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AIDS and Treatment of Hemophilia Patients

Margaret V. Ragni, MD

The story of AIDS in the hemophilia population has been scientifically fascinating, while at the same time medically devastating. The treatment successes of plasma in the 1950s, cryoprecipitate in the mid-1960s, and clotting factor concentrates in the 1970s which progressively enabled the hemophiliac to live a normal life, free from the shackles of frequent hospital treatment and disabling crippling arthritis, were, ironically, the very conduit of transmission of perhaps the most serious complication of hemophilia therapy, AIDS. Although hemophiliacs account for fewer than 1% of the total number of patients with AIDS, the incidence of AIDS in hemophiliacs is quite high (greater than 3 cases per 100)[1,2] and, unfortunately, AIDS has become a leading cause of death in this group.[3,4]

Scientifically, the problem of AIDS in hemophilia is unique because unlike other high-risk groups, they are a very well-characterized group, in whom close and frequent medical evaluation and followup is typical, sexually-transmitted diseases and herpesvirus infections are rare, monogamous heterosexual behavior is the norm, and a decline in further HIV exposure is expected. Thus, hemophiliacs are an ideal group to study transmission, genetic and epidemiologic co-factors, and ultimate prognosis of chronic HIV infection.

From the Pittsburgh Hemophilia Center, Pittsburgh Central Blood Bank, 812-5th Avenue, Pittsburgh, PA 15219, U.S.A.

What has arisen from this devastating problem of hemophilia-associated AIDS has been an intensified impetus to the long-standing quest to find new technologies to produce purer, safer replacement products, devoid of contaminating viruses and proteins to ensure the safest, most effective state-of-the-art treatment for hemophiliacs into the 21st century.

It is the purpose of this chapter to review the impact of AIDS on the treatment of hemophiliac patients, the timeframe of HIV seroconversion and seroprevalence, the risk of HIV transmission to household and family contacts, the safety of heat-treated factor concentrates, and the prognosis of HIV seropositive hemophiliacs.

I. IMPACT OF EARLY HEMOPHILIA-ASSOCIATED AIDS CASES ON TREATMENT

With the first report of AIDS in three hemophiliacs in July, 1982[5] arose the initial concern that AIDS might be caused by a transmissible agent. Although the occurrence of AIDS in intravenous drug users had been recognized since 1981, there was reason to believe that transmission in that group might be linked to other risk factors, e.g. homosexuality, and that isolated blood transmission in the absence of other risk factors was unlikely. Thus, the occurrence of AIDS in hemophiliacs provided the first strong support of the theory of

blood-borne transmission, but only in the context of frequent exposure to thousands of donor plasmas in the form of clotting factor concentrates. However, 5 months later, the recognition of AIDS in a transfusion recipient[6] raised serious questions about the potential transmission of AIDS through any blood products, whether multiple- or single-donor. This possibility became a major concern of the hemophilia community and triggered questions about the safety of the blood supply, not only for those whose risk was multiple-donor exposure, e.g. factor VIII and IX concentrates, but also for those whose potential risk was a single-donor transfusion, e.g. cryoprecipitate and fresh frozen plasma.

Without the benefit of identification of the presumed transmissible etiologic agent of AIDS, nor a screening test to protect the blood supply (Table 1), the National Hemophilia Foundation (NHF), in collaboration with the Centers for Disease Control (CDC) and Food and Drug Administration (FDA), established a surveillance mechanism to determine patterns of AIDS transmission among hemophiliacs and determine guidelines for hemophilia treatment based on these data. In December, 1982, after a total of eight cases of hemophilia-associated AIDS, all in concentrate-treated patients, the first NHF directive was issued, recommending that concentrates should not be introduced in those not previously exposed, including newborns and children through age 4 years, in newly-diagnosed patients, and in those with mild hemophilia (≥0.05 U/mL F VIII:C or F IX:C).[7] The following month, January, 1983, it was further recommended that manufacturers of clotting factor concentrates exclude high-risk donors who might transmit AIDS, and further, that they expedite processing methods to inactivate potential infectious or viral agents present in concentrates.[8] In response to these directives, several concentrate manufacturers developed questionnaires to screen plasma donors for high-risk characteristics and began to consider the possibility of using the technique of heat-treatment in concentrate production, a procedure proven effective in neutralizing transmissible agents in other products, e.g. albumin. These strategies were just beginning to be developed to reduce hepatitis viruses in concentrates, and presumably could reduce the infectivity of other infectious agents, including a possible AIDS agent.[9]

At the same time as attempts were initiated to make blood products safer, studies were beginning to show similar patterns of immune dysfunction in hemophiliac patients with AIDS as well as asymptomatic hemophiliacs. These included abnormal T helper/suppressor ratios, abnormal *in vitro* lymphocyte responsiveness to nonspecific mitogens and specific antigens, and diminished natural killer cell activity;[10–13] the latter appeared to be more common in factor VIII and IX concentrate-treated than cryoprecipitate- or plasma-treated (single

