Comparison of immunodeficiency and AIDS defining conditions in HIV negative and HIV postive men with haemophilia A

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Abstract

***Objective*—To investigate the hypothesis that high usage of clotting factor concentrate, rather than HIV infection, is the cause of immunodeficiency and AIDS in men with haemophilia.**

***Design*—A comparison of AIDS defining conditions and CD4 counts in HIV positive and HIV negative patients with haemophilia matched for usage of clotting factor concentrate.**

***Setting*—A comprehensive care haemophilia centre.**

***Subjects*—17 HIV positive and 17 HIV negative male patients with haemophilia A (age range 12-60 at beginning of study period) who had received similar amounts of clotting factor concentrate yearly over the years 1980-90.**

***Main outcome measures*—Clinical events listed as AIDS defining in the Centers for Disease Control AIDS definition; CD4 lymphocyte counts; death.**

***Results*—Of 108 HIV positive male patients with haemophilia A, only 17 could be matched to an HIV negative patient. This was due to the much higher average usage of factor VIII in the HIV positive group. Between 1980 and 1990, 16 clinical events occurred in nine of the 17 HIV positive patients. No event occurred in the 17 HIV negative patients. In each pair the mean CD4 count during follow up was, on average, $0{\cdot}5\times10^9$/l lower in the HIV positive patient.**

***Conclusion*—These data reject the hypothesis that high usage of clotting factor concentrate, rather than HIV infection, is the cause of immunodeficiency and AIDS in men with haemophilia.**

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Introduction

There has recently been debate about the pathogenic role of HIV infection in AIDS. It has been suggested that HIV is neither necessary nor sufficient to cause severe disease definitive of AIDS.[1-4] On the basis that transfusion of clotting factor concentrates could be related to the development of AIDS-like diseases in haemophilic patients, it has been suggested that the use of factor VIII concentrates is the "cofactor" essential for the development of AIDS in patients with haemophilia A.[2]

We studied the effects of HIV infection on the development of conditions listed in the Centers for Disease Control AIDS definition and on the immune systems of a group of men with haemophilia A. In order to control for the use of clotting factor concentrates we performed a follow up study with HIV positive patients matched to HIV negative patients on the basis of concentrate usage.

Patients and methods

Between 1979 and 1985, 111 men with haemophilia registered at the Royal Free Hospital's haemophilia centre—including 108 with haemophilia A, one with haemophilia B, and two with von Willebrand's disease—became infected with HIV after treatment with contaminated factor VIII concentrate.[5,6] Hospital records identified 152 men with haemophilia A who were registered at the centre in 1979, when infected batches of factor VIII started to be used, who had remained HIV negative after repeated regular testing. Clinical events that meet the Centers for Disease Control AIDS definition are routinely recorded in all patients at the centre.

Computerised yearly total treatment records were available for each patient since 1980. At the end of 1990 all HIV positive patients at the centre were switched to monoclonally purified factor VIII concentrate.[7] These concentrates seem to preserve the immune system of recipients,[8,9] though their effect on clinical end points is not clear.[10] As only HIV positive patients receive this treatment, we focused on CD4 lymphocyte counts and clinical events occurring before the end of 1990, when all patients received only intermediate purity factor VIII. We report separately clinical events which occurred in 1991-4. These data, however, should be interpreted with caution, as the patients may no longer have been comparable in terms of the factor VIII concentrate received.

Measurement of CD4 lymphocyte subsets began at the hospital in late 1982, and CD4 counts are recorded for each patient at each clinic visit. In general, HIV positive patients are seen every three to six months and HIV negative patients every six to 12 months. The measurement of CD4 cells in this cohort has been described.[11]

Eligible patients were 12 years and older at the start of 1980 and had at least two CD4 counts recorded during 1982-90. Patients aged below 12 were excluded, as CD4 counts decrease naturally from birth to 14 years.[11] Hence by the time of the first CD4 measurement in these patients in 1982 all patients were aged 14 or older.

