Transfusion-transmitted viral infections: building bridges to transfusion medicine to reduce risks and understand epidemiology and pathogenesis

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Transfusion medicine is a rapidly evolving field that now interfaces with virtually every clinical discipline and with many of the basic medical and social sciences. Transfusion medicine has been heavily influenced by, and has had a major impact on, public health and political, regulatory, and legal systems around the globe. When I was a young resident in pathology and laboratory medicine at the University of California in San Francisco (UCSF) in the early 1980s, I recall first encountering what was then referred to simply as blood banking. I was struck and intrigued by the relatively primitive understanding of the molecular basis of blood groups and by the simple methods used for compatibility and infectious disease testing. At the time, infectious disease concerns were minor and revolved primarily around possible residual risk of viral hepatitis following the recent implementation of hepatitis B surface antigen (HBsAg) screening. I had just completed my MD and PhD degrees at the University of Southern California (USC), where my thesis focused on the role of feline endogenous retroviruses in embryogenesis and lymphoma. Despite large-scale viral discovery studies at UCSF, USC, and elsewhere, no convincing evidence for a human retrovirus existed at that time. Certainly no one suspected that we were already in the early stages of a major global pandemic due to a novel class of retrovirus (lentivirus) that spread efficiently by blood transfusions. And then came acquired immunodeficiency syndrome (AIDS), and blood banking, indeed the entire world, changed forever!

Fortunately for me, I was in the right place, at the right time. Thanks to relevant training, supportive mentors, wonderful collaborators, and a home base in the epicenter of the US AIDS epidemic, I was able to participate in the early characterization of transfusion-AIDS and help develop and execute many of the studies that have advanced our understanding of infectious risks associated with blood transfusions over the past 25 years.

The theme of this Cooley Lectureship is "building bridges to transfusion medicine," referring to the success that has been achieved by application of novel approaches and findings from other fields to study transfusion complications, while at the same time deriving insights from investigations of viral infections in blood donors and recipients to develop broader understanding of the epidemiology, diagnosis, and pathogenesis of relevant infections diseases with far reaching implications beyond our field. This lecture traces the history of transfusion risk analysis, demonstrating how thoughtful and creative individuals both in academia and in industry have responded to once unimaginable challenges posed by emerging transfusion-transmitted infections which, unfortunately, we now view as routine.

EVOLUTION OF APPROACHES TO ESTIMATE TRANSFUSION RISKS

Concern over transfusion-transmitted viral infections (TTVIs) began with case reports of posttransfusion hepatitis (PTH) during World War II. Studies to accurately measure risks of PTH, however, were not initiated for several decades, since the requisite epidemiologic approaches and laboratory tools only became available in the 1970s. Even then it was not possible to appreciate the risks due to previously uncharacterized yet highly prevalent agents like hepatitis C virus (HCV), and there was no way to anticipate the enormous impact of emerging viruses like

ABBREVIATIONS: ARC = American Red Cross; FT = first-time (donor); IR-WP = incidence rate-window period; MP = minipool; NANBH = non-A, non-B hepatitis; PTH = posttransfusion hepatitis; RPT = repeat; TA = transfusion-associated; TSS = Transfusion Safety Study; TTVI(s) = transfusion-transmitted viral infection(s); TTVS = Transfusion-Transmitted Viruses Study; WNV = West Nile virus; WP(s) = window period(s).

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human immunodeficiency virus (HIV) and West Nile virus (WNV).

Discovery of novel agents like HCV and HIV and their associated transfusion risks depended on basic research in viral discovery and diagnostic assay development and on government funding of large-scale studies that established donor-recipient cohorts. Such studies were first initiated in the late 1970s, and the era of prospective and/or retrospective cohort studies was launched (Fig. 1). In addition to prospective donor and recipient enrollment and follow-up, these cohort studies established large-scale frozen specimen repositories. These repositories enabled investigation of clinically overt as well as asymptomatic infections by retrospective application of increasingly sensitive laboratory methods including immunoassays and later nucleic acid–based tests. The repositories also allowed for more broadly relevant research into determinants of transmission and pathogenesis of relevant viral infections.

As the major TTVI agents were discovered and interventions implemented, it was initially possible to directly measure residual TTVI risks by quantifying rates of infection in screened donors or rates of breakthrough transmissions to recipients. This era of direct risk measurement was short-lived, as improved donor risk factor screening and increasingly sensitive laboratory testing reduced risks to such low levels that direct measurement was no longer possible.

Novel approaches were needed to meet this challenge. The most important advance was the development and progressive refinement of the incidence-window period (WP) model, leading to the current era of risk modeling. By development and application of appropriate models we can now precisely estimate the residual risks of the major TTVIs and accurately project the yield and cost-effectiveness of additional screening measures for these agents. Modeling has also proved very useful for effective responses to new and emerging agents that are associated with transient viremia such as WNV.

ERA OF RETROSPECTIVE AND PROSPECTIVE COHORT STUDIES: 1960s THROUGH 1985

Early studies of PTH

Development of methods to identify etiologic agents and measure risks of their transmission by blood transfusion can be traced to the earliest studies of PTH. PTH was first reported in the United States by Beeson in 1943.[1] In 1964, Grady and Chalmers[2] reported the results of a 10-year retrospective study of PTH in nine Boston teaching hospitals. The incidence of clinically overt (i.e., symptomatic or icteric) PTH in recipients of blood products from volunteer blood donors was calculated at 0.6 cases per 1000 units, compared with 2.8 cases per 1000 units in recipients of blood products from a mixture of volunteer and commercial (paid) blood donors. In 1970, results of the first prospective study to determine the incidence of hepatitis in patients undergoing open-heart surgery scientists at the National Institutes of Health (NIH) were reported.[3] Icteric and anicteric hepatitis developed in a startling 51 percent of the recipients of commercial blood, whereas no hepatitis occurred in patients who received blood only from volunteer donors. These authors estimated the hepatitis carrier rate for commercial blood donors to be 6.3 percent while among volunteer donors the rate was less than 0.6 percent. These findings led the Food and Drug Administration (FDA) to mandate an all-voluntary blood donor system in 1975.[4]

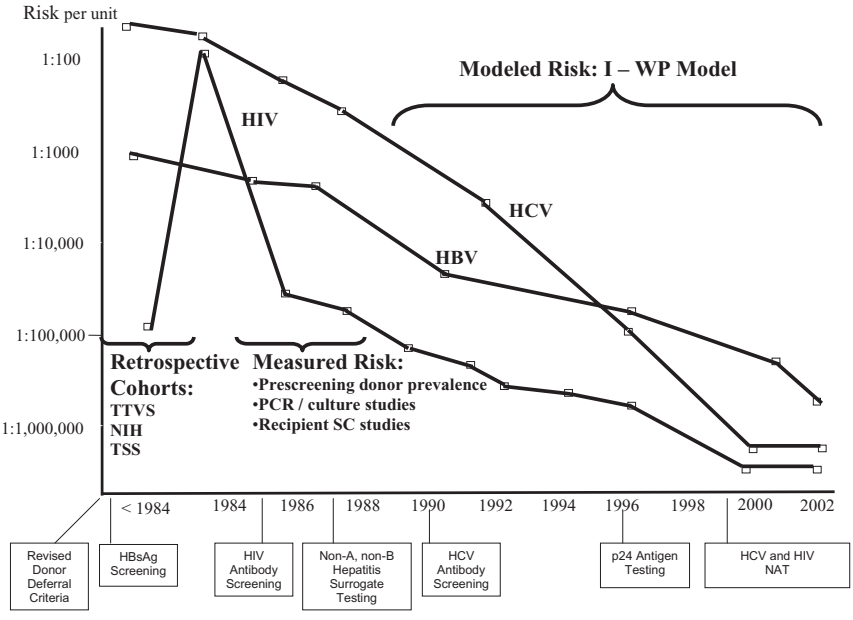

Fig. 1. Evolution of approaches to estimating the risks of transmission by blood transfusion for HIV, HBV, and HCV. Major interventions to reduce risks are indicated below the time line on the *X*-axis.

