positive results in light of these published data.

The objectives of our own study were:

1. to examine sera for anti-hepatitis C virus by enzyme immunoassay and to confirm the positives. The patients were selected by the general physician population based on their knowledge of the literature.
2. Positive and negative patients were followed and inquiry made to their physician concerning biological, clinical, behavioural and environmental risk factors.
3. The results would be analyzed towards formulating recommendations for testing.

From March 1 to October 1, 1990, 173 patients were entered; 125 (72.3 per cent) were negative and 48 (27.7 per cent) were repeat reactive in the Abbott enzyme immunoassay. Of the 48, 45 (94 per cent) have been confirmed in the competitive blocking test.

Twenty-six per cent of the men fell into the anti-HCV positive group and 28 per cent of the women. A relatively equal percentage, about half of each HCV group consisted of men or women. There were no patients in the positive group below the age of 23, although the negative group had younger patients.

The clinical risk factors were revealing comparing acute, chronic or no symptoms in the anti-HCV positive or negative groups compared to the total population. An approximate equal percentage in all groups were without symptoms (28.3, 31.7 and 30.7). More of the HCV negative group had acute symptoms and significantly more in the HCV positive group had chronic symptoms.

Equal numbers of all three groups were biopsy positive and had raised ALT.

The HCV positive group had a higher percentage with cirrhosis compared to the others.

Behavioural or environmental risk factors suggested that intravenous drug use and a history of transfusion are more predominant in the anti-HCV positive patients. The numbers of dialysis and haemophilia patients and health care workers are too small at this point to draw any conclusions, but I think it points to the fact that we need to include more.

Forty-five of the 48 patients' sera were neutralized in the blocking assay, being reduced between 55 and 95 per cent.

The non-neutralized patients were two women and a man. All lacked other risk factors, except the male, who had had a history of receiving one blood transfusion earlier in life.

In summary, during the last seven months we entered 173 patients, 48 had antibodies to HCV by the Abbott test and 45 were confirmed. The sex distribution is equal and the age accumulation is even across all the age groups beginning in the second decade.

Risk factors associated with anti-HCV positivity and were chronic symptoms, cirrhosis and IV drug use.

We need a larger entry to formulate recommendations.

Table 1
Anti-HCV positivity rates according to risk groups

Risk Group	% HCV Antibody Prevalence†
Chronic NANA Hepatitis	89
Acute NANB Hepatitis	58
Acute HBV Hepatits	41
Chronic HBV Hepatitis	57
Paid plasma donors	96
Intravenous drug users	48-92
Hemodialysis patients	1-33
Hemophiliacs	53-89
Hepatocellular carcinoma	43-72
Crytogenic chronic hepatitis and cirrhosis	80
Alcoholic cirrhosis	25-80
Institutionalized mentally handicapped children	3-11
Male homosexuals	4
Volunteer blood donors	0.3

† unconfirmed

Experiences with HCV Serologic Tests

DR. F.R. BISHAI (Ontario Ministry of Health, Toronto)

Two immunoassay kits for the detection of hepatitis C antibody were approved in 1990 by the Food and Drug Administration in the U.S.A. Abbott HCV EIA and Ortho HCV Antibody ELISA Test System were used to screen patients' sera for anti-HCV. Supplementary tests were used to help us assess the results. These additional tests were Ortho recombinant immunoblot assay (RIBA), Abbott anti-HCV neutralization EIA test, and antibodies to synthetic peptide C100 EIA. All procedures were performed according to the manufacturers' procedures.

The specificity of the screening assays were evaluated by testing anti-HCV in patients infected with other viruses. The results in Table 1 show that less than 3 per cent of the anti-flavivirus-positive sera recorded false positive for anti-HCV. Again, two out of ten sera samples obtained from patients infected with Epstein-Barr virus (EBV) which were positive for early antigen (EA) of EBV recorded false positive for HCV antibody by both HCV EIA kits.

The prevalence of anti-HCV in 487 non-A, non-B (NANB) hepatitis patients using Abbott EIA and Ortho EIA screening tests is shown in Table 2. The specimens were freshly collected and kept at 4 degrees Celsius until the tests were performed (usually within 72 hours). The results indicate a 97 per cent concordance between both assays. Five patients' sera were positive by Abbott EIA, but negative by Ortho EIA. These five were retested by recombinant immunoblot assay (RIBA) and by Abbott HCV neutralization EIA. Three of the five specimens were confirmed positive by neutralization EIA, but not by RIBA. One of the five specimens showed indeterminate results by RIBA and negative by Abbott HCV neutralization EIA, and one specimen was negative by both confirmatory tests.

On the other hand, ten patients' sera were positive by Ortho EIA screening test, but negative by Abbott EIA screening test. Three out of ten were confirmed positive by RIBA, 4 out of ten showed indeterminate results, and three out of ten were negative by RIBA.

Analysis of the 102 HCV antibody-positive specimens by both EIA screening tests is illustrated in Diagram 1. All of the 84 out of 102 positive sera which recorded a ratio of ≥ 3.0 (OD of specimen/OD of cut-off value) were confirmed positive by neutralization EIA test. Twenty specimens were randomly selected, then tested by RIBA. All 20 sera were also positive by RIBA.

On the other hand, 18 sera were positive by both EIA screening test, but recorded a ratio less than 3.0. Eleven of 18 were confirmed positive by the Abbott neutralization test and seven of 18 were not neutralized. In spite of the fact that the seven specimens could not be confirmed by neutralization test, two of the seven were confirmed positive by RIBA, three showed indeterminate results, one was negative, and one was not tested. It is evident that false positive results are more likely to occur among patients' specimens which registered ratio (OD of specimen/OD cut-off value) less than 3.0.

Table 1
Detection of HCV antibodies in patients infected with other viruses

Laboratory Diagnosis of Specimen	Number of Patients Tested	Positive for HCV-Ab		Remarks
		Ortho EIA	Abbott EIA	
Flavivirus Positive	70	2*	1**	* Positive for Dengue antibody ** Acute specimen of a confirmed flavivirus infection. The convalescent specimen of the same patient was negative for HCV.
Flavivirus Negative	20	2***	0	*** Had meningitis myelitis, but negative for flavivirus markers.
Rubella IgM Pos	5	1*	0	* High positive (13-month-old post-vacc. patient).
Rubella IgG Pos	10	0	0	
Hepatitis B Anti-HBc-IgM Pos	7	0	0	
EBV Infections: VCA-IgM	5	0	0	* Same patients. Symptoms: jaundice, elevated liver enzymes, lower abdominal pain.
EA-Pos	10	2*	2*	
CMV Pos by CMV IgM	8	0	0	

Table 2
Prevalence of anti-HCV in non-A, non-B hepatitis patients

ABBOTT EIA SCREENING TEST	ORTHO DIAGNOSTICS EIA SCREENING TEST	
	POSITIVE	NEGATIVE
POSITIVE	102†	5
NEGATIVE	10	370

RIBA		
POS	INDET	NEG
3	4	3

NO. OF SPECIMENS	RIBA	ABBOTT HCV NEUTRALIZATION EIA
3	NEG	POS
1	INDET	NEG
1	NEG	NEG

† See Diagram 1

The discrepant results for some of the fresh sera tested are summarized in Table 3. In this table, the results of an additional test were added. The additional test was the Abbott EIA test for the detection of antibodies to synthetic peptide C100. This test was kindly performed by Abbott Laboratories in Chicago. The results show that some patients' specimens were detected as positive by one test and negative by another. The reasons for this could not be fully explained. Factors such as the sensitivity and specificity could be implicated in these discrepancies; it is quite evident that the problems of discrepancy could not, so far, be resolved by performing the additional complementary or confirmatory assays.

In Table 4, are results of serum specimens from 402 NANB patients, collected throughout the past ten years, and stored at -20 degrees C., which were tested for anti-HCV. These sera were negative for all other markers for viruses which cause hepatitis symptoms such as hepatitis A and B, EBV, and CMV infections. The results show that the concordance between the two screening EIA tests was only 80 per cent. Our preliminary results showed that long-term storage of the specimens at -20 degrees C. caused false-positive and false-negative results when testing by EIA screening tests. An example is presented in Table 5, in which a few selected test results for anti-HCV by five different tests are recorded.

It is possible that freezing at -20 degrees C. for a long period could interfere with one test more than the other, resulting in the discrepancy in the results obtained from both Abbott and Ortho EIA kits. This phenomenon is being investigated further.

It is evident that the development of more specific and sensitive tests for the detection of HCV antibody and antigen which will overcome these interfering factors is urgently needed.

The above mentioned tests were performed to study the prevalence of anti-HCV in intravenous drug users (IVDU) from Ontario as shown in Table 6. Although the number of patients tested was small, the prevalence of HCV antibody was approximately 62 per cent. These results are similar to those previously published by other investigators in the United States and Europe.

In Table 7 is shown the prevalence of HCV antibody in sexually active individuals (heterosexuals aged between 20 and 35 years with multiple partners) at approximately 5 per cent. All the positives in this group had a history of intravenous drug abuse. Therefore, the possibility exists that HCV had been transmitted parenterally rather than sexually in this group. Again, although the number of subjects tested was small, the results indicated that none of the other 97 sexually active individuals who had no history of drug abuse was positive for anti-HCV. This leads us to believe that HCV infection is poorly transmitted by sexual contact.

Table 8 shows that of 33 post-transfusion hepatitis patients 12 were positive by both tests and 2 were positive only by Abbott EIA screen test, but negative by Ortho EIA test. One of the latter two specimens was confirmed by Abbott HCV neutralization test.

Summary

A small percentage of sera collected from patients infected with other viruses such as Dengue or EBV registered false positive results for anti-HCV by EIA screening test.

Specimens registered positive (ratio ≥ 3.0) for anti-HCV by Abbott and/or Ortho EIA screening tests were confirmed positive by Ortho-RIBA and Abbott anti-HCV neutralization EIA tests.

Table 3
A few selected test results for anti-HCV

(fresh specimens)

Specimen Number	Ortho EIA	RIBA	Abbott EIA	Neutralization EIA	Synthetic * Peptide C100
N27091	POS	POS	POS	POS	Moderately reactive
N65658	POS	POS	POS	NEG	Reactive
N70907	POS	POS	POS	NEG	Nonreactive
N58339	POS	POS	NEG	NEG	Nonreactive
N61819	POS	INDET	NEG	NEG	Reactive
N62948	POS	INDET	NEG	NEG	Nonreactive
N68355	POS	NEG	NEG	NEG	Nonreactive
N68880	NEG	NEG	POS	POS	Reactive
N57200	NEG	NEG	POS	POS	Reactive

*Antibody to synthetic peptide C100 was tested by Abbott Laboratories, Chicago, Illinois, U.S.A.

Table 4
Detection of hepatitis C antibody (Anti-HCV) in non-A, non-B hepatitis patients*

Abbott EIA	Ortho EIA	
	Positive	Negative
Positive	100	57
Negative	23	222

* All patients were clinically diagnosed as hepatitis patients. Symptoms: jaundice, elevated liver enzymes, etc.
All sera were negative for hepatitis A, B, CMV and EBV markers.
(Specimens stored at -20° C for several years.)

Table 6
Presence of anti-HCV in intraveneous drug users in Ontario

Abbott EIA	Ortho EIA	
	Positive	Negative
Positive	17*	1***
Negative	2**	12

* 10 results confirmed by neutralization EIA; 7 not yet examined.
** 1 positive and 1 indeterminate by RIBA
*** Confirmed by neutralization, but negative by RIBA.

Table 5
A few selected test results for anti-HCV
(old specimens stored at -20° C for several years)

	Ortho tests		Abbott tests		
Ref. no.	Screening EIA	RIBA	Screening EIA	Neutralization	Synthetic* peptide C100
11462	POS	NEG	POS	NEG	Nonreactive
13453	POS	POS	POS	NEG	Nonreactive
15037	POS	POS	POS	NEG	Nonreactive
15038	POS	NEG	POS	NEG	Nonreactive
16464	POS	NEG	POS	NEG	Nonreactive
17088	POS	IND	POS	NEG	Nonreactive
17597	NEG	IND	POS	NEG	Nonreactive
18085	POS	IND	NEG	NEG	Nonreactive
18634	POS	POS	POS	NEG	Nonreactive
18911	POS	IND	POS	NEG	Nonreactive
19991	POS	POS	POS	NT	Nonreactive
11665	POS	NEG	POS	NEG	Nonreactive
11675	NEG	POS	POS	NEG	Nonreactive
16665	POS	IND	POS	NEG	Ractive
20690	NEG	NEG	POS	NEG	Nonreactive
21004	POS	POS	POS	NEG	Nonreactive
21859	POS	POS	NEG (borderline)	NEG	Nonreactive
25154	POS	POS	POS	NEG	Nonreactive
H36731	POS	POS	POS	NEG	Nonreactive
H38224	POS	NEG	POS	NEG	Nonreactive
H40228	POS	NT	POS	NEG	Reactive
H41025	POS	POS	POS	NEG	Nonreactive

NT = not tested
* Antibody to synthetic peptide C100 was tested by Abbott Laboratories, Chicago, Illinois, U.S.A.

Table 7
Presence of anti-HCV in sexually active patients

Abbott EIA	Ortho EIA screening test	
	Positive	Negative
Positive	5*	0
Negative	0	97

* 1. Intraveneous drug users
2. OD of specimen/OD of cut-off value = ≥ 4
3. Confirmed positive by neutralization

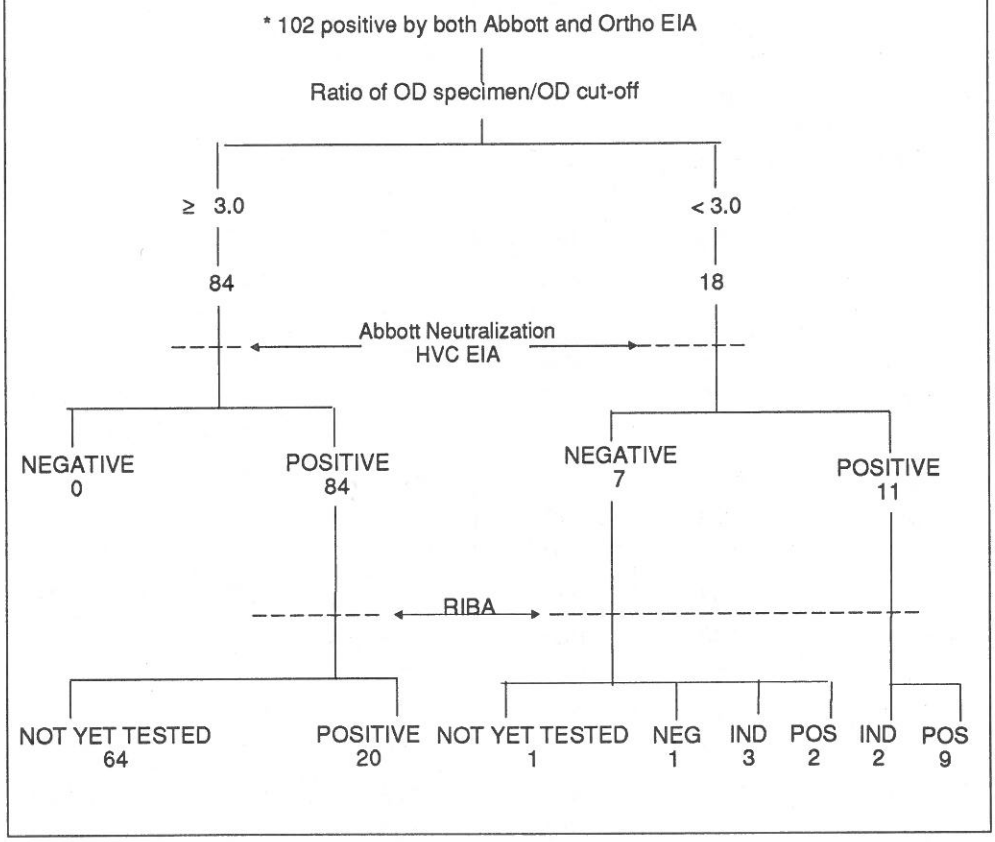
Table 8
Prevalence of anti-HCV in post-transfusion hepatitis patients

Abbott EIA	Ortho EIA screening test	
	Positive	Negative
Positive	12*	2**
Negative	0	19

* Confirmed by Abbott HCV neutralization EIA

** Only one was confirmed by Abbott HCV neutralization EIA

Diagram 1
Analysis of 102 fresh specimens positive for HCV antibodies



Positive for anti-HCV by both tests registering ratio <3.0 (OD of specimen/OD of cut-off value) showed contradictory results when RIBA and Abbott neutralization tests were used as confirmatory tests.

A higher percentage of false positives was recorded when frozen sera were tested for anti-HCV by EIA screening tests.

The confirmatory test (Abbott anti-HCV neutralization test) and other additional tests for anti-HCV (RIBA and anti-synthetic peptide C100 region) could not totally resolve the issue of false positive and/or false negative results obtained by using the EIA screening tests.

The prevalence of anti-HCV among intravenous drug users (IVDU) and transfusion patients was high but very low among sexually active patients.

Conclusion

Although the first generation EIA tests are useful as screening for anti-HCV, the use of some of the available supplementary tests such as RIBA, HCV -neutralization, and antibodies to synthetic peptides C100 are mandatory to eliminate some of the false positives and negatives. It is evident that the existing confirmatory tests cannot completely solve the problems. It is, therefore, essential to consider carefully the clinical history of each patient while analyzing the results of tests for anti-HCV.

It is obvious from our results that the development of more sensitive and more specific tests is urgently needed.

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Experience with HCV Serologic Tests

DR. R.K. CHAUDHARY (Laboratory Centre for Disease Control, Ottawa)

The major causative agent for post-transfusion hepatitis is designated as hepatitis C virus [1]. Infection with HCV is not only associated with transfusion, but sporadic cases are also reported [2]. Chronicity is a major problem associated with this virus and about 40 to 50 per cent of the patients become chronic carriers [3] and develop cirrhosis.

An ELISA test is now commercially available from Ortho Diagnostic and from Abbott Laboratories for the detection of anti-HCV. The test utilizes HCV proteins expressed in yeast cells. The validity of the test was established by examining well-documented test panels of sera. A confirmatory test is also available from Ortho Diagnostic.

The prevalence of anti-HCV was studied in risk groups in Canada. The number of samples tested from different groups is shown in Table 1. Samples were tested by Ortho Diagnostic ELISA and the recommended protocol was followed.

High prevalence of anti-HCV was detected in most of the groups tested except homosexuals, healthy individuals, and individuals without HAV or HBV infections. The complete results are shown in Table 2. There was a high prevalence (68.8 per cent) of anti-HCV in Canadian hemophiliac patients, which is similar to findings previously reported for Germany and Spain [4,5].

Among the hemodialysis group (1980-82), 58.3 per cent were positive for anti-HCV antibody. This group showed many cases of non-A, non-B (NANB) hepatitis, diagnosed by exclusion, between 1980 and 1982. This explains the reason for a prevalence rate in this group that is higher than those reported from other centres [4,5]. Nevertheless, only 17.4 per cent of hemodialysis patients were positive for anti-HCV in 1990. This difference could be explained by the fact that NANB activity was low during 1990.

Table 1
Analysis of Ortho and Abbott EIA test kits for hepatitis C virus (HCV) antibodies to the C100 antigen of HCV in various populations

Group Tested	Number Tested	Ortho and Abbott Positive (%)	Ortho Negative Abbott Positive (%)	Ortho Positive Abbott Negative (%)
Hepatitis A and B Negative	335	30 (8.9)	5 (1.5)	15 (4.5)
Intraveneous Drug Abusers	40	18 (45.0)	0	1 (5.2)
Female Prisoners	77	24 (31.1)	1 (1.2)	0
Hemodialysis* Patients	297 (Samples)	128 (43.1)	11 (3.7)	1 (0.3)
Homosexuals	86	2 (2.3)		
Normal Individuals	256	5 (1.9)		
TOTALS	1,091	207 (18.9)	17 (1.5)	17 (1.5)

* From some patients there were 5 - 10 consecutive samples.

Table 2
Immunoblot confirmation of anti-HCV positive samples from different groups

Group Tested	Number Tested	Ortho confirmatory test results		
		Reactive (%)	Indeterminate (%)	Non-reactive (%)
Hepatitis A and B Negative	27	15 (55.5)	3 (11.1)	8 (29.6)
Intravenous Drug Abusers	19	18 (94.7)	1 (5.26)	
Female Prisoners	24	24 (100.0)		
Hemodialysis Patients	28	22 (78.5)	6 (21.4)	
Homosexuals	2	2 (100.0)		
Normal Individuals	5	3 (60.0)	1 (20.0)	1 (20.0)

Table 3
Correlation between EIA OD values and immunoblot results

Number of Samples Tested	OD Values by Ortho or Abbott Kits	Ortho Confirmatory Test Results		
		Reactive (%)	Indeterminate (%)	Non-reactive (%)
46	> 2.0	40 (87.0)	4 (8.6)	2 (4.3)
13	1.0 - 1.9	6 (46.1)	4 (30.7)	3 (23.0)
15*	< 1.0	1 (6.6)	4 (26.6)	10 (66.6)

* One sample in this group could not be read because of dark background and has been included in the non-reactive group.

Canadian intravenous drug abusers and female prisoners had a prevalence rate of 45.2 per cent and 31.2 per cent, respectively. Although the numbers were small, these rates may be indications of the cases in general. The prevalence rate for anti-HCV was low (3.5 per cent) in homosexuals. A report from Spain also showed a low prevalence of anti-HCV in the homosexual population [5] in that country. Of the sera tested from hepatitis patients negative for recent infection with HAV or HBV, 13.4 per cent were positive for anti-HCV. In contrast, in a random serum set from normal healthy individuals, only 2 per cent of sera tested were positive for anti-HCV. In the Canadian blood donor population, only 0.39 per cent were positive for anti-HCV antibodies (M. Buchner, Canadian Red Cross, Ottawa: personal communication).

