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HIV-Antibody Seroconversions in Dutch Haemophiliacs Using Heat-Treated and Non Heat-Treated Coagulation Factor Concentrates

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Key words

Acquired immune deficiency syndrome – Haemophilia – Factor VIII heat treatment – Seroconversion rate

Summary

A national multicentre study was performed to investigate the effects of donorselection and the use of heat-treated plasma products on seroconversion to HIV in 157 Dutch haemophiliacs. All patients included in the study were seronegative for HIV antibodies in 1983.

Thirteen percent (20/157) seroconverted between 1983 and 1986. Nineteen of 20 seroconversions could be related to the use of non heat-treated products in the year preceding HIV antibody seroconversion. One seroconversion occurred in a person using heat-treated non donor screened product.

Seroconversion rate decreased as a result of the policy to discourage high risk blood donors and no seroconversions were observed following the introduction of donor screening in 1985.

Introduction

Transmission of the human immunodeficiency virus (HIV) by blood and blood products has been widely recognized (1). In haemophilia patients infection occurs through coagulation factor concentrates (2, 3). This infection is demonstrated by the presence of HIV-antibodies in the patients serum. In the USA (2, 3) and some European countries, e.g. Denmark (4), France (5) and the United Kingdom (6) HIV antibody prevalences of 60–90% among haemophiliacs have been reported.

In the Netherlands haemophiliacs are treated with coagulation factor concentrates of different origin according to different treatment schedules of the Dutch haemophiliac treatment centres. In this paper we analyse the seroconversion rate in different treatment groups. Since USA imported commercial factor concentrates were heat-treated approximately two years before the nationally produced factor concentrates, also the effect of heat-treatment could be studied in this group in a long term follow-up study.

Patients, Materials and Methods

Patients

In the Spring of 1983 a follow-up study was started in 180 haemophiliacs in the Netherlands. Serum samples of each patient were frozen and stored. Second, third, fourth and fifth serum samples were collected during the Summer 1984, Spring 1985, Autumn 1985 and Spring 1986, respectively. Periods were numbered as period I (1983 – Summer 1984), period II (Summer 1984 – Spring 1985), period III (Spring 1985 – Autumn 1985) and period IV (Autumn 1985 – Spring 1986).

In this group, of which none belonged to other AIDS high-risk groups, 13% (23/180) of the serum samples were positive for antibodies to HIV at entry of the follow-up study. These results were confirmed by immunoblot analysis. Since only the relation of seroconversions with the administered blood product was considered suitable for analysis these positive patients were excluded from the follow-up study.

Of the remaining 157 patients, 123 had haemophilia A (of which 85% of a severe type), 19 had haemophilia B (of which 95% of a severe type), 6 had haemophilia A and circulating antibodies to factor VIII concentrate, 5 had von Willebrand's disease, 3 had a factor XIII deficiency and 1 had a factor VII deficiency.

Transfusion histories were recorded from the beginning of 1982. Patients remaining seronegative throughout the study were considered belonging to a particular treatment group when one product or a combination of products was administered from one year prior to serum sampling until the end of the study.

Assuming a lag time of one year maximum for antibodies to HIV to occur after infection, patients seroconverting during our study were considered belonging to a particular treatment group when they exclusively used one product or a combination of products from one year prior to the last seronegative sample until the first positive sample.

Treatment Products

The following treatment products were administered:

A. cryoprecipitate, heat-treated since December 1985;

B. nationally produced factor VIII concentrate (Central Laboratory of the Red Cross Blood Tranfusion Service, The Netherlands), heat-treated since June 1985;

C. imported factor VIII concentrate (Hemofil-T, Hyland, Travenol, USA, heat-treated since May 1983. Factorate, Armour, Kankakee Ill., USA, heat-treated since September 1983);

D. imported activated prothrombin complex concentrate (Feiba Immuno AG, Wien, Austria, prepared from Dutch volunteer donor-plasma since late 1983), heat-treated since Spring 1985;

E. nationally produced factor IX concentrate (Central Laboratory of the Red Cross Blood Transfusion Service, The Netherlands), heat-treated since July 1985;

F. imported factor IX concentrate (Proplex, Hyland, Travenol, USA), heat-treated since October 1985;

G. fresh frozen plasma.

Since May 1983, individuals belonging to AIDS-risk groups have been discouraged by the dutch bloodbanks to donate blood. In Summer 1985 screening of all donors by anti-HIV antibody testing and heat treatment of locally produced factor VIII and IX concentrates was introduced in the Netherlands. Heat treatment procedures were as follows: Hyland factor-VIII concentrate was 72 hrs at 60° C, Armour factor VIII concentrate for

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Table 1 Haemophilia patients seroconverting for HIV-antibodies by treatment product

