Hepatitis C Virus: From Hippocrates to Cure

Nobel Lecture, December 7, 2020 by
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I had a vision, but not a dream. My vision was to better understand Transfusion-Associated Hepatitis (TAH) and to find ways to ameliorate this potentially devastating consequence of blood transfusion. I did not dream that this would lead to the discovery of a new viral agent, the near elimination of TAH and the potential to eradicate a virus chronically infecting 70 million persons world-wide. Certainly, I never dreamed of a Nobel Prize. Instead the Prize emerged, not from a eureka moment, but from a series of incremental observations that, together with the more basic discoveries of my co-recipients, Michael Houghton and Charles Rice, built a compelling story that in summation made my non-dream come true. It is the beginning of that story that I will relate in this lecture.

The hepatitis story actually began about 2000 years ago, when Hippocrates observed patients turning yellow, a condition he termed ikterus, and others that had hardening of the liver that he termed kirros. He considered these conditions the consequence of an imbalance in the four humors, in this case, an excess of yellow bile. While this hypothesis did not ultimately hold up to peer review, it emphasized Hippocrates great powers of observation and established him as the Father of Medicine. In truth, Hippocrates was making a profound statement, namely that disease was not the result of divine punishment, but rather the effect of external elements that perturbed the bodies delicate balance. He did not know that

some of those external elements were infectious agents nor that icterus could be caused by a virus, but he made the critical distinction between divine intervention and earthly perturbations.

Nothing much else was learned in the realm of hepatitis for the next 2000 years except that there were recurrent wars and in every one, large numbers of combatants turned yellow and developed the classic symptoms of what we now call viral hepatitis. Yellow bile continued to be out of balance for centuries. Before any virus was observed and before any virus specific assays had been developed, epidemiologic investigations (1) discerned that there were two patterns of hepatitis, one highly infectious and transmitted by contaminated water and food, designated hepatitis A, and the other less contagious, primarily transmitted by sexual or percutaneous exposure and of longer incubation that was designated hepatitis B.

While clinical observations were numerous and liver enzyme tests and liver biopsies established hepatic inflammation, no specific viral marker emerged in those 2000 years. The breakthrough came serendipitously in 1962 when as an NIH fellow, I collaborated with Baruch Blumberg and, while looking for lipoprotein polymorphisms using Ouchterlony gel diffusion, we noted a precipitin line in agar that did not take up the usual lipid stain, but instead stained red for protein. (2) That immune-precipitate represented a reaction between the serum of a multiply-transfused patient with hemophilia and that of an Australian aborigine. This aboriginal serum was selected from a large repository of samples from indigenous populations that Blumberg had collected and stored and, by chance, was being tested that day. That antigen, originally called the "Red Antigen" for its staining characteristics was later renamed the Australia antigen in deference to the practice of naming new hemoglobin variants after the country or city of origin of the designated individual. The ultimate linking of that antigen to the hepatitis B virus is a fascinating story that resulted in a Nobel Prize for the late Baruch Blumberg and has been chronicled in the Nobel Lectures of 1976. The relevance to my treatise herein is that the Australia antigen, later called the hepatitis B surface antigen (HBsAg), provided the first serum marker for any hepatitis virus and laid the foundation for the events that ultimately led to the discovery of hepatitis C.

In the late 1960s, an NIH prospective study was initiated by Robert Purcell, Paul Holland and Paul Schmidt. I assumed responsibility for that study in 1970. The study aims were straightforward and in essence were to determine the incidence and causes of TAH and, if possible, to identify interventions that could reduce hepatitis incidence. To achieve this goal, transfused patients undergoing open heart surgery were enrolled and sampled weekly or bi-weekly for three months and then monthly for an additional three months. This population of cardiac surgery patients was

chosen because they were heavily transfused, rarely had confounding liver diseases and were not immunosuppressed by disease or medication. Since there were no specific hepatitis viral markers when the study was initiated, hepatitis was diagnosed on the basis of liver enzyme abnormalities, namely an elevation of alanine amino transferase (ALT) to two and a half times the upper limit of normal (ULN) followed one or more weeks later by a second elevation at least two times ULN. Other measurements included aspartate amino transferase (AST), bilirubin and alkaline phosphatase. Non-viral causes of ALT, AST or bilirubin elevations were excluded to the extent possible. Importantly, all collected samples were stored frozen for later analyses. In addition, donor samples were similarly stored for donor-recipient linkage in patients who developed hepatitis in temporal relation to their blood transfusion.