When a supplementary test called RIBA HCV assay (Ortho Diagnostic) became available, we tested all our ELISA-positive samples, except samples from haemophiliacs. It is an immunoblot test where protein antigens C100-3 and 5-1-1 were immobilized on nitro-cellulose strips. The recommended protocol was followed.

The supplementary test showed that some of the samples positive by ELISA were non-reactive by immunoblot test. The proportion of non-reactives varied from group to group. In the HAV and HBV negative group, only 33.3 per cent were confirmed. Among hemodialysis patients, intravenous drug abusers and female prisoners, 80 to 96 percent were confirmed. Results are shown in Table 3. In the high risk groups, there was no significant difference between ELISA and immunoblot results.

These results show that HCV is an important cause of hepatitis in high risk groups tested. The diagnosis of an acute infection, however, is still not possible and confirmatory tests are required.

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Experiences with HCV Serologic Tests

DR. P. GILL (Canadian Red Cross Society, Ottawa)

I was asked if I would discuss some of our experiences with the actual test methods we had used. I will do that in the light of some tests we did for surrogate markers in our earlier evaluation of HCV and the comparison of those sera with the Abbott screen test and neutralization test.

When we were first asked to evaluate the Ortho test, we persuaded the Ottawa Centre to send us a series of routine blood donation specimens. We were also fortunate in being able to get the ALT levels done in the Ottawa General Hospital Clinical Chemistry Unit. From my earlier presentation, it was noted that, among the blood donations, we did get some with elevated ALTs. We got some anti-core positive and one donation which was anti-HBc and ALT elevated. When we came to do the HCV screen test, however, all six reactives were in the normal category of negative anti-HBc and normal ALT.

We also looked at HBsAg-positive donors having a bank of serum specimens from HBsAg confirmed positive and HBsAg positive on the screen test, but not confirmed specifically.

In Table 1, one can see that in the 90 HBsAg donors we had three which were also reactive on the screen test for HCV. In the 82 non-specific reactive HBsAg test donations we had zero reactivity.

In terms of performance of the HCV test when we first evaluated it, we had very good results from the comparison of strips and plates showing the reproducibility between non-reactive, weakly reactive and strongly reactive samples.

In recent experience using the actual test on blood donors we have compared the initial reactive rate and the repeat reactive rate over a series of master lots in the system and as can be seen in Table 2, it is fairly consistent.

Now, when we went back to look at those 1,544 donations which had been stored for over a year, we evaluated

Table 1
Canadian volunteer blood donors

Group	Initially Reactive		Repeatably Reactive	
	N = Total Tested	Rate %	N = Total Tested	Rate %
Blood Donors	1,544	0.52	6	0.39
High-Risk Population				
Acute Hepatitis (NANB)				
Other Population				
(Stipulate kind of population)				
HBsAg-Positive Donors	90	3.33	3	100
HBsAg Non-specific Donors	82	0	0	0

Table 2
Anti-HCV Initial and repeat reactive rates (lot by lot)

Master Lot	# Tested	% IR	% RR
119	12,662	1.45	0.58
128	39,033	1.20	0.58
137	42,349	1.04	0.53
152	58,549	0.98	0.49
147	48,451	0.93	0.47
157	81,459	1.01	0.50
162	36,722	0.94	0.55
316	34,088	1.13	0.52
Total	353,228	1.04	0.52
	Range	0.93-1.45	0.47-0.58

Table 3
Summary of Abbott and Ortho anti-HCV EIA and their corresponding confirmatory assays
Volunteer Blood Donors

Assay		Routine Blood Donors		
		No. Tested	No. Positive	% Positive
[a]	Ortho EIA:	1544		
	RR		6	0.40
	"Confirmed"		0	----
[b]	Abbott 1.0:	1544		
	RR		12	0.80
	"Confirmed"		1	8.30

RR = Repeat Reactive
[a] Ortho anti-HCV EIA uses HCV-C100 as antigen; "confirmatory" = supplemental RIBA assay (C100 + 5-1-1)
[b] Abbott 1.0 Generation anti-HCV EIA uses HCV-C100 as antigen; "confirmatory" assay uses solution neutralization

Table 4
Summary of Abbott and Ortho anti-HCV EIA and their corresponding confirmatory assays
Post-transfusion NANB hepatitis recipients and their implicated donors

Assay		PT-NANBH					
		Recipients			Implicated Donors		
		No. Tested	No. Positive	% Positive	No. Tested	No. Positive	% Positive
[a]	Ortho EIA:	71			295		
	RR		24	33.8		18	6.1
	"Confirmed"		20	83.3		17	94.4
[b]	Abbott 1.0:	71			295		
	RR		30	42.3		20	6.8
	"Confirmed"		30	100.0		20	100.0

RR = Repeat Reactive
[a] ORTHO anti-HCV EIA uses HCV-C100 as antigen; "confirmatory" = supplemental RIBA assay (C100 + 5-1-1)
[b] Abbott 1.0 Generation anti-HCV EIA uses HCV-C100 as antigen; "confirmatory" assay uses solution neutralization

them against the Abbott assay. We also used the specific "confirmatory" test that was provided by Ortho as a RIBA test, and from Abbott we used their so-called neutralization assay.

As shown in Table 3, in the Abbott test, we found 12 which were repeat reactive. Of those 12 donations which were repeat reactive, one confirmed. That one confirmed happened to be the one which was ALT positive and anti-core positive, which we had missed on the Ortho test.

When we went to look at some cases of post-transfusion hepatitis by both tests and looked at the implicated donors involved in those cases, we tested 71 cases by the Ortho EIA. As is shown in Table 4, 24 were found positive for a rate of 33.8 per cent. Of those 24, 20 were "confirmed" by the RIBA test to give a confirmation rate of 83.3 per cent in actual cases of post-transfusion hepatitis.

When we looked at the same 71 cases by Abbott tests, we got 30 "reactive" for a 42.3 per cent rate by EIA of post-transfusion hepatitis. Of those 30, 30 were also confirmed by the neutralization test for 100 per cent confirmation of the Abbott screen test.

In terms of the implicated donors, we looked at 295 with the Ortho EIA and found 18 were reactive. Of those 18, which is 6.1 per cent of all donors and, therefore, does not mean too much, 17 were confirmed by RIBA giving a 94.4 per cent confirmation rate.

In terms of the Abbott performance on the same 295 implicated donors, we picked up 20 which were repeat reactive and all 20 confirmed with the Abbott neutralization test.

So, this is our experience in blood donors, in post-transfusion hepatitis cases, and in implicated donors with the Ortho EIA with its companion supplemental test along with the Abbott EIA and its companion "confirmatory" test using the neutralization assay.

DR. BARR: Why in these last results are the implicated donors so low compared to the other? How can you explain it?

DR. GILL: We are looking at the total number of donors in a pool of donors in which they were all implicated. One of them was going to be the donor, but they were all implicated. When we tested them, we get some sense of specificity as well as sensitivity from these data.

When we looked at the implicated donors which were reactive on an EIA test and selected out that group and then did a RIBA on it, we were confirming 90 to 100 per cent of the time. But, in the total pool of implicated donors, frequently only one donation would be infective in any pool. Thus, if you have 10 donations going into a single individual case, only one of the 10 would be expected to be reactive.

That is why I mentioned, referring to the table, that the last column is not that significant for the first figure, as opposed to the second figure where you are getting the confirmation rate of a reactive donor.

DR. PURCELL: Any other questions?

SPEAKER: I am puzzled by the difference between the neutralization and the RIBA assay. Is there anything in technology which is more sound than the former?

DR. GILL: Neutralization is not true neutralization. It is really a competitive assay in solution between an antibody and a corresponding antigen. It is a quantitative EIA assay using the Abbott technology read by machine. RIBA is an immunoblot strip in which the strip is impregnated with two recombinant proteins and evaluated in terms of intensity of the blot. One senses, because of this requirement for two recombinant peptides to show an intensity of reactivity above a certain threshold level, that you probably get indeterminants in the RIBA assay which on a subsequent serum sample will move from an indeterminate to positive.

So, there is a difference in terms of a machine-read EIA type assay using neutralization in solution versus a visual evaluation of a strip of intensity by eye.

There probably is also a difference, in terms of the presentation of the antigen, in other words, the conformation of the antigen, when that antigen is presented on a strip versus when it is presented in solution. There may be physically a different interaction of antibodies in a patient's serum which will account for differences in sensitivity.

DISCUSSION

DR. PURCELL: We are open for discussion of this afternoon's topic, the immunodiagnosis of HCV, an exciting but in some respects, controversial topic.

SPEAKER: This a comment rather than a question because of what has been said about the retrospective studies utilizing sera which have been stored over a number of years at -20 degrees C. Some 30 to 40 years ago it was the practice to use stored sera whenever a retrospective study was undertaken. In the last few decades I think most of the time we stored sera at -20. We have data to show a decline in infectivity and loss of infectivity of viruses over a period of time when stored at -20, and also loss of antigenic potency. Furthermore, it is known that alpha viruses do require, for neutralization, soluble factors which deteriorate on storage and which can be restored by the addition of serum.

The same thing is observed in neutralization kinetics of herpes 1 and I wonder if any of the investigators would entertain the idea of trying to restore the loss of such factors by the addition of negative sera for hepatitis C, which have been detected by the system?

DR. PURCELL: Would anyone wish to answer that question?

I assume these are complement-like components that are lost on storage.

DR. CHAUDHARY: We do not know right now what factor might be responsible for the loss of the reactivity in the case of hepatitis C antibody, so it is too early to answer that. There may be caution at this point as to what factor might be responsible for this and what precaution we might be able to take.

DR. PURCELL: Here, of course, we are not dealing with loss of antibody, but dealing with the *de novo* appearance of pseudo antibody which occurs when the sera are stored at -80 as well as -20.

SPEAKER: Do current batches of gamma globulin have high titres of anti-HCV?

DR. BISHAI: We tested, I think, one or two. One was positive for both 5-1-1 and C100 and that is the reason for one of my questions in the morning, whether positivity indicates infectivity. I would like to hear an answer from the panel after I heard Dr. Mushahwar tell us in the morning that all who are positive by EIA or by RIBA or by neutralization tests, that their sera are infectious.

However, in the afternoon Dr. Mushahwar indicated that that was not necessarily so. So, I would like to ask, which is the case?

DR. MUSHAHWAR: I think a group in Italy has tested about 22 immunoglobulin preparations for the presence of anti-HCV. They found that almost 50 per cent of them were positive and these were confirmed repeat reactives.

Of course, they have not done the PCR on them and the question is whether those are infectious or not. Obviously, in the Cohen procedure in the preparation of the immunoglobulin there is a heating step that renders the preparation non-infectious, I hope.

DR. OVERBY: My opinion - I guess I better call it my bias - is that the C100 antibody is more associated with the presence of infectious virus. When the C100 antibody is positive, the chance that there is infectious viremia, I think, is very high. There is no evidence that that antibody, I am convinced, is associated with protective immunity.

But, the fact that immune globulins and plasma products are heat inactivated is good evidence that heat is effective and minimizes infectivity.

SPEAKER: Immunoglobulins have one of the best safety records of any biological sterility. There are two or three reports in the literature of transmission from globulin preparations and I think in each case it was shown that they were not prepared properly.

DR. GITNICK: We tested 30 lots of immune globulin, both ISgN and HBIg, made by different manufacturers, both by Abbott and Ortho. Of the 30 lots in Los Angeles, 28 were positive repeat reactive and confirmed by both assays.

DR. PAPPAS: I was doing all right this morning, but by the end of the afternoon I am not quite sure what to understand or, more important, what to believe.

I was interested in Dr. Chaudhary's data with the group that were suspected of having hepatitis, that were negative for HAV and HBV markers, and in the discrepancy between the initial test and the confirmatory test, because this seemed to approach the discrepancy seen in the blood donor population. I wonder if we could find out a little more about that patient group. Were they acute or chronic?

DR. CHAUDHARY: Actually, there are two points which I would like to make.

Some of these patients had symptoms of hepatitis, but were negative serologically for recent infection for hepatitis A or for hepatitis B. Some of them had a past history of infection with hepatitis B, but were not serologically positive for a recent infection with hepatitis A or B.

One other problem is that a large number of the ELISA positive samples had a low OD value and most of these were negative on the RIBA confirmatory test.

When we divided our samples into different OD values, as Dr. Bishai was showing, we found that in 2 and over, 90 per cent of them were confirmed, and between 1.2 to 1.9 there were about 50 or 40 per cent confirmed, and less than 1, there were very few which were confirmed.

These patients did come with a diagnosis of hepatitis, but we did not know when the sera were taken, or at what point in the acute stage of infection they were. Does it answer your question?

DR. PAPPAS: Yes. One of the things I think we have to consider is that when you are looking at a diagnostic test where there is no good gold standard, the test is really only useful if it increases the post-test probability.

DR. CHAUDHARY: That is going to be a real problem in the low incidence group.

DR. LARKE: This is an interesting meeting because it acknowledges sponsorship from both Ortho and Abbott. The inevitability is, as we have seen this afternoon, they are going to be scoring toe to toe as to which is the better product.

I suppose to some extent it depends on what you are trying to assay. The person who is looking for the disease in drug users has less of a problem than somebody who is in the blood transfusion business.

The problem with a meeting like this is that what we are comparing is the

sort of kits that were available when things first came out. If I am faced today with making a decision based on, for example, a blood transfusion, my big concern, of course, is the false negative. We can live with false positives. You throw out a few donors you do not need. Well, we need them all, but you know what I mean. It is the false negatives that give you nightmares just as it is with HIV.

The problem now is that we have already heard that Abbott has a second generation kit and I have to presume Ortho is refining their products.

So, what I take away from this meeting today may help me not at all with what is available next week or next month. This is an art that is moving ahead almost weekly - how do we make our decisions?

DR. PURCELL: Who would like to tackle that question?

DR. MUSHAHWAR: I do not think the problem is new. The first hepatitis B test, the first generation test, was licenced if it detected 20 mg of hepatitis B surface antigen or less. The third generation test that came almost 10 years after detects 0.3, 0.2 mg. Does that mean for 10 years you have to wait to make a decision to use the best test available?

The answer to that is, no. But when the second generation test is available, and I think it will be soon, I think you should use it.

DR. BISHAI: I may add just one point here. I think that through the results which we have discussed this afternoon, I would suggest something: I would like to have ALT tested in all cases along with Abbott and Ortho.

I do not know whether somebody will shoot me for that, but I think that it will add some good information and help us to make a decision about those which are positive or negative and it will eliminate some of the false negatives and some of the false positives if we have ALT along with the test of EIA or Abbott or Ortho.

DR. MUSHAHWAR: ALT testing, and this is my own opinion, may cause more confusion than you have now. The reason for that, ALT among volunteer blood donors is affected by exercise, by what you eat the night before, what you have done the night before, the region of the country where you are from, and all that is going to make the picture more confusing than it is now.

DR. PURCELL: Any other comments from our panel concerning that?

DR. BARKER: I like that last question. Mine is a lot easier, I think, but it is really the same question in a sense. I have a feeling that we are reliving, as Dr. Mushahwar just said, the previous experiences that we have had with new tests and we do not wait for perfection. Obviously, we start with the best that we can start with and then things improve from there, which is a pretty nice situation.

The tests change, but the other thing that obviously changes is the people who are being tested, and that is another approach, as everybody here knows, I am sure, that we have taken to the indeterminate anti-HIV Western blot people with whom every blood transfusion service has a lot of experience. That is to say, we go back six months later and see what turns up at that point.

So, my question is, maybe it is too early yet, but does anyone have some experience on retesting some of these people who are producing the confusion, the people who do not clearly confirm or give contradictory results between the two tests? Because my hunch is that going back a subsequent time may clarify a lot of these people's status.

DR. CHAUDHARY: We have some experience. In the kidney dialysis unit we tested serial sera from each patient each month and we found that some of the patients who had a doubtful result in the previous testing, on the second sample after a month they became completely positive. So, if the patient's test is indeterminate, then a second sample at the one-month interval might be able to help.

YANG FA WANG: I have a question about the use of PCR to detect the HCV virus. Because of the long sequence of the HCV virus, according to your point of view, which part of the wider fragment will be best for selection of the PCR primer to detect HCV virus?

DR. PURCELL: The question was, which part of the HCV genome is best for making primers for PCR?

DR. OVERBY: Right now, now that the 5-prime untranslated region sequences are available, that looks to me like it would be a good region for primers if you want to rescue just a genome from a specimen.

I believe that there are some other regions that are thought to be well-conserved among the isolates that have been made. The NS-3 non-structural region would be a good region. My bias right now would be to the 5-prime untranslated region if you just want to see whether the genome is present or not and rescue it by PCR.

I think the one to stay away from right now is the presumed structural region.

SPEAKER: In the 5-prime non-coding region, if those strands could have been PCR amplified and looked at, there is about a 99 per cent identity which is higher than in any other part of the genome.

SPEAKER: The following question is, is it not right to use a nested PCR to detect HCV? Do you not think the nested PCR has enough sensitivity and specificity to verify the HCV virus without the hybridization, just using nested PCR.

DR. OVERBY: My understanding is from those who are using nested PCR, they are using it because they think it is an improvement. At least they have convinced me that that is a good technique, if you do two series with nested primers.

DR. PURCELL: Using two different regions of the genome which adds to the specificity and the sensitivity appears to be essentially the same as one set of primers plus hybridization.

DR. OVERBY: I think that will make up for some of the errors and so forth that might creep in because of personal skill, etc.

DR. TOFFMEYER: I might be jumping the gun a bit for some of the topics for tomorrow, but I am curious about the data that you have from the two hemodialysis units in Ottawa.

You have demonstrated that, in 1990, one patient in every six who were exposed to your dialysis units are hepatitis C antibody positive. I am just wondering, since you are continuing to check them on a monthly basis, if anything is being done with that data clinically, if the patients are isolated or more done?

DR. CHAUDHARY: Actually, if you go back to the table, in 1980-81 there was a suspected outbreak of non-A, non-B in that unit. At that time

those people who had elevated ALT, whom we suspected to be infected with non-A, non-B, had a different dialysis room virus established for them.

In 1990, we did not have any more new infections associated with new patients in that clinic after we instituted a separate room and separate dialysis unit for suspected non-A, non-B patients.

DR. TOFFMEYER: The separation in the early 1980s, was that done only on the basis of the liver enzymes?

DR. CHAUDHARY: Yes, as well as the fact that we also tested them for hepatitis A, B, EBV and CMV. If they were negative for these viruses, and had the elevated liver enzymes, we considered them as non-A, non-B and they were separated.

DR. SPENCE: I was just wondering if anybody knows when you administer gamma globulin-containing antibody to a person whether you can demonstrate the presence of antibody in this recipient following the administration?

DR. BISHAI: We have not done that, but it would be very good to do. The most important thing, I think, is to apply PCR on the gamma globulin to evaluate infectivity.

DR. MUSHAHWAR: In the chimp studies we have done, we have given plasma that was positive, by the C100. We did not know at that time, but it was the Hutchinson strain and when we gave - I think Dr. Overby may correct me here because he was involved with those experiments - I think we gave something around 30 to 40 mL and when the test came out we found persistence for that C100 from that amount of plasma positive the first 15 days. The volume I am not sure of, but it was passively acquired for a while. Then it became negative before the appearance of the actual C100 due to infectivity.

DR. OVERBY: I think a number of these prospective studies have shown the presence of passively transmitted anti-C100 picked up a day or two after the transfusion. I suppose they have involved ten units or something, only one of which was positive, but that is only a 1:10 dilution, while plasma pools, if somebody can figure out what the prevalence is, how many units would be in, say, a pool of 1,000 units. But, that would be considerably diluted in commercial preparations of IgG.

SPEAKER: I suppose most lots of plasma, pooled plasma, are really about 10,000 donors and I guess you can estimate 1 in 200 will have antibody to hepatitis C, roughly about one-half of one per cent.

If you administer gamma globulin to individuals and look for antibody to hepatitis A, in general, you cannot detect it with the RIA or ELISA tests, but you can detect it with neutralization tests which are more sensitive. I suspect that may be the case here.

DR. LARKE: As we mentioned this morning, we are screening all of our organ donors for hepatitis C antibody. We have a situation where a young man involved in a motor accident in northern Alberta was sent down to Edmonton where he subsequently died, became an organ donor, was tested for anti-HCV and was positive. That information went back to the referring hospital and one of the nurses had, indeed, stuck herself. The question comes up, if there is anti-HCV in commercial lots of gamma globulin, would you advise this nurse, who had stuck herself with a positive donor, to get gamma globulin?

DR. OVERBY: I am not a clinician, but I think it wouldn't hurt.

SPEAKER: You have to bear in mind that the antibody that is being measured, as you have already noted, is an antibody to a non-structural protein and not a neutralizing antibody.

Now, I think it was Isak who showed data on a proportion of individuals who had antibody to envelope. Do you recall what that was?

Until recently, the Chiron group had reported that they did not find antibody to envelope, but you do find antibody to NS-1 which is now thought to be another envelope protein.

DR. MUSHAHWAR: Yes, those were the only antibodies present based on a synthetic peptide. I think for the envelope antibody we saw - I do not remember what the last slide showed - either one or three that had no other markers but envelope.

SPEAKER: What proportion had antibody plus other markers, had antibody to envelope plus other markers?

DR. MUSHAHWAR: That varied from around 59 to 94 per cent, I think.