Discovery of hepatitis B virus and its role in PTH

In 1965, Blumberg and coworkers[5] described the Australia antigen (subsequently renamed HBsAg) and showed that this antigen could be identified in the sera of many persons with hemophilia who had received multiple transfusions. Retrospective studies of specimens from the NIH cohort, led by

Paul Holland and Harvey Alter, subsequently confirmed an association between HBsAg in donor blood and occurrence of PTH.[6,7] These studies estimated that exclusion of HBsAg-positive blood donors through either first- or second-generation assays (e.g., agar gel diffusion or counterelectrophoresis) would decrease the rate of PTH by approximately 25 percent. A prospective study on the effect of exclusion of commercial donors and use of HBsAg-screened blood was reported in 1972 by Alter and associates.[8] This study confirmed the predicted 25 percent reduction in PTH by HBsAg screening and showed that elimination of commercial donors resulted in a 70 percent reduction in the PTH; the simultaneous exclusion of commercial and HBsAg-positive donors reduced PTH to 7.1 percent of the prior rate.

On the basis of these studies, routine screening of blood donations for HBsAg was inititated with so-called second-generation assays in 1971, with transition over the next several years to third-generation tests (i.e. radioimmunoassay or enzyme-linked immunoassay [EIA]). Although this led to further reduction in hepatitis virus (HBV)-related cases, PTH cases continued to be observed and particularly subclinical cases manifested only by persistent hepatic enzyme elevations in recipients.

Non-A, non-B hepatitis and surrogate markers

When it became clear that PTH cases continued to occur that were not caused by infections with HBV or other known viral agents (e.g., hepatitis A Virus, Epstein-Barr virus, and cytomegalovirus [CMV]),[9-11] the term non-A, non-B hepatitis (NANBH) was coined by Dr Harvey Alter at NIH. Retrospective analysis of NIH cohort data from the late 1970s and early 1980s suggested that NANBH represented 90 percent of residual PTH cases in the United States, with rates in multiply transfused patients as high as 10 percent. Several large studies were launched at that time to establish the incidence, correlates, and clinical consequences of NANBH, as well as to help identify the etiologic agent. The initial results of the multicenter Transfusion-Transmitted Viruses Study (TTVS), led by Jim Mosley, were published in 1981.[12] This report showed a substantial association between recipients with NANBH and donor alanine aminotransferase (ALT) concentrations, which was quickly confirmed by the NIH group and others.[13] TTVS subsequently reported an association between occurrence of NANBH in recipients of blood that tested positive for antibody to the core protein of HBV (anti-HBc).[14] The research group at NIH also confirmed this observation and showed that anti-HBc testing of donors, in concert with ALT testing, would eliminate 30 to 50 percent of recipient NANBH.[15] On the basis of these studies, in the late 1980s blood collection agencies in the United States began screening donated blood for anti-HBc and ALT as surrogate markers of NANBH.[16] The rate of PTH among recipients followed at the NIH subsequently dropped to 2 percent.[17]

Discovery of HCV as etiologic agent of NANBH

Extensive research was conducted in the 1970s and 1980s to identify the etiologic agent(s) of NANBH. More than 25 preliminary reports of associated agents were determined to be false. Then, in 1988, the HCV was identified with then-novel molecular biology techniques by Michael Houghton's group at Chiron Corporation in collaboration with investigators at the Centers for Disease Control and Prevention (CDC) and Harvey Alter at NIH.[18] The process of virus discovery involved construction of a cDNA expression library in a bacteriophage with high-titer plasma from a chimpanzee inoculated with PTH plasma. The library was screened for clones expressing putative viral antigens with serum from a recipient with chronic NANBH as a presumed source of viral antibodies. Screening more than 10,000 clones from this library led to the identification of a positive cDNA clone expressing what was termed 5-1-1 antigen.[19] The entire viral genome was sequenced within 1 year, and antigens were expressed for development of antibody detection assays. Early studies established that HCV was the etiologic agent of at least 80 percent of residual NANBH.[17] The era of molecular discovery of TTVI had been launched.

Subsequent studies by TTVS and NIH investigators, and many groups from around the world, quickly established the prevalence of HCV infection in donor and recipient populations and the rates and correlates of transmission of the virus by seropositive transfusions and plasma derivatives.[20,21] Studies of HCV infection in donor and recipient populations also contributed substantially to our understanding of the natural history, virology, immunology, and pathogenesis of liver disease in HCV infection.[22-24] Investigations of PTH thus were critical to the discovery of HCV and have continued to yield important scientific insights related to this important pathogen to this day.

AIDS and HIV

The success with progressive eradication of PTH in the 1980s was unfortunately overshadowed by the concurrent emergence of transfusion-associated AIDS. AIDS cases were reported in transfusion recipients and persons with hemophilia in 1982 and 1983, spawning global concern over the safety of the blood supply. In the United States, the Transfusion Safety Study (TSS) was launched by the National Heart, Lung, and Blood Institute in 1983 to investigate etiology of transfusion-associated AIDS, establish the magnitude of risk, and identify possible deferral policies, tests, or other measures to reduce the risk. This study was conceptualized and led by Jim Mosley at USC and

included many key blood bankers including Steven Kleinman, then at the American Red Cross in Los Angeles. One component of TSS was establishment of a repository of 200,000 donation specimens collected in late 1984 in five high-AIDS-prevalence cities.[25] Subsequent testing of these specimens for anti-HIV (and later for antibodies to human T-lymphotropic viruses [HTLV-I and -II] and HIV-1 p24 antigen) allowed for determination of the prevalence of HIV infection in the donor pool at that critical juncture and subsequently to studies that established rates of transmission to recipients and course of infection in donors, recipients, and persons with hemophilia.

A major contribution of TSS, and my first lead author article with that group, estimated the risk of HIV transmissions by transfusions over the decade before implementation of serologic screening in 1985.[26] This estimate was achieved by combining data from the TSS donor repository collected in late 1984 with HIV-1 seroincidence data among homosexual men and data from lookback investigations that traced recipients of prior donations from donors who later developed AIDS. With a model developed to integrate these data sources, it was possible to piece together an accurate picture of the risk of HIV-1 transmission by transfusion in the San Francisco Bay Area between 1978 and 1985 (Fig. 2). The analysis showed that the risk of transfusion-associated (TA) HIV-1 infection rose rapidly from its first occurrence in 1978 to a peak risk of approximately 1.1 percent per transfused unit in late 1982. This peak was followed by a marked, progressive decline in risk beginning in 1983 as a result of declining numbers of blood donations from at-risk individuals. This decline was attributable to increasing awareness of the infectious nature of AIDS in the homosexual community and implementation of donor education and deferral measures by blood banks.[26,27] These data illustrate the effectiveness of donor education and self-deferral measures at safeguarding the blood supply in the absence of specific testing options.