Initial findings were both striking and surprising (Fig. 1). Approximately one-third of patients undergoing open heart surgery developed hepatitis based on the ALT elevations as defined above. This inordinately high incidence was predicated on three factors. First, the patients received large volumes of blood, averaging 17 units per case, because the heart lung machine used at the time required seven units of blood simply for priming. Second, the majority of cases were asymptomatic and occurred after hospital discharge. Such cases would not have been recognized if prospective follow-up post-discharge had not been in place. The third critical element for the inordinately high incidence proved to be the source of donor blood. Prior to 1970, a large proportion of transfused

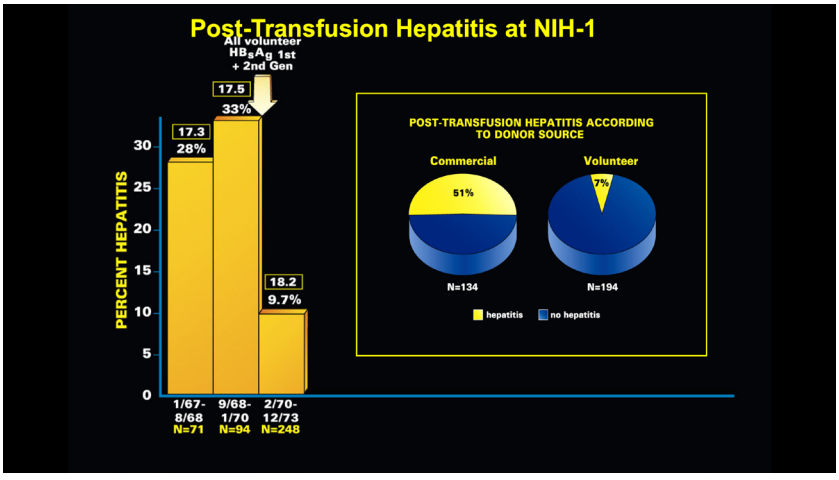

Figure 1. Bar graph showing early years of the prospective study. Rates of hepatitis exceeded 30% prior to 1970. Based on demonstrated risk of commercial donor blood (right figure), transition to an all-volunteer donor supply and introduction of hepatitis B virus (HBV) donor screening was instituted in 1970, resulting in a 70% reduction in hepatitis incidence.

blood at NIH was obtained from commercial sources. A study led by John Walsh (3) showed that if a patient received at least one unit of paid-donor blood, the risk of TAH was 51% whereas among those who received solely volunteer donor blood, only 7% developed hepatitis, even though the volume of blood received was similar. The use of paid donor blood at that time was predicated on limited supply.

In 1970, with these data in hand, the NIH blood bank was able to transition to an all-volunteer blood supply. Simultaneously, we introduced an unlicensed, home-brew assay for hepatitis B surface antigen. These combined measures (Fig. 1) resulted in a dramatic 70% reduction in TAH incidence to a new level of approximately 10%. (4) While we introduced two interventions simultaneously, extrapolation indicated that the major determinant of the marked decline in incidence was the conversion to an all-volunteer donor source. Indeed, nothing done since that time, including specific viral testing, has had such a dramatic impact.

In 1973, sensitive third generation assays for HBsAg became available and we tested stored samples from identified TAH cases. This revealed (Fig. 1) that only 25% of cases were related to hepatitis B virus (HBV) infection, leaving causation of the non-B cases yet to be explained. A breakthrough occurred in 1975 when Feinstone, Kapikian and Purcell at NIH discovered the hepatitis A virus using immune electron microscopy (5). We immediately sent our non-B case samples to their laboratory where testing revealed that not a single non-B hepatitis case was due to HAV infection. It was then, in a brilliant execution of deductive reasoning, that we decided that if these cases were not due to hepatitis A or hepatitis B infections, that we would call them non-A, non-B hepatitis (NANBH). (6) Actually, there was some rationale for this obtuse terminology in that we had not yet proven that we were dealing with a transmissible virus and, if so, how many there might be. Thus, we demurred on using “C”, the next letter in the hepatitis alphabet.

The next critical step was to perform transmission studies using the chimpanzee model. We are no longer able to use chimpanzees in US government-sponsored research, but at the time they were an invaluable model and some suggest that a chimpanzee should have been the fourth recipient of this HCV-based Nobel Prize. Chimps were an ideal model for NANBH in that they were readily infected, developed elevated but low-level ALT levels, did not manifest any clinical illness or deleterious effects of the study and often became chronic asymptomatic carriers. Thus, their infection closely mimicked that of the majority of NANBH/HCV infected patients. In our initial chimpanzee studies (7), we were able to transmit hepatitis by inoculating only one milliliter of plasma, not only from patients with acute or chronic NANBH, but importantly, also from asymptomatic blood donors who had been implicated in human hepatitis