Table 1. Impact of AIDS on Hemophilia Treatment: Chronologic sequence of events

1st cases of AIDS in hemophiliacs	7/82
1st case of transfusion-associated AIDS	12/82
NHF directive: cryoprecipitate/FPP for newborns, children <4 yr, newly diagnosed and mild patients	12/82
NHF directive: DDAVP in mild/moderate hemophilia A patients, delay elective surgery, manufacturers advised to exclude high risk donors	1/83
1st reports of abnormal immune function in asymptomatic hemophiliacs	12/83
HTLV-III/HIV identified as etiologic agent of AIDS (Gallo)	5/84
HIV is shown to be heat sensitive	3/85
NHF directive: heat-treated concentrates are preferred products. HIV antibody screening of all blood donors recommended.	4/85

donor) patients, and suggested a greater risk for AIDS in hemophiliacs treated with concentrate, e.g. multiple donor exposure. However, long-term exposure to concentrates was not necessary for the occurrence of AIDS, as illustrated by the development of AIDS in hemophiliac children with only a few years of concentrate exposure. Further, the fact that immune abnormalities occurred in asymptomatic hemophiliacs receiving single or multiple-donor products suggested that immunologic defects might arise from blood product treatment alone.[13,14] These findings also introduced concern about the role of underlying chronic immune defects and susceptibility to AIDS.

Based on these data and on a 1983–4 collaborative study by the NHF and CDC which revealed no common lots of concentrate among the small but growing number of AIDS cases in hemophiliacs,[15] the NHF, as a precautionary measure, began to recall certain lots of clotting factor concentrates, in which a plasma donor had subsequently been identified with AIDS.[16,17] A delay in elective surgery in hemophiliac patients was also advised, to avoid exposure to large amounts of concentrate typically required with surgery, to avoid exposure to a potential AIDS agent.

Thus began a series of guidelines for hemophilia treatment, early on in the AIDS epidemic, which were and continue to be issued and regularly updated by the NHF, in collaboration with the CDC and FDA, based on careful review of the latest medical and scientific information in the areas of AIDS surveillance, AIDS-related clinical observations, and advances in product safety. However, the identification of the etiologic agent of AIDS, the Human Immunodeficiency Virus (HIV) was not to come for another year and a half (Table 1), and the initial demonstration of heat-inactivation of HIV in concentrates was not to occur for another 2 years (see Part IV).

II. TIMEFRAME OF HIV SEROCONVERSION AND SEROPREVALENCE IN HEMOPHILIACS

Unfortunately, at the time that the first cases of AIDS in hemophiliacs were being recognized, the majority of hemophiliacs had already been exposed to the AIDS virus (HIV).

Retrospective studies of stored frozen hemophiliac blood samples, using AIDS antibody assays[18,19] developed shortly after the isolation and characterization of the AIDS retrovirus,[20–24] revealed that the peak in hemophiliac seroconversion occurred in 1982 and 1983, with the earliest seroconversions in 1978[25–28] (Table 2), shortly before the beginning of the AIDS epidemic among homosexual men and intravenous drug users. Thse data also fit well with the first known cases of AIDS in hemophiliacs in late 1981 and early 1982.[29] The majority of hemophiliacs who had developed AIDS were treated with factor VIII concentrate, with only a minority treated with factor IX concentrate,[30,31] and only rarely cryoprecipitate[30,32] HIV antibody prevalence was 80–90% amongst those treated with factor VIII concentrates, 30–40% in those treated with factor IX concentrates, 10–15% in

Table 2. Timeframe of HIV Seroconversion in Patients with Hemophilia: Cohort of 78 HIV Antibody Positive Hemophiliacs in Western Pennsylvania

	No.	Cumulative %
1977	0	0/78 (0%)
1978	2	2/78 (2.6%)
1979	2	4/78 (5.1%)
1980	2	6/78 (7.7%)
1981	13	19/78 (24.4%)
1982	28	47/78 (60.3%)
1983	19	66/78 (84.6%)
1984	3	69/78 (88.5%)
1985	7	76/78 (97.4%)
1986	1	77/78 (98.7%)
1987	1	78/78 (100%)
	78	

those treated with cryoprecipitate, and 0% amongst those receiving fresh frozen plasma[26,28,33–40] (Table 3). These figures were similar across the U.S. and abroad, consistent with world-wide distribution of concentrates within a short timeframe of production.