HIV positive patients were matched to HIV negative patients in two stages. Firstly, each HIV positive patient was matched for his median yearly usage of concentrate to an HIV negative patient whose usage was closest to and within 5% of that of the HIV positive patient. Secondly, the mean yearly amounts of concentrate received were compared in cases and controls and considered a good match if they were within at most 30 000 units. All matching was blind to patient outcome. Matching on the basis of both median and mean usage ensured that patients were treated similarly in terms of the number of years of treatment with concentrate and the overall amount of concentrate received. As most patients with severe haemophilia—and therefore the highest users of clotting factor concentrate—were seropositive to HIV, only a few matches could be identified. However, the patients who could be matched in this manner were comparable in their usage of concentrate.

Since August 1987 HIV positive patients at the centre have routinely been treated with zidovudine after the development of AIDS or, more recently, once their CD4 count falls below $0{\cdot}2\times10^9$/l. Since 1988 patients have also been given prophylaxis against *Pneumocystis carinii* pneumonia (with pentamidine or co-trimoxazole) and candidiasis (with fluconazole). Primary prophylaxis against both conditions is begun once the CD4 count falls below $0{\cdot}2\times10^9$/l.

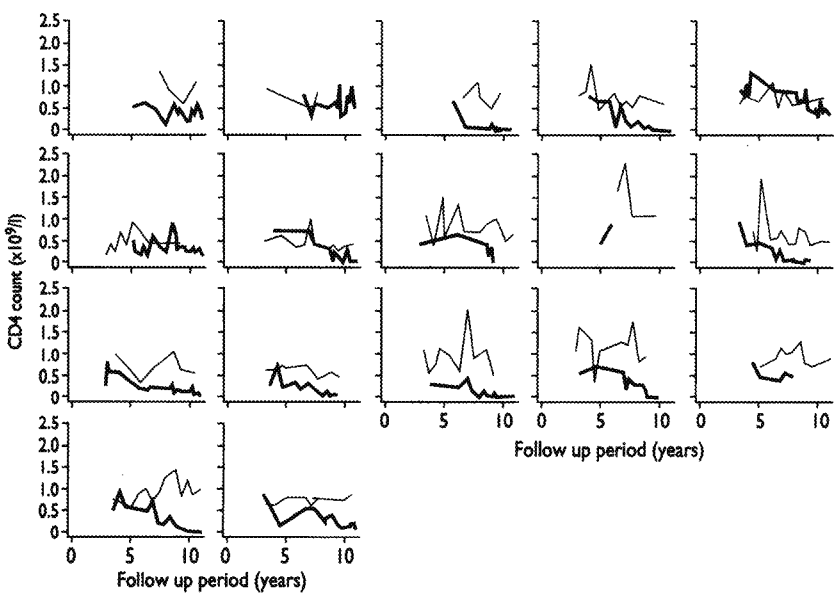
Statistics—Paired comparisons between HIV negative and HIV positive patients were tested for significance with McNemar's test for the development of AIDS and the sign rank test for the mean CD4 count during follow up.

Results

Seventeen matched pairs were identified. There was wide variation in the patients' ages, though the distribution was similar in the HIV positive and negative groups. The patients' ages at the start of 1980 ranged from 14 to 60 years (median 26) in the HIV positive group and 12 to 50 years (median 20) in the HIV negative group. In almost all cases the mean yearly factor VIII usage was higher than the median (table 1), reflecting the skewed nature of factor VIII usage. In general the mean yearly usage of factor VIII was slightly higher in the HIV positive patients, though as a result of the matching procedure in no pair was the difference more than 30 000 units. One HIV positive patient (pair 1) seroconverted as a result of exposure to clotting factor concentrate received before the introduction of computerised treatment records in 1980. This patient did not require factor VIII subsequently.

Table 1—*Median and mean yearly factor VIII usage (units) and ages (on 1 January 1980) of 17 HIV positive and 17 HIV negative patients*

	HIV positive			HIV negative		
Pair No	Median	Mean	Age (years)	Median	Mean	Age (years)
1	0	0	20	0	0	35
2	2 870	4 539	14	2 920	6 483	24
3	8 530	43 874	60	8 868	17 676	14
4	10 200	41 430	37	9 724	19 415	45
5	10 270	51 207	50	10 581	25 330	36
6	10 870	15 045	18	11 101	25 365	25
7	15 763	22 097	14	16 170	20 856	29
8	17 570	23 001	26	17 765	17 550	18
9	28 450	35 017	32	28 610	23 714	47
10	29 965	40 480	21	30 295	37 880	14
11	37 425	60 791	29	37 400	40 753	25
12	38 896	73 576	20	38 520	44 177	20
13	39 480	62 874	24	39 900	43 146	13
14	40 200	64 779	16	40 415	61 456	50
15	59 992	73 169	43	60 490	64 687	12
16	92 435	97 964	41	91 920	86 704	18
17	101 920	106 371	56	104 240	106 359	16