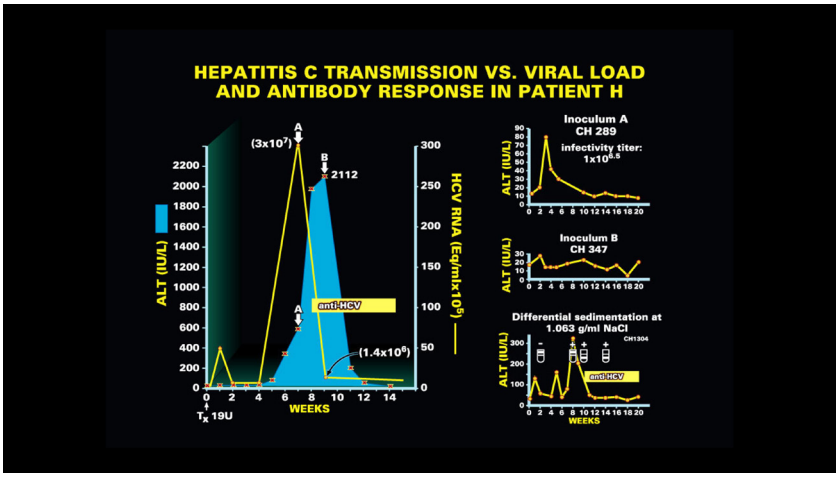

Figure 2. Graph depicting ALT elevations (blue) and HCV RNA levels (yellow line) in patient H. ALT levels began to rise 5 weeks post-transfusion and reached a peak of 2112 IU/ml. HCV RNA levels (yellow line) increased prior to ALT elevations and reached a peak of 3x10e7 copies/ml. A 500 ml plasmapheresis unit was obtained at point A and 1ml was shown to be infectious in a chimpanzee (upper panel right). Plasma obtained at the peak of ALT (point B) was not infectious in a second chimp (right middle panel) presumably because viral load was rapidly falling and residual virus was immuno-complexed (right lower panel).

transmission. Tabor and coworkers at the Food and Drug Administration (FDA) (8) performed parallel transmission studies and my primary collaborator, Robert Purcell, serially passaged NANBH from chimpanzee to chimpanzee. Hence, the presence of a blood transmissible NANB agent was firmly established in this animal model.

In 1977, the most important patient in my career came to NIH and was enrolled in our prospective study. The patient, Mr. H, had trailblazing as his avocation. One day, while marking a mountain trial he collapsed from a heart attack. His wife, who accompanied him, performed CPR and brought him back to life and ultimately to NIH where he was found to have triple vessel coronary disease and underwent one of the first coronary bypass surgeries. Six weeks after surgery, his ALT levels began to rise rapidly concomitant with classic symptoms of hepatitis and the onset of jaundice (Fig. 2). It had been my goal to obtain a large volume of blood from a patient in the early phase of acute NANBH, at which time the viral load was presumed to be at a high level. Thus, we obtained 500ml of plasma by apheresis on the ascending limb of his ALT curve. As seen in Figure 2, that plasma transmitted hepatis to a chimpanzee (upper panel on right). Subsequently, his ALT level continued to rise to a peak of 2112 IU/ml or 50 times the upper limit of normal, at which time we obtained a second apheresis unit. Surprisingly, that unit, obtained at point B, was not

infectious in a second chimpanzee (middle right panel). In retrospect, we believe that unit was not infectious because the viral RNA level was falling precipitously and because by that time antibody had appeared that was complexing the virus and making it less infectious. Complexing was confirmed by ultracentrifugation as shown in the lower right panel. Purcell then took the plasma from point A, henceforth referred to as the H77 inoculum, and performed titration studies based on infectivity in chimpanzees. H77 was shown to have a titer of 10e6.5 chimp infectious doses per ml. Interestingly, this was almost identical to the HCV RNA titer of 10e7 copies/ml found in the patient sample when PCR subsequently became available. Hence, at this point we had two critical elements for further study, namely an infectious inoculum of known titer and a reliable animal model.

Armed with these critical elements, Steve Feinstone (9) performed chloroform extraction studies and showed that infectivity was abrogated by chloroform treatment whereas a mock chloroform extraction resulted in hepatitis transmission to a second chimp. This study implied that the NANBH agent had essential lipid in its envelope, narrowing the spectrum of potential viral etiologies. Le Fang He (10) in the Purcell lab then performed filtration studies wherein the H77 inoculum was passed through various size filters and the filtrates inoculated into chimps to assess residual infectivity. These experiments revealed that the NANBH virus was between 30 and 60 nanometers in diameter. Combining these studies, one could deduce that the NANB agent was small and lipid encapsulated, narrowing the taxonomic possibilities to that of a small RNA virus in the Togaviridae family, a hepatitis B-like agent or a new class of viral agents. We already had considerable evidence that NANBH was not related to the hepatitis B Hepadna family. My recollection is that Dan Bradley at the Centers for Disease Control (CDC) was the first to postulate that the NANBH virus was most likely a flavivirus, as it later proved to be.