SPEAKER: Had an antibody to envelope or NS-1?

DR. MUSHAHWAR: Yes.

SPEAKER: That is very high. So, you would assume, then, that commercial gamma globulin would have antibody to those proteins also.

DR. MUSHAHWAR: Yes, I think the other antibodies, as tests become available, should be measured.

DR. HAMMOND: I wonder whether the panel could help the diagnostic lab with a take-home message. We understand that it is difficult to choose the test, but who should be tested and how frequently, and what do we do to inform the clinicians?

DR. PURCELL: The panel is stuck with that one.

DR. CHERNESKY: I will answer partly anyway and tell you what we are doing in Hamilton. The gastroenterologists in Hamilton are very interested in testing patients that they suspect have non-A, non-B hepatitis. So, I think that is the number one group that this test can help by testing.

Up to now, before we had the confirmatory test available, what we were doing was trying to give the clinician a probability that, if they could slot their patient into a particular risk group, the chances of a seropositive being a genuine positive would be very high, in a low-risk group, very low.

So, I think the number one group to be tested would be these patients with chronic ALT elevations, classical cases of non-A, non-B and then work down from there.

SPEAKER: I would like to add a comment, I think, to answer this question of infectivity.

I think we need to generate data to determine how many repeat reactives confirmed by the C100 assay are PCR positive, how many repeat reactives that are confirmed are PCR negative. This would be good data.

DR. CHAUDHARY: Dr. Mushahwar, in one of your slides you showed that 13.7 per cent of samples were positive by Abbott and Ortho ELISA and then 16.2 per cent were positive in your second generation ELISA. Do you think that it is a difference? And the second question I have for you is that you call your supplementary test a neutralization test, although it is not a neutralization test. It is a competition test as Dr. Gill mentioned. Why do you people give it a name which is not appropriate?

DR. MUSHAHWAR: As you know, it is not commercialized yet and the names are mixed. It is a blocking test.

As far as the first question, whether this is a significant number of positives, the 13.3 of the 16? Of course it depends on how many you tested and the number was small. But, you have to look at the picture as a whole, not to pick on a small number like that. If you want me to repeat the results, I am ready to do it. Like the Oklahoma Blood Bank, that was very significant.

SPEAKER: I have, perhaps, one last question. As far as I can determine, all of the tests and supplementary and confirmatory tests of both Ortho and Abbott are double antibody tests. Can we look forward to a test that uses some other format and that might get rid of some of the problems that we have seen here today, perhaps some type of antibody capture test?

DR. MUSHAHWAR: Yes, I think the biotin, anti-biotin assay that I discussed will be highly specific.

As far as confirmatory tests, I think now we do have specific monocloning antibodies and we have a very good polycloning antibody. There is no reason whatsoever to do competitive antibodies for the antigen in the solid phase. This is the principle of Havav and Korav and we need to investigate that and see what type of data we get with competing two antigens for antibody, as compared to competing two antibodies for an antigen. We are currently doing that.

DR. OVERBY: I would just like to say, as long as we are going to be dependent on recombinant-derived proteins and a combination of them, 2, 3, 4 and so forth, that puts some logistic constraints on anything other than the anti-globulin type formats. It can be done, but –

SPEAKER: If we label the protein, for instance, and use that as the detector –

DR. OVERBY: That is right, yes. Logistically, it requires a lot more work. But, if it gives you the specificity and sensitivity, why not?

SESSION III

CLINICAL FEATURES OF HCV INFECTION

Chairpersons:
DR. S. C. PAPPAS (Sunnybrook Hospital, Toronto)
DR. S. MONTPLAISIR (Sainte Justine Hospital, University of Montreal)

Association of HCV with Chronic Liver Disease

DR. V. FEINMAN (Mount Sinai Hospital, University of Toronto)

I would like to state at the beginning, that there is a difference in assessment of laboratory tests and especially in the interpretation of anti-HCV tests, between transfusion medicine and clinical medicine.

Whereas in transfusion medicine sensitivity is the primary objective and over-diagnosing potential sources of infection is justified, in clinical medicine, sensitivity and specificity are of equal importance. As far as hepatitis C diagnosis is concerned, we are in a transition period at present. We have available relatively non-specific, but very sensitive testing systems for the detection of a nonprotective antibody. New testing systems which will enable us to follow the virus into circulation and in tissues will become available soon, which will facilitate the diagnosis of hepatitis C, assessment of infectivity, and follow-up of antiviral therapy.

In Table 1 is a list of the test systems presently available and the substances they measure as well as the test systems under development (marked with an asterisk).

The ELISA tests available currently are using as a target antigen a product of the nonstructural part of the hepatitis C genome called C100. So-called "second generation" ELISA tests using target antigens other than C100 will go into clinical trials soon.

A recombinant immunoblot assay RIBA-1 (Chiron Corp.), which employs two target antigens C100 and 5-1-1, can be used as a supplementary test and a method for the confirmation of the presence of antibodies to C100 (Abbott Laboratories) is available as well. These are not confirmatory, but supplemental tests. The only confirmation test is the polymerase chain reaction for HCV RNA (PCR)[1,2]. At present, this test is available in research laboratories only.

My task today is to discuss HCV as a cause and contributor to chronic liver disease. I have divided the topic into two parts:

Table 1
Test systems for hepatitis C diagnosis

	TEST SYSTEM	SUBSTANCE MEASURED
1.	ELISA assays	
	a) 1989-90	Antibody to C100-3
	b) 1990-91*	Various target antigens other than C100-3
2.	Recombinant immunoblot assay (RIBA)	Antibodies to C100- 5-1-1
3.	Polymerase chain reaction (PCR)**	Specific RNA sequences
4.	Immunofluorescence**	HCV antigens in tissues
5.	*In situ* hybridization**	RNA or cDNA sequences
6.	Antigen detection methods**	HCV antigens

* to be released in 1991-92
** tests available in research labs only

I. HCV as a primary agent causing a specific disease: acute and chronic hepatitis C, the most common type of blood-related and sporadic hepatitis non-A, non-B (NANB);

II. the co-existence and impact of HCV infection on liver disease of different etiologies.

I. HCV as a primary agent, may cause the following conditions:

1. asymptomatic carriage
2. acute resolving hepatitis C
3. fulminant hepatitis C
4. chronic hepatitis C (CPH and CAH)
5. chronic hepatitis C with progression to cirrhosis
6. hepatocellular carcinoma (only epidemiological association is established).

As the clinical features of hepatitis C will be discussed by Dr. Gitnick, I shall limit my remarks to asymptomatic carriers and to chronic hepatitis NANB.

Together with Professor M. Blajchman from McMaster University and the Directors of the Red CRoss Centres of Toronto, Winnipeg and Hamilton, I am coordinating a prospective, randomized double-blind controlled study with the aim of assessing the efficacy of so-called surrogate tests (alanine aminotransferase ALT and anti-HBc), in addition to anti-HCV testing of blood donors in the prevention of post-transfusion hepatitis.

We have collected considerable data on "healthy donors" prior to the introduction of anti-HCV testing by the Canadian Red Cross at the end of April, 1990[3].

As seen in Table 2, of 2,781 donors, 11 (0.4 per cent) were anti-HCV positive by the ELISA (Ortho). Of the 11 anti-HCV positive donors, three had elevated ALT (>1.5 x ULN) and one of the three with elevated ALT was also anti-HBc positive. On the other hand, in an additional cohort of anti-HCV positive donors, the frequency of ALT elevation (higher than 1.5 x ULN) was only 2 of 36 (5.5 per cent). The exact relationship between anti-HCV positivity and raised ALT will be established after large numbers of anti-HCV positive donors have been investigated.

Of the 11 anti-HCV donors, only 4 (36 percent) were positive by RIBA, which means these donors had antibodies to two target antigens derived from the nonstructural part of the HCV genome: C100 and 5-1-1. We have observed that only the anti-HCV ELISA positive tests with a sample to negative cut-off ratio (S:C) higher than 4.0 were RIBA positive. On the other hand, there are some anti-HCV positive individuals

Table 2
Anti-HCV, elevated ALT and anti-HBc in Canadian donors prior to April 1990

Location	No. of Donors Tested	^ALT (> 1.5 x ULN)	Anti-HBc +	Anti-HCV +
Toronto	1707	19	45	7 (0.41%)
Hamilton	768	9	16	2 (0.26%)
Winnipeg	306	2	8	2 (0.65%)
Total	2781	30 (1.08%)	69 (2.48%)	11 (0.40%)

with high S:C values (>5.5) who are RIBA negative. The significance of these findings is under investigation.

Of 600 randomly admitted patients to the Mount Sinai Hospital, 12 (2 per cent) were positive for anti-HCV by ELISA (Ortho) but only three of the 12 (25 per cent) were positive by RIBA (done in collaboration with Drs. A. Simor and D. Low).

In order to assess the significance of anti-HCV results we have compared the results of anti-HCV testing of healthy donors and of random hospital admissions of pedigreed patients with chronic NANB hepatitis and anti-HCV-positive individuals to recipients ("gold standard").

Fifty-eight patients with chronic blood-related NANB hepatitis who were followed from one to six years in our liver clinic, were examined. In each patient, in addition to the history of blood contact (blood transfusion in 50 and IV drug abuse in eight), other causes of chronic liver as listed in Table 3, were ruled out by proper tests.

Table 3
Causes of chronic hepatitis-like conditions to be ruled out in each case of chronic hepatitis non-A, non-B

- Viral hepatitis (B; Delta; CMV; EBV)
- Autoimmune chronic active hepatitis
- Hemochromatosis
- Alpha-1-antitrypsin deficiency
- Wilson's disease
- Primary biliary cirrhosis
- Sclerosing cholangitis
- Drug-induced hepatitis

Fifty of the 58 patients had liver biopsies that showed chronic hepatitis. Of the 58 patients with chronic NANB hepatitis, 49 (84 per cent) were positive for anti-HCV by ELISA. On repeat testing of numerous sequential samples, the sample to negative cut-off ratio (S:C) was always higher than 4 (most of the patients had S:C ratios higher than 5.5). Of 45 serum samples tested by RIBA, 41 (91 per cent) were positive.

The significance of RIBA testing is that in addition to C100, it also detects antibodies to 5-1-1. It seems that the presence of antibodies to 5-1-1 is of importance for both chronic liver disease and infectivity. In a preliminary report from Finland[4] anti-HCV donors were divided into RIBA positive and RIBA negative. Donor samples which were RIBA positive were associated with hepatitis in the recipients as opposed to the ELISA-anti-HCV positive but RIBA-negative donors who were not infectious.

In a study of post-transfusion hepatitis[5] in 1985, we identified 52 patients with post-transfusion hepatitis. Of 181 implicated donors we were recently able to examine 111 donors for anti-HCV. Four donors were found to be anti-HCV positive[6] by ELISA and RIBA. A unit of blood from each of these donors had been given to a different recipient. All four recipients developed post-transfusion hepatitis, 3 chronic and one acute resolving.

As far as infectivity is concerned, it would be dangerous to assume that anti-HCV ELISA positive but RIBA negative individuals are not infectious. This aspect is under intensive study in our laboratory and by others.

Of the 52 pedigreed patients with post-transfusion hepatitis[6], 18 developed chronic hepatitis and 34 had acute resolving hepatitis. Whereas of the 34 patients with acute resolving hepatitis only 9 per cent became anti-HCV positive (seroconverted), 11 of the 18 patients who developed chronic hepatitis (61 per cent) became anti-HCV positive. It is possible that the presence of anti-HCV in post-transfusion hepatitis is of prognostic significance and denotes a chronic course.

II. The co-existence and impact of HCV infection on liver disease of different etiologies:

1. Hepatitis B
2. Hepatitis B and Delta
3. Cryptogenic cirrhosis
4. PBC
5. Autoimmune chronic active hepatitis
6. Alcoholic liver disease
7. Post-transplantation
8. Hepatocellular carcinoma.

Simultaneous hepatitis B and C infections

1. Co-infection: simultaneous acute hepatitis C and acute hepatitis B. The patient was infected with an inoculum containing both hepatitis B and C and, in rare cases, the Delta agent as well. This is quite common in intravenous drug abusers.
2. Superinfection: acute hepatitis C superimposed on HBsAg carrier state or on chronic active hepatitis B. Acquired by blood transfusions or, more commonly, in intravenous drug abusers.
3. Superinfection in reverse: acute hepatitis B can also be superimposed on chronic hepatitis C.

When the anti-HCV test became available (1st conference on hepatitis C organized by Chiron and Ortho in September 1989, in Rome), large numbers of HBsAg-positive persons were also reported to be anti-HCV positive. There are two aspects to this problem. First, one can expect that in intravenous drug abusers the simultaneous infection by hepatitis B and C viruses is a common occurrence. The second problem is, does the simultaneous infection with hepatitis B and C viruses (and in some, additional Delta infection) produce more serious liver disease?

Table 4
Results of RIBA tests in anti-HCV positive blood donors, random admission to a general hospital, and in patients with documented chronic hepatitis non-A, non-B (RIBA positive means positive for antibodies to 2 target antigens: HCV C100 and 5-1-1)

	No. ELISA Positive	No. RIBA Positive
Donors	11	4 (36%)
Hospital admissions	12	3 (25%)
Chronic NANB hepatitis	45	41 (91%)

Table 5
Features differentiating autoimmune from viral chronic hepatitis

Features	Chronic hepatitis	
	Autoimmune	Viral
History of blood contact (transfusion, IV etc)	No	Yes
Sex	Predominately female	Either
Age	15-25 or menopausal	Any
Amenorrhea	Frequent	No
Ten-fold elevation of serum transaminase	Yes	No
Yo-yo transaminases	No	Yes
Autoantibodies (ANA;SMA;AMA)*	>1:40	Absent
LKM antibody	May be present	Absent
Viral hepatitis markers	Absent	Positive
Overlap autoimmune disorders	Thyroiditis	No
	Diabetes	
	Ulcerative colitis	
	Skin rashes	
	Pulmonary infiltrates	

* ANA = antinuclear antibody
SMA = smooth muscle antibody
AMA = antimitochondrial antibody
LKM = liver and kidney microsomal antibodies

Table 6
Anti-HCV by ELISA in autoimmine chronic active hepatitis

Country	Al-Cah	No. anti-HCV+	%	Reference
Italy (Palermo)	22	13	59	Hepatology 1990;12:424
Japan	18	9	50	Hepatology 1990;12:430
Italy & France *after treatment	38	23	60	Lancet 1990;5:1160-61
England *after treatment	53	21	40	Lancet 1990;335:754-757
Canada (Toronto)	19	5	26	

*Note the marked drop in anti-HCV frequency in some patients after treatment

In our clinic we are following a number of patients with chronic hepatitis B who are also positive to anti-HCV by both ELISA and RIBA, and are positive for HCV RNA by PCR, and at the same time are HBeAg and HBV DNA positive as well. Many have a history of intravenous drug abuse and one cannot establish what came first, hepatitis B or C, or both simultaneously. It is therefore difficult to assess if superinfection with an additional virus interferes with the viral replication of the first virus. Some of these patients will be treated with Alpha Interferon and sequential measurements of both HBV DNA and HCV RNA will be made.

A. Lok, et al.[7] reported that 20 per cent of asymptomatic HBsAg carriers, 30 per cent of patients with chronic hepatitis B, 28 per cent with HBV-related cirrhosis and 28 per cent of patients with HBsAg-positive hepatocellular carcinoma were also positive for anti-HCV by ELISA. The prevalence of anti-HCV was similar among HBeAg positive and HBeAg negative patients. The authors concluded that the concomitant HCV infection did not produce more serious liver disease in patients with hepatitis B. Forty per cent of the anti-HCV positive patients had a history of blood transfusions and 75 per cent had a history of intravenous drug abuse. In spite of this report, caution against hasty conclusions is recommended, especially as we have no specific viral markers for hepatitis C to follow the virus in tissues and in blood. This is the stumbling block in our understanding of these conditions. On the other hand, I am cognizant of the fact that progress will be made within the next several years in this very dynamic field.

The material presented in this symposium is primarily from our Liver Study Unit, except the patients with primary biliary cirrhosis (PBC) - the major part of which is from a therapeutic study on PBC coordinated by Dr. J. Heathcote - and the liver transplantation patients (Drs. G. Levy and P. Greig).

The patients to be described are all pedigreed patients; they were followed for at least one year, and in most cases, many years, and all patients had liver biopsies.

Cryptogenic cirrhosis

Of ten patients with cryptogenic cirrhosis, seven were positive for anti-HCV by ELISA (70 per cent), and five of those seven were positive by RIBA as well. Cryptogenic cirrhosis was in the past a "wastebasket" of diseases of different etiologies. Since the introduction of viral testing, the number of these patients is decreasing. I am of the opinion that patients with cryptogenic cirrhosis who are anti-HCV positive are mostly likely sequelae of chronic hepatitis NANB.

Primary biliary cirrhosis (PBC)

Of 52 sera of 52 proven patients with PBC, only three were positive for anti-HCV and two of those gave a history of blood transfusions in the past. We can therefore assume that hepatitis C virus plays no role in PBC.

Anti-HCV in autoimmmune chronic active hepatitis

About 20 per cent of patients with chronic active hepatitis are the autoimmune type.

The pathogenetic basis of autoimmune chronic hepatitis is a reaction of the immune system of the host against "self components". This altered reaction may be caused by either defective suppressor functions or altered "self components", or both. These changes are usually altered by genetic causes but viruses may play a role as well. Another possibility is an aberrant presentation of a normal "self component" to the immune system. The differentiation between autoimmune chronic hepatitis and viral induced hepatitis is important for both diagnosis and therapy. Whereas Alpha Interferon is of value in the treatment of viral hepatitis, it may be detrimental when given to patients with autoimmune hepatitis.

The differences between autoimmune and viral chronic hepatitis are outlined in Table 5.

Whereas autoimmune hepatitis is primarily a disease of females, viral hepatitis affects both sexes. In hepatitis B there is a predominance of males. In autoimmune hepatitis, the patients are between the ages of 15 and 25, or menopausal. Amenorrhea is quite a frequent symptom in patients with autoimmune hepatitis. There is no specific age for viral hepatitis but the perinatal transmission of hepatitis B is well known. The intermittent elevation of the transaminases (yo-yo) pattern is characteristic for NANB, but it is occasionally seen in chronic hepatitis B as well. In autoimmune hepatitis, the elevation of the transaminases is usually persistent.

The presence of autoantibodies in high titres (antinuclear, smooth muscle and antimitochondrial) is characteristic for autoimmune hepatitis. By contrast, as early as 1985, MacKay, et al.[8] found that in chronic NANB hepatitis, autoantibodies to nuclear, smooth muscle, mitochondrial and liver membrane antigens were low, being not greater than that recorded for a normal population, and usually in low titres.

Liver and kidney microsomal (LKM) are present in the so-called Type II autoimmune chronic active hepatitis and may be present without antinuclear antibodies. On the other hand, the frequent detection of LKM antibodies in documented HCV infection indicates that HCV may be associated with or even cause an autoimmune phenomenon (F. Bonino, personal communication). Viral hepatitis markers compatible with viral replication are usually absent in autoimmune hepatitis. Antibodies as evidence of remote infection with hepatitis B may be present and the same applies to anti-HCV as well. With the routine tests available at present, it is impossible to differentiate an antibody as evidence of a remote infection with the hepatitis C virus from ongoing HCV replication. In addition, patients with autoimmune hepatitis are known to have a tendency to overproduce antibodies of any type. This may explain the high incidence of anti-HCV positivity by ELISA in this condition as seen in Table 6.

The anti-HCV positivity by ELISA ranged from 26 to 60 per cent in five studies from various parts of the world. MacFarland, et al.[9] have pointed out that the high rate of ELISA anti-HCV positive results in autoimmune hepatitis may be false positive due to high levels of IgG. The authors have found that only the polyclonal IgG caused by liver disease and not the monoclonal type as in multiple myeloma as in multiple myeloma is responsible for the false anti-HCV results. MacFarland[9] and a group in Italy[10] observed that there was a marked drop in anti-HCV positivity in patients treated with steroids following a response. This drop of anti-HCV positivity as seen in Table 6 in line 5 and 6, was accompanied also by a drop of serum IgG. On the other hand, in another Italian study[11] the anti-HCV positive tests by ELISA were also positive by RIBA.

In our own material, we have found that 26 per cent of patients with autoimmune hepatitis were anti-HCV positive as well. One patient has lost anti-HCV after treatment with steroids; the remaining four were also RIBA-positive and their sera contained HCV RNA as detected by PCR. Two had a history of blood transfusion. This means that some patients with autoimmune hepatitis may be infected with hepatitis C virus as well.

One cannot state if the infection with hepatitis C was a superinfection or the hepatitis C infection antedated the development of autoimmune disease. In order to answer these questions, more specific viral markers are required. For practical purposes it is important to note that in patients with chronic forms of hepatitis and cirrhosis, one has to pay attention to the levels of serum IgG, especially when higher than 30g/L (normal 7.0-17.0 g/L) as this may cause false positives. Very rarely, antibodies to human superoxide dismutase, which are exceedingly rare in the normal human population, may be present in autoimmune hepatitis. This can be detected by the RIBA immunoassay.

The association of anti-HCV with alcohol-induced liver disease

Reports from Italy and Spain[12,13] showed a high rate of anti-HCV by ELISA in patients with alcoholic liver disease (52 per cent in Italy and 27 per cent in Spain). A strong relationship between anti-HCV frequency and the severity of alcoholic liver disease was found. E. Schiff (personal communication) found about 30 per cent of patients with chronic alcoholism were anti-HCV positive by ELISA. The significance of this observation, however, could not be assessed.