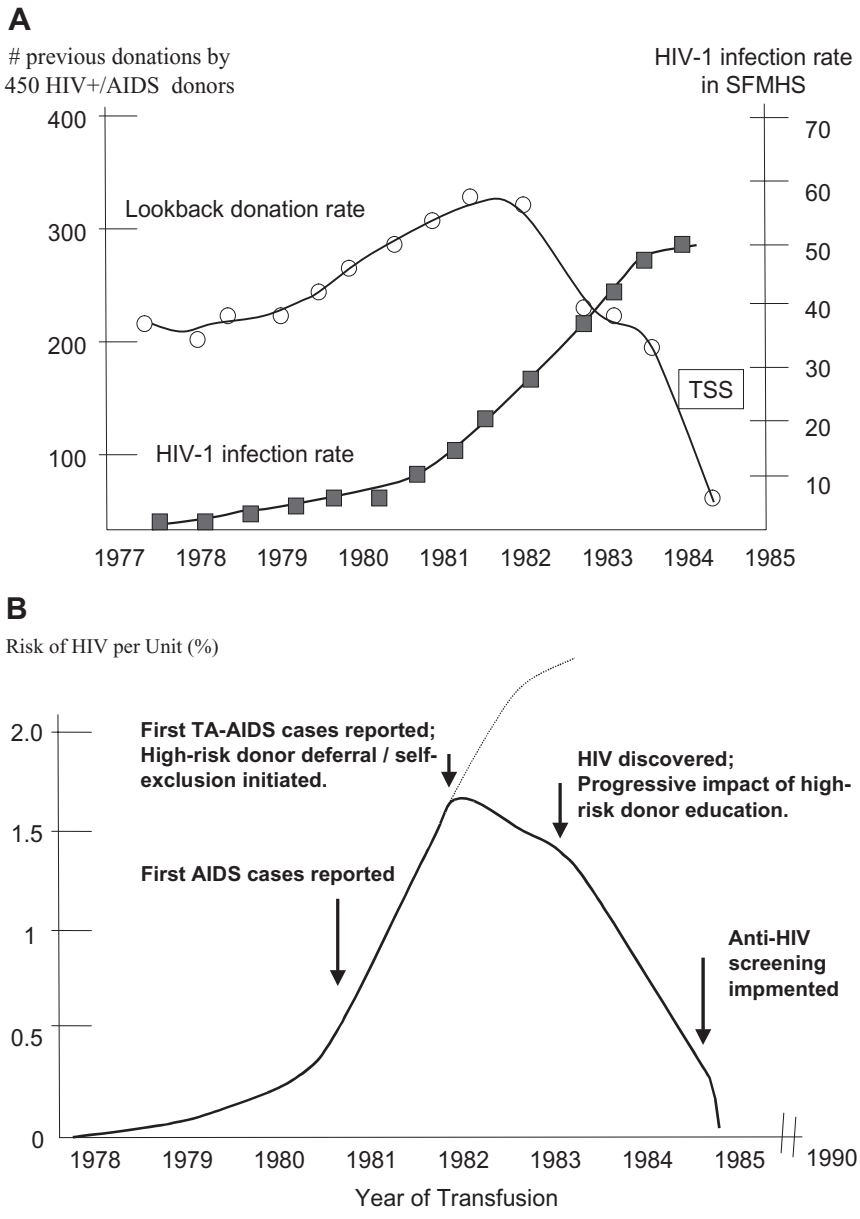

Fig. 2. Risk of HIV transmission by blood transfusion, before the implementation of HIV-1 antibody screening. (A) Source data, including the rates of prior donations by donors later identified as HIV-infected and hence triggering lookback and the accruing prevalence of HIV infection among high risk subjects in the San Francisco Bay Area (SFMHS). The period of collection of the TSS repository, from which HIV prevalence immediately before implementation of routine antibody screening was derived, is indicated by a box. (B) Results of the risk projection and demonstration of the dramatic decline in HIV risk coinciding with progressive implementation of high-risk donor qualification and deferral measures and preceding the availability of prospective antibody screening. Reprinted with permission from Busch et al.[26]

This temporal profile for the risk of HIV from blood components and derivatives was corroborated in a report from the CDC, in which 4619 TA AIDS cases reported from 1982 through 1991 were reviewed.[28] When plotted by year of implicated transfusion that led to HIV infection, the

number of cases rose from 56 in 1978 to 714 in 1984, then dropped sharply to 288 by 1985 when screening for antibody began, and decreased to a mean of 20 per year from 1986 through 1991. More than 95 percent of AIDS cases diagnosed subsequent to screening were attributable to transfusions received before implementation of anti-HIV screening in early 1985. Upon further investigation, the patients in most of the cases diagnosed from post-1985 transfusions were found to be infected from sources other than HIV-seronegative blood transfusions.[28]

The TSS was the only study that traced and enrolled recipients of known seropositive units, which allowed for direct measurement of HIV transmission rates and correlates of transmission by seropositive blood. We documented that 89.5 percent of recipients transfused with anti-HIV-1–positive blood components seroconverted to anti-HIV-1 positivity.[29] Variables that correlated with likelihood of HIV-1 transmission were type of blood component transfused and duration of red blood cell (RBC) component storage.[30,31] With subsequent development of viral load assays in the early 1990s, the TSS repository was further studied to establish the importance of the level of viremia for HIV transmission in cases of transfusion-acquired infection[32] as well as for heterosexual transmission from persons infected through transfusion to their sexual partners.[33]

TSS also enrolled infected donors, recipients, and persons with hemophilia into prospective follow-up to establish the rate of progression to clinical disease and clinical and laboratory correlates of progression. The rate of AIDS in recipients was found to be faster than the rate of progression among infected donors and persons with hemophilia followed in parallel.[34,35] Once age and underlying disease were controlled for, however, progression rates to clinical AIDS were virtually identical for TSS donors, recipients, and persons with hemophilia.[36] This suggests that factors such as route of infection, inoculum size, and proposed cofactors such as other viral infections (e.g., CMV or HBV) are not highly significant in determining the course of HIV disease.

Archived samples from the TSS also served to investigate the role of coreceptors for HIV-1 in parenteral transmission of the virus.[37] Recipients of HIV-seropositive blood units and coagulation factor concentrates who had a 32-bp deletion in the CCR5 gene were less susceptible to infection, although those who were infected progressed at the same rate as recipients with a normal CCR5 gene. TSS samples were also critical for the demonstration of rare cases of dual-strain HIV infection and subsequent recombination and to understanding the rate and determinants of molecular evolution of the same strain of virus in multiple donors and recipients in transmission clusters.[38-40] The significance of an HIV virulence gene, nef, was also demonstrated by Australian scientists based on a cohort of a blood donor and eight transfusion recipients who were infected with an HIV-1 viral strain lacking a functioning nef gene and who consequently had markedly prolonged disease-free survival without therapy.[41] Thus, as with HBV and HCV, studies of HIV-infected blood donors and recipients resulted in significant contributions to our understanding of viral transmission and pathogenesis that continue to this day.

Interestingly, transfusions not only proved to be an efficient mode of transmission for HIV, but, according to some evidence, may also affect the course of disease in patients with AIDS. Observations from retrospective studies and small prospective studies suggested that allogeneic transfusions from noninfected donors might accelerate disease progression in HIV-infected patients.[42] In vitro experiments from my group provided evidence that transfusion of allogeneic white blood cells (WBCs), presumably through immunologic activation of lymphocytes and macrophages, can enhance replication of HIV, whereas transfusion of autologous WBCs and allogeneic RBCs, platelets, and plasma did not show this effect.[43] The Viral Activation [by] Transfusion Study (VATS), a multicenter US clinical trial funded by the National Heart, Lung, and Blood Institute, addressed this issue and investigated the benefits of providing leukoreduced blood to patients with late-stage HIV infection. The study found no evidence of significant activation of HIV replication or accelerated disease progression in recipients of either leukoreduced or nonleukoreduced transfusions, perhaps due to the advanced state of immunodeficiency in the enrolled patients.[44]

ERA OF DIRECT RISK MEASUREMENT: 1985 THROUGH 1990

Direct measurement of HIV risk from antibody-screened transfusions

Shortly after introduction of HIV antibody screening in 1985, rates of infected donations declined markedly as seropositive donors were identified and deferred, and risk factor screening was enhanced based on findings from interviews of infected donors (Fig. 3). These data led to initial optimism that the problem of transfusion-transmitted HIV had been solved. Cases of AIDS linked to screened blood transfusions, however, continued to be observed. In one early study in the United States, 14 (13%) of 106 AIDS cases initially reported to CDC as possible infections from screened blood transfusions were confirmed as transfusion transmissions by linkage to donors who later seroconverted.[45] Although the CDC used these data to estimate that 10 overt TA-AIDS cases had occurred annually from 1986 through 1991 due to anti-HIV–screened blood transfusions,[28] it was recognized that this was an underestimate of the national recipient HIV infection rate, given poor case ascertainment due to underlying recipient