While continuing to pursue the elusive direct assay for NANBH virus detection, we examined in greater detail the clinical consequences of chronic NANBH infection in collaboration with the NIH's National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) liver disease research branch, then under the leadership of Jay Hoofnagle. This collaboration continued for decades and included Adrian DiBesceglie, Marc Ghany, Jake Liang and a cadre of hepatology fellows, indeed too numerous to name individually, many of whom are now the current generation of leaders in the hepatology world. The key to this long collaboration was the accession of liver biopsies from chronically infected patients, most of whom were asymptomatic. As shown in Figure 3, initial biopsy of 39 individuals revealed that the majority had only mild histologic lesions, but that 10% already had cirrhosis and 13% had what was then termed severe

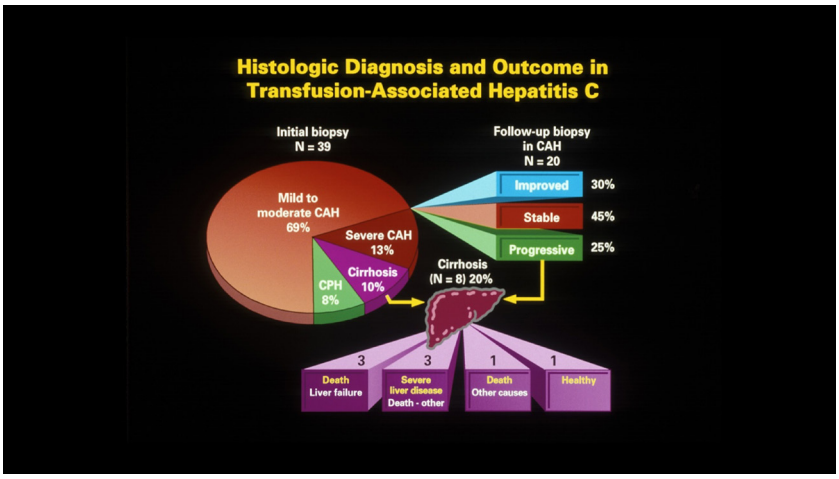

Figure 3. Liver biopsies were obtained from 39 patients. On initial biopsy, most had only mild to moderate inflammation, but 13% had chronic active hepatitis (CAH) and 10% had cirrhosis. Follow-up biopsy showed additional cases of cirrhosis such that overall, 8 of 39 (20%) had severe fibrosis progression. 3 died of liver failure and 3 others had severe liver disease when they died of their underlying heart disease.

chronic active hepatitis (CAH). Repeat liver biopsy, after varying intervals, in those with CAH showed that 25% progressed to cirrhosis while under observation resulting in a cumulative incidence of 20% (8 of 39) who developed severe fibrosis progression, terminating in cirrhosis. This 20% incidence of cirrhosis in chronic NANB/HCV hepatitis has withstood the test of time though the final number may be closer to 30%. Among the eight patients with cirrhosis, 3 died of liver failure and 3 others had severe liver disease when they died from complications of the heart disease that led to their initial surgery. This biopsy study led by Marvin Berman (11) dispelled the notion that NANBH was simply a benign transaminitis and proved that this primarily asymptomatic infection could lead to cirrhosis and death. From that point forward, the intensity of global study of this agent and its disease accelerated exponentially.

As we continued to prospectively follow the incidence of TAH, we found that the rate had declined from near 10% in 1970 to about 6% in 1985. The lowered incidence was probably related to the decreased volume of blood transfused, declining from an average of 18 units per patient in 1970–1973 to an average of 9 units per patient in the 1980s. During this long interval, we attempted several interventions to further decrease incidence, including ALT testing of blood donors, but this had no discernible impact. When HIV testing of donors was instituted in 1985, we thought this might serve as a surrogate marker for hepatitis carriers, but it did not do so because HIV at that time was transmitted primarily by sexual con-

tact while NANBH was almost exclusively transmitted by percutaneous exposure. In the mid-80s we performed a retrospective analysis of this prospective cohort which suggested that donor screening for antibodies to hepatitis B core antigen (anti-HBc) might serve as a surrogate for the NANBH virus carrier state since HBV and NANB had overlapping routes of transmission. This retrospective study (12) and another independent study by the Transfusion Transmitted Virus Study Group (TTVS) (13) predicted that the introduction of anti-HBc donor screening might reduce the incidence of TAH by 25–30%. Hence, in 1987 we instituted routine donor screening for anti-HBc and indeed observed the predicted decline in TAH incidence to a level of 4% by 1989.

While the clinical severity of NANBH had become increasingly evident over the interval from 1975–1985, no serologic, enzymatic, radio-immunologic or early molecular method led to a specific NANBH assay or further elucidated the nature of the agent. It was then that I wrote a poem of frustration titled "I Can't See The Forest for the HBAgs":

I think that I shall never see
This virus called non-A, non-B
A virus I cannot deliver
And yet I know it's in the liver
A virus that we often blame,
But which exists alone by name
No antigen or DNA
No little test to mark its way.
A virus which in our confusion
Has forced us into mass collusion
To make exist just by exclusion
But is it real or an illusion?
O' Great Liver in the sky
Show us where and tell us why
Send us thoughts that will inspire us
Let us see this elusive virus
If we don't publish soon
They're going to fire us
Let us find this little beastie
Give us a sign ---- a star from the Easty
Thus, today, our quest we will begin
For this agent that plagues us like original sin
And perhaps someday, we all will agree
That indeed there exists a non-A, non-B