Another report, by Rumi, et al.[14]) has shown that in alcoholics only 50 per cent of the ELISA anti-HCV positive sera were positive by RIBA. The presence of RIBA-positive anti-HCV correlated with the histological signs of viral hepatitis such as periportal necrosis and lobular lymphocytic infiltration. There was no correlation with age, sex, abnormality of liver function tests and duration of alcoholism. In other words,

hepatitis C infection contributed to the severity of liver disease in alcoholics.

In assessing the contribution of hepatitis C to liver disease in alcoholics, one has to keep in mind two aspects. First, the epidemiology; in chronic alcoholics one has to consider a history of blood transfusion, intravenous drug abuse, horizontal spread, and other risk factors of viral hepatitis. Another aspect is the fact that alcohol is a general toxin and it also has immunosuppressive effects. It is known that alcoholism facilitates infection of any kind (bacterial and viral) and, therefore, one can assume that hepatitis C superimposed on alcoholic liver disease will add to the liver damage caused by the alcohol, but at present we are unable to prove this hypothesis without more specific HCV markers.

HCV and hepatocellular carcinoma (HCC)

Seroepidemiologic data linking HCC with HBV infection are well known. HCC following NANB infection with an interval between the acute NANB hepatitis and the development of HCC was quite long (17 or 18 years). In 1984, Kiyosawa, et al.[15] described a 39-year-old woman who developed post-transfusion hepatitis NANB two months after blood transfusion which progressed to chronic active hepatitis then to cirrhosis, and finally to hepatocellular carcinoma documented by sequential liver biopsies. The patient was hepatitis B negative.

Okuda, et al.[16] have postulated that in patients with chronic NANB hepatitis, and in those with markers of a remote hepatitis B infection, the development of hepatocellular was related to NANB hepatitis and not to HBV. In Japan there is an increased death rate from hepatocellular carcinoma but a decrease of HBsAg positivity. Whereas in Japan, the number of deaths from HBsAg-positive liver cancer in 1988 was 6,000, the number of anti-HCV positive patients with HCC who died was 14,000. According to K. Nishioka (The Japanese Red Cross, Japan: personal communication) the incidence of HCC among the HCV-antibody positive population is four times that of the HBsAg-positive population. In other words, the relative carcinogenicity of anti-HCV positive individuals was four times higher than in HBsAg patients. A high prevalence of anti-HCV positive in patients with HCC and cirrhosis of the liver was also reported in Italy and in Spain[17,18].

In our own material, of four patients with HCC, two (50 per cent) were positive for anti-HCV by ELISA. The patients were HBsAg negative but anti-HBs and anti-HBc positive. In contrast to the statistics of Japan and Italy, in a study from South Africa 48 per cent of 380 patients with HCC were HBsAg positive, and 30 per cent were anti-HCV positive and HBsAg negative[19]. Similar data were found in Mozambique[20].

These reports showed a higher contribution of HBV than HCV. Most of the data of anti-HCV positivity were based on ELISA results alone. It is difficult to decide whether the HCV virus plays an etiologic role in HCC or if the HCC is a result of liver cirrhosis only. The lack of methodology for measuring HCV markers in tissues precludes a final opinion in this regard. HCV-RNA was detected in tumour tissues of patients with HCC as well as in surrounding cirrhotic tissues by PCR and identical sequences were obtained from the cancerous and non-cancerous tissues from the same patients. This suggests a strong association between HCV infection and HCC (T. Niyamura, National Institute of Health, Tokyo, Japan: personal communication). Further studies based on molecular biology will explain the role of hepatitis C virus, hepatitis B virus, and other factors in the etiology of HCC.

HCV and liver transplantation

The available data on anti-HCV presence prior to transplantation and in orthotopic liver transplants (OLT) recipients are based on first generation testing only and therefore are of limited value.

One could expect that the prevalence of anti-HCV in cryptogenic cirrhosis and in patients with chronic NANB hepatitis will be high. The more important question, however, is this: is there a *de novo* or recurrent HCV infection following OLT? And a second problem is the differentiation between hepatitis C and rejection reactions.

We retrospectively tested sera of 50 patients who underwent OLT (Drs. G. Levy and P. Greig). Eleven patients had cryptogenic cirrhosis. Four of the eleven patients were positive for anti-HCV by ELISA. One additional patient with chronic hepatitis B and hepatocelluar carcinoma was also positive for anti-HCV. It was of interest to note that all five patients remained positive for anti-HCV following OLT (six to 24 months post-OLT). In addition, six of 45 patients (13 percent) who were anti-HCV negative prior to OLT seroconverted to anti-HCV in the post-operative period. The pre-transplant diagnoses of the patients were: four had PBC and two had autoimmune chronic active hepatitis with cirrhosis. In three of the six patients, clinical and histologic evidence of hepatitis was present in the post OLT period. These are preliminary data only and no supplementary test or PCR was done. A prospective study using second generation tests and PCR will soon be initiated.

Summary

Hepatitis C infection plays an important role in the etiology of chronic liver disease, especially in chronic NANB hepatitis where hepatitis C seems to be the predominant or only type of chronic NANB hepatitis. The high frequency of anti-HCV and RIBA positivity in cryptogenic cirrhosis suggests that most patients with cryptogenic cirrhosis are experiencing a result of chronic hepatitis C.

We have shown that donors who are anti-HCV positive in high titres transmitted hepatitis to recipients. Attention is drawn to the fact that only 25 to 36 percent of asymptomatic individuals who are positive for anti-HCV by ELISA are also positive by RIBA, which means that they have antibodies to two target antigens, products of the nonstructural part of the genome of hepatitis C virus.

One cannot answer the question as to whether hepatitis C contributes to other chronic liver diseases (alcoholic, autoimmune, and hepatocellular carcinoma) without the availability of testing systems for the presence of specific viral RNA in cells and in the circulation. PCR is at present the most reliable test to assess infectivity. This methodology may also answer the question, what is the significance of anti-HCV positive tests by ELISA with low titres?

Since the description of the HCV genome by Dr. M. Houghton and his group in 1988[21], considerable progress has been made in the diagnosis and prevention of hepatitis C. I am sure that within one or two years, the ability to assess viral replication of the hepatitis C virus will enhance our understanding of

this disease, it will help in the detection of hepatitis C, and it will facilitate the follow-up of antiviral therapy.

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DR. GITNICK: Victor, in your alcohol data, and in yours, Gene, when you tell us about the 25 to 30 per cent frequency of HCV positivity, can we rely on that as being confirmed positives, or is this the initial screening assay?

DR. FEINMAN: When you say confirmed –

DR. GITNICK: A secondary assay for specificity, either RIBA or the Abbott blocking test.

DR. FEINMAN: I think that the Italian reports were done only on the basis of the first generation test. Maybe Gene has –

SPEAKER: He says that three of his were reactive by RIBA, six were indeterminate and two were negative. So, out of eleven patients, three were RIBA-positive, six were RIBA-indeterminate and the rest were negative. So, we know that we have three of eleven that confirmed.

Did you do second generation RIBA?

DR. FEINMAN: But, Gary, even if they are positive, what is the significance?

SPEAKER: I don't know what the significance is. You would think that if a person has underlying alcoholic liver disease and gets a superinfection, that it should make the disease worse and cause progression, but to say that we have any data to support that - we don't.

DR. FEINMAN: We need the other test to do that really.

SPEAKER: Since we are dealing in anecdotes, we just had an opportunity to look at our first alcoholic cirrhosis, a Danish patient who was anti-HCV positive. We examined by PCR. That patient was positive by PCR but, of course, many more have to be done, and this relationship is really a screwy one.

DR. SCHIFF: The VA Cooperative Study on Alcoholic Hepatitis, headed by Charles Mennanhall, looked at this. They found, unlike our own series, a correlation with HCV sero-positivity and severity of liver disease, as you outline with these other two. We couldn't find that in our study.

DR. FEINMAN: We have here in Toronto a very large Addiction Research Institute, and there are lots sera stored for this. We are just waiting to do all that when we have the real machinery to answer some of these questions. They are all ready.

Clinical Features of Hepatitis C Infection

DR. G. GITNICK (UCLA School of Medicine, Los Angeles)

Thomas Carlyle once said, "They only are wise who know that they know nothing."

For several decades we have known of the existence of the hepatitis C virus and yet we have known little about it. The advent of the hepatitis C virus antibody test makes possible finally, comprehension of this elusive agent. Researchers have assessed its genomic organization, its taxonomic classification, and its pathogenicity in the remarkably short time since the creation of the test for antibody. Serological studies have yielded new insights into the spectrum of disease caused by this remarkable agent.

I will attempt to summarize and to integrate much of what is currently known about the relationship of hepatitis C to human disease, including its transmission route, evolving serology, and implications for the pathogenesis of acute and chronic hepatitis.

Hepatitis C in perspective

Pioneering work by Dr. Saul Krugman[1] and his predecessors led to the differentiation of infectious hepatitis from serum hepatitis. The hepatitis B surface antigen assay, begun with the work of Blumberg[2], led to the differentiation of hepatitis B from other forms of hepatitis. The association of hepatitis B infection with chronic active hepatitis and cirrhosis was subsequently proven[3]. Feinstone and others[4] demonstrated the presence of the hepatitis A virus. This discovery led to the realization that a significant number of people with viral hepatitis had a disease unrelated to hepatitis A or hepatitis B.

In 1973, Koretz, et al.[5] described the course of 14 of 24 post-transfusion hepatitis patients who developed non-B hepatitis after receiving hepatitis B surface antigen-negative blood. This was one of the earliest reports of post-transfusion viral hepatitis transmitted by an agent serologically unrelated to hepatitis B. In 1974, Prince, et al. published a similar report describing 36 of 51 post-transfusion hepatitis cases which were serologically negative for hepatitis B[6]. In 1975, Knodell described 30 of 34 post-transfusion hepatitis cases which were not associated with hepatitis B[7]. In that same year, Alter reported the results of the National Institutes of Health prospective post-transfusion hepatitis study, and identified cases serologically unrelated to hepatitis A or hepatitis B. Alter first utilized the term "non-A, non-B hepatitis" in this report[8].

The absence of a specific assay for non-A, non-B (NANB) hepatitis hindered progress. Nevertheless, our understanding derived in large part from our ability to transmit infected blood specimens to chimpanzees that subsequently developed NANB hepatitis. The chimpanzee experience made possible the characterization of certain aspects of the virus and a better understanding of disease transmission and pathogenesis[9,10,11].

A second transfusion-associated agent has been postulated, that is not chloroform-sensitive[13]. Chloroform-sensitivity implies that the virus contains lipid, presumably a lipid coat as a component of the surface membrane. Filtration studies determined that the major hepatitis C agent was a small virus of approximately 30 to 60 nanometers in diameter[14]. Thus, before the development of the hepatitis C virus test and the application of molecular biologic techniques, research had discovered that the major NANB agent was a 30 to 60-nanometer, lipid-enveloped virus, probably of RNA origin and, by virtue of these characteristics, possibly related to the arthropod-borne viruses.

Prospective studies of transfusion-associated hepatitis revealed that 80 to 90 per cent of cases were due to the NANB hepatitis agent[9]. They further determined that between one and one and a half per cent of the units of blood taken from voluntary blood donors were likely to transmit NANB hepatitis[15]. Eventually, it was shown that screening blood with ALT and the hepatitis B core antibody assay (anti-HBc) would identify a significant number of infectious donors. This screening was considered likely to reduce the transmission of NANB hepatitis blood products by as much as 40 per cent.

Koretz[20] and subsequently others revealed that 40 per cent to 70 per cent of NANB hepatitis cases progressed to chronic hepatitis. Furthermore, within three years of infection, approximately 20 per cent of patients with chronic NANB hepatitis could be found to have histologic evidence of cirrhosis[21]. However, this was an inordinately indolent form of cirrhosis. A prospective study reported by Koretz revealed that even after 15 years of follow-up, only 9 per cent of patients actually developed clinical evidence of portal hypertension[21]. Thus, this chronic illness could be characterized as histologically progressive but with an exceptionally slow clinical progression. At the end of 15 years of follow-up, 91 per cent of patients initially found to have chronic hepatitis lacked any evidence of liver failure.

Transfusion-associated viral hepatitis

Our appreciation of the course of transfusion-associated viral hepatitis developed from several long-term prospective studies of post-transfusion hepatitis[5,9,10,20,23,24,25]. In the 1960s and 1970s when our national blood supply utilized many paid donors, the frequency of post-transfusion hepatitis in major medical centres was reported to exceed 30 per cent[5,26]. This fact had not been appreciated because the illness that developed was clinically mild, although it led to an unusual frequency of chronic hepatitis. Thus, it was generally detected only by prospective studies that followed transaminase elevations. These cases initially were thought to be related to "serum hepatitis", subsequently identified as hepatitis B. It was later determined that these cases were unrelated to the hepatitis B virus[5,9], and that "serum hepatitis" must represent other causative agents as well.

In the decades that followed, the incidence of transfusion-associated

hepatitis decreased from more than 30 per cent to less than 5 per cent. In large part this was due to the elimination of paid donors and the introduction of donor screening with the surrogate markers, ALT and anti-HBc. The introduction of screening with the anti-HCV assay has further reduced the transmission of transfusion-associated hepatitis[31]. Patients receiving clotting factors commercially prepared from the sera of paid were found to have an unusually high frequency of chronic hepatitis. For example, 65 per cent of hemophiliacs had NANB chronic liver disease[27]. Anti-HCV testing of this patient group revealed that between 60 and 90 per cent carried antibody to HCV[23,24]. Of interest is the fact that intravenous drug abusers also had a 60 to 90 per cent prevalence of anti-HCV[23,24].

Modes of transmission

Parenteral

Hepatitis C virus transmission by blood products and by shared contaminated needles among drug users is well established. Accidental needle stick transmission, at least in theory, may also occur. It has been demonstrated in most studies that 80 to 90 per cent of transfusion-transmitted NANB hepatitis is due to HCV. High risks groups include multiple-transfusion patients, especially haemophiliacs among whom it has been shown is a 60 to 90 per cent rate of positivity to anti-HCV[23,24], and renal dialysis patients.

Most hemophiliacs who are HCV positive have been found to have chronic hepatitis[22,34]. Renal dialysis patients frequently have been found to carry anti-HCV. In a recent study reported by our group, 16 per cent were found to be anti-HCV positive[32].

Sexual transmission

If sexual transmission exists, it is not yet well documented. Since the epidemiology of HCV is so similar to that of hepatitis B virus, it would seem that sexual transmission would occur with ease. In fact, among homosexual males, where there is an exceptionally high prevalence of hepatitis B viral markers (60 to 80 per cent), only 4 to 8 per cent have antibody to hepatitis C virus[24]. This prevalence if five to ten times that of the blood donor population, but is exceedingly low compared to the 60 to 80 per cent prevalence of hepatitis B virus infection among the same group. Furthermore, one study of partners of anti-HCV positive homosexuals showed that most were anti-HCV negative[33].

The Centers for Disease Control sentinel county studies revealed that approximately 50 per cent of community acquired NANB hepatitis has no demonstrable route of transmission. It is presumed that sexual transmission occurs in these patients; however, convincing data has not yet been reported. In fact, in one study, only one of 18 sexual partners of intravenous drug addicts and none of the 19 partners with NANB transfusion-transmitted hepatitis were found to carry anti-HCV[23]. In another study, none of 42 sexual partners or 20 household contacts of transfusion-associated NANB cases was anti-HCV positive. These differences may reflect the relatively low viral load among patients infected with HCV as compared to that of patients with hepatitis B virus.

Perinatal transmission

Whereas perinatal transmission of hepatitis B virus infection is well documented, this is not the case with hepatitis C virus infection. It is possible that perinatal transmission may occur but available data do not support this hypothesis[33]. Infants born of anti-HCV positive mothers may be transiently anti-HCV positive due to transfer of passive antibody. In one study which prospectively followed the offspring of 37 anti-HCV positive women, there was no evidence of infection[34]; a second study confirmed this finding[35]. A recent report in abstract form revealed that three infants born of anti-HCV positive mothers developed increased levels of anti-HCV six months after delivery when ALT also became elevated[36].

Arthropod vectors

Since HCV is thought to be a flavivirus, it would seem reasonable to presume that arthropod vectors may transmit this virus as they do other viruses related to this group. There is no evidence, however, to support this assumption. The high level of community-acquired hepatitis C raises the possibility of currently unknown vectors.

Chronic hepatitis C

Just as hepatitis refers to inflammation in the liver, chronic hepatitis may be considered a persistence of this inflammation for a prolonged period. To many clinicians and pathologists, however, the term has a more narrow meaning, namely the presence of one of several distinct histologic patterns on a liver biopsy.

"Chronic hepatitis C" refers here to an abnormal serum transaminase which has been present for more than six months in association with a positive hepatitis C antibody (anti-HCV) test. Such a definition is quite broad, and does not consider such aspects of the illness as etiology, actual date of onset, presence or absence of symptoms, or prognosis.

Chronic carriers

Some individuals who are exposed to hepatitis C develop the chronic carrier state; that is, the virus persists in their bodies. Hepatitis A and NANB carriers are known to exist because serial specimens of their blood have been shown to cause hepatitis when injected into humans (blood transfusions) or experimental animals. Most carriers are symptom free. Those with underlying chronic liver disease may be symptom free or complain of fatigue or jaundice.

Carriers may have underlying liver disease. Subjects with abnormal liver tests, whether they are symptomatic or not, usually have abnormal liver biopsies. The situation is less clear, however, in asymptomatic carriers with entirely normal liver studies. Liver disease in such individuals sometimes has been recognized.

Does acute viral disease become chronic?

In the case of viral hepatitis, it is assumed that when the patient first comes into contact with the virus, acute disease results. Since histologic verification of a pattern of acute hepatitis is rarely obtained (usually because this period is entirely asymptomatic), this assumption cannot be documented.

In a similar vein, an episode of clinically apparent (symptomatic) "acute" hepatitis, with the subsequent development of a chronic carrier state and chronic hepatitis, may not be acute at all. If the pre-morbid serology and histology is unknown, the "acute" episode may in fact be the first clinical exacerbation of an already established chronic disease!

Thus, the following data must be interpreted in the light of these caveats. Hepatitis C is said to progress to chronic disease in 40 to 70 per cent of cases;

these data were largely derived from observations based on clinically apparent acute disease where no preexistent information was available. In prospective studies of NANB post-transfusion disease (where at least pre-morbid sera was available, but where the patient was challenged with a very large dose of viral particles), about 50 per cent developed chronic hepatitis.

Types of chronic hepatitis

The biological definition of chronic hepatitis previously described can be used to subdivide the entity based on histology. A number of hepatitis C patients, when biopsied, demonstrate a resolving acute hepatitis or other less specific findings (e.g. fatty infiltration, focal hepatocyte necrosis). Based on pathological differences chronic inflammatory disease of the liver can be divided into two entities: chronic persistent hepatitis and chronic active hepatitis. The chronic persistent form generally has a good prognosis. The disease does not progress and may spontaneously remit. The chronic active form, if untreated, carries a more guarded prognosis of postnecrotic cirrhosis in most symptomatic patients. The prognosis in the asymptomatic patient is less well defined.

Chronic persistent hepatitis

In chronic persistent hepatitis, the clinical picture is not very dramatic. Patients usually feel completely well. Some may complain of fatigue or a vague sensation of pressure in the right upper quadrant of the abdomen. The condition does not progress to cirrhosis. This benign, nonprogressive disease is usually characterized, therefore, by an asymptomatic patient with transaminase elevations. The liver biopsy demonstrates "portal triaditis" (inflammation confined to the portal tract) with otherwise normal hepatic architecture.

Chronic active hepatitis

The association of cirrhosis, plasma cell infiltration of the liver, and hypergammaglobulinemia in young women was described by Waldenström in 1950 and by Kunkel and associates in 1951. Saint and co-workers applied the term "active chronic hepatitis" to this condition in patients of both sexes. Bearn and associates emphasized the prominence of systemic features, such as fatigue, fever, arthralgias, acne and striae. In 1956, MacKay and co-workers introduced the terms "lupoid hepatitis" and subsequently "autoimmune hepatitis" to describe comparable cases with a positive reaction to the lupus erythmatosus (LE) test. The terms "aggressive hepatitis", "subacute hepatitis", and "juvenile cirrhosis" are additional nomenclature to describe this spectrum of disease.

Many of the histologic features (piecemeal necrosis, fibrosis, and intralobular hepatotcyte necrosis), that occur in this presumably autoimmune disorder have been found in liver diseases due to other causes, in particular viral infections such as hepatitis C. Interestingly, in patients with viral illness, symptoms are much less prominent and transaminase levels are relatively low.

Patients with the autoimmune variant of chronic active hepatitis and high grade transaminase elevations frequently progress to cirrhosis and end stage liver disease. Although many patients with the viral variant also develop cirrhosis, they do so over a longer period of time and in a more predictable fashion.

The pathogenesis of this disease is unknown. It is even uncertain if the same processes occur in all patients, or if the histologic lesion of chronic active hepatitis is merely the final, common end point of a number of different mechanisms.

What about the progression to cirrhosis? The ultimate scarring is likely due to the intense inflammatory response. Yet it is disconcerting that occasional patients with clinical evidence of marked inflammation (symptoms and high grade transaminase elevations) do not develop cirrhosis, while other individuals with little evidence of clinical disease march on over the years to a picture of end stage liver disease.

The future

We really remain on the ground floor in our understanding of hepatitis C virus infection. Clearly, more sensitive and specific assays need to be developed, as does a vaccine to prevent the spread of this disease. Of even greater importance, strategies must be developed to interdict the high degree of chronic liver disease that follows this infection. Obviously, preventing the primary disease would solve the problem. If prevention is impossible, then efforts must be made to prevent the development of chronicity. Interferon treatment may help in this regard, but long term results are still lacking. Once a vaccine is developed, universal vaccination programs must be instituted in order to avoid the consequences of infection of this clinically indolent but prognostically serious infection.