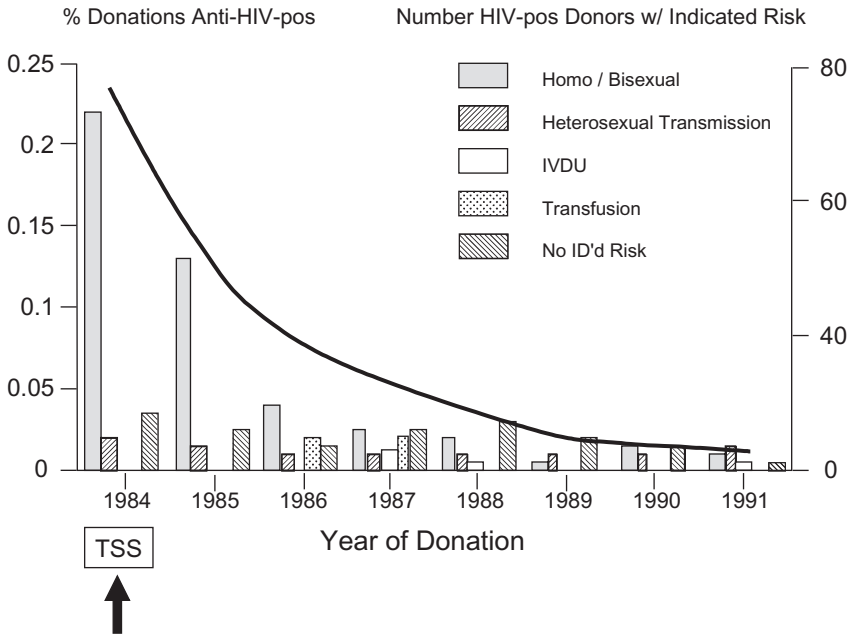

Fig. 3. Rates and risk factors of HIV-infected blood donors in San Francisco, California, through 1991. The dramatic decline in rates of infected donations was attributable to deferral of infected RPT donors and to improved questions for exclusion of high-risk donors and particularly men who had sex with men. Reprinted with permission from Busch.[64]

mortality and the long incubation period from infection to clinical AIDS.

In the United States, two prospective studies were launched in 1986 to specifically measure residual HIV risk from antibody-screened units. The first study (the Frequency of Agents Communicated by Transfusions Study [FACTS] led by Kenrad Nelson and Paul Ness at Johns Hopkins University) was carried out in Baltimore and Houston hospitals and involved testing patients for anti-HIV-1 before and after cardiac surgery.[46] Cases of recipient seroconversion were investigated to rule out other risk factors, and donors were traced to document the source of infection. Two cases of HIV-1 infection were documented after transfusion of 120,000 units of blood; seroconverting donors were identified in both cases. In the other study, Girish Vyas and I led a study that employed sensitive viral culture and polymerase chain reaction (PCR) assays to detect HIV-1 in pooled peripheral blood mononuclear cells derived from 200,000 anti-HIV–negative donations given in the San Francisco Bay Area from 1987 to 1990 (a prelude to subsequent introduction of minipool (MP) nucleic acid amplification testing [NAT] a decade later).[47] A single seronegative donation, collected in 1987, was identified as infected by both PCR and culture. After adjusting for assay sensitivity and the pooled analyses, a risk estimate of 1 in 160,000 donations was calculated.

In the late 1980s, those of us involved in blood safety began to realize that a more practical approach for estimating risk of HIV infection from transfusions was needed. The first seminal study that moved the field forward was led by Lyle Peterson at the CDC.[48] The approach involved the use of data from "lookback" investigations to estimate the rate of HIV-1 transmissions that occur from donors who donate in the "window period" preceding detectable antibody. This approach used data compiled by collaborating US blood bankers who traced previous recipients of 701 blood donors who had seroconverted to anti-HIV positivity before 1991. Recipients of preseroconversion donations were tested for 179 of the seroconverting donors; the most recent seronegative donation resulted in infection in 36 (20%) of these cases. Infection rates correlated indirectly with the time interval between the seropositive and seronegative donations (i.e., intervals of <90 days, 76% transmission; 91-180 days, 28% transmission; >180 days, 11% transmission). Mathematical modeling of these data indicated a median 45-day infectious WP for the overall period (1985-1990). Separate analysis of donations given prior and subsequent to March 1987 (when improved sensitivity EIAs were implemented in US donor centers) indicated a reduction in the median infectious seronegative window from 55 to 42 days. This study represents the predecessor of the incidence-WP model discussed later.

The p24 antigen controversy

Documentation of significant residual risk of HIV-1 transmission by WP donations fueled concern over blood safety that had been ignited by widening appreciation of the extent of infections that had occurred among persons with hemophilia and component recipients in the United States and elsewhere before antibody screening. Additional screening measures to interdict window-phase donations were considered. Seminal studies of serial plasma donor specimens (seroconversion panels) by Susan Stramer and Jean Pierre Allain, then at Abbott Laboratories, were reported in 1989, which documented detection of HIV-1 p24 antigen by screening EIAs 1 to 2 weeks before antibody seroconversion, leading to recommendations that this test be implemented into donor screening.[49] Two large studies were launched in the United States to directly evaluate the utility of p24 antigen screening. The first was a prospective, multicenter study led by Harvey Alter at NIH and Jay Epstein at FDA that

tested 500,000 donations for p24 antigen in parallel with antibody testing.[50] The second study, led by Jim Mosley and me at USC, involved p24 antigen EIA screening of 8597 specimens selected from the TSS repository based on the corresponding donors being male, less than 45 years old, with residence in high HIV prevalence zip codes; based on the 1.5 percent seroprevalence in the selected donation subset, these samples were equivalent to more than 2 million contemporary donations.[51] Both studies failed to detect p24 antigen–positive, antibody-negative donation, indicating very low potential yield. Furthermore, concerns were raised over a possible magnet effect, related to high-risk persons going to blood banks to get a more sensitive HIV assay, which could lead to increased rather than decreased risk, were antigen screening introduced (a theory later discounted by an analysis led by Jim Korelitz and me for the Retrovirus Epidemiology Donor Study [REDS] group).[52] Although these data led to an initial decision against p24 antigen screening, political pressure continued to mount and the FDA eventually mandated p24 antigen screening in the United States. Subsequent results from 5 years of national p24 antigen screening, compiled by Susan Stramer at the American Red Cross (ARC) and me, confirmed the findings of the earlier studies, with only 1 antigen-positive, antibody-negative donation detected per 6 million donations.[53] This experience highlighted the dilemma of trying to directly measure extremely low-level residual risk or predict the yield of new assays in the context of growing public expectation and political pressure for a zero-risk blood supply.

Direct measurement of transfusion risks of other viruses in the late 1980s

The FACTS repository discussed above was also used to directly measure risks of hepatitis viruses and human T-cell leukemia viruses in the late 1980s.[46,54,55] The data were particularly informative for HCV, since the study was conducted during the time period when NANBH surrogate markers and HCV antibody testing were first implemented. These researchers found that the risk of HCV infection was 0.45 percent per unit before the implementation of any testing. After the implementation of testing for ALT and anti-HBc, the rate of infection dropped to 0.19 percent per unit. Finally, once the first-generation EIA test for anti-HCV was implemented the rate dropped to 0.03 percent or 1 per 3300 units.[54] A subsequent reevaluation with the more sensitive second-generation EIA on blood recipients indicated that the risk was closer to 1 in 1700.[55] Once the second-generation test was implemented for blood donor screening, the frequency of residual infection declined precipitously, such that transmission cases could no longer be documented by prospective follow-up studies.