I do not know if this poem influenced Michael Houghton and his research group at the Chiron Corporation, but shortly after I recited it at a national meeting, Chiron announced that after a 6-year effort they had cloned the NANBH agent and called it hepatitis C virus (HCV). The description of the cloning experiments will be provided in a separate lecture by my Nobel co-recipient, Michael Houghton. This was a monumental breakthrough, utilizing a then novel cloning technique involving a gt-11 expression vector and immune-recognition of expressed antigens (14). Houghton assumed that antibody would be present in chronic carriers and/or recovered subjects even though such antibodies had not been detected in the previous decade. Although various accounts have been given, it is believed that 6 million clones were immune-screened before a single clone tested positive. By subcloning, the Chiron team, and principally Qui-Lim Choo, identified a genetic sequence that allowed them to express an antigen that then led to an antibody assay developed by George Kuo (15).

Subsequently, I received a call from Kuo requesting that I send him samples from what was called the Alter Non-A, Non-B panel. George implied that Chiron had developed an antibody test for NANBH virus carriers that he wished to validate with the notorious panel that had stymied 19 other laboratories claiming a specific NANBH assay. The panel was small, but not easy to decipher because every sample was present in duplicate and every duplicate was in a random position. The positive samples were all proven infectious in chimpanzee transmission studies and the negative controls were from blood donors who had donated at least 10 times and had never been implicated in hepatitis transmission. Kuo returned his test results promptly, but I was slow to analyze them because I expected another false claim. To my surprise and delight, Chiron broke the code perfectly, detecting 3 patients with chronic NANBH in 6 random positions and 2 implicated donors in 4 random positions (Fig. 4). They failed to detect 2 patients with acute hepatitis because they were testing for antibody rather than virus. Later samples from these patients tested positive in the Chiron antibody assay. Importantly, all 7 negative controls tested negative in 14 random positions.

With the Chiron Anti-HCV assay in hand, I then tested 15 of our most definitive NANBH cases and found that all 15 had been anti-HCV negative in their pre-transfusion sample and had seroconverted in post-transfusion samples (16). It was then evident that the NANBH virus and HCV were identical, as later confirmed by molecular sequencing. Further, in testing donors to 25 TAH cases, we detected an anti-HCV positive donor in 80% by a first-generation antibody assay and 88% by a more sensitive second-generation assay (Fig. 4). It was then evident that the introduction of anti-HCV testing to routine donor screening might prevent nearly 90%

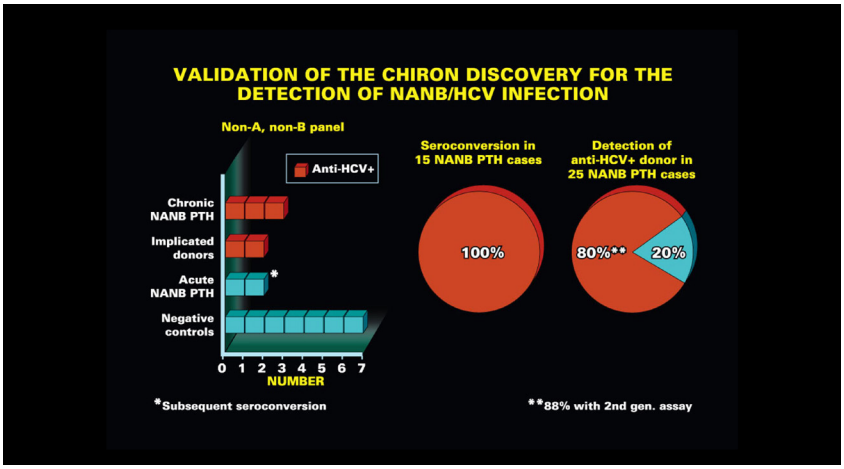

Figure 4. Testing of a coded NANBH panel by the Chiron anti-HCV antibody assay. All coded samples were in duplicate that were placed in random positions. The assay detected 3 chronic carriers and 2 implicated donors in all duplicate samples. Two patients with acute NANBH tested negative for antibody during the acute phase, but later seroconverted to antibody positive. 7 pedigreed negative controls were negative in all duplicate samples. 15 cases of NANBH in the NIH prospective study were all anti-HCV negative pre-transfusion and Anti-HCV positive post-transfusion. Donors to 25 NANBH cases were tested and an antibody positive donor was found in 80% of cases by initial testing and in 88% by a more sensitive 2nd generation anti-HCV assay. This predicted that anti-HCV donor screening might pr event nearly 90% of post-transfusion hepatitis (PTH).

of transfusion-associated hepatitis. We began such testing in 1990, and it was FDA-mandated nationally by 1992. Real life experience confirmed our prediction (Fig. 5). After the implementation of routine anti-HCV donor screening in 1990, hepatitis C incidence fell to 0.6% and then to zero when a more sensitive second-generation assay was introduced in 1992. Interestingly, a previously postulated, but never delineated, non-A, non-B, non-C form of hepatitis also disappeared after testing for HCV. I now believe that the relatively small number of presumed “Non-ABC” cases with generally mild post-transfusion ALT elevations were not representative of viral infection, but rather were the result of intercurrent events related to surgery, medications, or non-alcoholic fatty liver disease.