Patient	Seroconversion-period[1])	Disease[2])	Treatment-product
1	I	Haemophilia A	CLBC
2	I	Haemophilia A	CLBC
3	I	Haemophilia A	CLBC
4	I	Haemophilia A	CLBC
5	I	Haemophilia A	CLBC
6	I	Haemophilia A	CLBC
7	I	Haemophilia A	CLBC/Armour
8	I	Haemophilia A	CLBC/Armour
9	I	Haemophilia A	CLBC/Hyland/cryo
10	I	Haemophilia A and circ. α-VIII	CLBC/FEIBA
11	I	Haemophilia A	CLBC/cryo
12	I	Haemophilia A	Hyland
13	I	Haemophilia A	cryo
14	I	Factor VII-deficiency	PPSB
15	II	Haemophilia A	CLBC
16	II	Haemophilia A	CLBC
17	II	Haemophilia A	CLBC
18	II	Haemophilia A	CLBC/Armour
19	II	Haemophilia A	Armour
20	III	Haemophilia A	CLBC/cryo

[1]) The seroconversion periods were defined as follows: period I: 1983–1984, period II: 1984 – Spring 1985, period III: Spring 1985 – Autumn 1985.
[2]) All haemophiliac patients had haemophilia of the severe type (factor VIII concentrate below 0.01 U/ml)
Abbreviations used: Circ. α-VIII: circulating antibody to factor VIII, CLBC: nationally produced factor VIII concentrate, Hyland: imported commercial factor VIII concentrate, Armour: imported commercial factor VIII concentrate, PPSB: nationally produced factor IX concentrate, cryo: cryoprecipitate

30 hrs at 60° C dry-heat and locally produced factor VIII and IX concentrates for 72 hrs at 60° C. Proplex factor IX concentrate for 144 hrs at 60° C.

Assays

All serum samples were collected at the patients' own treatment centre and tested blindly at the Human Retrovirus Laboratory of the Department of Virology (Academic Medical Centre, Amsterdam, The Netherlands). Antibody (IgG) to HIV was measured by a commercially available ELISA (Abbott recombinant HTLV-III EIA, Abbott, Chicago). All ELISA positive sera as well as previous negative samples of the same patients were subjected to Western blot confirmation, as described (7).

Results

Fourteen of 157 haemophiliacs (9%) seroconverted for HIV during 1983–1984 (period I) and another five of 143 (4%) seroconverted during 1984 – spring 1985 (period II). Only one of 138 (1%) seroconverted during spring 1985 – autumn 1985 (period III). No seroconversion occurred during autumn 1985 – spring 1986 (period IV). There were no discrepancies between the ELISA-assay and the Western blot (Table 1).

Of the 157 patients 122 belonged to the one product treatment group and 35 belonged to the combination product treatment group (Table 2, 3).

In 51 patients exclusively using cryoprecipitate (of which 72% had haemophilia of the severe type) only one seroconversion (2%) occurred. In 17 patients exclusively using non heat-treated local factor VIII concentrate (88% severe haemophilia) nine seroconversions (53%) were observed. In 27 patients exclusively treated with imported factor VIII concentrate (96% severe haemophilia) two seroconversions (7%) occurred. Of the 17 patients exclusively receiving local factor IX concentrate only one seroconverted (6%). None of the patients receiving imported activated prothrombin complex concentrate (n = 5) or imported factor IX concentrate (n = 3) seroconverted.

Table 2 Seroconversions (1983–1986) for HIV antibodies in Dutch haemophiliacs exclusively receiving one treatment product

Treatment-product	Number tested	Sero-converters	Seroconversion rate (1983–1986)
Cryoprecipitate	51	1	2% (1/51)
Local factor VIII concentrate (non heat-treated)	17	9	53% (9/17)
Imported factor VIII concentrate (USA)	27	2	7% (2/27)
Imported activated prothrombin complex concentrate (Austria)	5	0	0% (0/5)
Local factor IX concentrate	17	1	6% (1/17)
Imported factor IX concentrate (USA)	3	0	0% (0/3)
Fresh frozen plasma	2	0	0% (0/2)

Three combinations of treatment products were distinguished (Table 3). 25 patients used a combination of local factor VIII concentrate and cryoprecipitate or activated prothrombin complex concentrate of which three seroconverted (12%). 8 patients used a combination of local factor VIII concentrate and imported factor VIII concentrate of which four seroconverted (50%). 2 patients used a combination of imported factor VIII concentrate and cryoprecipitate, none of them seroconverted.

The mean amount of product (IU/year) used by the seroconverting factor VIII users was higher than the factor VIII users who remained seronegative, although the amount varied greatly in both groups.

The mean amount of product used by the local factor VIII concentrate users was higher than the mean amount of the imported factor VIII concentrate users (Table 4).