ERA OF RISK MODELING: 1991 THROUGH PRESENT

Principles and initial application of incidence rate: WP model

The large-scale studies that directly measured risk were discontinued in the early 1990s because of their high cost and the realization that they were too insensitive to assess residual risks of viral transmission from screened transfusions. An alternative approach had to be developed for estimating risk of screened agents. That approach is now known as the incidence rate-WP (IR-WP) model.[56] To appreciate this model, it is important to first understand the distinction between prevalence and incidence of infectious agents in the donor pool. Prevalence measures the percentage of donations that test positive on screening and confirmatory tests, including old and recently acquired infections; in contrast, incidence measures the rate at which new infections develop in a population at risk. Since donations from donors with prevalent infections are detected by serologic screening tests and interdicted, they pose minimal if any risk to recipients. The major threat to the safety of the blood supply are donations by donors in the infectious WP, that is, the time between infection, or more precisely infectivity, and detectability by screening tests. Additional sources of transfusion risk attributable to variant viral strains, immunosilent carriers, and testing errors[57-64] contribute only minimally to risk, especially when dual testing systems are in place, that is, serology tests targeting antibodies and antigen or NATs to detect viremia.

The IR-WP model was developed in parallel in the early 1990s by George Schreiber, Steve Kleinman, Jim Korelitz, and me working for the NLHBI-REDS study; by Eve Lackritz, Glen Satten, and Lyle Petersen from the CDC; and by Roger Dodd and colleagues at the ARC.[56,65-67] The model estimates the probability of a potentially infectious donation being released into the blood supply, or residual risk, by multiplying the IR by the number of days during which an infection may be present but not detectable by current screening assays (i.e., the length of the WP). The model therefore requires that the relevant viral IRs and WPs are known with accuracy. IRs have been classically derived by dividing the numbers of known confirmed incident cases (detected by serologic or NAT screening) by the number of person-years or the sum of the periods of time during which donors are at risk (i.e., are not infected yet). This derivation is usually confined to repeat (RPT) donors because this population is followed for a certain period of time allowing evaluation of how many donors are incident, that is, first tested negative on all assays and then tested positive for a particular marker on a subsequent donation. The minimum statistics required for IR calculations are the number of incident cases (the numerator), the total number of RPT donations, and the mean interdonation

interval length (multiplication of the last two entities provides the number of person-years or denominator). Alternatively, if donation histories are available for all RPT donors, we can derive person-years by summing up the length of all interdonation intervals. The interested reader should refer to recent reviews by Steve Kleinman, Simone Glynn, David Wright, and me for detailed discussion of adjustments, derivation of confidence intervals (CIs), and other factors impacting the accuracy of IR estimates.[56,67,68]

The second key statistic needed to estimate residual risk is the length of the WP for each test. WP estimates for HCV and HBV initially used in the IR-WP model were based on analyses of repository specimens from PTH cases.[64-66] Serial samples from HBV- and HCV-infected recipients were tested, and the time from transfusion to development of a positive test was calculated. Multiple studies involving testing of serial posttransfusion samples from more than 200 HCV cases demonstrated that the WP from transfusion to HCV antibody detection ranged from 60 to 80 days, depending on sampling intervals and whether second- or third-generation EIA was used to detect seroconversion (Fig. 4).[69-73] The WP for HBV was much less well defined, because the commonly cited estimate of 59 days from transfusion to HBsAg conversion was based on a series of only seven evaluable TTVS cases.[74] By combining these WP estimates with IR estimates from 1990 through 1993, the REDS group derived residual risk estimates for HCV of 1 in 103,000 and for HBV of 1 in 63,000.[65]

The WP estimate used for HIV in the initial IR-WP models was derived from the mathematical modeling of transfusion-transmitted infections occurring from HIV-seronegative units donated by persons who subsequently seroconverted.[48,64] Further laboratory studies by my group

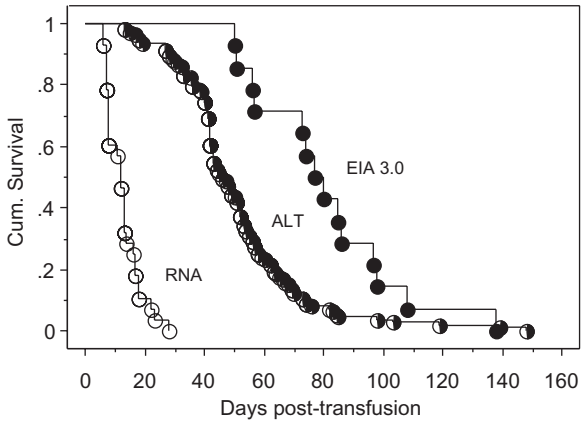

Fig. 4. Estimated times from transfusion to development of detectable RNA by NAT assays, elevated ALT, and antibody by third-generation EIAs (EIA 3.0). Results are based on Kaplan Meyer survival analysis of serial data from 94 HCV-infected recipients enrolled into the TTVS. Adapted from data in Operskalski et al.[20] and Mosley et al.[22] and unpublished analyses.

with samples from seroconvertors in high-risk cohort studies demonstrated that the HIV-1 WP had been reduced from a median of 45 days for the period from 1985 to 1990, derived from the lookback data, to approximately 22 to 25 days after introduction in 1992 of new format anti-HIV-1 and -2 EIAs, which detect HIV-specific immunoglobulin M (IgM) antibody 10 to 15 days earlier than previously available assays.[75] By combining the 25-day WP estimate with HIV IRs derived from RPT donors in large US donor populations, two independent studies derived point estimates for the risk for HIV transmission during the 1992 to 1995 period of 1 in 450,000[66] and 1 in 495,000,[65] respectively.

Although the early models used these WP estimates, we quickly realized that these estimates lacked precision and perhaps more importantly failed to focus on the subset of the exposure to seroconversion WP during which a donation is infectious. For example, it is unclear whether donations given in the early stages after exposure, that is, during the so-called eclipse (RNA-negative), pre–ramp-up intermittent RNA-positive, and very early ramp-up viremia phases of infection, contain enough virus to be infectious.[64] In particular, the biologic basis and infectivity of the low-level intermittent "blips" of RNA and/or DNA positivity, which we and others have documented in plasma donor seroconversion panels in the pre–ramp-up phase of HIV, HBV, and HCV infections, are not yet defined (Fig. 5).[76-78] Further studies including animal transmission experiments in appropriate model systems,[79] donor lookback studies[80,81] (where prior units from seropositive or NAT-positive RPT donors are traced and recipients of these units tested), and recipient traceback studies[82] (investigation by recall or testing of stored donation specimens of donors whose blood products were transfused to recipients who subsequently acquired an infection after transfusion) are now in progress to answer these fundamental questions and permit better estimation of risks. For example, Nico Lelie's group in the Netherlands[83] used minimum chimpanzee infectious doses data to predict human infectivity, relative to viral load and NAT assay performance, in a recently published mathematical model of WP risk. Although this approach is reasonable and rational, the animal infectivity data employed in the model were very limited, and it is unclear whether the animal data are directly transferable to humans. Further work in this area is now in progress by my group in collaboration with Harvey Alter, Kris Murthy, and Chris Miller here in the United States, as well as by Hitoshi Yokizawa's group in Japan, to define the minimal infectious dose and the infectivity of "blip" viremia for each of the major agents.