It can be estimated by extrapolating the incidence figures in our prospective study to the US population as a whole that blood transfusion may have transmitted 4.8 million cases of hepatitis in the two decades from 1970 to 1990 and, conversely, that an additional 2.4 million cases may have been prevented by HCV testing in the two decades from 1990 to 2010. Most of the transmitted cases prior to 1990 would have been asymptomatic and unrecognized, but 75% to 80% would have become chronic HCV carriers and 20% of those would have been at risk of ultimately developing cirrhosis if they did not first succumb to another illness.

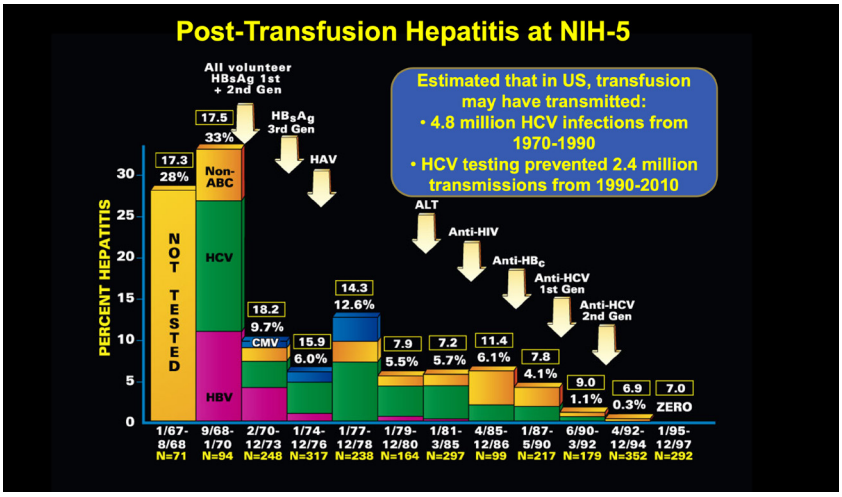

Figure 5. Bar graph showing the declining incidence of post-transfusion hepatitis over time and the interventions associated with that change. Anti-HCV donor testing was introduced in 1990, reducing incidence to 1.1% and a second generation assay introduced in 1992 resulted in the absence of HCV cases (green bar). No hepatitis cases were seen in the interval from 1995–1997. Estimates of cases caused by transfusion and cases prevented by testing are shown in the rectangle above the graph.

As previously noted, there was frustration in not being the first to unequivocally identify HCV, but I got over it fairly rapidly and wrote this poem of conciliation, titled, "There's No Sense Chiron Over Spilt Milk":

After more than a decade of severe frustration
and endless serologic constipation, the whole field in a state
of stagnation
the scientific equivalent of Faustian damnation.
At last, non-A, non-B has achieved emancipation
and enters an era of new creation
with enormous impact on blood donation.
But is this real or an aberration?
is it true love or just a flirtation?
Are we ready for delivery after this prolonged gestation?

The non-A, non-B story began to get brighter
When Bradley squirreled away chimp plasma of very high titer.
Seeking this agent with the passion of a zealot,
Houghton centrifuged particles to a concentrated pellet.
And the science moved forward from a state very placid
when the pellet released its ribo-nuclear acid;
A nucleic erection in a field formerly flaccid.

it was then Dr. Houghten's anti-viral prescription
to make clones by reverse transcription
While Chiron's finance department had another conniption.
But Michael said this was not a rehearsal
The RNA had to be changed by a transcription reversal.

Like Jack the Ripper at his most vindictive, he chopped up
the codon with an enzyme restrictive
Could these tiny pieces be of an antigen predictive?
Corporate executives prayed that it would on their knees
benedictive
Showing that science and religion are not contradictive.

With the hope these pieces would become a viral detector
they inserted them with care into an expression vector,
Could these invisible codes recreate non-B architecture?

So that codon insertion might be most benefactory
they packaged the plasmid in an E. coli factory;
Man and bug in a collusive compactory
praying the result would not be anti-climactory.
Then out of the agar there spewed forth a clone
that for years of frustration would fully atone,
The non-A, non-B virus was suddenly known
and HCV became the name it would own
A viral peasant had ascended the throne.

Suddenly there was antigen expression,
Followed by detection of antibody and genome in rapid
succession;
A major advance for our profession,
A triumphal ending to the non-B obsession.
Chiron opened a cloning concession;
9/10ths of the law is clearly possession.

As for Chiron I hold no resentment
in the tests support of my claims, I'll find my contentment.
For coming in second, I make no apologies;
I can always turn to the bench and do blood bank serologies.
For me there will be no Nobel prizen,
but there's always another virus on the horizon.