The low prevalence of HIV antibodies in patients treated with factor IX concentrate was of interest because it suggested that the frequency of transmission of HIV might be lower with factor IX concentrate than with factor VIII concentrate. This, in turn, pointed to differences in the manufacture of these concentrates which might account for a differential ability to transmit virus. Although both factor IX and VIII concentrates do transmit hepatitis B and nonA, nonB hepatitis, there are differences in the production of these products. Both are prepared from frozen-thawed plasma, factor IX concentrate from the supernatant of frozen-thawed plasma, with subsequent diethylaminoethyl (DEAE) ion exchange absorption,[41] and factor VIII concentrate from the precipitate of frozen-thawed plasma, from which the Vitamin K-dependent factors and fibrinogen are removed by cold ethanol fractionation and precipitation.[42] This difference suggested that the plasma precipitate from which factor VIII concentrate was derived might contain cellular debris which might contain HIV in higher concentrations than the supernatant of plasma from which factor IX concentrate is prepared; alternatively the DEAE absorption might serve to inactivate HIV in FIX concentrate. However, this issue is still unresolved, for a number of reasons, not the least of which is the inability to detect HIV viral particles, viral antigens or antibodies in concentrates.[43,44]

Some of the early serologic studies of hemophiliacs suggested that HIV seropositivity might not be caused by infectious virus but rather by immunization with noninfectious HIV proteins derived during the processing of plasma into factor concentrates.[37] However, the increasing AIDS incidence with increasing duration of seropositivity (Table 4)[26–28] and the development of HIV-related symptomatology in 50% of an infected cohort[28] confirmed that hemophiliacs were exposed through clotting factor concentrates to infectious viruses and not just viral antigens.

Thus, within a short period of time following isolation of the AIDS retrovirus and development of an AIDS antibody test, serologic studies in hemophiliacs across the country and abroad established strong evidence that infectious retroviruses were transmitted through clotting factor concentrates, that concentrates probably contained the AIDS virus as early as 1978, that the peak transmission occurred in 1982 and 1983, and that transmission of HIV accounted for the development of AIDS and HIV-related symptomatology and isolation of HIV in hemophiliacs.[35,21]

Table 3. HIV Seroprevalence in Patients with Hemophilia

	Blood Product Treatment			
	FVIII	FIX	CRYO	FFP
Melbye 1984[33]	12/21 (57%)	1/1 (100%)	—	—
Ramsey 1984[34]	18/25 (72%)	0/4 (0%)	—	—
Koerper 1985[35]	28/28 (100%)	—	2/14 (14%)	—
Goedert 1985[36]	51/69 (74%)	0/12 (0%)	0/22 (0%)	
Evatt 1985[37]	18/21 (85%)	2/6 (33%)	—	—
Lederman 1985[38]	18/23 (78%)	—	0/26 (0%)	—
Gjerset 1985[39]	20/26 (77%)*	—	6/15 (40%)	—
Ragni 1986[26]	61/82 (74%)	9/30 (30%)	10/59 (17%)	0/19 (0%)
Goldsmith 1986[40]	10/18 (56%)	0/5 (0%)	0/3 (0%)	0/12 (0%)

* Includes a small number of FIX concentrate-treated patients. FVIII is factor VIII concentrate; FIX is factor IX concentrate; CRYO is cryoprecipitate; and FFP is fresh frozen plasma.

Table 4. AIDS Incidence in HIV Ab(+) Hemophiliac Cohort, Western Pennsylvania

Year Seroconversion	No. Seroconverting	AIDS Incidence by Yr of Seroconversion	Cumulative AIDS Incidence	No. Years Seropositive
1978	2	1/2 (50%)	1/2 (50%)	
1979	2	1/2 (50%)	2/4 (50%)	
1980	2	2/2 (100%)	4/6 (67%)	
1981	13	4/13 (31%)	8/19 (42%)	
1982	28	3/28 (11%)	11/47 (23%)	
1983	19	3/19 (16%)	14/66 (21%)	
1984	3	0/3 (0%)	14/69 (20%)	
1985	7	2/7 (29%)	16/76 (21%)	
1986	1	0/1 (0%)	16/77 (21%)	
1987	1	0/1 (0%)	16/78 (21%)	
	78	16/78 (21%)		

III. RISK OF HIV TRANSMISSION TO HOUSEHOLD AND FAMILY CONTACTS OF HEMOPHILIACS AND RISK TO SEXUAL PARTNERS

1. Risk to Sexual Partners

Very early in the AIDS epidemic, there were suggestions that the disease could occur in female sexual partners of men in high risk groups, including intravenous drug users[45,46] and bisexual men.[46,47] However, the majority of these women belonged to high-risk groups themselves, through their own lifestyles, e.g. prostitution and intravenous drug use.[47,48] Because the development of AIDS in these women could be attributed to the latter risk factors alone, the importance of heterosexual transmission of AIDS as a risk factor alone remained unclear. In contrast, the female sexual partners of hemophiliacs, in general, were healthy and had no other AIDS risk factors, and hemophiliacs themselves rarely had multiple sexual contacts, sexually transmissible diseases, or herpesvirus infections,[49] potential cofactors associated with development of AIDS in other high-risk groups. Further, the paucity of AIDS cases in hemophiliacs early in the AIDS epidemic and the absence of immunologic abnormalities in female sexual partners of hemophiliacs[50,51] as compared with the marked immunodeficiency in female sexual partners of men in other high risk groups,[45–46,52] suggested that the risk of AIDS in female sexual partners of hemophiliacs was small. However, the 1984 report of AIDS in the wife of a hemophiliac[53] and of AIDS-like symptoms in the sexual partner of a hemophiliac[54] and the subsequent demonstration of a small but growing (10–15%) prevalence of antibody to HIV in some female sexual partners of hemophiliacs[49,55–58] confirmed that the risk of heterosexual transmission, although low, was real. Subsequent to these reports, HIV was isolated in semen,[59] vaginal and cervical fluids,[60,61] and saliva,[62] and heterosexual transmission was shown to be bidirectional.[63] Although early reports by Melbye *et al.*[64] suggested that anal intercourse was a possible factor in heterosexual transmission, the lack of anal intercourse in HIV antibody positive hemophiliacs and their female sexual partners, some of whom became HIV antibody positive, provided evidence that anal intercourse was not necessary for heterosexual transmission of HIV.[49] Studies showing continuing low prevalence of HIV transmission from HIV infected hemophiliacs to their female sexual partners[49,55,57,64,66] confirmed that vaginal intercourse was a definite, but not a very effective mode of transmission.