International application of IR-WP model

The IR-WP model has now been applied successfully worldwide to provide country specific viral-TTI esti-

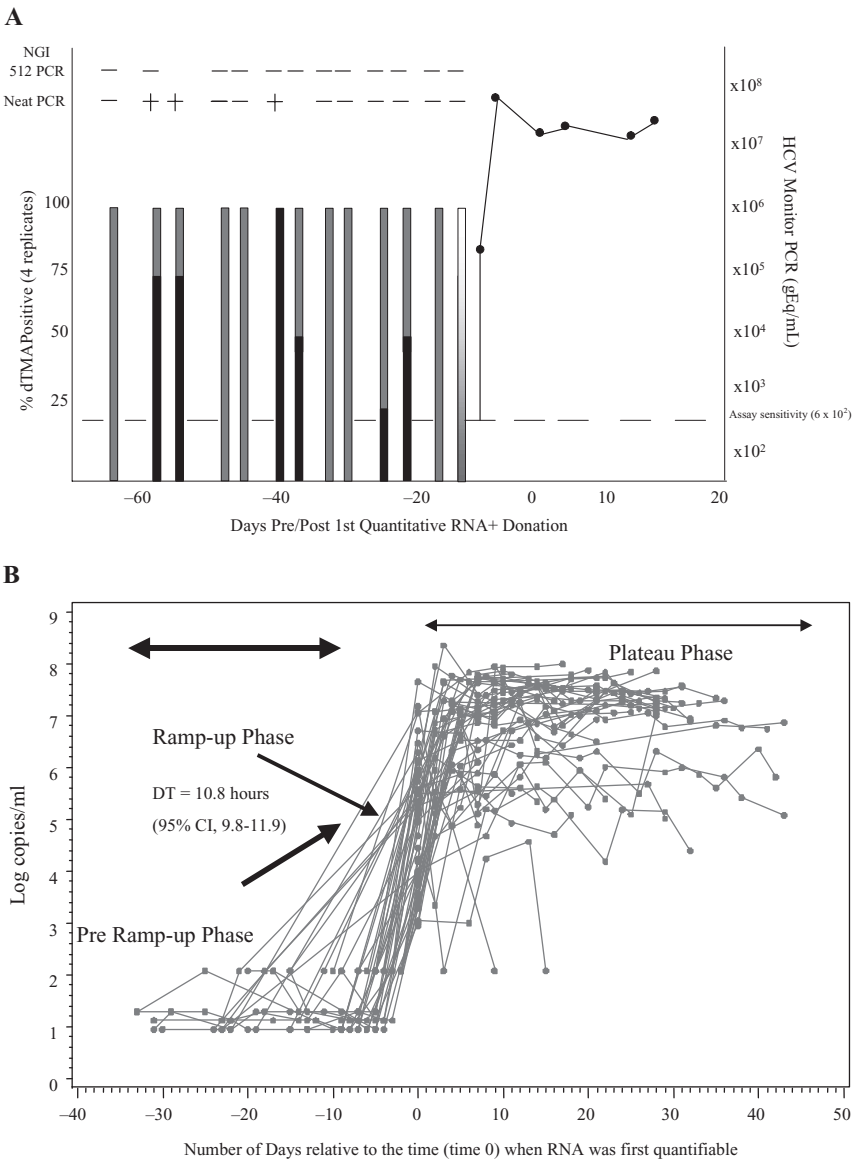

Fig. 5. Dynamics of early HCV infection based on analysis of plasma donor seroconversion panels. (A) Low-level intermittent viremia preceding "ramp-up" phase. (B) Viral load evolution in early HCV infection based on longitudinal regression analysis of data from 55 donors. Reprinted with permission from Glynn et al.[77]

mates.[84-92] In developed countries in Europe, North America, and the Asia-Pacific regions, the residual risk estimates are uniformly low and suggest that the donor selection process (self-selection, behavioral screening) and test screening adopted over the past several decades were very effective. More importantly, reports with the model clearly demonstrate that HIV and HCV residual risks have been further reduced to extremely low levels after MP-NAT implementation. For HIV, the estimated probability of having a potentially infectious donation being released in the blood supply varied from about 1 in 450,000 (Italy) to 1 in 3 million (Australia) donations before NAT, whereas residual risks after NAT varied between 1 in 900,000 (Italy) and 1 in 5 million. Even more marked was the reduction for HCV, with risks decreasing from approximately 1 in 120,000 (Australia, Italy) to 1 in 800,000 (France) donations before NAT (third-generation EIA) to approximately 1 in 1 million (Australia, Italy) to 1 in 10 million (France) after NAT. Although HBV risk estimates need to be evaluated with some caution, data from most reports suggest that of the major viral TTIs evaluated, HBV remains most likely to be transmissible by transfusion, with residual risks of 1 in 75,000 (Spain) to 1 in 500,000 (France) donations. Unfortunately, MP HBV NAT testing only reduces the WP by approximately 10 days[93] because the ramp-up viremia phase for HBV is characterized by a much slower doubling time (2.6 days) than for HIV (20.5 hr)[94] or HCV (14.9 hr).[76,77] The difficulty in decreasing HBV risks also stems from the fact that no risk factor can be identified in 30 to 40 percent of HBV cases[95] and consequent inability to develop donor screening criteria that are sensitive enough to effectively screen out HBV-infected donors and specific enough to avoid unnecessary deferral of healthy donors.

My team has been fortunate to collaborate with several groups in developing country settings to apply the IR-WP model to estimate residual risk and project the yield of NAT or p24 antigen screening. These studies documented substantially greater risk than exists in developed countries. For example, in collaboration with Ester Sabino and her colleagues, we estimated HIV risk in Brazil at 1 in 66,000;[96,97] with similar methods we worked with Anthon Heyns and colleagues in the Republic of South Africa to estimate HIV risk at 1 in 38,000 in the context of screening for both HIV antibody and p24 antigen.[98,99] Given the relatively high HIV incidence and residual risk levels in such countries, the yield and safety impact of NAT screening would be predicted to be relatively greater than in developed countries, albeit still small from a net yield and cost-effectiveness perspective.[97,99] Unfortunately, the financial resources to support NAT screening in developing country settings are limited. Nonetheless, NAT screening on individual donation (ID)

specimens has recently been implemented in the Republic of South Africa as a result of criticism and consequent discontinuation of their previous blood safety strategy that included use of race as an eligibility criterion.[99]

Limitations and refinement of IR-WP Model

The basic assumptions of the IR-WP model are that: 1) WP donations represent the most dominant source of risk, 2) overall IR in RPT donors is constant throughout the time period of the study, 3) a recently infected donor is as likely to donate in the early interval after infection as he or she is to donate subsequently, and 4) if no further adjustment is made, first-time (FT) donors have the same IR as RPT donors. The first assumption appears valid for HIV and HCV with WP-risk probably accounting for 90 percent or more of the total risk and other sources of risk (viral variants, chronic seronegative carriers, and testing error) for 10 percent or less.[64] This is also the case for HBV in countries performing anti-HBc screening, whereas HBsAg-negative and/or anti-HBc–reactive chronic carriers may contribute a level of risk equal or greater to WP risk in the absence of anti-HBc screening.[81,100] The second assumption is valid so long as the time period of the analysis is short; for longer periods of analysis it is important to consider changes in IRs over time that result from changes in the epidemiology of the agent in the population or revision of screening criteria.

The third assumption may be more problematic, because recent studies have noted that the interdonation interval preceding seroconversion may be longer than expected for HIV[101] or HCV incident cases.[102,103] These data imply that some donors delay their return around time of infection or are deferred at the donation site based on recent risk or symptoms or signs suggestive of recent TTVI infections. Consequently the model (which assumes a steady-state rate of donation) may tend to overestimate risk and the yield of tests that detect infection earlier (i.e., NAT or HIV p24Ag). Consistent with this concern, Seed and coworkers[102] reported that the duration of the seroconversion interval for HCV seroconverters in Australia was much longer than for the other viruses, and consequently the IR-WP model predicted three times the yield of HCV NAT–only donations than were observed. Similarly, an apparent difference in observed versus expected HCV yield was noted for several countries in Central Europe as recently reviewed by Juliet Coste, Henk Reesink, and others.[89,104] In contrast no significant difference has been found in predicted versus observed HCV yield data in France or the United States, compatible with the finding of no delay in return for HCV seroconverters in the United States.[101] Further, no significant differences were found in any study between observed (0.0-0.6 per 10^6 donations) and predicted (0.2-0.4 per 10^6 donations) yields for HIV.[89] Thus, the model seems to predict reasonably well the yield of new assays unless donors significantly delay their return around the time of infection and/or seroconversion.