I like to think of the year 2000 as the end of the beginning. It marked the elucidation of a novel transfusion-transmitted agent (Non-A, Non-B hepatitis virus); the proof of its persistence in most infected individuals; the clinical description of its chronic sequelae, including cirrhosis and hepatocellular carcinoma; its cloning and characterization as a flavivirus and renaming as HCV; the development of assays for antibody detection and their implementation in blood donor screening, and; the virtual eradication of transfusion-associated hepatitis. There was still much more to come in the HCV story, but this was the end of the phase of discovery, the recognition of its global spread, its major role in chronic liver disease and liver-related mortality and its impact on blood transfusion safety. This was the end of 25 years of clinical observation and molecular characterization. It was a period of accelerating and often unexpected enlightenment, an end that was not anticipated at the beginning, but which played out in small increments, a cascade of observations that built one upon the other into what was ultimately a compelling story when curative therapies emerged from earlier genomic characterizations and the development of infectious clones. Charles Rice, the third recipient of this Nobel Prize, will describe the pathway to drug cure in a subsequent lecture.

While I have outlined the role that NIH played in the discovery phase and in the elimination of transfusion-associated hepatitis, I would now like to spend some time on ancillary studies that defined other aspects of HCV pathogenesis. We, and many others, have been interested in the persistent nature of HCV infection. What are the elements of the virus-host interaction that prevent viral clearance in the majority of HCV infected patients? One element is clearly the quasispecies nature of RNA viruses that lack efficient proof-reading capability for the elimination of evolving variants. Hence, HCV exists not as a single virus, but as a family of closely related, yet genomically and often antigenically distinct variants. Patrizia Farci, my closest collaborator over the past two decades, showed by sequencing 105 clones derived from a single sample of patient H's plasma, that 57% of clones were identical, but that 19 other variants were simultaneously present in smaller proportions (17). Hence, even if this patient had mounted a good immune response to the dominant strain, any of the other 19 variants could emerge to dominance and escape the antibody response. Farci also showed the rapid emergence of variants by cloning sequential samples over the course of the first 16 weeks of acute infection. Variants continued to appear in each successive sample and the rate of appearance accelerated once the anti-HCV response engaged and put additional immune pressure on the HCV envelope receptor sites, particularly in the envelope 2 region. (18).

Jens Bukh, a major collaborator in Denmark, has worked extensively with the H77 inoculum and shown that antibodies that effectively neutralized the initial H strain inoculum failed to neutralize strains that evolved at a later time. (19). Shimizu, et al documented this neutralization escape in the chimpanzee model (20). Hence, rapidly evolving viral variation and escape from antibody neutralization combine to enhance viral persistence. In addition, Wedermeyer et al (21) have shown that CD8 T cell responses are blunted during HCV infection, presumably by antigen excess, and that T-cell activity recovers in those who spontaneously clear HCV. This blunting of the T-cell response is another characteristic element of HCV persistence. In another collaborative study, von Hahn in Jane McKeating's lab (22) showed that mutations that produced a change in only two amino acids in the E2 envelope protein resulted in escape from T cell immunity that was highly effective against the wild type strain. Hence, there is mutational escape from both antibody and T-cell immune responses that contribute to viral persistence.

In our prospective studies we observed that 60–70% of chronically infected patients had an indolent course with only minimal or moderate fibrosis progression over decades of observation whereas 20–30% progressed to cirrhosis in 15–40 years and 5% had rapid fibrosis progression resulting in cirrhosis and death in less than 10 years. Farci (23) examined the differences between rapid and slow progressors in my patient population and showed that sIow progressors demonstrated initial containment of the virus with diminution in HCV RNA to very low levels followed by a rapid rise in HCV RNA as a presumed escape mutant became the dominant strain. In contrast, rapid progressors never showed immune containment as evidenced by persistently high-level HCV RNA from early on and throughout their chronic course. In addition, rapid progressors had high levels of monocyte chemotactic protein (MCP-1, CCL-2) a profibrogenic chemokine that both attracts and is produced by hepatic stellate cells, the most critical cells in hepatic collagen deposition. In a separate study (24), Kentaro Matsuura from the laboratory of Yasuhito Tanaka in Nagoya, Japan did an advanced fellowship in our laboratory and showed that the micro RNAs Let-7a and Let-7c were at low level in patients with advanced fibrosis and declined further in parallel with progression to cirrhosis. This made physiologic sense in that Let-7 is a suppressor of TGF-β, a major driver of hepatic stellate cell activation and concomitant fibrous tissue deposition. Thus, one can postulate that the production of MCP-1 and the release of TGF-β from Let-7 inhibition can combine to activate stellate cells and lead to rapid fibrosis progression. Clearly other factors may be involved but these were the key factors revealed in cytokine-chemokine arrays and in micro-RNA array screening.