Based on these studies and the growing, but small, number of cases of heterosexually transmitted AIDS, the NHF

issued a series of directives[67–72] beginning in August, 1984, to hemophiliacs to decrease the risk of transmission of HIV to their sexual partners and future offspring (see below), including the consistent use of condoms, and deferral of pregnancy. In addition, efforts were begun by NHF to provide education and risk reduction counselling programs for hemophiliacs and their families. However, despite these efforts, there has been recent evidence that only a minority of couples are complying with condom use.[49] These data suggest that great strides in educational and counselling efforts must be made if HIV transmission is to be prevented.

Why some female sexual partners become infected and others remain uninfected is unknown. Sexual frequency, condom use, and assistance with blood product infusion do not appear to be related to seropositivity in female sexual partners of hemophiliacs.[49] At least one study suggests the importance of the immune status of the hemophiliac, specifically a low T_4 lymphocyte count, in transmission of HIV from infected hemophiliacs to their female partners.[65] However, the heterosexual transmission to sexual partners of hemophiliacs has been documented when the T_4 exceeded 500/mm^3, and has occurred in partners of asymptomatic hemophiliacs (Table 5), suggesting that a low T_4 count is not necessary for heterosexual transmission of HIV.

Although low rates of HIV transmission to sexual partners of hemophiliacs were initially attributed to low isolation rates of HIV from peripheral blood lymphocytes of hemophiliacs,[49,55] more recent studies (Ragni, *et al.*, unpublished), show nearly 100% HIV isolation from peripheral blood lymphocytes of hemophiliacs with AIDS and ARC and 75%+ in asymptomatic hemophiliacs. Thus, other host or risk factors must play a role in heterosexual HIV transmission to partners of hemophiliacs. This remains an area of intense study.

2. Risk to Offspring

Approximately 1 year after the initial cases of AIDS and immunodeficiency were described in female sexual partners of high risk men,[45,46,52] the first AIDS cases in pediatric patients were recognized.[73–75] It was postulated that AIDS in these young children occurred via transplacental[76,77] or perinatal[78] transmission from mothers at risk for AIDS. This was corroborated by the subsequent isolation of HIV from amniotic fluid,[79] fetal tissue,[80] and breast milk,[81] and by

Table 5. HIV Transmission in Female Sexual Partners of Hemophiliacs, Western Pennsylvania

By Blood Product Treatment

	HIV Ab Prevalence	
	Hemophiliac	*Female Sex Partner*
FVIII	31/31	4/31 (13%)
FIX	8/8	1/8 (13%)
CRYO	1/1	0/1 (0%)
	40/40	5/40 (13%)

By Hemophilia Diagnosis

	HIV Ab Prevalence	
	Hemophiliac	*Female Sex Partner*
AIDS	11/11	2/11 (18%)
ARC/Other*	8/8	1/8 (13%)
Asymptomatic	21/21	2/21 (10%)
	40/40 (100%)	5/40 (13%)

* Other includes CDC Class IV HIV infection (Herpes zoster, oral candidiasis).

the fact that the epidemiologic characteristics of most children with AIDS reflected those of the female heterosexual risk group, e.g. intravenous drug use. In 1983, however, the low incidence of AIDS in hemophiliacs, the initial absence of immunologic abnormalities, ARC, or AIDS in spouses of hemophiliacs,[50,51] and the lack of other risk factors in hemophiliacs and their spouses/partners suggested very little likelihood of transmission from hemophiliacs to their offspring. In fact, only a few cases of AIDS have since occurred in children of hemophiliacs, the first in 1984[82] and HIV seropositivity has been detected in fewer than a dozen children of hemophiliacs,[83,84] one of whom has lymphadenopathy and recurrent infections.[83] Studies have shown that the risk of transmission in offspring of hemophiliacs appears to be high if the mother is HIV antibody positive: between 60 and 69% of children born to HIV antibody positive female sexual partners of hemophiliacs are HIV antibody positive.[83,84] Similarly, up to 50% of children of infected mothers in other risk groups have become HIV antibody positive.[85–87] However, it is clear that the numbers of infected patients are still too small to draw any conclusions about the risk of AIDS and the mode of delivery or breast feeding. Some preliminary data do, however, suggest that the risk of HIV infection in the infant is higher when the mother has symptoms of HIV infection during pregnancy. Clearly, prospective studies are needed to further delineate the risk factors associated with the development of AIDS in offspring of women in high risk groups, including the sexual partners of hemophiliacs.