CD4 lymphocyte counts in HIV positive patients (bold tracing) and HIV negative controls (light tracing) measured since 1982

Table 2—*Clinical events listed as part of Centers for Disease Control AIDS definition and deaths during 1980-90 in 17 HIV positive and 17 HIV negative patients matched for factor VIII usage*

Pair No	HIV positive	HIV negative
1		
2		
3	Died	
4	Toxoplasmosis, died	
5		
6		
7	*Pneumocystis carinii* pneumonia	
8	Toxoplasmosis, mycobacteriosis, died	
9		
10	*Pneumocystis carinii* pneumonia (twice), lymphoma, died	
11	*Pneumocystis carinii* pneumonia	
12	Oesophageal candidiasis, cytomegalovirus retinitis, died	
13	*Pneumocystis carinii* pneumonia, oesophageal candidiasis	
14	*Pneumocystis carinii* pneumonia, cytomegalovirus disease, died	
15	Died	
16	Wasting syndrome, *Pneumocystis carinii* pneumonia	
17		

Before the end of 1990 no condition listed as part of the Centers for Disease Control AIDS definition or death occurred in the 17 HIV negative patients. However, nine of the 17 HIV positive patients had developed AIDS by the end of 1990 and seven had died. Four of the seven patients died of AIDS; deaths in the three remaining patients were due to pneumonia, cerebral haemorrhage, and cirrhosis. All other deaths in this group were due to AIDS related causes. Among the nine HIV positive patients with AIDS, 16 AIDS events occurred during the study period. No such events occurred in the HIV negative patients ($P<0{\cdot}01$, McNemar's test) (table 2).

The figure shows the CD4 lymphocyte counts of patients during follow up. Mean counts ranged from $0{\cdot}07$ to $0{\cdot}70\times10^9$/l (median $0{\cdot}28\times10^9$/l) in HIV positive patients and from $0{\cdot}45$ to $1{\cdot}55\times10^9$/l (median $0{\cdot}79\times10^9$/l) in HIV negative patients. On average, mean CD4 counts over the study period were $0{\cdot}5\times10^9$/l lower in HIV positive patients ($P=0{\cdot}0001$, sign rank test; 95% confidence interval for difference $0{\cdot}35$ to $0{\cdot}66\times10^9$/l).

All nine HIV positive patients with AIDS received zidovudine. However, in seven zidovudine was begun only after an initial AIDS defining event. Three HIV positive patients received zidovudine but had not developed AIDS by the end of 1990.

After 1991 no deaths or conditions that would be AIDS defining in HIV positive patients occurred in the 17 HIV negative patients. Though there were no new incident cases of AIDS in the HIV positive patients, there were a further six AIDS events in patients who already had AIDS and a further six deaths in this group.

Discussion

Though factor VIII concentrates have been implicated in immune modulation, several workers have shown that the immune response is only subtly affected by the infusion of these concentrates in HIV negative

subjects.[12 13] However, in general, the patients in these studies have mild haemophilia and do not receive anywhere near as much factor VIII as HIV positive patients, who more commonly have severe haemophilia. Consequently, the possibility that factor VIII causes some of the immune deterioration in HIV positive haemophilic patients has not previously been ruled out.

We matched patients on the basis of their average yearly factor VIII usage so that any differences in clinical events and laboratory markers could not be attributed to factor VIII usage. Though we made our matching criterion as stringent as possible, small differences remained in the amount of clotting factor concentrate received between HIV positive and HIV negative patients. In order to match more closely at both stages a much larger number of patients would be required. It is unlikely, certainly within the United Kingdom, that this amount of detailed information on factor VIII usage would be available for many more patients. The possibility that these differences might explain the differences in clinical events and CD4 counts must be considered.