Lastly, I want to discuss insights into hepatocellular carcinoma (HCC), a devastating consequence of HCV infection that occurs in 1–4% of patients who progress to cirrhosis. Yasuhito Tanaka and Masashi Mizokami in Japan performed a molecular clock analysis of samples from Mr. H, whose virus was HCV genotype 1a, and from a Japanese patient with genotype 1b infection (25). Sequencing serial samples from these patients allowed for mathematical analysis of when a virus diverged from a common ancestor and when it spread rapidly in a given population. The molecular clock estimated that genotype 1b emerged in Japan in 1880 and then persisted at low level until the 1930s and 40s when it spread rapidly within the population. This spread was coincident with the Pacific wars in which Japan engaged, at first with China and then with the US and it allies in World War II. We now learn that it was common for Japanese soldiers to "shoot-up" before battle, generally with amphetamines, and it was this needle sharing that was at the root of rapid HCV dissemination among Japanese combatants, ultimately involving much of the male population. In the US, genotype 1a emerged in 1910 and then lay dormant until the 1960s and 70s when it spread rapidly in the US population due to our illicit drug-use epidemic. HCV continues to spread by this mechanism to the current day and indeed would dissipate to large measure if the drug epidemic could be contained. Importantly, there is an approximate 30-year lag between the spread of HCV in the US and that in Japan. This is critically important because the incidence of HCC is 8 to 10-fold higher in Japan compared to the US and it has been postulated that the US incidence will ultimately match that of the Japan when the virus persists in our population for several more decades. Indeed, that appears to be the case. El-Serag (26) has shown that the incidence of HCC in the US tripled between the late 1970s and the late 1980s and that virtually all this increase was attributable to HCV infection. Hence, the difference between HCC incidence in Japan and the US appears to be primarily a function of time because the evolution to cirrhosis and then to HCC is a multi-decade phenomenon, generally exceeding 40 years. Given the trajectory of HCC in the US, it has been estimated that HCC incidence will increase 4-fold from a 2009 baseline to the year 2029, if there is no change in the standard of care. Fortunately, there has been a major change in the standard of care as direct-acting, HCV-specific antivirals have been developed that can now cure 95–100% of chronically infected individuals over an 8–12 week course of oral therapy with minimal side effects. Such high cure rates were unimaginable prior to 2010, when a combination of pegylated interferon and ribavirin were the standard of care. It is now conceivable that every identified HCV carrier can be treated to cure, with the caveat of the obstacles discussed below.

We have now reached the point where the eradication of HCV can be contemplated. With cure rates approaching 100%, from this time forward, no one with identified HCV chronic infection should develop cirrhosis or die from hepatitis C sequelae. However, there are caveats to this optimistic forecast. First, in the US, it is estimated that no more than half of HCV carriers have been identified and this proportion is significantly less in the developing world. Hence, intensive population screening for anti-HCV and/or HCV RNA is the first goal in global eradication. Second, the high cost of these life-saving drugs is a major impediment. Indeed, in the first years of DAA deployment, only a minor portion of identified patients were treated because insurance companies and national health services found the costs prohibitive. With time, there has been the realization that the cost of cirrhosis, end-stage liver disease, HCC and liver transplantation exceed the cost of 8–12 weeks of therapy and an increasing number of patients in the developed world have been treated, but there are huge gaps in developing nations even though the cost in these countries is markedly reduced. Cure is no longer constrained by science, but is a matter of dollars.

Given these caveats, is eradication possible? The usual path to viral eradication is vaccination. However, there is no current vaccine that has been licensed or even entered into clinical trials. Michael Houghton, in an accompanying lecture, will describe an HCV envelop protein vaccine that has been shown in the chimpanzee model to significantly decrease the frequency of chronic infection even though it does not prevent HCV infection per se. The success of messenger RNA-based vaccines for Covid-19 raises the hope that this approach might also be effective for HCV, but no such data are available at the time of this publication.

It is intriguing to speculate whether HCV could be eliminated globally in the absence of a vaccine. This would be a difficult task, but given that we have almost optimally sensitive screening assays and highly curative therapies, it is a goal that could be achieved over the course of the next 2–3 decades. The first essential step would be to develop a highly sensitive HCV screening assay that could be used in the field and provide results within minutes. A positive test would immediately trigger the provision of 1–3 month's supply of oral therapy at a price commensurate with the GNP of the nation. Starting therapy simultaneously with testing obviates the loss to follow-up that would occur with delayed treatment. To implement such a test and treat strategy on a global scale would be labor-intensive and costly, but it could be done in compliant nations if there was the political, corporate, philanthropic and moral will to make it happen. Chronic HCV is different from chronic HBV or HIV, because there is no need for life-long follow-up and treatment. It will be tragic if the cure of millions will be blocked, not by technologic capability, but by

moral inertia and high cost. Vaccine development and this test and treat strategy are synergistic and can proceed in parallel. Of course, other preventive measures are also needed to reduce new infections, primarily by limiting shared needle and drug paraphernalia use among injection drug users and by standardizing global health care practices to insure single-needle use and proper sterilization of reusable instruments, to foster universal precautions, and where needed, to change practices of tattooing and ritual scarification.

Thus, the end of HCV is now in sight, even if one has to use a telescope to view it. Unless one can envision this goal, it will not happen. It is a long staircase to ascend, but will not happen unless we take that first step.

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