Currently, the NHF recommends that hemophiliacs and their sexual partners use condoms consistently and defer pregnancy (see above)[67–72] to prevent HIV transmission to partners or offspring. The continuing birth of children to hemophiliacs and lack of consistent condom use in a majority of hemophiliacs[49] despite counselling efforts, strongly suggest that increasing efforts must be made to improve and intensify the approach to education and risk-reduction counselling for both hemophiliacs *and* their partners to prevent further HIV transmission.

3. Risk to Household Members

A number of investigators have shown that the only household contacts of adults with AIDS who are at risk for HIV transmission are their sexual partners or infant offspring exposed *in utero*.[63,66,88,89] This appears to be true for hemophiliac households as well as for other high-risk households. Close contact with AIDS and/or ARC patients does not appear to be an efficient mechanism for HIV transmission: specifically, there appears to be no risk between an infected patient and family member, nor does there appear to be any risk associated with assistance of hemophiliacs with home infusion of blood products. However, at least one report has documented HIV transmission between an infected infant with AIDS and his mother whose chapped hands and cuts presumably exposed her to the child's infected body fluids.[90] This latter case, and several reports of nonneedle stick exposure in health-care workers[91] underline the importance of adherence to infection control guidelines for those assisting with home factor concentrate infusion.[92] The NHF has also recommended that personal items such as razors, toothbrushes, or drinking or eating utensils not be shared, and that persons assisting hemophiliacs in blood product infusions should use bleach to clean up spills.[69]

IV. SAFETY OF HEAT-TREATED CLOTTING FACTOR CONCENTRATES

In response to the mounting evidence for HIV transmission through plasma-derived clotting factor concentrates, a number of physical and chemical inactivation procedures were developed to eliminate contaminating viruses from these blood products (Table 6). Because concentrates are manufactured from

Table 6. Safety of Inactivated Clotting Factor Concentrates

Inactivation Technique	Product Safety	
	NANB	*HIV*
I. *Physical Inactivation*		
A. Dry heat[98]	Yes [108–110]	No [99–101] Yes [102–107]
B. Dry heat with chloroform[110]	Yes [11]	No [118]
C. Wet heat, pasteurized[44,108]	No [112,113]	No [108,112,113]
D. Wet heat, n-heptane suspension[115]	No [116] Yes [114,117]	No [118]
E. Wet heat, steam heat[118]	No [118*]	No [118]
II. *Chemical Inactivation*		
A. β-propriolactone and u.v. irradiation	No [118,120]	No [108,118]
B. Tri (n-butyl) phosphate with detergent[121]	No [121,122]	No [121,122]
C. Monoclonal antibody affinity chromography[123]	No [124]	No [124]

* HBV was transmitted.

plasmas of thousands of donors, the transmission of hepatitis B[93,94] nonA, nonB hepatitis,[95,96] and delta hepatitis,[97] in addition to HIV, is common in concentrate-treated hemophiliacs. Attempts to reduce the potential infectivity of these products were made for a number of years before the occurrence of AIDS and HIV transmission through blood products. In 1983, prior to isolation of HIV, the FDA and various manufacturers of concentrates began to develop a heat-treatment technique to reduce the level of hepatitis viruses, both hepatitis B and nonA, nonB hepatitis. Heat treatment had been previously shown to be effective in neutralizing transmissible agents in other blood products, e.g. albumin, and thus it was logical to consider this technique for concentrates. One problem in determining efficacy of any inactivation technique, however, was the inability of various isolation and serologic assays to detect HIV viral particles, viral antigen or antibody in concentrates.[43,44] Thus, products had to be spiked with infectious virus and the log reduction of virus established for *in vitro* studies; for *in vivo* studies, patients had to be followed for HIV seroconversion, hepatitis B markers or transaminase elevation (for nonA, nonB hepatitis), in order to demonstrate safety or adequate inactivation.

A number of physicochemical inactivation techniques have been studied (Table 6). We shall consider each briefly, specifically focusing on the safety of each product in terms of HIV and nonA, nonB hepatitis transmission.