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DISCUSSION

Dr. LARKE: On one of your earlier slides, you showed a range of incubation periods for post-transfusion non-A, non-B that went, I think, from eight to 208 days. Do you have any kind of a minimum time frame in which you might say that something as short as eight days may be a previous insult that was undetected, or is that a case where, perhaps, there was a massive transfusion of highly infectious donor blood, for example.

DR. GITNICK: That was one case, one case that truly had appeared to have viral hepatitis, did receive - in our patient population everybody receives a lot of blood - but that patient received something like 30 units of blood, and we never were able to prove the source of that viral hepatitis.

It could well be that that person came into the hospital infected, but his pre-transfusion specimens failed to show any evidence of viral hepatitis. His liver tests were normal, and he had no HCV antibodies. So, we had to include him in the study, but I personally have great doubts about that short an incubation period. I have never seen it, except in that one situation.

DR. FEINMAN: The shortest incubation period in our prospective study was two weeks, and 80 per cent of patients were between two and 12 weeks in 1984 to 1985.

SPEAKER: Dr. Feinman, I was a bit unclear about the hepatocellular carcinoma data that you presented. Did the data indicate that there was a greater propensity for the development of hepatocellular carcinoma with hepatitis C than with hepatitis B, or was it the other way around?

DR. FEINMAN: It depends in which country it is. In Japan, there is a greater propensity for anti-HCV-positive individuals. I mean, patients with hepatocellular carcinoma are more anti-HCV positive. And, this is a country where HBV is in decline.

DR. PAPPAS: I was a bit unclear, and Dr. Purcell may want to comment on this. At the recent meeting at the NIH on hepatoma, the Japanese data presented there, as I understood, showed that the cumulative incidence of hepatocellular carcinoma in HCV carriers was less than that of hepatitis B virus carriers.

Dr. Okuta presented a 24 per cent cumulative incidence over four years in HCV-positive individuals and contrasted that to a 59 per cent incidence in their hepatitis B carriers.

DR. BARKER: I need to understand that data better. Now, what I really want to know is, I understand that if you look at 100 who have hepatocellular carcinoma and you test them for B and C, you get an incidence of positivity. But, if I get hepatitis C, what is my risk of getting hepatocellular carcinoma? You're not telling me it's a 24 per cent risk?

DR. PAPPAS: These were, as I understood, and again, Dr. Purcell may want to comment on these data, (they were presented verbally) that these were cirrhotic patients. These were patients with established cirrhosis followed prospectively.

DR. BARKER: Again, if I get hepatitis C, what is my risk of getting cancer?

SPEAKER: From day one?

DR. BARKER: From day one, right.

DR. PAPPAS: Does anybody know that answer? Is there any prospective study that has addressed that?

SPEAKER: I think not.

DR. FEINMAN: I think retrospectively, it would not be determinable.

SPEAKER: I think the Japanese data convincingly showed a progression, but those are retrospective studies, starting with a patient with either severe or cirrhotic hepatitis, and then looked back at retrospectively for time of exposure, transfusion and such.

I don't think it represents the general patients that you have described who have the very mild disease. I don't believe, I think it's still true that Harvey Alter has yet to see a case of hepatocellular carcinoma in patients who have been followed in the NIH since 1964, over half of whom had chronic hepatitis.

SPEAKER: We have not seen a single case in our study either.

DR. BARKER: I think you have almost answered my question, but having followed this story for a while, I understand that hepatocellular carcinoma is a serious disease and so is decompensated cirrhosis. What I don't know, and I wonder if either of you would like to speculate, or maybe Dr. Alter will, how many people in either of our countries are dying from this condition?

You know, we like to tell ourselves that we are dealing with a very serious disease here, and we have to do everything possible to prevent it, and I happen to subscribe to that. But, I am not really sure how many people are either suffering significant morbidity or dying from, let's say, post-transfusion chronic HCV infection. Would either of you like to comment?

DR. GITNICK: I would love to, Lew. You bring up, I think, a very important point.

We, in fact, have a paper at the liver meetings in November that addresses this, because in our cohort, although we don't deny that you see a lot of cirrhosis, and you see a lot of chronic hepatitis, if you look at the patients, the patients are not sick.

Now, with 13 to 20 years of follow-up, most of our patients, though they may have histologic abnormalities, are leading normal lives, have no symptoms and have no evidence of liver failure.

It is a slowly progressive indolent disease that moves over decades, and if you get the disease in your forties or fifties, you probably - at least if you're in Los Angeles and UCLA - you are probably going to die from something else!

I think, for those of us who are now looking at this issues: how do you select people for treatment; how do you select people for transplantation; how do you select for Interferon treatment, we've got to keep in mind the fact that for a large number of people, you have an asymptomatic disease that is not associated with liver failure.

For a small number of people, you do have a progressive disease. There is a small segment of people with hepatitis C, who look like the common situation

with hepatitis B, where you have relatively rapid progression.

DR. ALTER: I have two comments to make. The first one is on the association of hepatitis C with liver cancer. To date, there have been no control studies retrospective or prospective that can definitively associate hepatitis C with primary liver cancer. Although several countries have published studies showing high rates of hepatitis C among individuals with liver cancer, particularly Japan, there have been no control populations, and until control studies are done, we cannot positively make that association, even though obviously there are some data that point in that direction.

The second comment has to do with the risk of dying or of suffering severe consequences from this disease. I just want to remind you that the transfusion associated part of this disease makes up a very small percentage of the overall burden.

I agree with Dr. Gitnick that many, many people will probably die of something else before they die of this, but on the other hand, chronic liver disease is the ninth leading cause of death in the United States among adults, and of that a couple of population based studies ongoing now point to the fact that 30 per cent of that chronic liver disease may, in fact, be due to hepatitis C.

SPEAKER: What percentage did you say?

DR. ALTER: Thirty per cent may be due to hepatitis C, with less than ten per cent due to hepatitis B. Preliminary data were actually presented in Houston.

SPEAKER: You are saying that 30 per cent of all chronic liver disease is hepatitis C?

DR. ALTER: That may be, yes.

SPEAKER: And, what's the mortality associated with that 30 per cent, versus the 60 per cent who have alcoholic disease.

DR. ALTER: I'm not so sure 60 per cent have alcoholic disease. These are based on population-based studies of which there are very few, which can differentiate the etiologies of a population in whom chronic liver disease is being diagnosed.

I have a feeling some of our preconceptions about the etiology of chronic liver disease may be changed when data from these studies are published.

SPEAKER: I think the other thing we have to keep in mind is that if one looks at unselected autopsy series from the late fifties and early sixties, there is, variously reported, about five to eight per cent incidence of cirrhosis in patients dying for other reasons, without a pre-mortem suspicion of liver disease.

So, clearly, there is a huge group of patients dying from other causes who have unsuspected liver disease.

DR. ALTER: I don't deny that. Nor do I deny that a tremendous number of people with cryptogenic cirrhosis probably have hepatitis C as the etiology. My point is that to get that cryptogenic cirrhosis, it literally takes decades. It doesn't take months or weeks or years.

Now, a small number of people have rapidly progressive disease. But, for most people, you have a slowly progressive disease, and in choosing a strategy for management, a clinician has to keep that in mind.

DR. SCHIFF: Yes, I just want to emphasize that Dr. Barker's question, which was a very important one - does chronic viral C hepatitis really impact on life expectancy? - is going to be answered by a follow-up study coordinated by Leonard Seeff on patients that were in the TTVS, in Harvey Alter's NIH study, in Bob Knodell's study and in the VA Cooperative Study on Post-Transfusion Hepatitis.

There has been some 14 years of follow-up, and they are just starting to generate data that are interesting. But, the final report should answer your question, if indeed this impacts on survival.

DR. FEINMAN: I would like to look at the spectrum the other way around, looking at a number of patients who have cirrhosis in teaching hospitals in this city. About eight years ago, we examined this aspect. More than 55 per cent were of alcoholic etiology. Thirty per cent were associated with hepatitis B. They had hepatitis B markers, but we cannot say that hepatitis B was even the culprit.

In our prospective study from 1984 to 1985, only 36 per cent of patients with acute non-A, non-B became chronic. Only two of those have chronic active hepatitis. It is already five years now, and one is refusing to be biopsied now. The disease was quite mild and four patients out of these 18 patients improved spontaneously within two years. They developed normal transaminase.

So, the disease in Canada is much more benign.

DR. LARKE: I realize this is a symposium principally on hepatitis C, but I can't resist asking this question with two experts like Dr. Gitnick and Dr. Feinman.

For someone who has chronic carrier state of viral hepatitis, be it C or B, what sort of follow-up do you recommend?

DR. FEINMAN: At Sinai we have been following 700 HBsAg carriers since 1971. Once a year we do an alphafetoprotein and once a year or once in two years, an abdominal ultrasound.

Now, these are asymptomatic patients, with normal transaminases. However, when a patient has chronic hepatitis, quite active, or has cirrhosis, I would do an ultrasound every four months, because it takes four months to enlarge a space-occupying lesion by one centimetre.

DR. GITNICK: I don't follow people that closely. I do not get ultrasounds every four months, even in cirrhotics, Victor, and you may be right and I may be wrong. But, I have not found that the rate of pick-up is that great with that close a follow-up. I get alphafetoproteins annually and ultrasounds when I think there is a clinical indication.

DR. SCHIFF: Well, the important fact to keep in mind is that with early recognition, hepatocellular carcinoma can be surgically resected. Now that is true of non-B as well, and what we do is when we have a cirrhotic that we feel would be a surgical candidate, we get ultrasound and alphafetoprotein every six months. This is B, or C, and predominantly C, because it looks like it is going to make a difference in survival.

There were some earlier data that suggested there wasn't a difference, but Chris Pappas, who was at the recent NIH conference, tells me there were two reports that said it makes a difference, and I think, particularly, if it's an encapsulated hepatocellular carcinoma, it would be tragic to miss that at a time when it was resectable.

Surgeons are very aggressive now, and I think we can do more with

hepatocellular carcinoma superimposed on cirrhosis.

SPEAKER: Yes, I think the data clearly are very weak. The best data, I think, say that if the tumour is confined to a segment and you can do a segmentectomy, then your chance for prolonging life more than 12 months is fairly good.

If the tumour is beyond that size, then your chances are less significant. However, I wasn't at that conference, and perhaps there are prospective data. I won't buy retrospective data, but if there are prospective data, perhaps I would be convinced.

DR. PAPPAS: This would be a good time to close the discussion. It reminds me that there are two types of people in investigation. There are those with lots of data and no enthusiasm and there are those with no data and lots of enthusiasm.

SESSION IV

PREVENTION AND CONTROL

Chairpersons:
DR. M. CHERNESKY (St. Joseph's Hospital, Hamilton)
DR. R. MARUSYK (University of Alberta, Edmonton)

Anti-HCV Testing Experience in the American Red Cross: the Role of the Laboratory in Control of HCV Transmission

DR. L. BARKER (American Red Cross Society, Washington)

It is self-evident that blood donors are not a random sample of any population or of its distribution of risk factors for HCV infection. They do, however, provide us with a very large testing experience over many years, and sometimes this provides interesting clues to various virus infections.

For example, after a few years of hepatitis B surface antigen testing, we could see what the prevalence was in various parts of the country through the window of testing Red Cross blood donors with the first modern virus test that was introduced in 1971 (Figure 1). Testing blood donors is an important measure in making blood transfusion as safe as we can, and so we have added a good many tests since HB surface antigen, as shown in Table 1. We don't do anti-CMV testing on all of our blood, but we do all of the rest of the tests, except for the HIV antigen test. So far we do not see a solid scientific justification for introducing that test or the HIV-2 test, both of which were licensed in the past year by the U.S. Needless to say, we did add the anti-HCV test, which appears to be a very valuable contribution to making blood transfusions safer.

Table 1
Transmissible disease tests currently used in blood collection in the U.S.

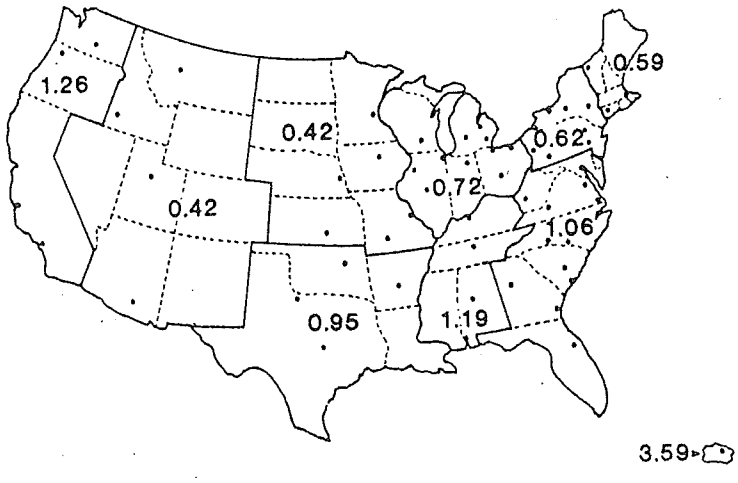
Tests	Agents
STS	*Treponema pallidum*
HBsAg	Hepatitis B virus (HBV)
ALT	Non-A, non-B hepatitis
Anti-HBc	Non-A, non-B hepatitis, HBV
Anti-HIV	HIV-1, HIV-2
Anti-HTLV-I	HTLV-I, HTLV-II
Anti-HCV	Hepatitis C virus
Anti-CMV	Cytomegalovirus

From past experience that we will see certain patterns when we introduce new tests, and one of these is a relatively sharp drop in the prevalence of positive results early on in testing. This is what we saw with anti-HIV in the first couple of years. As shown in Figure 2, there was a sharp drop in the number of Western blot confirmed positives per 100,000 donors, which is the result of the repeat donor population making the transition to a fully-tested population in which we see only incident or new infections.

The actual evolution of testing results of blood donors for the last several years of ARC anti-HIV experience is shown in Figure 3. The first time male donors represent a relatively small proportion of our total donor population -- less than ten per cent of the total donors -- but they have a strikingly higher prevalence of confirmed positive results than do repeat males, or the overall donor population. The prevalence of positives is exceptionally low in repeat female donors.

In the case of HCV antibody testing, this kind of data is not yet available as it's too early in our testing experience. But we do have some real time experience which starts when the FDA approves a test, and we promptly install it in all of our blood centres. Those events occurred in May, 1990, with the anti-HCV test, which we obtained from Ortho Diagnostics, the first manufacturer licensed in the U.S.

Figure 1
Distribution of HBsAg detection rates among Red Cross blood donors, 1975-1978



Source: Szmuness W, Alter HJ, Maynard JE, (eds). Viral Hepatitis 1981 International Symposium. Philadelphia: Franklin Institute Press, 1982:145-55.)

The results I am going to show you are data supplied by my co-authors: Thomas O'Brien of Ortho and Alan Polito of Chiron Corporation.

The test results in Table 2 are from the first month of official testing with the licensed reagents, in the Northeast region covering Maine and Massachusetts. In 26,479 samples, we found a repeat reactive rate of one per cent, an initial reactive of which roughly 70 per cent were repeat reactive.

It has been possible, with the help of Ortho and Chiron, to do RIBA testing on this collection, and what you see is that 37 per cent of the repeat reactives are RIBA positive, and another 15 per cent are indeterminate. So, roughly half are either RIBA positive or indeterminate, and the remainder are clearly RIBA negative. The positive RIBA results have a strong tendency to cluster in donors who have high sample to cut-off ratios (Table 3) and the opposite applies to the negative RIBA results.

A look at the correlation between surrogate markers and anti-HCV results in donor samples (Table 4) reveals several striking findings. About 50 per cent of the RIBA-positive samples have either an ALT elevation or they are anti-HBc positive. Among the RIBA-positive group, compared to the overall donor populations, samples with ALT elevations are roughly 20-fold more common, and samples with anti-HBc are roughly 15-fold more common. In the RIBA-indeterminant and RIBA-negative samples, there are also some individuals who have elevated ALTs or anti-HBc, and using the second generation RIBA, some of these appear to be clearly positive.

Looking at the percentage of HCV test results among donor samples by surrogate marker status (Table 5), we see that of the people with elevated ALTs, 5.6 per cent are repeat reactive, and most of those are RIBA positive. In the case of anti-HBc, almost the same, 5.4 per cent are repeat reactive. Slightly less of those repeat reactives are RIBA positive. There were only four samples in this population that were positive for both the surrogate markers, and all four of those people were repeat reactive and RIBA positive.

The positive predictive values, then, for combinations of tests are shown in Table 6. A relatively low percentage of donors volunteer donors who are positive for anti-HBc or have ALT

Figure 2
Frequency of HIV Western blot-positive donations among American Red Cross blood donors, 1985-1989

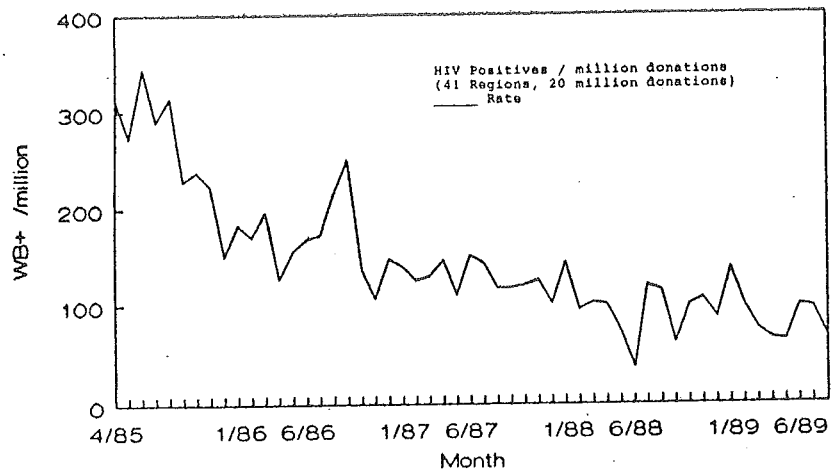


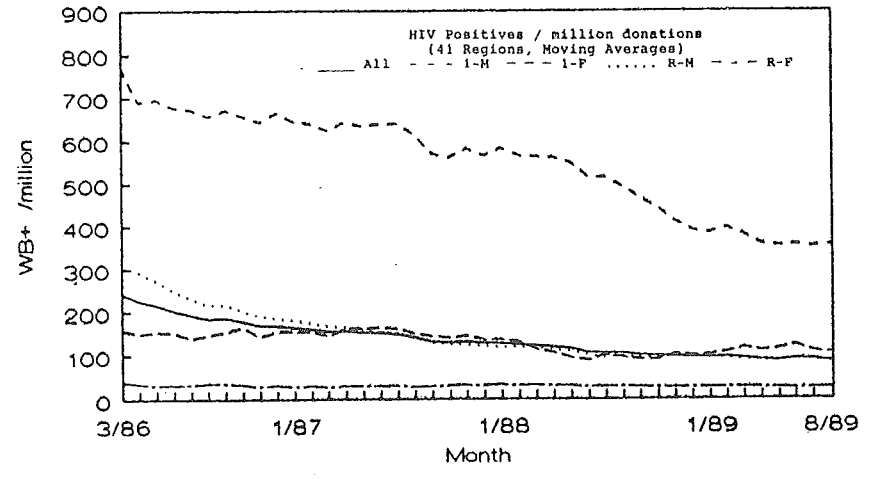
Table 2
Anti-HCV test data American Red Cross northeast region, May 1990

(n=26,479)

Test/result	Number	% of total	% of PR
EIA/IR	268	1.01	
EIA/RR*	190**	0.72	
RIBA/Pos	69	0.26	37.1
RIBA/Ind	29	0.11	15.5
RIBA/Neg	88	0.33	47.3

* RR/IR = 70.1%
** 4 samples QNS for RIBA

Figure 3
Frequency of HIV Western blot positive donations among American Red Cross donor subsets, 1985-1989



elevations only are, in fact, people with anti-HCV that is RIBA positive, in contrast with people who are repeat reactive for anti-HCV (37 per cent of those are RIBA positive).

Examination of confirmed positive results provides striking support for the interaction between these markers. Sixty-three per cent of these repeat reactives with anti-HBc are RIBA positive. Ninety-two per cent of the repeat anti-HCV reactives with ALT, although it is a very small group so far, and 100 per cent of those with both surrogate markers, are confirmed with the RIBA.

The age distribution by anti-HCV test is shown in Table 7. Fifty-eight per cent of the RIBA positives are in the 30-39 age group. In contrast, roughly half of the indeterminants and negatives are in the older age group. There may be a clue here about factors that contribute to non-specificity.

The prevalence of anti-HCV test results by sex shows (Table 8) a higher prevalence in both first time and repeat male donors over female donors for both repeat reactive and RIBA positive test results.

Figure 4 shows the overall distribution at the end of our second month of system-wide testing. It is interesting to note the higher prevalence numbers on the HBsAg map are in the same places that they are on this anti-HCV map. That is to say, they are in the southeast, with a slightly higher numbers in the West, and the lowest prevalence rates are in the middle of the country.

Now that we have a specific test, the laboratory has a much more valuable role in the control and transmission of HCV than it has had before. There have been many years of frustration during the absence of a specific test and the need to rely on non-specific surrogate markers. But, now we are past that point and we can look forward to the improvements in the anti-HCV test that will further strengthen the contribution that the laboratory can make in improving blood safety.

At the same time, it is important to point out that there are a number of other things that we can do. For example, in the U.S. overall, the transition from a portion of our blood supply being collected from paid donors and supplied by commercial blood banks, to an all volunteer system in the

Figure 4
Distribution of anti-HCV test results, Red Cross donors, June, 1990.