This study included patients with a wide range of factor VIII usage, including some patients receiving very low amounts and some receiving very high amounts. If a 30 000 unit difference in mean yearly factor VIII usage explained the clinical differences it would be likely that at least some clinical events would occur in the HIV negative patients receiving the highest amounts of factor VIII. Furthermore, a dose-response relation might be expected in both HIV positive and HIV negative patients. This did not seem to be the case in our study. Consequently, it is unlikely that the differences in mean yearly factor VIII usage could explain the clinical findings.

By matching on the basis of concentrate usage we restricted our analysis to only 17 patient pairs. However, despite these small numbers we found a statistically significant difference in overall CD4 cell counts between HIV positive and HIV negative patients. The development of clinical conditions listed in the Centers for Disease Control AIDS definition was restricted to HIV positive patients (also a highly significant difference) despite the reported associations between factor VIII usage and opportunistic infections.[1 2] The development of such conditions in patients thought to be HIV negative would prompt further investigation in addition to their regular HIV testing. Hence it is highly unlikely that any such conditions went unnoticed in patients known to be HIV negative.

RELEVANCE OF AGE AND OTHER FACTORS

Though the range of ages was similar, the median age of the HIV positive patients in our study was six years greater than that of the HIV negative patients. Age is associated with increased progression of HIV disease,[14] so the possibility that age differences led to the increase in AIDS conditions and deaths in the HIV positive patients should be considered. As the overall distribution of ages was similar, it is unlikely that a six year difference in median age could explain such large differences in clinical events. CD4 lymphocyte counts in uninfected patients remain fairly stable over the age of 14,[11] so it is also unlikely that the age difference could have resulted in such large differences in the CD4 counts of the patients.

It might be expected that the introduction of high purity concentrates for HIV positive patients would lead to a reduction in clinical disease in these patients. But after 1991 a further six AIDS events and six deaths occurred in the HIV positive patients. No clinical events occurred in the HIV negative patients. Though these data should be interpreted with caution (as the patients may no longer have been comparable in terms of the amount of factor VIII received), they add further support to the finding that clinical events which met the Centers for Disease Control AIDS definition occurred only in patients infected with HIV.

Antiretroviral agents for HIV infection became available at the centre from 1987. In this study most of the HIV positive patients who received zidovudine did so only after an initial AIDS diagnosis. More recently some HIV positive AIDS free patients with CD4 counts below $0{\cdot}2\times10^9$/l received the drug. However, it is clear that differences in the patients' CD4 counts were apparent long before the counts dropped to this low level. Consequently, these data are not consistent with the suggestion that AIDS is caused by zidovudine.[3 4]

In conclusion, we have shown that in a group of haemophilic patients matched for factor VIII usage the development of conditions listed as part of the Centers for Disease Control AIDS definition was restricted to those patients who were HIV positive. Furthermore, CD4 counts were lower and more likely to decline in these patients. It is unlikely that differences in factor VIII usage, patient age, or zidovudine use can explain the findings. We conclude that HIV infection leads to progressive immune deterioration and AIDS irrespective of clotting factor usage.

Key messages

- HIV infection in patients with haemophilia was transmitted in unheated clotting factor concentrates; hence patients who are infected with HIV tend to be those who have received high amounts of these concentrates
- This confounding is removed in studies comparing the development of immunodeficiency and AIDS defining diseases in patients matched for usage of clotting factor concentrate
- In this study clinical events listed as part of the AIDS definition were restricted to HIV positive patients, and CD4 counts were lower in these patients
- Present data reject the hypothesis that high usage of clotting factor concentrate, rather than HIV infection, is the cause of immunodeficiency and AIDS in men with haemophilia

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Commentary: non-HIV hypotheses must be studied more carefully

Peter Duesberg

Patients with haemophilia treated with commercial factor VIII and zidovudine are at risk of developing AIDS defining immunodeficiency diseases like pneumonia, candidiasis, and toxoplasmosis but not Kaposi's sarcoma or dementia. Their risk of these diseases is proportional to their lifetime dosages of factor VIII and cytotoxic DNA chain terminators like zidovudine.[1 10]

Haemophilia specific AIDS diseases have been explained in terms of two hypotheses. A non-infectious hypothesis holds that the long term administration of immunosuppressive foreign proteins contaminating commercial factor VIII and zidovudine causes AIDS. An infectious hypothesis holds that HIV causes AIDS.[1]

Still no evidence for infection

Based on inadequate tests, Sabin *et al* reject the foreign protein-zidovudine hypothesis.[2] However, their data and those of others actually support the hypothesis.