Table 3 Seroconversions (1983–1986) for HIV antibodies in Dutch haemophiliacs receiving a combination of products

Treatment-products	Number tested	Sero-converters	Seroconversion rate (1983–1986)
Local factor VIII concentrate (non heat-treated)/ Cryoprecipitate or Activated prothrombin complex concentrate (Austria)	25	3	12% (3/25)
Local factor VIII concentrate (non heat-treated)/ Imported factor VIII concentrate (USA)	8	4	50% (4/8)
Imported factor VIII concentrate (USA)/ Cryoprecipitate	2	0	0% (0/2)

Table 4 Average amount of product (IU/year) used by haemophiliacs exclusively receiving factor VIII concentrates[1])

Local factor VIII concentrate	Imported factor VIII concentrate
105.254 (range: 5.950–234.230)	78.090 (range: 26.250–203.000)
Seronegatives	Seroconverters
78.897 (range: 5.950–203.000)	118.891 (range: 40.470–234.230)

[1]) calculated on product use over the period 1982–1985.

Discussion

Of the investigated cohort, of which the majority (86%) had haemophilia of the severe type, 13% (23/180) was seropositive for HIV-antibodies in 1983. This low overall prevalence of anti-HIV seropositive haemophiliacs, in contrast to the high prevalence of HIV-antibodies among haemophiliacs in the USA at that time (2, 3), indicates a lower grade of contamination of the Dutch donor pool before 1983. However, more haemophiliacs used cryoprecipitate in the Netherlands than in the USA.

Prevalence of HIV seropositivity rose to 21% in 1984 (37/180) and 24% in 1985 (43/180). Individuals, in the Netherlands as well as in the USA, at risk for AIDS have been discouraged to donate blood since May 1983. In our study 6 out of 17 (35%) recipients of local non heat-treated factor VIII concentrate exclusively, seroconverted in period I, 3 out of 11 (27%) in period II and 0 out of 8 (0%) in period III. This decrease in seroconversion rates is probably a result of the policy of the bloodbanks to discourage high risk blood donors.

Also in 1983, heat treatment of the commercial factor VIII concentrate was introduced in the USA (Hyland May 1983, Armour September 1983), while approximately two years later (Summer 1985) this was followed in the Netherlands.

Since Dutch haemophiliacs received different regimens of therapy, heat-treated as well as non heat-treated, the effect of heat treatment was particularly suitable to observe.

18 of the 20 seroconversions (90%) observed in our study could be related to non heat-treated factor products. In 9 of 20 seroconversions (45%) the local factor VIII concentrate was the source of infection, whereas in 4 seroconversions (20%) this source could be excluded. The higher amount of product used by the local factor VIII concentrate users could possibly contribute to the difference in seroconversion rates between recipients of local non heat-treated factor concentrates and recipients of imported heat-treated factor concentrates, but it will not be a major factor considering the amount of units used (Table 4).

In the haemophiliac group who received local non heat-treated factor VIII concentrates in combination with cryoprecipitate and/or activated prothrombin complex concentrate, a relatively low (12%) seroconversion rate was found. This could be contributed to a lower amount of concentrate used in this group, which varied greatly from one single dose to over 90% of the total amount of units used.

Only two seroconversions were observed in the recipients of heat-treated products. One patient (nr. 12) seroconverting in period I was followed elsewhere more frequently and was found seropositive in June 1983, i.e. when heat-treatment of the commercial factor VIII concentrate (Hyland) just started (9). So this seroconversion could be related to non heat-treated product usage. The other patient (nr. 19), seroconverting in period II, was still seronegative in September 1984, whereas he was found seropositive in January 1985. He exclusively used imported heat-treated intermediate and high purity factor VIII concentrate (Factorate, Armour, USA) from September 1983 on (10, 11). A similar case was described by White et al. (12). The heat treatment procedure of the product he received may be characterized as less vigorous since it is only performed for 30 hrs, whereas donorscreening was not yet introduced. Tersmette et al. (13) described a significantly lower reduction of infectious virus after 30 hrs compared to the result obtained after 72 hrs. The stabilizer used by Armour during the heat treatment process may also play a role, since it possibly would not only stabilize factor VIII but also infectious agents.

HIV-antibody screening of blood donors was introduced in the Netherlands as well as in the USA as late as Summer 1985.

To date, after the triad discouragement, heat treatment and donor-screening no seroconversions have been described (14).

Whether all seropositive haemophiliacs are actually infected with virulent virus or a proportion is immunized with non-infectious virus particles is still open for debate and therefore the prognosis for the individual is difficult to predict.

The cumulative incidence of AIDS among seropositive haemophiliacs in the United Kingdom is 5% (15). In the USA the cumulative incidence of AIDS is approximately 3% [calculated on an estimated 12,000 haemophiliacs at risk (16) and 364 reported patients with AIDS, as of 14 August 1987 (17)]. However, in the USA cumulative AIDS incidence varies from region to region and has been reported as high as 32% in a group of haemophiliacs seropositive for 5 years (18).