HIV was first shown by McDougal *et al.* in 1985,[98] to be heat-labile when subjected to temperatures between 60 and 68° for between 20 and 72 hr. The greater the temperature and the longer the time of heating, the greater the log reduction in HIV. Early studies of the dry-heat inactivated concentrates (heating in the lyophilized state) by Rouzioux *et al.*, Felding *et al.* and Gazengel and Larrieu[99–101] revealed no HIV seroconversion in hemophiliac recipients. This led to the recommendation in March, 1985, by the NHF that heat-treated concentrates should be the preferred choice of treatment.[68–69] However, subsequent studies by van den Berg *et al.*, Mannucci *et al.* and Ludlam *et al.* and others revealed a small but definite risk of HIV seroconversion among heat-treated concentrate users,[102–107] as well a definite transmission of nonA, nonB hepatitis, based on alanine aminotransferase (ALT) elevation in recipients as reported by Spire, Colombo, and others.[108–110] Because of the persisting nonA, nonB hepatitis risk with heat-treated concentrates, the NHF suggested in its April, 1985, directive that treatment with single-donor products

(cryoprecipitate or fresh frozen plasma) might be preferrable in individuals especially from areas of low AIDS incidence.[32] Further, because of the small but potential risk of HIV transmission through heat-treated concentrates, the NHF recommended that all donor plasmas be screened by the newly developed and licensed AIDS antibody screening test.[32]

Because of the inability of dry heat to completely inactivate HIV, Spire and Hilfenhaus[108,111] separately began to evaluate a heating technique in the liquid or "wet" state at 60° for 10 h, termed pasteurization, a process which had been used previously to inactivate hepatitis B in concentrates and other plasma proteins. *In vivo* studies by Schimpf, Mosseler, Carnelli and Spire demonstrated absence of HIV transmission and a marked reduction in nonA, nonB hepatitis transmission in recipients of pasteurized concentrates.[108,112–114] When lyophilized concentrates were suspended in n-heptane and then heated "wet", as developed by Hildenbrant *et al.*,[115] there also appeared to be no HIV transmission and a marked reduction in but not complete elimination of nonA, nonB hepatitis transmission.[114,116,117] The use of steam heat to inactivate transmissible viruses in concentrates[118] also appeared to eliminate HIV and nonA, nonB hepatitis transmission, but did not completely eliminate hepatitis B virus transmission. Thus, in contrast to dry-heated products, "wet"-heated or pasteurized concentrates resulted in no HIV transmission and a marked reduction in nonA, nonB hepatitis transmission.

The value of HIV antibody screening as an adjunct to heat-inactivation of concentrates has been demonstrated in several studies. In Western Pennsylvania, of 48 HIV seronegative hemophiliacs treated with heat-inactivated concentrates, two (4%) seroconverted during a 2-year monitoring period; both were exposed exclusively to heat-inactivated, unscreened concentrates (Ragni, unpublished) (Table 7). A CDC study of 13 hemophilia treatment centers in Canada, Australia and Europe revealed seven

Table 7. HIV Seronegative Hemophiliac Cohort: Use of Heat-treated FVIII and IX Concentrates

	No. HIV Ab(+)
FVIII	2*/28 (7%)
FIX	0/20 (0%)
	2/48 (4%)

* Includes two patients who received unscreened, heat-treated concentrate.

(<1%) HIV seroconversions, all occurring in association with heat-inactivated, unscreened concentrate treatment 107. A third study by Lawrence *et al.*[119] reported four seroconversions among 1494 HIV seronegative hemophiliacs treated with heat-inactivated, unscreened concentrate, as compared with no seroconversions among 1365 patient-years of heat-inactivated, donor-screened product. Thus, these studies clearly underscored the importance of HIV antibody screening in combination with viral inactivation procedures and the apparent inadequacy of heat-inactivation alone in eliminating HIV from clotting factor concentrates.

Several other physicochemical approaches to viral inactivation of concentrates, including the use of β-propriolactone with ultraviolet (u.v.) light inactivation[120] and tri (n-butyl) phosphate (TNBP) inactivation,[121] have also been recently evaluated. The use of u.v. light and β-propriolactone has shown success in preventing both HIV and nonA, nonB hepatitis transmission,[108,120,121] but there continues to be concern for the oncogenic potential of this chemical, thus limiting its usefulness. TNBP inactivation of concentrates, which depends on lipid inactivation of lipid-containing enveloped viruses,[121] has eliminated HIV and nonA, nonB hepatitis transmission in recipients of these products,[122] however, TNBP might not be expected to inactivate non-lipid enveloped nonA, nonB hepatitis serotypes. This will require further long-term evaluation.

One of the newer techniques recently developed by Zimmerman and Fulcher,[123] murine monoclonal antibody affinity chromatography, using monoclonal antibody to von Willebrand's factor, has produced a purified factor VIII concentrate devoid of both contaminating proteins and transmissible viruses. Early clinical studies of this material has indicated no HIV or nonA, nonB transmission, and there appears to be some short-term stabilization of immunologic parameters, possibly related to the absence of contaminating foreign proteins.[124]

With the introduction of various new viral inactivation techniques for clotting factor concentrates, there were concerns about the effect of inactivation techniques on hemostatic potency, inhibitor development, possible foreign protein exposure, or other adverse effects. However, a number of clinical studies by Heldenbrant,[115] in recipients of heat-inactivated products, and Levine,[124] with the monoclonally-derived products, have indicated that, when compared with recipients of standard non-inactivated concentrates, there was comparable efficacy and factor recovery, no increase in inhibitor formation, no excess immunoglobulin or immune complex levels, and no clinically adverse reactions. Thus, while these newly manufactured inactivated products do not appear to increase the risk of complications, they do appear to add a dimension of safety through the reduction or elimination of HIV and nonA, nonB hepatitis transmission. Hopefully, the continuing development of new technologies will provide increasingly safer treatment products for hemophiliacs and thus eliminate the infectious complications of clotting factor concentrates for future generations.