Sabin *et al* claim that HIV causes AIDS because all of their haemophilic patients had antibodies against the virus. But HIV cannot be enough for AIDS because six of their 17 HIV positive patients (table 2) remained healthy for 10 years. Likewise 12 000 out of 15 000 HIV positive American haemophilic patients have remained AIDS free since 1985.[1 3]

Sabin *et al* observe that AIDS diseases occur, if at all, only five to 10 years after the appearance of antibodies to HIV. However, "viral" AIDS should occur within weeks after infection because viruses replicate exponentially. A single infected cell produces over 100 HIV virions within 48 hours,[4] generating 10^{14} viruses within two weeks after infection—enough to infect every cell in the human body. Moreover, viral AIDS should be neutralised—not caused—by the antiviral immunity that is detectable in haemophilia.

By contrast, long time periods are required to accumulate pathogenic doses of foreign proteins via transfusions.[1] Because HIV is a rare contaminant of factor VIII, it seems to be just a surrogate marker for high dosages of foreign proteins. Sabin *et al* confirm this: "Most patients with severe haemophilia—and therefore the highest users of clotting factor concentrate—were seropositive to HIV. . . ."[2] Thus their results support the foreign protein hypothesis.

An appropriate test of the foreign protein hypothesis would compare HIV positive patients with HIV negative patients matched for the lifetime dosage of factor VIII. By contrast, Sabin *et al* matched patients for current usage. Plainly, a 60 year old and a 14 year old would not be an appropriate match (table 1).[2]

The HIV hypothesis predicts the same pattern of AIDS diseases in haemophilia as in other AIDS patients, but Kaposi's sarcoma and dementia were not observed in the haemophilic patients studied by Sabin *et al* (table 2). However, the foreign protein-zidovudine hypothesis predicts the restriction to immunodeficiency diseases observed by Sabin *et al* exactly.

The HIV hypothesis predicts sexual transmission of AIDS. However, in the United States the wives of 15 000 HIV positive haemophilic men have only the normal background prevalence of AIDS defining diseases.[1 5]

Strong temporal evidence

HIV is now said to cause AIDS 10 years after infection, and infections by transfusions were stopped in 1985. Hence AIDS in patients with haemophilia should have peaked long before 1995. Instead, cases declined until 1986[1] and then sharply increased from 1987 both in the United States and in Britain when zidovudine and other drugs were introduced as treatment for and prophylaxis against HIV infection and AIDS.[6 7]

Zidovudine kills all growing cells, particularly fast growing blood cells, and therefore must cause immunodeficiency. Even the manufacturer acknowledges, "It is often difficult to distinguish adverse events possibly associated with zidovudine administration from underlying signs of HIV disease."[8] An epidemiological study cited by Sabin *et al* directly confirms that zidovudine treated HIV positive haemophilic patients have a 4·5-fold higher annual AIDS rate and a 2·4-fold higher annual death rate than untreated controls.[9] This explains why the morbidity of American[6] and British[7] patients with haemophilia has sharply increased since 1987, when most HIV positive haemophilic patients began zidovudine.[1 10] Sabin *et al* confirm the zidovudine hypothesis, as "all nine HIV positive patients with AIDS received zidovudine" in addition to the "toxic" pentamidine,[10] co-trimoxazole, and fluconazole.[2]

The foreign protein hypothesis predicts that AIDS in patients with haemophilia can be prevented if not cured by using factor VIII that is free of foreign protein. Sabin *et al* endorse this: "These concentrates seem to preserve the immune system. . . ." Indeed, not only were the immune systems of HIV positive haemophilic patients treated with pure factor VIII and no zidovudine "preserved" but their T cells increased by up to a quarter over three years—despite the presence of the hypothetical T cell killer HIV.[11 12]

Conclusion

The foreign protein-zidovudine hypothesis provides biochemically plausible candidates for pathogenicity—that is, large amounts of foreign proteins transfused over decades and DNA chain terminators prescribed

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