Because haemophilia AIDS incidence may relate to date of seroconversion, it is of interest to know in what year the 23 seropositive haemophiliacs at entry of our study seroconverted.

In the present 1987 seropositive group of which 23 of the 43 haemophiliacs are seropositive for more than four years no AIDS has occurred.

Acknowledgements

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References

1 Feorino P M, Jaffe H W, Palmer E, Peterman T A, Francis D P, Kalyanaraman V S, Weinstein R A, Stoneburner R L, Alexander W J, Raevsky C, Getchell J P, Warfield D, Haverkos H W, Kilbourne B W, Nicholson J K A, Curran J W. Transfusion associated acquired immunodeficiency syndrome. N Engl J Med 1985; 312: 1293–6.

2 Goedert J J, Sarngadharan M G, Eyster M E, Weiss S H, Bodner A J, Gallo R C, Blattner W A. Antibodies reactive with human T cell leukemia viruses in the serum of haemophiliacs receiving factor VIII concentrate. Blood 1985; 65: 492–5.

3 Evatt B L, Gomperts E D, McDougal J S, Ramsey R B. Coincidental appearance of LAV/HTLV-III antibodies in hemophiliacs and the onset of the AIDS epidemic. N Engl J Med 1985; 312: 483–6.

4 Melbye M, Biggar R J, Chermann J C, Montagnier L, Stenbjerg S, Ebbesen P. High prevalence of lymphadenopathy virus (LAV) in European haemophiliacs. Lancet 1984; ii: 40–1.

5 Allain J P. Prevalence of HTLV-III/LAV antibodies in patients with hemophilia and in their sexual partners in France. N Engl J Med 1986; 315: 517–8.

6 Jones P, Hamilton P J, Bird G, Fearns M, Oxley A, Tedder R, Cheingsong-Popov R, Codd A. AIDS and haemophilia: morbity and mortality in a well defined population. Br Med J 1985; 291: 695–9.

7 Lange J M A, Coutinho R A, Krone W J A, Verdonck L F, Danner S A, Van der Noordaa J, Goudsmit J. Distinct IgG recognition patterns during progression of subclinical and clinical infection with lymphadenopathy associated virus/human T-lymphotropic virus. Br Med J 1986; 292: 228–30.

8 Evatt B L, Ramsey R B, Lawrence D N, Zyla L D, Curran J W. The acquried immunodeficiency syndrome in patients with haemophilia. Ann Intern Med 1984; 100: 499–504.

9 Van der Meer J, Daenen S, Van Imhoff G W, De Wolf J T M, Halie M R. Absence of seroconversion for HTLV-III in haemophiliacs intensively treated with heat treated factor VIII concentrate. Br Med J 1986; 292: 1049.
10 Van den Berg W, Ten Cate J W, Breederveld C, Goudsmit J. Seroconversion to HTLV-III in haemophiliac given heat-treated factor VIII concentrate. Lancet 1986; I: 803.
11 Breederveld C, Van den Berg W, Ten Cate J W, Smit L, Goudsmit J. Seroconversion for HIV in patients using heat-treated factor VIII concentrates. II Int Conference on AIDS, 1986, Abstract.
12 White G C 2nd, Matthews T J, Weinhold K J, Haynes B F, Le Roy Cromartie H, McMillan C W, Bolognesi D P. HTLV-III seroconversion associated with heat-treated factor VIII concentrate. Lancet 1986; i: 611–2.
13 Tersmette M, De Goede R E Y, Over J, De Jonge E, Radema H, Lucas C J, Huisman H G, Miedema F. Thermal inactivation of human immunodeficiency virus in lyophilised blood products evaluated by ID50 titrations. Vox Sang 1986; 51: 239–43.
14 CDC. Survey of non-U.S. hemophilia treatment centers for HIV seroconversions following therapy with heattreated factor concentrates. MMWR 1987; 36: 121–4.
15 Jones P. Haemophilia, AIDS, and no fault compensation. Br Med J 1987; 295: 944–5.
16 Eyster M E, Gail M H, Ballard J O, Al-Mondhiry H, Goedert J J. Natural history of human immunodeficiency virus infections in hemophiliacs: effects of T-cell subsets, platelet counts and age. Ann Intern Med 1987; 107: 1–6.
17 CDC. Update: Acquired immunodeficiency syndrome – United States. MMWR 1987; 36: 522–6.
18 Ragni M V, Winkelstein A, Kingsley L, Spero J A, Lewis J H. 1986 Update of HIV seroprevalence, seroconversion, AIDS incidence, and immunologic correlates of HIV infection in patients with hemophilia A and B. Blood 1987; 70: 786–90.

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