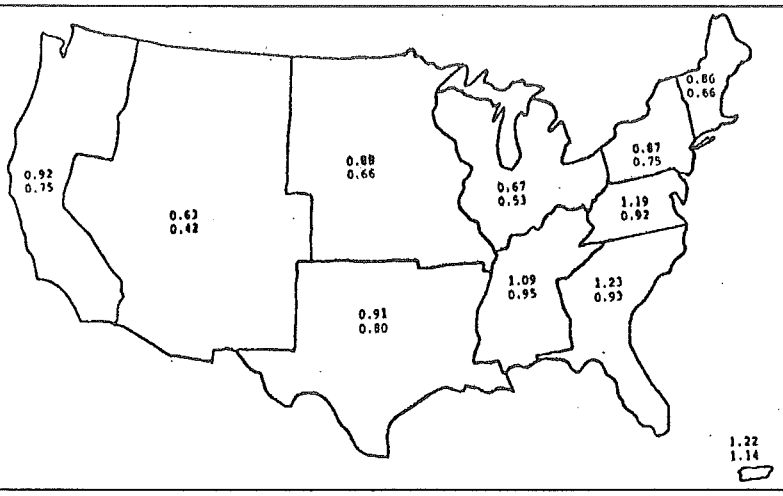


Table 3
Distribution of EIA S/C ratios*

EIA S/C Ratios	RIBA Results		
	Pos.	Ind.	Neg.
0.5-1.0	0	1	6
1.0-1.5	4	3	27
1.5-2.0	1	1	11
2.0-2.5	1	3	14
2.5-3.0	0	3	5
3.0-3.5	1	4	4
3.5-4.0	2	2	0
4.0-4.5	1	1	2
4.5-5.0	22	4	5
5.0-5.5	31	7	11
5.5-6.0	5	0	1
> 6.0	2	0	1

* average of repeats

Table 4
Percentage of surrogate markers among donor samples
(by HCV test Status)

HCV test	n	ALT Elev.	Anti-HBc Pos.	Either
All donors	26,479	1.2	2.2	
EIA-RR	190	9.7	16.7	24.2
RIBA-P	69	24.6*	30.4*	49.3**
RIBA-I	29	0.0	13.8	13.8
RIBA-N	88	1.1	6.8	8.0

Remarks:
* among the RIBA-P population, compared to the overall donor population, samples with ALT are 20 times more prevalent; samples with anti-HBc are 14 times more prevalent.
** close to 50% of RIBA-P samples also have either ALT elevation or anti-HBc positivity.

1970s was the most effective thing that we did over an extended period to improve the safety of the blood supply. The HBsAg test provided very helpful laboratory support to make the case for that switch to an all-volunteer donor system. So, we now have an all-volunteer system, with the exception of plasma for further manufacturing into plasma derivatives.

We have learned in recent years that there are several ways we can seek to improve the safety of the blood supply that we obtain from volunteer donors, and we may be at the point where some of these measures have more to contribute over the next few years, or at least as much to contribute as further enhancements in the quality of laboratory testing and additional laboratory tests.

We have seen over and over again the phenomenon of the highest prevalence of infectious disease markers turning up in first time male donors. It is very likely, however, that not all first time male donors contribute to this phenomenon. In fact, a great many first time male donors contribute blood at high school and college drives, and they have a very low prevalence of markers. When we move up a little bit in the age bracket of these first time male donors, we begin to see the problem, and you also see it in some specific donor group settings.

So, we need to do more about how we select, screen and accept first-time donors, and specifically we need to be a little more attentive to deferring some of those people and keeping them in the donor pool (Table 9). It is clear that what we are dealing with at this point, with all of the agents we are concerned about, and hepatitis C is just one more example, is what we call a window phenomenon in which people are infected but there are not any positive markers. So, we need some better disincentives for people with risk factors. I don't know exactly how that is going to be done, but it appears now that we need at least as much behavioural science these days as we do laboratory science in the field of making blood transfusion safer.

A notion that is a little bit out of line with the way we do our blood drives in the Red Cross is the notion of a pedigreed pool of donors, but the weight of evidence these days may be supportive of that approach. Also, there is clear appeal in collecting blood in

Table 5
Percentage of HCV test results among donor samples (by surrogate marker status)

Marker	n	EIA-RR	RIBA-P	RIBA-I	RIBA-N
All donors	26,479	0.72	0.26	0.11	0.33
ALT elev.	321	5.61	5.30	0.00	0.31
Anti-HBc+	572	5.42	3.67	0.70	1.05
Both	?	(4)*	(4)*	(0)	(0)

*4 samples only

Table 6
Positive predictive values of test combinations for a RIBA positive result

Test Results	%
Anti-HBc positive only	3.7*
ALT elev. only	5.3*
ALT elev. and anti-HBc +	?
EIA-RR only	37.6*
EIA-RR and anti-HBc +	63.0**
EIA-RR and ALT elev.	92.9**
EIA-RR, ALT elev., anti-HBc +	100.0**

Remarks:
* predictive values for surrogate tests are much lower than those of the specific test (HCV EIA)
** when we put the surrogate tests and HCV EIA together, the predictive values become very high (63-100%).

Table 7
Age distribution (%) of donors group (by HCV test status)

Donor Group	n	Age Group		
		< 30	30-39	> 39
All donors	26,479	45.0	23.0	32.0
EIA-RR	190	24.7	33.9	41.4
RIBA-P	69	17.4	58.0	24.6
RIBA-I	29	17.2	27.6	55.2
RIBA-N	88	33.0	17.0	50.0

Table 8
Prevalence (%) of HCV test results (by sex)

	1st time donors	Repeat Donors	Total
I. EIA-RR Prevalence:			
Male	1.18	0.74	0.82
Female	0.60	0.53	0.55
Total	0.89	0.65	0.72
II. RIBA-Pos Prevalence:			
Male	0.57	0.28	0.33
Female	0.18	0.16	0.17
Total	0.37	0.23	0.26

Table 9
Possible new approaches to donor selection

Avoid (some) first-time donors
Disincentives
Pedigreed donor pool
Market research: neighbourhood selection
("Greenlining")
Epidemiologic characterization

places where we have a relatively high level of confidence that the infection prevalence will be low. In order to approach perfection, an unlikely goal, we would have all repeat donors, who are very unlikely to be carrying the variety of agents that we are concerned about.

In closing, I would like to say that we are very fortunate these days in the relationship that has developed, particularly in the last five or ten years, between people engaged in blood transfusion and people engaged in virology and epidemiology and places like our Centers for Disease Control and your Laboratory Centre for Disease Control. I think that is a very promising relationship from the standpoint of patients who are going to need blood transfusions and are going to depend not only on the quality of the testing that we do, but on all the other things we do to make sure we have safe blood from our donors.

DR. JAMES: Your concept of greenlining and marketing is germane to to some matters that we were discussing yesterday privately. But, how do you avoid the charge that this elicits, in effect, blood-banking, redlining the insurance practices of the early 1970s where you give higher rates to people based on their zip codes.

If you move to a system of marketing that was sensitive to sex and potentially other demographic factors like race that might indicate higher sero-prevalence rates in those populations, do blood collection agencies not run the risk treading some very dangerous lines in terms of selecting donor populations?

I am thinking also of Paul Cummings' paper last year in Najim, where black donors were shown to have higher anti-HIV reactivity rates. How do you avoid extending that logic to marketing particular racial groups, for example?

DR. BARKER: I want to say that I really enjoyed talking to Robert Chambers, because he has done a lot of work on the notions that were on that last slide that we have been talking about for a few years.

I don't have the answer to your question. I will make a couple of comments, though.

You know, I think it is very nice and appropriate and desirable and everything else to say that health is a right. I don't think we have to say that being a blood donor is a right, and I really think that people who are going to need to receive blood would probably prefer that we do.

Some of these broad brush labels are not all that applicable, really, and I think some of the papers that say we have a problem with first time donors and we've got a problem with blacks and so forth and so on, are somewhat misleading in that it is much more segmented than that.

There are plenty of people in the black population in the U.S. who would qualify unquestionably to be blood donors, just as there are males, as I mentioned, that would qualify unquestionably, if we were more selective, and did this greenlining or whatever you want to call it.

So, my feeling is that you've got to look at that from two directions, and I think if you look at it from the patient's direction, you do what you can do to keep out the high risk donors.

DR. ALTER: I just want to inject a little lighter note concerning your slide on what you would consider to be the ideal blood donor population and remind you of a study published from Colombia, South America, in which they were looking at the sexual transmission of hepatitis B. They compare prostitutes to nuns, making an assumption that they had control for important risk factors.

What they found was that the rate of HBV infection was similar in both groups, because the nuns were health care workers.

DR. MUSHAHWAR: I refer to your figures and association between repeat reactives and ALT; yesterday Dr. Bishai mentioned perhaps that would be a good idea, but I really knocked it down.

The predictive value of the positive repeat reactive plus ALT is 92.9 positive. So, Dr. Bishai, I think it's a good idea, because now we have data to say so.

DR. BARKER: Dr. Bishai has been right a lot of times in my experience.

DR. CHERNESKY: What is happening as far as drawing up national guidelines for advising physicians and patients who are picked up through the Red Cross screening program?

DR. BARKER: We have a lot of material that we give our blood centres. We put it together with the medical directors in our centres. In fact, before we introduce a test like this I can't tell you a lot of detail about what's in there. Of course, our first leading concern is to

tell people we will not be able to continue to collect blood from them, and we don't want them to come back to donate again.

Beyond that, we are not any smarter than anyone else as far as telling them, and we depend to a considerable degree on CDC. So, I am happy that Dr. Alter is here to talk about the other kinds of guidance that we would like to give to people and want to give to them, but you know, we have to go through a period with these tests where we have a lot of uncertainty about what it all means and what to tell them to do.

DR. ALTER: Public health service in the United States isn't smarter either, but unfortunately needs to publish guidelines or recommendations. An interagency group has gotten together and drafted recommendations for screening notification and counselling of donors.

We were supposed to have an outside consultants' meeting to comment on a draft of these recommendations next week, but because the Congress has decided not to pass a budget, the meeting has been cancelled.

So, I don't know how many months it will be before these recommendations come out. But basically, they do follow many of the FDA recommendations; that is, that any donor who tests repeatedly reactive on the EIA has to be indefinitely deferred from donation.

We are saying optimally before donor notification a supplemental test for specificity should be used so that you are a little more certain as to whether this donor is positive.

We are saying that all donors should be notified of these results, that all other results of any testing done be provided either to the donor or to their physician. Then, depending on a variety of situations that are too detailed to discuss right now, we are giving a different message, depending on how strongly we think the donor actually has hepatitis C.

DR. FEINMAN: I would like to make a comment. We have two problems here.

First is to protect the recipient, and I think this is the most important thing. Therefore, people who are anti-HCV positive are being eliminated from the donor pool.

The second thing is that we are very afraid that we are offending the public, or we are doing something wrong, by not giving a complete explanation.

Now, we can only give complete explanations when we know the explanations ourselves. I think in all the centres we have just organized a centre together with Dr. Hurst in Toronto that we are examining these people in depth and also their contacts. When we have 200 to 300 people examined in an objective way, and we know the answer ourselves, then we will tell the public what to do. I think we tell the public that we are in a transition period where we do not know what to do, and they have to wait until we have something definite.

Management of HCV Infections by Treatment

DR. E. SCHIFF (University of Miami, Miami)

I am going to focus on interferon treatment of chronic viral hepatitis, and I want to make five points.

One, I want to make the point that some patients with chronic viral hepatitis, in particular viral C hepatitis, can be successfully treated with interferon alpha 2B treatment.

Two, that interferon treatment is associated with side effects, and side effects are common. Fortunately, they are almost always reversible.

Three, and this is very important, that no one has shown that interferon treatment - or from that standpoint, no treatment - has prevented the progression of chronic active viral hepatitis to cirrhosis or has increased the survival rate. That depends on long-term follow up studies, and no one has the data that demonstrates that.

Four, I want to re-emphasize the importance of making an accurate diagnosis of chronic viral hepatitis, in this case chronic viral C hepatitis, before treating someone with interferon.

And, finally, although you are going to be bombarded with various algorithms from various companies marketing these products - with good intentions - any physician that treats patients knows that the final decision to treat has to be tailored to that patient. And, you weigh all these things in the algorithm, but you've got to tailor it to that patient.

Now, to orient the discussion, let's pick up where Gary Gitnick left off, with a patient I followed who illustrates a very common phenomenon. A 68-year-old woman who had diabetes, had undergone a coronary bypass operation about eight years previously. At that time, she had normal liver chemistries. She was subsequently noted, by her cardiologist, to have ALT elevation, on routine follow-up exams. In those days they called it transaminitis. Nobody got very excited about it.

She had right upper quadrant discomfort. I often call it liver ache. We see it in many patients with chronic liver disease, but in this case she was found to have cholelithiasis, and appropriately, she underwent a cholecystectomy. Well, to everybody's surprise, at the time of surgery, they found that she had cirrhosis of the liver. A liver biopsy showed that this was chronic active hepatitis, superimposed on the cirrhosis. They had problems postoperatively. She developed hepatic encephalopathy. Fortunately, she responded favourably to treatment, and went on another seven years and finally succumbed.

Now, the point here is that this is a very common type of patient. What you have is an older individual who underwent elective surgery and got blood, and the result is the insidious evolution of a serious liver disease - cirrhosis of the liver - without ever having an episode of icterus, manifested itself only by transaminase elevation.

She did not complain of fatigue and her right upper quadrant discomfort may well have been related to her gallbladder disease, because after surgery she never complained of it again. This is a common phenomenon.

Now, whether or not we treat this remains to be seen, but you would want to consider that if you intervened early in the course of this illness that you might have prevented the evolution of cirrhosis. We cannot document that right now.

When we see somebody with persistent transaminase elevation, before we begin to call it chronic, we want to see this elevated for at least six months. The anti-HCV test becomes positive as early as 12 to 15 weeks, but as we have seen, usually it's about six months before you can be sure if someone is negative that they don't have positive C antibody.

We have no predictive parameters of the severity of the disease or of chronicity - none of the symptoms. And, as has been pointed out over and over again and as illustrated by this case, these people are often asymptomatic.

Liver biopsy is necessary to assess the extent of liver disease. Once this product (interferon) is licensed, and it will be in the United States certainly by the first of the year, there will be a reluctance to biopsy patients. But certainly for the time being patients ought to be biopsied because the blood bank people, in particular, know that about 20 per cent of these patients that have ALT elevation that persist, don't have chronic hepatitis. They have fatty metamorphosis of the liver. It would be tragic to treat somebody for a fatty liver with interferon.

Now, if we look at someone who has a persistence of transaminases in excess of six months, for the purpose of this discussion, and we run through the differential diagnosis, it is going to come down to the following points. You are going to be able to eliminate these fairly easily.

Is this C? Is this somebody who has chronic viral hepatitis who is C negative? It may be highly suspect if it followed a transfusion.

Although most often when you see these patients, you don't see them within six months of the transfusion, you see them years later and there is a history of a transfusion. So is it another virus, or is this C? We don't have the proper way of detecting it right now.

Is this an autoimmune chronic hepatitis?

You are going to hear much more of that in the following talk, but this is very important, because when we talk about interferon, you are going to see that one of the properties of interferon is that it is an immuno-modulating type of drug.

If you have somebody who is either predisposed to autoimmune disease, or has a sub-clinical form, and you treat it with interferon, you are just going to turn on the whole disease, and the liver disease is going to get worse.

I find, when patients come to me with chronic non-A, non-B (NANB) hepatitis, that usually there is a history of some sort of parenteral route of transmission.

Now, Dr. Miriam Alter has said that most of the acute NANB is sporadic - I am being anecdotal here - but most of what I see that comes, particularly for

treatment, has a good history of parenteral transmission. These are people who would be categorized as NANB.

What you commonly see in private practice is an individual, often in his/her mid-forties, who may very well be successful, and who was picked up as a blood donor, or was picked up on an insurance exam. They are called sporadic, because they have no history of a risk factor. It isn't until they get into the room with you in discussion that you find out that they actually were parenteral drug abusers in their early twenties, and forgot about it, really kind of repressed it. You know, these are good citizens, and they want to give blood. They don't want to transmit hepatitis to anybody, but they don't even think of themselves as parenteral drug abusers, but they did play with drugs many years before. We have yet to determine how many of these patients there are, but I think the number is significant.

Now, when you see a patient with chronic hepatitis whose symptoms don't correlate with severity of disease, but symptoms are very important to the patient, and particularly if you are going to start a drug that's going to have side effects. If the patient has symptoms and your treatment can eventually get rid of the symptoms, that's going to be important to him. And, I think it's something that you still have to weigh when you decide whether or not you're going to treat the patient.

These are the common symptoms of chronic viral C hepatitis, when, indeed, people have symptoms. Clearly, the most common is fatigue.

The other thing that we've discussed is whether or not that patient is going to progress to cirrhosis of the liver, and if this is going to impact on his/her survival.

The preliminary data that are coming out of this trial of Leonard Cease would suggest that life, like survival, is no different in long-term follow-up. When you do long-term follow-up of people with established post-transfusion hepatitis versus those who don't, it is very preliminary. But, it's going to confirm some people's suspicions that many people have cirrhosis that is silent and doesn't impact on their survival.

You are going to biopsy the patient; what you see is inflammation confined to the portal triad. You don't see significant piecemeal necrosis. If you are doing blind liver biopsies, there is a sampling error of, perhaps, as high as 30 per cent. (We do a great deal of laparoscopy in our centre to avoid sampling error.) Even if the patient's condition is consistent with what we used to call chronic persistent hepatitis, there is a lot of controversy as to whether this may evolve into a more active type of disease and even go onto cirrhosis.

Now, if you have someone who is asymptomatic and had this type of histologic picture, should you treat with interferon? It's controversial. If you believe that this is going to go on to cirrhosis, and that interferon could prevent it, even if the person was asymptomatic, and unless there was a contraindication, you would want to treat him.

On the other hand, if the disease never impacts on their survival and they don't have symptoms, you are giving them a very expensive drug with significant side effects.

However, if they have bridging necrosis or they have early cirrhosis, you can be confident that this disease is likely to progress. And, certainly if they are symptomatic, I don't think anybody would argue with treating them with interferon.

What are the properties of interferon? In essence you can break them down into two categories.

One is an antiviral effect. This is particularly important in the treatment of chronic viral C hepatitis. Interferon will turn on this 2', 5' - oligoadenylate synthetase, which leads to increased ribonuclease activity, which is antiviral.

In C, and we haven't definitively characterized this, there seems to be much more cytopathic effect than in B. Whereas in the case of B, as we all know, the injury seems to come primarily from the host immune response to the virus replicating in the liver cells. But in C, as you will see when we look at patterns of ALTs following treatment with interferon, this seems to exert primarily an antiviral effect.

On the other hand, interferon, when it is used in B, has an antiviral effect, but the immuno-modulatory effect is very important, because interferon is capable of turning on T-helper cells, cytotoxic T cells, natural killer cells, the expression on the surface of the hepatocyte of histo-compatibility antigens, and what is thought to be core antigen, that then induces the immune response, which in turn, will destroy hepatocytes that have replicating virus.

As I will demonstrate to you, unlike C, when you treat someone with chronic B with interferon, you get a flare in the transaminases. You get an enhancement of the hepatitis prior to clearance of the virus, because you are turning on this system.

In C, we don't see clinical evidence of that.

I can give you as an example two types of cases, both of which are B, in which there was immuno-modulation and how interferon, in particular, will induce a flare before clearing hepatitis B; this does not happen with C. In C, we don't look for a flare, we look for normalization of aminotransferase.

Now, I am going to give you data which come from the multi-centre trial, which was published in the New England Journal of Medicine, coordinated by Gary Davis. We were participants in this trial of interferon in chronic NANB hepatitis, 86 per cent of which turned out to be C positive.

At the time, we did not have a marker, and what we did was to take people with post-transfusion hepatitis where transaminases persisted for a year - whether it was a biopsy that demonstrated chronic hepatitis, or more. There were some health care workers in the group, but no parenteral drug abusers.

There were 166 people entered. They were initially screened to demonstrate that they weren't spontaneously clearing the transaminase, and they were randomized to 3,000,000 units, three times a week for six months, or one million units - this is interferon alpha 2B, subcutaneously - three times a week for six months. Or, they were assigned to an untreated control group.

The controls did not get a placebo. They were just followed, and at six months, they were evaluated. They were rebiopsied. If they had responded, they were just followed over the next six months. If they relapsed during follow-up, they were re-treated. The non-responders were just followed.

The untreated controls were randomized either to three or one million units, three times a week, and that was added to this experience.

As I mentioned, the important parameter to follow is the ALT elevation. Now, we hope that we will have assays for HCV RNA in the future, and use it as we used HBV DNA to look at viral activity in a more sensitive way.

When they responded, the transaminases came down in a matter of weeks. I might say that a complete responder was normalization of transaminases. Partial responders were people who decreased their ALT by more than 50 per cent, and it fell below one and a half times the upper limit of normal.

Among the responders, 85 per cent were complete responders.

Now for the proportion who responded, clearly the 3,000,000-unit regimen was shown to be the superior one. It is superior to one million, but one million was significantly better than the controls. It was also noted that, if patients haven't responded by three months, they are unlikely to respond. This is very important in adjusting the dose. There are ongoing trials now looking at 3,000,000 versus 5,000,000 versus 10,000,000. The final results are not in, but it looks like 5,000,000 may offer some additional benefit, and, besides, people can't tolerate the 10,000,000 three times a week.