Although these new inactivation technologies allow for some optimism regarding improved product safety, efforts to enhance the safety of source plasmas from which concentrates are made by development of second and third generation AIDS screening tests are crucial. For now, screened, heat-inactivated clotting factor concentrates remain the treatment recommended by the NHF.[32] It is hoped that newer, safer products will continue to be forthcoming as a result of continuing scientific and technological advances.

V. PROGNOSIS IN HIV SEROPOSITIVE HEMOPHILIACS

With the new technologies to inactivate factor concentrates, those hemophiliacs who are now seronegative and treated with screened, heat-inactivated concentrates, appear to have very little likelihood of HIV exposure.[28,107,119] However, predictions regarding the clinical outcome among those already infected, which constitutes the majority of hemophiliacs, continue to be speculative for several reasons. First, as the incubation period for AIDS is 5 or more years,[125] and peak hemophilia seroconversion occurred just 4–5 years ago,[16,25] we are just now approaching the 5-year mark. Moreover, there has been a slowing in the rate of new cases of hemophilia-associated AIDS during the last year[1,4,126] and although it is expected that AIDS cases will continue to occur, projections for numbers of cases developing in seropositive hemophiliacs are difficult to estimate. Second, the course of AIDS in hemophiliacs appears to differ from other risk groups, as person-to-person spread is not characteristic, and continuing exposure to HIV has presumably ceased with the implementation of screened, heat-inactivated blood products. These differences further complicate AIDS predictions for this population.[4]

A number of factors, however, appear to be associated with or contribute to the outcome of HIV infection in hemophiliacs. Each will be considered separately.

(1) Time from seroconversion. Studies of several hemophiliac populations have shown increasing AIDS incidence with increasing duration of seropositivity[27,28] (Table 4), with an 18–23% AIDS incidence at 5 years and

upwards of 50% AIDS incidence at 7 years following seroconversion. Of those not developing AIDS at 7 years following seroconversion, the remainder have developed AIDS-related problems,[127] suggesting that most if not all HIV infected hemophiliacs may develop symptoms related to HIV infection. However, these predictions must be tempered by the small number seropositive longer than 7 years and the geographic variability of AIDS incidence in U.S. hemophiliacs (see below).

(2) Age at seroconversion. Older age at seroconversion, specifically above 21–22 years has been associated with significantly higher AIDS incidence in hemophiliacs[27] (Ragni, unpublished) (Fig. 1). Whether this relates to the longer number of years of exposure to HIV through concentrates, worse baseline immune status relating to chronic antigenic exposure through chronic blood product treatment,[13,128,129] or longer exposure to transmissible viruses, e.g. hepatitis B and nonA, nonB hepatitis viruses through product use, remains unknown. A number of studies have shown that the chronic use of concentrates can suppress immune responses to specific antigens[130,131] and down regulate monocyte function.[132] Whether the chronicity of exposure to foreign proteins or foreign viruses contributes to immunologic de-