Regarding the fourth assumption, the recent development of less-sensitive ("detuned") HIV-1 assay[106] and evaluation of NAT-yield data[105] have permitted direct assessment of the relative IRs in FT and RPT donors. Janssen and associates[106] reported a 2.4-fold higher IR in FT donors (7.18 per 100,000 person-years) than in RPT donors (2.95 per 100,000 person-years) in the United States with the sensitive and/or less-sensitive HIV-testing strategy. With NAT-yield data, Dodd and coworkers[87] and Stramer and coworkers[88] have confirmed this 2-fold differential in IRs between FT and RPT donors for HIV and extended this finding to HCV where the NAT yield ratio was 2.4. It is therefore now possible to estimate IRs in all donors if the relative proportion of FT and RPT donors is known and RPT donor IR are measured. This approach works well in developed countries where 70 to 80 percent of donations are from RPT donors. In contrast, in many developing countries a very high proportion of donations are from FT donors, and hence new approaches such as the less-sensitive EIA method are particularly useful to estimate FT donor incidence and derive risk estimates.[96-99]

"New strategy" for estimating risk based on viral dynamics and rates of detection of donors with incident infections

A new strategy for risk estimation has recently been developed with my colleagues in REDS and ARC. The strategy employs more accurate estimates of infectious and marker positive WPs for HIV, HCV, and HBV and then uses WP ratios and rates of detection of donors with incident infections to project risk.[107] The first component of this approach is based on back-extrapolation of data documenting increasing concentrations of viral nucleic acid in plasma during the ramp-up phase of primary viremia. The approach involves several steps. The first step is testing of plasma or blood donor seroconversion panels to determine the exponential viral growth rate during the ramp-up phase preceding serological marker reactivity—this is expressed as the number of days for the viral load to double (i.e., viremia doubling time).[76,77,94] The second step is to determine the number of viral nucleic acid copies present at the time that the relevant donor screening assays become positive. In the case of an HBsAg assay, this is defined as the number of HBV DNA copies present when the HBsAg signal-to-cutoff ratio is 1.0.[108,109] For a NAT assay, this is defined as the reported 50 percent limit of detection.[107-109] These viral load parameters are derived by regression or probit analyses and are dependent upon the sensitivity of the particular antigen or NAT donor screening assay used in each country or screening program.

Then, based on an assumption that infectivity first occurs when virus is present at a specified concentration (e.g., one viral copy per 20 mL of plasma, which is the amount of plasma assumed to be in additive-solution RBCs), an extrapolation (with the doubling time) is performed from the copy number at the HBsAg or NAT threshold of detection to the time at which the minimal infectious dose of virus would be present; this is the imputed length of the infectious WP. Figure 6 summarizes the estimated lengths of relevant WPs for HIV, HBV, and HCV.

The second component of the new strategy uses NAT screening data and/or HIV-1 detuned EIA data to determine the rate of donations in the early stages of infection detected by these assays. Table 1 summarizes representative NAT yield data from the United States and uses ratios of the pre-NAT infectious WP relative to the NAT yield WP for each agent to transform the NAT yield rates into residual risk rates (see Busch et al.[107] for details on derivation of these ratios). The table demonstrates that these rates projected from NAT-yield data are very similar to those derived with the now classical IR-WP model. This approach can also be used to project that conversion from MP- to ID-NAT would detect only 2 to 3 additional HIV-1 and HCV infectious units annually in the United States, whereas for ID HBV NAT would yield 25 to 30 infectious donations missed by HBsAg and MP-NAT.[107-109]

The new strategy approach for risk and yield projection is attractive because it 1) does not require discrimination of FT from RPT donations and therefore does not require adjustment factors to account for differential incidence 2), avoids biases related to the assumption that infected donors donate at a steady state during the preseroconversion WP, and 3) employs more accurate yet still conservative estimates for the infectious WPs. A collaborative project is now under way, under the auspices of the Infectious Diseases Working Party of the International Society of Blood Transfusion, to apply the new strategy approach to global NAT yield and less-sensitive EIA data.

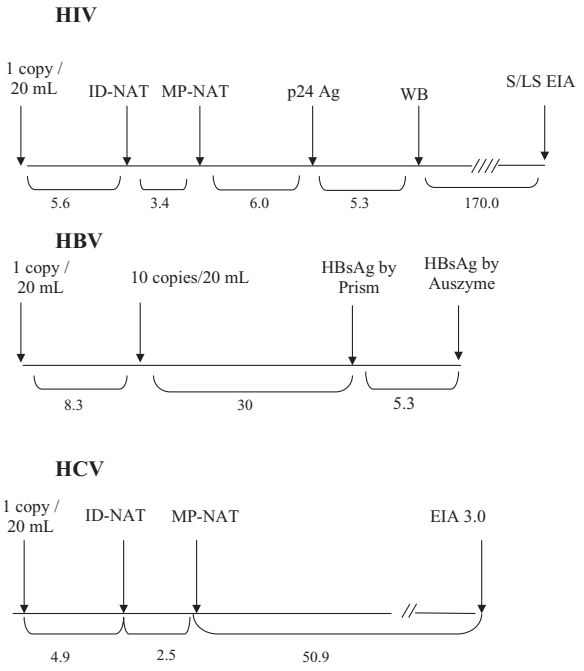

Fig. 6. Timelines from projected development of infectious viremia to earliest detectability of HIV, HBV, and HCV infections by different screening assays and resulting WPs between respective markers. Compiled from Busch et al.[107] and Busch and Kleinman.[109]

Use of IR-WP models in cost-effectiveness analyses and donor eligibility policy development

In addition to establishing residual-risk estimates, the estimates of test utility from IR-WP models have been widely used in cost-effectiveness analyses to inform policy debates over the merits of implementing NAT screening, converting from MP-NAT to ID-NAT, or introduction of pathogen inactivation.[110-112] They have also been used to inform discussions over donor recruitment and deferral policies such as acceptance of male donors who have had sex with other men in the past[113,114] or policies related to eligibility of donors from racial-ethic groups with different risk profiles.[99] These applications of models to inform policy development, although beyond the scope of this review, should prove increasingly important in the future as debates increase around the world over resource allocations for blood safety and ethical policies regarding donor qualification and deferral policies.

TABLE 1. Application of "new strategy" approach, based on NAT yield data and WP ratios, to project risks of transfusion HCV, HIV, and HBV, and comparison with risks derived from classic IR-WP model*

Virus	Number of cases	Number of units screened	MP-NAT yield	Risk derived from MP-NAT yield	Risk from IR-WP model
HCV	145	37.5×10^6	1 in 2.6×10^5	1 in 1.55×10^6	1 in 1.61×10^6
HIV	10	36.7×10^6	1 in 3.7×10^6	1 in 1.67×10^6	1 in 1.78×10^6
HBV	4	1.4×10^6	1 in 352,000	1 in 174,000	1 in 269,000

* Adapted from Busch et al.[107]

RESPONSE TO NEW AND EMERGING PATHOGENS: THE CHANGING PARADIGM OF TRANSFUSION PATHOGENS

Despite the dramatic progress over the past several decades in understanding and reducing risk from major TTVIs, there continues to be pressure to further enhance the safety of transfusions. A small number of cases of HIV transmission by units that had screened negative by current MP-NAT assays has lead to renewed pressure to implement even more sensitive ID-NAT screening methods. It is likely that NAT for HBV, HAV, and parvovirus B19 will be added to donor screening over the next several years, despite very limited clinical utility and poor cost-effectiveness. Concern over transmission of variant Creutzfeldt-Jakob disease (CJD) by transfusions contributed to the decision for accelerated implementation of WBC reduction and consideration of prion filtration methods, as well as to exclusion of donors with extended residence or transfusions in Great Britain and other European countries.