Unfortunately, in contrast to people with chronic viral B hepatitis who have responded to interferon therapy with the loss of DNA and e antigen (as has been shown by J. Hoffnagle of Chicago), they lose more surface antigen, rather than relapse, as is the case for HCV.

With HCV, there is a 50 per cent relapse rate. We need further data on what happens to those who relapse. In other words, was it just undetectable in the blood and it's still in the liver and it starts replicating again? We need those data, and I am sure they will be forthcoming soon.

But, regardless, 50 per cent will relapse over a six-month period. Fifty per cent do not relapse, and so, if you start from the beginning you get 25 per cent who seem to be home free six months after interferon is discontinued.

If you continue to follow those, although some will relapse, most don't. If they do relapse, and you re-treat them with the same dose of interferon, they respond again, and go back into remission. In our study, there were 12 patients that were re-treated, and 11 went back at the same dose to complete remission.

One of the problems that you are going to find is that when you are following people that you have treated with interferon and you see a rise in transaminases with cessation of therapy, don't be so quick to reinstitute interferon, because some of these people have what looks like a flare. Now, I am not talking about a flare prior to clearance. I am talking about a flare when the interferon was stopped. Those people will spontaneously come back down. Now, why that flare occurs is not clear, but that is not an example of somebody you want to treat with interferon. You just want to watch them.

If, however, they have a rise in transaminases which persists for longer than a month, then you want to consider re-treatment with interferon.

Some patients will rebound, rather than relapse, and there is concern about that. We didn't see that in our study, but it has been reported by others. That means that the transaminases actually go up higher than they were prior to treatment, and if there is underlying cirrhosis of the liver, or someone is close to decompensation, you are afraid you could actually do them harm.

Of our group of 166 patients, 55 per cent had cirrhosis of the liver. A minority had chronic persistent hepatitis - 18 per cent.

I would like to discuss side effects, which are common and they are dose related. Now, this information is not taken just from our study, it is taken from all studies with interferon.

A very common side effect is fatigue. You almost always will get a flu-like illness when you first start interferon, i.e. fever, chills, and myalgia. For that reason, we give them acetaminophen before the dose, after the dose, and usually at night. However, another common problem is irritability, and some of these people can't sleep at night; if you have someone like that, you give the interferon to them in the daytime, rather than at night.

I might say that when we look at late effects here, this is a very common problem. Now, I am being anecdotal, but in our centre where we treat these patients, the nurses can spot someone that's on interferon. They are very restless. They are jumpy, irritable. They can get very depressed and, therefore, someone who has had a history of a severe depression prior to treatment, should not be a candidate for interferon therapy.

The fatigue, malaise, asthenia are common. Alopecia occurs and, fortunately, is usually reversible.

When you follow these patients, it is very common to have granulocytopenia, thrombocytopenia, but neither of these usually gets you into trouble at all. We assign cut-offs to it, but it is uncommon to see someone get into real trouble, and the situation is reversible.

You are going to hear a discussion of auto-antibodies, particularly autoimmune phenomena with interferon. I don't think there is any question that what you don't want to do is to treat somebody who is a sporadic case. If they instead have underlying autoimmune disease, a worsening of the liver disease will occur.

If you look at some of the earlier literature, there was a report by Vento of two patients. One of the patients had clearly what was consistent with post-transfusion hepatitis - two months after transfusion there resulted a hepatitis that persisted; the biopsy was chronic active and the patient was negative for ANA parameters.

They treated the patient with interferon, and the patient decompensated, and then clearly autoimmune phenomena evolved.

So, that is going to happen. When you give interferon, it is common to get low titre ANA positivity. Myet showed that. But, in his case, that didn't really relate to significant underlying liver disease or other target organs. You just got a weakly positive ANA.

The main thing would be if you saw transaminases rising as you gave interferon, and particularly if you saw IgG going up, or ANA with it, you would want to stop therapy immediately.

Another problem is autoimmune thyroiditis. We think that these people often have underlying autoimmune thyroid disease that is sub-clinical and the interferon can bring it out. This happened in our study with one patient who got very hypothyroid.

Tests for antibodies to interferon will be presented in marketing wars, but I think the bottom line is that they don't impact significantly on the clinical response.

There is one paper by Anna Locke which suggested that that might happen. But, I think, in general the development of antibodies to interferon *per se* has not been a problem.

To conclude, I am going to review the points that we made in our study.

First, interferon is effective in the treatment of chronic NANB hepatitis. I might point out that 14 per cent of these patients were anti-HCV negative by ELISA test, yet they responded just as well as the ones that are C positive. Do they really have C? Is it another virus?

Patients responding to treatment with interferon demonstrated reduction in hepatic inflammation, histologically, as well as biochemically.

I did not mention the histology, and I should. Patients were biopsied initially, obviously, and at six months. What was demonstrated was a significant reduction in peri-portal inflammation and lobular inflammation. No significant difference in fibrosis.

Now, remember, this biopsy was done six months after therapy. If you, particularly the gastroenterologists and hepatologists, go back to the experience with autoimmune hepatitis when corticosteriods were used, there is no question that corticosteriods get rid of symptoms. Corticosteriods will normalize the transaminases, but the last thing to occur is histologic remission. And, if you biopsy somebody at six months, you are still going to see activity. So, I think what is important is what happens over the long term, not just after six months.

But, there was a significant difference in the inflammatory changes.

Responding patients may relapse following withdrawal from interferon therapy; this is a major shortcoming.

One of the things we are looking at with the higher-dose interferon regimen is, will we keep people in remission longer, when interferon is stopped? We can't answer that question right now.

Re-treatment results in a response similar in kind and degree to the initial response. When they relapse, if it's a true relapse, they are very likely to go into remission with re-treatment.

Now, one of the unanswered questions is, when you are following these patients, and you re-treat them and they go back into remission, and they relapse again, should you establish some sort of maintenance regimen? We don't have data for that yet. In general, what a rational approach would be is, if it takes them a long time to relapse, then wait for the relapse. But, if they are relapsing very shortly after cessation of therapy, you might want to try a lower dose maintenance regimen. But, we don't have the data to justify that statement. I am just telling you what you might do with what data are available right now.

I think it is clear that interferon is safe, but there are a lot of side effects associated with it. It is well tolerated from the standpoint that we had only one person who could not go back to work. Everybody continued in their walk of life, although they had side effects. But, there are side effects and you have to respect that.

Also, this is not inexpensive treatment. It is estimated that interferon will cost about $7.00 per million units. That adds up if someone is going to get 3,000,000 units three times a week for six months, over $1,000. How the third party payers are going to respond to this isn't quite clear. It is hoped that they'll help out in recovering some of these costs.

Conclusion

I wanted to make the point that interferon will help some people with chronic viral C hepatitis. This is not penicillin for pneumococcal pneumonia, but it clearly is a breakthrough, because up until interferon we had nothing for these patients and now we have something.

I think what we need desperately is long-term follow-up studies to see if, indeed, interferon can prevent the progression to cirrhosis of the liver and above all can increase the survival rate. Right now, we don't know, but we hope it will do so.

SPEAKER: Do we know whether non-responders have a higher antibody titre to interferon than responders?

DR. SCHIFF: Well, there was one study by Anna Locke, where she compared interferon alpha 2B with interferon alpha 2A. With the latter, there were more antibodies against the interferon. In her study, there was a difference in patient response.

The ones with the other preparation that didn't get as much antibody did better, but that's the only study I have seen like that.

DR. GITNICK: I don't want you to interpret these questions as suggesting that I don't favour the use of interferon, because as you well know, I am an advocate of the use of interferon as a treatment.

The first question is, does interferon reduce or cause the disappearance of necrosis on liver biopsy?

DR. SCHIFF: Peri-portal inflammation which you are going to see with piecemeal necrosis is less, but in terms of fibrosis, no.

DR. GITNICK: What about liver cell destruction, necrosis?

DR. SCHIFF: I can't answer that, because if we look at lobular inflammation and we look at peri-portal inflammation, it is gone. As far as necrosis, it wasn't statistically significantly demonstrated, when I looked for it.

DR. GITNICK: The next question is, in trying to decide which of my patients to treat, it would be helpful to know, for example, what per cent of patients who are asymptomatic and whose liver biopsies show only an inflammatory infiltrate in the portal tracks, that is chronic persistent hepatitis, what per cent of those patients have been shown to have progression of their chronic persistent to the picture of chronic active, i.e. those who have cell destruction on liver biopsy.

DR. SCHIFF: I don't think anybody can answer that question.

This is one of the controversies. In other words, as you know, no one would argue with starting interferon in someone who is symptomatic, who has chronic active hepatitis or more.

If someone has symptoms and has chronic persistent hepatitis, perhaps some people would treat because the patient is a little more motivated. But, if the patient is asymptomatic, with chronic persistent hepatitis, there are some people I would treat, others I would not.

Myself, as you know, I would follow them. But if, indeed, chronic persistent can progress to cirrhosis, as many people are saying, then what you want to know in this asymptomatic person is: "Will interferon stop the progression?" And, if it does and if it impacted on survival or on morbidity or on complications, you would want to treat them.

We don't know the whole answer.

DR. GITNICK: You just said that some people would say that chronic

persistent will progress to cirrhosis, but if you read all of the world's literature and you look for proof, how many cases are there in which clear-cut chronic persistent hepatitis led to cirrhosis?

DR. SCHIFF: You said clear-cut chronic persistent. What we have found –

DR. GITNICK: I am talking about the asymptomatic patient –

DR. SCHIFF: I know. But, what we have not found on blind biopsy, are people who had chronic persistent, who later had cirrhosis. Now, was that a sampling error?

In our own experience, where we do laparoscopy extensively, we have not had a case like that.

DR. GITNICK: Nor in ours. I don't deny that 20 per cent of people with chronic active will spontaneously revert to a picture of chronic persistent, but if you look at a hundred chronic persistents, my question is: "How many will actually go on to cirrhosis?"

So, should I take away from this discussion the concept that if you were deciding whom to treat, you would not - at least this year - treat the asymptomatic clear-cut chronic persistent?

DR. SCHIFF: No, I wouldn't. But, some would. I would not.

D. V. FEINMAN: The answer is that in hepatitis NANB, you do not have another marker, only ALT. In hepatitis B, even if it is chronic persistent and the patient has HBV DNA and e antigen, you treat. So, we have to wait for better characterization of this disease to answer this question.

About your other question on chronic persistent: in 1975 together with Dr. Barrett, we coined a name, chronic benign persistent hepatitis, and I felt very sorry about it, because many years later we saw patients, called chronic benign persistent, who had liver cirrhosis.

Autoantibodies and Peak Transaminase During HCV Treatment with Interferon

DR. B. WILLEMS (Saint-Luc Hopital, Montreal)

Interferon has been shown to be a promising therapeutic approach to the treatment of chronic hepatitis C[1,2]. Recently, the development of autoantibodies has been reported to have occurred during the treatment of hepatitis B with interferon[3].

We report here the development of autoantibodies during the treatment of hepatitis C with interferon alpha 2B. The patients were enrolled in a multi-centre Canadian study. Other causes of liver diseases were systematically excluded, and no patient had autoantibody before treatment.

Of these 18 patients, five developed autoantibodies and high transaminase levels during, or immediately after, interferon treatment (see table below).

Discussion

Mayet, et al.[3] have reported that the treatment of chronic hepatitis B with interferon induces autoantibodies "not specific for autoimmune hepatitis". They did not observe anti-LKM or anti-mitochondrial antibodies. Twenty-seven out of 31 patients (87 per cent) developed autoantibodies; SMA and ANA were the most frequently seen. Some appeared during interferon treatment, like SMA; others, after treatment, like antithyroglobulin or antimicromial antibodies.

McFarlane, et al.[4] have recently reported false positive tests for anti-HCV in autoimmune chronic active hepatitis. Of 50 patients, 21 were positive for anti-HCV. However, when the hepatitis was inactive or treated with immunosuppression, only one of 22 patients was positive. The optical density of the anti-HCV assay was correlated with the level of gammaglobulin.

Vento, et al.[5] have reported two patients with chronic non-A, non-B (NANB) hepatitis who developed hepatic failure four and six weeks after interferon treatment. These patients had increasing levels of gammaglobulin during interferon treatment and responded to prednisone. This is in contrast to our patients who did not develop high gammaglobulin levels or hepatic failure, and did not have to receive corticotherapy.

A striking association of type 2 autoimmune hepatitis (anti-LKM positive) with anti-HCV seropositivity, found in 86.1 per cent of such patients, was reported by Lenzy, et al.[6]. This association should be confirmed by PCR technique[7] before incriminating HCV in the genesis of this disease.

Conclusion

Autoantibodies can occur during the treatment of chronic hepatitis C with interferon. Anti-LKM, AMA, ANA, and anti-microsomal antibodies have been observed in our patients, in association with elevation of transaminase level. One can speculate if the phenomenon is an autoimmune hepatitis induced by interferon.

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Patients	Liver Hist.	Anti-HCV Ortho/Abb	Chiron RIBA	Autoantibody	Peak ALT/Week (UI)
1	CAH, cirr.	+/+	+	ANA 1/20 anti-LKM 1/160	814/12
2	CAH, cirr	+/+	+	anti-LKM 1/640	310/12
3	CAH	+/+	+	AMA 1/160	280/20
4	CAH, cirr	+/+	C100+ 5-1-1 -	ANA 1/160	232/26
5	CAH	+/+	+	anti-microsomial 1/1600	280/28

CAH = chronic active hepatitis
cirr = cirrhosis
Risk factors for hepatitis C: IV drug -1,2,5; needle accident - 3; transfusion - 5. Patients received 3 MU of interferon alpha 2B twice a week for 24 weeks.

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SESSION V

CONCLUDING SUMMARIES

Chairpersons:
DR. F.R. BISHAI (Ontario Ministry of Health, Toronto)
DR. S. LEE (Victoria General Hospital, Dalhousie University, Halifax)

Epidemiologic Features of HCV

DR. L. SPENCE (Chairman, Session I)

The first speaker on the epidemiology symposium was Dr. Krugman, who provided an interesting account of the history of the disease, viral hepatitis, beginning in Greece in the fifth century B.C., and continuing up to the present time.

He described outbreaks associated with military operations and in medical clinics. Two distinct types, designated A or MS1, and B or MS2, are recognized by studies in human volunteers.

Differences in incubation periods and roots of transmission were described.

Important discoveries in the 1960s and 1970s include the discovery of Australia antigen by Blumberg and its association with hepatitis B, new epidemiological knowledge of hepatitis B; the discovery of the virion of hepatitis B by Dane, et al.; hepatitis B vaccine development and the use of the HBIG in prevention; the demonstration of hepatitis A in stool by Feinstone et al; the development of the IGM test for this virus; and the discovery of hepatitis D by Rizetto in Italy.

In the 1980s, the enterically transmitted non-A, non-B (NANB) agent, designated hepatitis C, was discovered. New vaccines for hepatitis B were prepared, and tests for hepatitis C were developed by the use of molecular technology. Studies of the natural history of hepatitis C were initiated.

Our second speaker was Dr. Mosley, who discussed post-transfusion hepatitis. He used the modal incubation period of cases studied by Allen and Sayman and himself to demonstrate that many hepatitis cases were probably not due to hepatitis A or B viruses.

He referred to the screening of blood for transfusion of hepatitis B and the optimism which existed that the screening would solve the problem of post-transfusion hepatitis. This proved not to be the case, and by 1973, cases were still occurring, of which only a small number were shown to be hepatitis B.

This generated arguments over the sensitivity of the test for hepatitis B surface antigen in identifying infected donors and diagnosing B cases.

In the middle to late 1970s, studies of post-transfusion hepatitis were initiated by the Transfusion Transmitted Viruses Study Group. These studies were designed to determine the incidence of post-transfusion hepatitis, clinical or sub-clinical, after blood was screened for hepatitis B; to apply serological techniques to the study of hepatitis B negative cases and to store serum samples for later evaluation, when new tests would become available.

Strict criteria were used to select all cases, including mild cases. In studies of more than 3,000 cases of post-transfusion hepatitis between 1974 and 1979, 10.2 per cent of cases of NANB were found in recipients of blood transfusions, and 2.9 per cent of cases in controls.

The rates for hepatitis B were 1.0 per cent and 0.1 per cent, respectively. The majority of the NANB cases were asymptomatic.

A panel of sera from post-transfusion hepatitis patients was prepared and used for the evaluation of new tests for NANB hepatitis. When this panel was tested for hepatitis C, an etiological basis for approximately half of the cases of transfusion associated hepatitis was demonstrated.

The range of the incubation period for hepatitis C was found to be 19 to 91 days, with a peak of 42 days. In other studies, the estimated incidence of hepatitis C was 3.3 per cent in blood recipients and 0.1 per cent in controls.

He then discussed surrogate testing of blood donors. Dr. Mosley's conclusion was that the surrogate ALT testing program in hepatitis C cases occurring in the U.S.A. did accomplish something.

The level of ALT could be used to predict chronicity in post-transfusion hepatitis due to hepatitis C. In hepatitis C cases, ALT was higher, and persistence of chronicity was longer than in hepatitis C negative cases, but there was no difference in the incubation period.

Hepatitis cases that were hepatitis C negative were generally very mild and not alarming, but had a potential for being a problem. Almost all hospital cases of hepatitis were associated with transfusions, and the severity of these cases and the propensity for chronicity were significant. Cases occurred in three to five per cent of recipients.

Dr. Mosley thought that the present tests should exclude most of the infected donors since the occurrence of hepatitis C in a blood recipient was overwhelmingly associated with donor HCV positivity.

Our next speaker was Dr. Alter. She said that NANB hepatitis is usually considered a transfusion disease, but other risk factors existed, including the fact that there is no known source.

The disease was poorly reported in the United States, and she had set up studies at sentinel control centres which indicated an overall incidence of seven cases per hundred thousand population. It accounted for 25 per cent of acute viral hepatitis.

The majority of cases were in the 15 to 39 age group. There was a high incidence in hispanics. Blood transfusion, drug abuse, and multiple sexual partners were important, but for 40 per cent there were no risk factors. The disease was low in health care workers. After 1985, multiple sex partners became a more important factor than blood transfusion.

The infection progresses to chronic disease in more than 50 per cent of cases.

Anti-HCV positive cases were more likely to have persistent ALT abnormal levels than anti-HCV negative individuals.

The liver disease may be chronic persistent or chronic active hepatitis.

Transfusion-acquired cases may be more severe.

Some cases may lose antibody in the first 12 months of illness, and she

felt that the question of persistence of antibody had to be resolved.

Seroconversions may occur 15 days after onset of symptoms, but the time may be much longer.

In some patients there were antibody fluctuations.

Among blood donors 0.6 per cent were positive for hepatitis C.

Intravenous drug users were 40-60 per cent positive.

In cases following transfusion, 20 per cent were from negative donors.

There were high antibody levels in haemophilia patients.

Using supplementary tests, she was able to confirm rates at 90 per cent in chronic liver disease, 80 per cent in IV drug users and 84 per cent in transfusion recipients.

Evidence was provided for sexual transmission by elimination of other factors, but homosexual activity was not a factor.

Hospitalization and international travel were not risk factors, nor was the consumption of shellfish.

Cases in which heterosexual activity had occurred with more than two partners showed a rate of 16 per cent for HCV; zero to two partners, the rates were 3 per cent.

Heterosexuals with STDs had a rate of five per cent, and blood donors had rates of about 0.5 per cent.

For homosexuals, the rates varied from three to 15 per cent in various studies.

Sexual partners of a drug user had antibody rates of about 20 per cent.

There were 170,000 cases annually in the United States; 85,000 chronic infections each year; 17,000 cases going on to chronic active hepatitis or cirrhosis.

Dr. Alter also answered a number of questions at the end of her talk:

Are all patients infectious? What are the long-term consequences of HCV infection? What is the role of preventive and therapeutic measures?

She said that the screening of blood would have a small impact on total disease because transfusions account for only a small proportion of cases.

Dr. Alter felt that the role of sexual transmission must be defined, and that perinatal transmission was unclear and that such transmission only occurred in HIV positive women.

The next speaker was Dr. Peter Gill, who described blood donor testing for hepatitis C virus in the Canadian Red Cross. All the donors were volunteers. The Red Cross had a monopoly, and he said that no surrogate tests were done in Canada.

Donors had to answer a questionnaire about jaundice or contact with jaundice, and there was a more detailed questionnaire to exclude AIDS.

Screening was done using the Ortho HCV antibody capture ELISA system. There were concerns about sensitivity and specificity, since the antibody response measure was not to the classical components of the virus.

Dr. Gill presented accumulated data up to August 23rd. The Red Cross had tested 353,228 specimens. Initial reactors were 1.04 per cent; repeat reactors were 0.52 per cent.

Rates were a bit lower in the Maritimes - about 0.31 to 0.33 per cent. Vancouver had 0.6 and Toronto, 0.64 per cent.

The Chiron RIBA HCV test system was the confirmatory test used, and in post-transfusion hepatitis, 86 per cent of cases were confirmed by RIBA. In implicated donors, RIBA confirmed 93 per cent, and in 2,974 EIA repeat reactive donors, 27.6 were positive by RIBA, 54 per cent were negative and others were indeterminate.

From the RIBA positives, it was shown that male donors were more likely to be positive. Results for males and females under age 40 were very similar.

The last paper was by Dr. Bryce Larke, who reported on the testing of blood by the Red Cross in the Province of Alberta.

The Ortho test was used for screening. He tested 37,946. In the initial reactives, there were 317 positives and in the repeat reactive the rate was 0.66 per cent. These figures agreed with the national average.