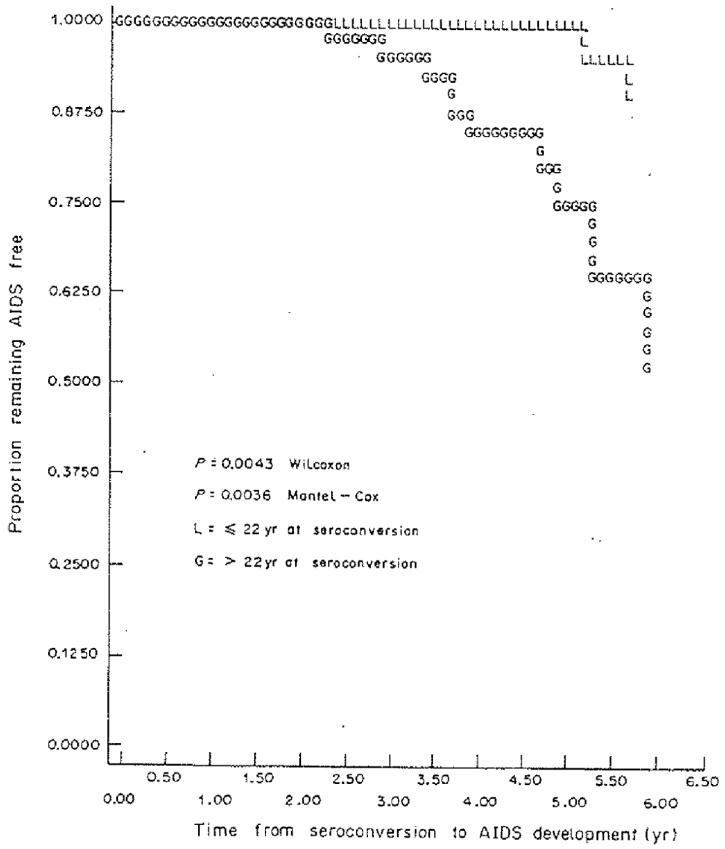

Figure 1. Relation of age at seroconversion to risk of developing AIDS in hemophiliacs, Western Pennsylvania.

cline and/or susceptibility to AIDS remains an unanswered question, and a subject of ongoing research.

(3) Level of T_4 lymphocytes. HIV exposure and infection in hemophiliacs appears to result in a progressive depression in cellular immunity with a decline in T lymphocyte function[28,133] and T_4 lymphocyte number,[27,28] similar to defects described in other high risk groups.[134] In one study, a T_4 lymphocyte count of less than 100/mm^3 was highly associated with AIDS, more than 60% of those below that level and none of those above that level developed AIDS.[28] Another study has shown a strong association between a T_4 count of less than 150/mm^3 and development of AIDS.[27] It is of interest that even prior to HIV exposure in hemophiliacs, both T_4 number and T helper/suppressor ratio have been markedly decreased, typical of changes found in multi-transfused patients.[128,129] Whether these changes, which have been attributed to chronic foreign antigen and foreign protein exposure through chronic blood product treatment, contribute to AIDS development in hemophiliacs is unknown. However, it is clear that the ability to intervene with antiviral therapy before such a level of T_4 depletion and dysfunction occurs is crucial and must be pursued. It is hoped that antiviral and immunomodulator therapy may help to slow or reverse what appears to be an inevitable immunologic decline in these patients.

(4) Pattern of antibody to specific HIV proteins. A decline in HIV antibody, specifically gag (p24) and pol (p53),[64] and appearance of HIV antigen in infected hemophiliacs have recently been shown to be predictive of the development of AIDS and AIDS-related complications.[135,136] The reason for this observation is unknown but may relate to the presence of increasing virus (antigen) production in patients with disease progression, resulting in antigen-antibody formation and consequent loss of free antibody.[135] These changes appear to be unrelated to T_4 number[135,136] and in at least one study appear to be a better predictor of AIDS than T_4 number.[135] Similar findings have been described in several cohorts of homosexual men.[141–143] The longitudinal evaluation of HIV antibody and antigen in infected hemophiliacs and other risk groups may serve as predictors of disease progression.

(5) Geographic variability. The geographic variability in the distribution of U.S. hemophilia-associated AIDS cases has been documented by the CDC[1,3] with some states reporting none and other reporting 20 or more cases. Presumably hemophiliac exposure to HIV through blood products, primarily clotting factor concentrates, occurred at about the same time, since these products were manufactured and distributed nationally. This assumption is corroborated by the first known HIV exposures in hemophiliacs in 1978, peak in seroconversion in 1982 and 1983,[25,26], and similar HIV antibody prevalence in geographically diverse hemophilia populations.[26,28,33–40] Thus, other factors must play a role in the observed geographic differences. A recent cooperative study of Pennsylvania hemophiliacs revealed a 10-fold difference in AIDS incidence in hemophiliacs from the west and central parts of the state as compared with those from the east. This difference was unrelated to HIV antibody prevalence, yearly blood product usage, or accuracy of reporting.[140] Although some speculation has arisen as to the possibility of more infectious or "bad lots" of concentrate, a recent CDC study by Jason and coworkers[141] has shown that hemophiliacs exposed to "recalled" lots of concentrate (a donor subsequently identified with AIDS) do not differ in HIV seroprevalence or T_4 number from hemophiliacs receiving nonrecalled lots of concentrate, thus arguing against a "bad lot" theory. However, another possibility to explain geographic differences in hemophilia-AIDS incidence may be the exposure of hemophiliacs in different geographic areas to different viral stains which could differ in virulence and thus potential outcome.[142] It is clear, based on the above preliminary studies, that prospective stu-

dies of large numbers of infected hemophiliacs are needed to determine the ultimate outcome of HIV infection in hemophiliacs and to establish if there are specific cofactors or host characteristics that make some hemophiliacs more susceptible to AIDS than others.

In closing, it is a tribute to the unfailing efforts of the NHF and its medical and scientific advisory committee, in collaboration with the CDC and FDA, that consistent, timely, and effective responses to the problem of AIDS in hemophilia were provided in the form of directives to both the hemophilia community and the plasma industry. These have led to new and safer products, and an expected cessation of further HIV exposure in this population. For that, patients and providers alike are most thankful, and we look forward to a time when transmissible agents are a thing of the past.

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