Table 2 compares the features of established and emerging pathogens of concern for blood safety. The previous paradigm for an emerging transfusion-transmitted pathogen was that it would cause a prolonged, asymptomatic carrier state, as was the case with HIV and HCV. The new paradigm includes a wide array of agents that do not fit the HIV or HCV model. The concept of a "global village" has emerged, which reflects the fact that a potential blood-borne infectious agent present in any region of the world could "traffic" to the United States overnight. This has led to increased concern over transfusion risk of parasitic agents such as *Trypanosoma cruzi*. There are increasing examples of zoonotic transmissions of infectious agents, with the potential for rapid adaptation of new agents to humans and subsequent spread to blood donors and then to blood recipients. Salient examples include probable origins of HIV-1 and -2 from and simian immunodeficiency viruses, HTLV-I and -II from primate T-lymphotropic viruses, and variant CJD from bovine spongiform encephalopathy. Proactive surveillance for such events is clearly important. There is intense research to identify new blood-borne infectious agents with novel molecular discovery strategies. Although recent examples of putative new agents have proven to be nonpathogenic (hepatitis G virus, TT virus, and SEN-V) or not transmitted at significant rates by transfusions (human herpes virus-8), every newly discovered agent requires serious investigation to assess its relevance to transfusion safety.

TABLE 2. Changing paradigm of "significant" transfusion pathogens

Classic transfusion pathogens (HBV, HIV, HTLV, HCV. *T. cruzi*)
Asymptomatic, persistent infections in donors
Proven transmission by transfusion
Proven disease associations
New agents of concern
Transient infections in donors (bacteria, HAV, B19, WNV)
Transfusion transmission not established (human herpers virus-8, enteroviruses, severe acute respiratory syndrom [SARS])
Zoonotic agents (simian immunodeficiency virus, HTLV-I and -II from primate T-lymphotropic virus, ecotropic recombinant virus, bovine spongiform encephalopathy, chronic wasting disease, WNV, SARS)
No established disease (GB virus/hepatits G virus, TTVs, SEN-V, human endogenous retroviruses)

To meet this challenge, investigations of putative transfusion-transmissible agents must be accomplished in a rigorous fashion. Such investigations must be rapidly executed and include: 1) determination of the prevalence and incidence of the agent in the population at large and the blood donor base (both historical and contemporary); 2) if the agent is present among donors, the probability of transmission by transfusion must be investigated through inoculation studies in animal models (if available) and testing of linked donor-recipient and hemophiliac samples in repositories; 3) if transmissible, studies must be conducted to determine the disease consequences of the agent in exposed and/or infected populations; and 4) if there is a serious disease risk to recipients, question- and/or test-based approaches for screening donors must be evaluated and implemented, and methods for removal or inactivation of the agent in plasma and/or plasma-derived (acellular) and cellular blood components investigated.

The recent example of the rapid and highly effective response of the transfusion medicine community to the WNV epidemic in the United States represents an excellent model for future responses to emerging infections.[115-120] The response included timely collaboration between blood collectors and public health agencies to document the extent of transfusion transmission and understand the dynamics of viremia and infectivity (Fig. 7), inventory control measures for frozen products, rapid development of NAT (rather than antibody detection) for blood donor screening, and implementation of a geographically and temporally targeted ID-NAT strategy until technology permits more universal application of ID-NAT.[119,120] It would be hoped that the extraordinary speed of response demonstrated in the US WNV epidemic (NAT screening implemented only 9-12 months after the first major year of the epidemic) can be duplicated for other emerging agents such as Dengue and pandemic influenza viruses.

The WNV story also illustrates how blood donor data and insights can have broad implications for public health. Because of the acute nature of WNV infection and the unpredictable nature of localized epidemics, blood collection agencies established liaisons with public health departments to monitor the extent of the epidemic in particular communities. Blood donor NAT screening data serve as an early indicator of regional epidemic activity and thereby compliment surveillance data from dead birds,

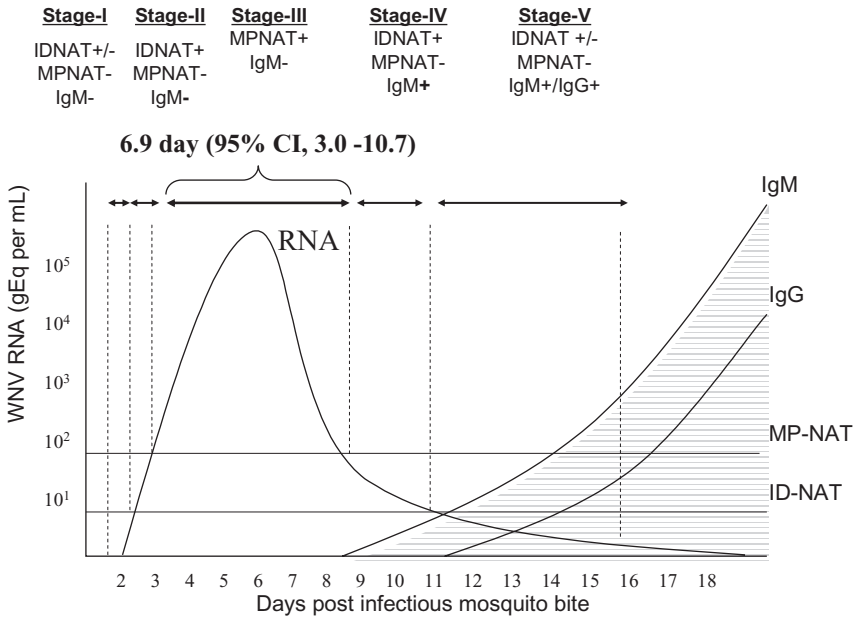

Fig. 7. WNV WP stages defined by results of MP- and ID-NAT and IgM and IgG serologic test results. The period of viremia detectable by MP-NAT (Stage III) lasts approximately 7 days. ID-NAT is able to detect low-level viremia missed by MP-NAT, both in the early "ramp-up" period (Stage II) and in the Postseroconversion Stages IV (IgM-only) and V (IgM and IgG detectable). MP-NAT breakthrough transmission cases have been documented following transfusion of units collected from donors in Stage II, but not in Stage I (ID-NAT–negative "eclipse" phase) or Stages IV or V (viremic "convalescent" phase). Based on data from Kleinman et al.,[115] Montgomery et al.,[116] Stramer et al.,[117] and Busch et al. Originally published as cover of TRANSFUSION; reprinted with permission.

sentinel bird flocks, and mosquitoes. Donor screening has a rapid turnaround time, infections are identified soon after WNV acquisition, many of these infections remain asymptomatic, and those that do become symptomatic are identified before illness onset. In addition to real-time surveillance, these donor data can be used for other epidemiologic purposes. A recent analysis of 2003 US blood donor data used an estimate of the mean time RNA is detectable by MP-NAT combined with MP-NAT yield in all US states to project the number of incident infections throughout the United States during 2003.[121] This number of mostly asymptomatic infections was then compared to neuroinvasive disease cases reported to the CDC. By use of this approach, it was estimated that 735,000 (95% CI, 322,000-1,147,000) WNV infections occurred in the United States in 2003 with 256 infections occurring per reported neuroinvasive case (95% CI, 112-401).

CONCLUSIONS

Our ability to understand, measure, and consequently reduce infectious risks from transfusions has evolved dramatically over the past 25 years. The rate of recent progress in developing modeling approaches for estimating risk and rational approaches to respond to new pathogens of concern are particularly noteworthy. Although there will continue to be new agents and new challenges, we now have a foundation based on science and open policy debate and formulation that should suit us well in the decades ahead. I am grateful to have participated in these exciting times and developments. And I sincerely appreciate the collaboration and support of my many colleagues in Blood Systems and Blood Centers of the Pacific, UCSF; the American Red Cross and Americas Blood Centers; and the FDA, CDC, and NIH.

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