The rates in first-time donors was 0.71 per cent; in repeat donors, it was 0.65 per cent. Females made up 42 per cent and males 58 per cent of the donors. The rates in males were 0.87 per cent and in females, 0.37 per cent.

Urban rates were 0.6 per cent; rural rates 0.46 per cent, the difference there not being significant.

There was no difference between whole blood and apheresis specimens, as both had a rate of 0.69 per cent.

On occasion, the same individual gave different results, and this worried Dr. Larke. He sometimes got a patient that was positive and then negative.

The highest rates were in males aged 30 to 39.

He also did a seroprevalence study of anti-HCV among pregnant women in Northern Alberta in 1990, August and September. He used the Abbott EIA test, and he found anti-HCV reactors. There were six in 1191, or 0.5 per cent, and the exact same number of positives for hepatitis B surface antigens: six in 1191, or 0.5 per cent, but the positive reactions were mutually exclusive.

In the discussion which followed the papers, one of the points raised was the interaction between HIV and HCV - "Would there be any problem if you were testing for HCV in a patient who had AIDS?" The answer given was that the only problem would be in the final stages of the disease when the patient began to show substantial immunosuppression.

But, Dr. Mosley said that there were no problems in testing hemophiliac patients who were positive for both infections.

The role of IGM was discussed. Some people, I think, had reported the presence of IGM. Surrogate testing is still being done in the U.S.A., and has been useful. This is not the case in Canada, however.

There were complaints about the sensitivity of current HCV-EIA tests, and we were assured that this would improve later. One should not be too worried about it; use the tests that were available.

There was a question of whether donors should be told the results. There was some discussion of that, and it was felt that before one went to the trouble of changing a person's lifestyle as regards to sexual practices, one should at least do supplemental testing and confirm that it wasn't a false positive, of which there are many.

In any case, it was felt that persons who have many partners should practice "safe sex".

Dr. Mosley did not agree with Dr. Alter that transmission occurs through sexual contact. He gave examples of contacts of hemophiliacs, who

presumably were positive, had low rates, one to two per cent positive. He contrasted this with hepatitis B, known to be sexually transmitted, where you have household contact positive rates of 10 per cent rate and sexual contact rates of 20 to 30 per cent.

These presentations provided a considerable body of knowledge of the epidemiology of hepatitis C. Much remains to be learned, however, about the methods of transmission and the mechanisms for virus survival. Such knowledge will provide the basis for prevention and control.

Immunodiagnosis

DR. R. PURCELL (Chairman, Session II)

I want to pick up on some of the challenges and opportunities that have been highlighted by these speakers, and expand on those.

At present there are only two ways of detecting the presence of HCV. One is direct, by measuring the viral RNA by polymerase chain reaction (PCR); and the other is indirect by measuring antibody to the virus. (A third, very expensive method is experimental transmission to a chimpanzee.) It is therefore unlikely, but not impossible, that we will have a direct test for antigen.

Nevertheless, at present we are limited to serologic tests for antibody. And, since most laboratories will be dealing with serologic tests rather than by testing by PCR, important questions to be answered include the size of the window of undetectability, and whether we will be able to close that window by serologic tests. The answer to the latter is, not entirely.

In a study carried out by Shimizu, et al.[1] in an attempt to see how early in the course of infection HCV RNA first appears *de novo*, chimpanzees were inoculated with three million doses of the Hutchinson strain of HCV and tested daily during the first ten days of the experiment and weekly thereafter. HCV RNA was not detected by PCR following inoculation, but first became apparent on the third day following inoculation in one chimpanzee, and on the fourth day in the other. Other indirect markers of HCV infection, the cytoplasmic tubular structures[2] and the cytoplasmic antigen[3] that Shimizu described previously, appeared approximately six and three days respectively, after the appearance of HCV RNA. Despite the fact that the virus first appeared *de novo* very early in the infection, it wasn't until the usual incubation period of about seven or eight weeks that hepatitis occurred, and it wasn't until about 15 weeks that antibody to HCV was detected with the Ortho ELISA test. Thus, I think it's safe to say that currently available serologic tests will never be able to detect the pre-acute or even the acute phase of HCV replication.

Are these observations limited to chimpanzees? No, they are not. In a subsequent set of experiments by Farci, et al.[4], patients who developed post-transfusion NANB hepatitis and chimpanzees that were inoculated with clinical materials from the patients, were studied for antibody to HCV with the Ortho ELISA test for anti-C100 and for HCV RNA by modification of the PCR technique that utilizes two pairs of "nested" primers. This technique was shown to be able to detect <0.1 infectious dose of the Hutchinson strain of HCV and thus, was as sensitive as the single PCR/hybridization technique of Weiner, et al.[5]. Only two of the five patients studied were PCR-positive when primers drawn from the NS3 region of the viral genome[5], were used. However, when primers from the NS4 region of the HCV genome were used, the other three patients were positive by PCR, as were the chimpanzees that were infected with HCV derived from them. Thus, we demonstrated genomic heterogeneity of HCV strains in the United States.

Like the chimpanzees studied previously, the patients became PCR positive for HCV RNA soon after transfusion, usually by the time the first sample was collected one week after transfusion. Most of those studied became chronic carriers of HCV and were still PCR positive as long as 14 years after transfusion. However, one of the five patients did have an acute, self-limiting infection, and became PCR negative after several weeks. All of the patients developed antibody by ELISA assay. One patient with acute hepatitis and one patient with chronic hepatitis subsequently became antibody negative and the other three patients, with chronic hepatitis, were still positive 10 to 14 years after transfusion. Similar results were obtained in the experimentally infected chimpanzees. In each instance the patients and the chimpanzees were positive for HCV RNA in the serum for many weeks before antibody was detected. Thus, there was a very large window of potential infectivity that was not detectable by serologic means.

In other studies (Farci, et al., unpublished data), we failed to detect antibody to C100 in some patients and chimpanzees with NANB hepatitis. When expressed proteins representing epitopes from other regions of the HCV genome (Abbott Laboratories, N. Chicago, Ill.) were used to test for antibody in these patients and chimpanzees, many were positive. Thus, antibody tests incorporating additional HCV epitopes can be expected to improve the screening and diagnostic efficiency of HCV detection.

Heterogeneity of HCV strains may complicate the development of broadly reactive diagnostic tests as well as vaccines. Comparisons of Japanese with U.S. strains has revealed a degree of heterogeneity comparable to that seen between different serotypes of poliovirus, suggesting that different serotypes of HCV might also exist[6].

Finally, non-specificity problems inherent in currently licensed tests make the use of these tests for serodiagnostic purposes unreliable at this time. This is particularly true when sera that have been stored for long periods of time are tested or when the sera have come from developing countries[7]. The use of currently available tests is not recommended for testing such sera.

In summary, great progress has been made in our understanding of HCV. However, fuller understanding will require the use of both serologic techniques and molecular techniques in order to have a clear picture of HCV infection.

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Clinical Features of HCV Infection

DR. S. C. PAPPAS (Chairman, Session III)

One only needs to reconsider the data that have been presented over the past day and a half to realize that we are really in an interim period of diagnostic decision-making at the clinical level. There are no good sensitivity and specificity data, but more important, there are no reasonable positive-predictive and negative-predictive value data because investigators are looking at populations where the prevalence of disease is varied, e.g. the data on the RIBA-positive confirmatory test in chronic hepatitis patients versus hospital admission patients, versus patients identified as asymptomatic blood donors.

I think one of the major messages to come from the session on clinical features, in combination with the other sessions, is that we really do need much more data on predictive values, and more important, the analyses of some of the diagnostic tests results in terms of likelihood ratios, comparing pre- and post-test probability analyses.

Dr. Feinman nicely reviewed some of the clinical material and presented some interesting information suggesting that the presence of anti-5-1-1 polypeptide antibody is correlated with infectivity.

He alluded to the co-existence of hepatitis B and hepatitis C infection in patients and remarked that the data appears to suggest that hepatitis C virus infection does not adversely influence hepatitis B virus infection.I think we can be fairly confident that hepatitis C virus does not play a major role in the etiology or pathophysiology of primary biliary cirrhosis.

Its role in alcoholic liver disease still remains unclear, although I think it is not going to be an important issue. Similarly, the precise role of HCV infection in autoimmune chronic active hepatitis remains unclear, although again I think the cumulative data suggest that this is not going to be an important consideration from the etiological point of view.

The importance of distinguishing autoimmune chronic active hepatitis from hepatitis C virus infection, however, was underscored this morning, by both Dr. Feinman and Dr. Schiff, because of the therapeutic implications of interferon therapy.

We do know that HCV antibody positivity does appear to be important in patients with cryptogenic cirrhosis, but precisely what this means is really not clear at this time. Again, while the data regarding hepatocellular carcinoma in hepatitis C virus are tantalizing, as Dr. Miriam Alter pointed out this morning, a precise tight link similar to that for hepatitis B is not available at the present.

One has to be careful because much of the data depends very much on the patient population studied.

Dr. Gitnick gave us a very nice overview of the clinical features of hepatitis C virus infection. He mentioned the fact that this disease seems to be an indolent disease, and alluded to the propensity for the development of chronic liver disease - something that was recognized, of course, long before the development of any type of serological testing.

The non-specificity of the clinical aspects of HCV infection comes through clearly when one looked at the various slides outlining the clinical features.

It is interesting that the severity of the initial disease does not appear to predict the development of chronicity, something that may be slightly different in contrast to the case for hepatitis B virus infection.

The precise incidence of chronic viral hepatitis following sporadic non-A, non-B hepatitis still remains unclear; however, it does not appear to be as high as the situation following post-transfusion hepatitis.

While all this clinical information is clearly important, there are many unanswered questions. Predominant among these is, what do we do with the blood donor who is found to be HCV positive? What do we do with the HCV-positive asymptomatic patient, who may or may not have liver disease?

I believe for the time being we must continue to rely on good clinical judgment and common sense, two things that historically usually have not led us astray in the practice of medicine.

It would be premature to alter radically our approach to those patients whom we have diagnosed as chronic non-A, non-B viral hepatitis at this time.

Many of you in the audience come from public health or blood banking backgrounds, and may have come to this conference hoping to come away with some type of policy recommendations. Unfortunately, because we are in an interim period, we are not going to be able to formulate many medical plans or policies at this time.

Prevention and Control

DR. M. CHERNESKY (Chairman, Session IV)

I thought what I would do is try to pick out some of the issues which were raised during this morning's session on prevention, treatment and control.

Although our speakers tried to be as complete as possible, in the section on prevention, treatment and control, there were a few areas that were left out.

To begin with, the screening of blood donors was covered and Dr. Barker very neatly came up with one important recommendation, which was to concentrate on looking carefully at first time male donors.

In addition, Dr. Alter mentioned that recommendations would be forthcoming from CDC about dealing with HCV serological positives and their contacts.

The issue of testing transplant donors did not get addressed at this meeting. The question was raised yesterday, and there was some discussion, but I think that no recommendations have been made.

Most of the session was spent on immuno or chemotherapy, and mainly the interferon studies.

The fourth area, that of immunization, was not mentioned and perhaps there are people in the audience who know of vaccine studies that are already underway.

As far as the interferon studies are concerned, Dr. Schiff made several very important points. In particular, he said that we need a correct diagnosis if people are going to be enrolled in interferon treatment studies, which might, perhaps, include the need for a biopsy.

And Dr. Alter also brought up the point later that there should really be a clear measurement of benefit to the patient in these studies, and perhaps that should be done by biopsy.

Dr. Schiff also mentioned that we need more markers to monitor during interferon treatment of HCV, and he suggested perhaps HCV RNA as a potential marker.

The issue of the role of prednisone was alluded to, and there were some data shown this morning that showed there may be some benefits in combination with interferon treatment. But, I think these are still issues which need to be addressed.

Dr. Schiff mentioned that in the two interferon studies that were published in the New England Journal of Medicine in 1989, there were a striking number of patients who benefited then relapsed at six months. Evidently, this is still a burning issue which needs to be addressed.

He mentioned that he is finding if patients don't respond within three months at a certain unit level of interferon, that perhaps increasing the level of interferon will sometimes do the trick. But, he also mentioned that many don't respond at all.

Side effects are inevitable when you are using interferon, and symptoms like fatigue and flu-like illness and irritability are problems.

He talked about reversible hematological problems, such as thrombocytopenia and, of course, Dr. Willems clearly showed some of the problems that have been arising in the Canadian studies as far as the production of interferon antibodies and auto-immune problems, which had previously been described in hepatitis B treatment using interferon.

And last, but not least, Dr. Schiff discussed the cost factor involved - several thousands of dollars in each case - when you use this drug. He concluded by saying that all these issues really need to be addressed at some point in time, which will probably be over the next few years.

DISCUSSION

SPEAKER: This question is addressed to Dr. Alter. Some 30 years ago there was an outbreak in an airforce base, reported by Heles, of hepatitis sub-human, primate associated. Until this minute, I am totally unable to pin down the mode of transmission simply because about 50 per cent of the handlers of these animals contracted hepatic disease.

Those who handled established animals that were kept on the base did not contract it. Now whether this was hepatitis A or hepatitis B or non-A, non-B, or transmitted by animal to human contact or aerosolization or parenteral, I can't decide.

In view of what you have described, is there something that shows anything related to that, especially for people who handle primates in experimental surgery and other interested institutions?

DR. ALTER: To tell you the truth, I really can't answer the question. I know what report you're talking about, but I don't remember the details of that outbreak. I am aware of hepatitis A being transmitted between primate, non-human primate and animal handlers.

In our case-control studies, although we have asked that question, we haven't gotten any association. That doesn't mean it doesn't happen.

So, I have no idea. I would be willing to state it is an aerosolization, that is in contact with blood or feces, depending on which virus we're dealing with.

DR. PURCELL: Almost all of the chimpanzees in captivity that were captured in the wild and were brought in, when the tests became available, were found to have antibody to hepatitis A. About 60 per cent also had antibody to hepatitis B, but these outbreaks occurred shortly after animals came into the facility, and in most of those cases the sera were no longer available.

But, in one outbreak that occurred among handlers of woolly monkeys that had been recently brought into the country, we did have the opportunity to do the serologic testing, and it was clearly A.

This is probably not surprising, since chimpanzees are sort of like non-toilet trained babies. This was probably fecal spread to the handlers who at that time never used precautions, and that I think almost surely was all A.

Much of the B, I think, got into chimpanzee colonies because they were used for generating reagents to blood proteins and such. They were often inoculated with human blood materials.

Then, they also spread it within the colonies among themselves, probably as Miriam said, by occult spread of blood, or sexual spread among the animals.

But, the epidemics, I think, were all A.

SPEAKER: Recently, I met with Dr. Margolis from CDC, and he told me of a possible association, not yet statistically proven, of what is called chronic fatigue syndrome and hepatitis C.

Could you tell me what Hal is talking about?

DR. ALTER: I don't what he's talking about. Now, it seems to me there was a report on the possibility of such an association, but as far as I know there is no basis for it.

DR. SPENCE: I was wondering if anyone can tell us anything about other possible candidate NANB agents and whether hepatitis E plays any role in NANB hepatitis in North America.

DR. ALTER: I'll answer the latter question.

We have not found any evidence of endemic hepatitis E in the United States. We have also not found any hepatitis E in Americans returning from countries where hepatitis E is endemic, who get acute hepatitis within, we'll say, seven weeks or so after returning from their travels.

So, we have yet to find it, other than in a couple of imported cases from individuals who are actually from the endemic countries.

DR. LEE: Maybe before Dr. Purcell responds to that, I'll ask Dr. Alter what test system is presently available for hepatitis E.

DR. ALTER: There are only research-based tests currently available. There is IEM, but again, that's research based and not something that is really available commercially.

DR. PURCELL: And Chris Chrishensky has an immunofluorescence test, but that's really limited to a couple of laboratories, another research base.

The question of another agent is an interesting one. Of course, your own Dr. Phillips here has reported on the giant cell hepatitis that he has studied, and that could conceivably be a sixth hepatitis virus, although it doesn't appear to be a major cause of chronic hepatitis.

Adrienne Debucelli had several patients who were in her interferon treatment study who, when they were tested serologically, were found to be anti-HCV negative, with the anti-C100 test, and to be PCR negative, yet they responded to interferon as though they had virus infection.

But, I understand that more recently Steve Feinstone, using a wide array of different primer pairs has found those to be positive and Chris Chrishensky I think has found liver biopsies from the two patients also to be positive with an antigen that he has detected in HCV-infected individuals.

So, I think it is premature, until we can sort out the heterogeneity of HCV, to say that there is another agent.

DR. LEE: I have a related question to Dr. Schiff. In this morning's talk on interferon therapy, regarding the non-responders: "What is the proportion of interferon non-responders?"

And the second related question is: "Does the non-responder relate to the level of ALT?"

DR. SCHIFF: Fifty per cent were non-responders. There were no parameters that could predict whether or not someone was going to respond.

That is in contrast to B, wherein B women are more likely to respond than men. Re the duration of illness, if it is shorter, you are more likely to respond; height of the HBV DNA, the lower ones are more likely to respond; the higher the transaminase, the more likely to respond. Both those latter two would reflect ongoing immune attack and they need a push to get over it.

Nothing like that for HCV. Perhaps when we get things like PCR for HCV RNA used in these studies, perhaps - I have no idea - that might be a helpful

predictor. But, we have nothing that helps predict response.

SPEAKER: Max Chernesky has mentioned that the issue of screening organ donors for anti-HCV had really not been addressed. I thought that was a given. I mean, we do it for blood; why would we not do it for organ donation? Is there anyone here who is not doing organ donors for anti-HCV?

DR. LEE: I can speak on behalf of Nova Scotia, in fact the entire Maritimes - we don't do HCV yet.

SPEAKER: Does anyone think that we shouldn't be?

DR. LEE: Anyone?

SPEAKER: What do you do with the results?

SPEAKER: Well, we don't transfuse blood that's got it in it, why would we transplant an organ?

DR. LEE: I am sure that those involved in transplantation will come up with certain guidelines that will come out telling us that we have to do it. I think we have to wait and see.

Existing Problems and Future Requirements

Dr. S. KRUGMAN (New York University Medical Center)

First of all, as many of my colleagues know, I have devoted the last 35 years of my career to studies on the natural history and prevention of hepatitis. These studies have been devoted almost exclusively to hepatitis A and hepatitis B.

As far as my experience with hepatitis C, I can state without any question it is negligible. And it is for that reason that I would like to express my appreciation to Dr. Bishai for inviting me to participate in this meeting, to attend this meeting, so that my continuing medical education will be such that at least I have some background as far as hepatitis C is concerned.

Listening to all of the presentations here the past two days has impressed me with the fact - and I am sure all of you are aware of this - that from the point of view of the natural history of hepatitis C,there are striking similarities between hepatitis C and hepatitis B.

Of course, we are all aware of the fact that not only are there similarities, but there appear to be differences. Whether these differences are significant or not, I think only time will tell.

Now, one of the important and exciting things that we were able to do during our studies on the natural history of hepatitis B and hepatitis A was for many, many years, working in an endemic situation, we had the opportunity to collect hundreds and possibly in some cases thousands of serum specimens. And, we had a serum bank.

In that serum bank there were specimens collected before, during, months, years after onset of hepatitis. Initially, we thought that all of these specimens that were being stored represented specimens from patients who had had hepatitis A. But, as time went on, and as more and more reagents and more and more identification of agents became available, it became very clear that what appeared to be a very cloudy situation, was becoming more and more clarified.

I would like to refer, as an introduction to C, to the situation with B.

When the first generation tests became available for hepatitis B, we very quickly went to our deep freezes and pulled out our serum specimens. It became obvious that if one observed patients from the time of exposure, positive tests were in the neighbourhood of five or six weeks, and sometimes as late as two months. When the second generation tests became available, we pulled the same serum specimens out of the deep freeze and, lo and behold, it wasn't six weeks. The sixth week was not the first time we detected the antigen. It had come down to about three weeks.

Then we came to the third generation test. And, to make a long story short, in some instances it became possible to detect antigen hepatitis B surface antigen as early as the sixth and seventh day after exposure to hepatitis B.

If we hadn't had that third generation test, we would have assumed that antigen, or evidence of infection, was not detectable until that period of time.

The same thing occurred with the detection of antibody.

Initially, using complement fixation to try to detect antibody, in looking at serial samples of serum, beginning from day zero, and going way out in this instance to about 70 days, it was obvious that by complement fixation there was no detectable antibody.

This happened to be a study where we had actually administered heat and activated MS-2. We had assumed that all of the recipients of the heat and activated MS-2 were susceptible.

Why? Because they did not have complement fixing anti- antibody.

It became very clear that within one week after exposure to this hepatitis B antigen, that there was a significant rise in antibody, but as time went on, it declined to undetectable levels.

Several years later, when passive hemagglutination assays became available, we went back into our deep freeze, selected out all of these specimens, and lo and behold, that first specimen, which obviously had no antibody with this lower generation test, had a very good level of antibody. With a more sensitive test, there it was.

Then, of course, there was radioimmunoassay, etc., etc.

The good news was that we didn't have a sensitive test and that enabled us to observe that it was indeed possible to use plasma as a source of antigen.

The bad news would have been that if we had had a sensitive test, we never would have been able to make this serendipitous finding; obviously, it's better to have that sensitive test.

I feel very confident that we are now in the second generation. We will then go to the third generation. I hope there will be a fifth and a sixth generation test, and I hope the time will come when instead of PCR, there will be the equivalent of HBsAg and HBeAg, the kind of tests that any routine laboratory can carry out.

And, as these tests become available to us in the future, the cloud, as far as hepatitis C is concerned, will clear up, and we will make the same progress that we have made over these years in regard to hepatitis B.

Much of the controversy and many of the differences that we have heard at this meeting, I am quite sure, will be clarified.

But we need more data.