Lower genital tract neoplasia in women infected with the Human Immuno-deficiency Virus.

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Declaration

I declare that the submitted manuscript is with the undernoted exceptions the sole work of the author, J R Smith. Laboratory investigations performed for HIV virology have been performed by Ms D Gor, the remaining virology, haematology, and bacteriology have been performed by the respective laboratories in St Mary's Hospital, London W2, whilst EBV and HPV detection have been performed by Mr M Botcherby of the Jefferiss Wing at St Mary's Hospital.

T4 / T8 counts were performed in the haematology laboratories of the Middlesex Hospital, London. Histopathology was performed by Dr M Anderson and cytology by Ms A Morse. Local immunology studies have been performed, in conjunction with Dr S E Barton, St Stephen's Hospital, London and Mr P Maddox and Dr D Jenkins, the Whittington Hospital, London. Ten of the HIV seropositive patients were enrolled by Dr M Hepburn, Royal Infirmary, Glasgow and, with the exceptions of EBV and HPV analysis, their respective laboratory investigations performed there.

Ethical committee approval was sought and obtained for this study from both St.Mary's Hospital and Glasgow Royal Infirmary.

Summary

This thesis is an account of studies designed to test the hypothesis that Human Immunodeficiency virus (HIV) infection in women predisposes to the development of cervical intraepithelial neoplasia (CIN) by allowing the unchecked action of oncogenic viruses. Fifty HIV seropostive women, for 43 of whom there were HIV seronegative case matched controls were studied by cytology, colposcopy, and histology. Investigations were carried out to detect the presence of oncogenic viruses Human Papilloma virus (HPV), Epstein Barr virus (EBV) and Herpes Simplex virus (HSV). A secondary hypothesis, that the development of CIN is related to immunosuppression was tested at both a systemic and local level. At a local level immune funtion was investigated by measuring the Langerhans' cell number, and relating it to the presence or absence of CIN and to generalised immunosuppression.

There was no significant difference in the prevalence of CIN (6/43 (14% (95% Confidence Interval: 5-28%)) versus 4/43 (9% (95% Confidence Interval: 3-28%))) or detectable cervical infection with oncogenic viruses between HIV seropositive and HIV seronegative patients. However, if the HIV infected women showed signs of immunosupression, as detected clinically, or measured by T4 count, beta 2 microglobulin, and p24 antigen the incidence of CIN was increased. An association between depressed local cervical epithelial immunity and HIV related immunosuppression was demonstrated, but this was not shown to be related to CIN. In this study HIV infection

per se did not appear to increase significantly the risk of CIN developing, but immunosuppression as a result of HIV infection did increase this risk.

Abbreviations

AIDS related complex: ARC

Anal intra-epithelial neoplasia: AIN

Acquired immune deficiency syndrome: AIDS

Beta 2 microglobulin: B2M

Candida albicans: CA

Centre for Disease Control, Atlanta, Georgia, USA staging system for HIV disease: CDC stage

Cervical intra-epithelial neoplasia: CIN

Chlamydia trachomatis: CT

95% Confidence Interval: CI

Deoxyribonucleic acid: DNA

Drug Dependency Unit: DDU

Enzyme immuno assay: EIA

Enzyme linked immunosorbent assay: ELISA

Epstein Barr virus: EBV

Fluorescent treponemal antibody: FTA

Full blood count: FBC

Genitourinary medicine: GUM

Gardnerella vaginalis: GV

Hepatitis B: Hep B

Herpes simplex virus: HSV

HIV P 24 antigen: P24 Ag

Human immunodeficiency virus: HIV

Human papilloma virus: HPV

Intravenous drug user: IVDU

Kaposi's sarcoma: KS

Litre: l

Millilitre: ml

Milligram: mg

Neisseria gonorrhoea: NG

Open reading frame: ORF

Perianal intra-epithelial neoplasia: PAIN

Persistent generalised lymphadenopathy: PGL

Polymerase chain reaction: PCR

Radio immuno assay: RIA

Ribonucleic acid: RNA

Royal College of Obstetricians and Gynaecologists: RCOG

Sexually transmitted disease: STD

Syphilis treponemal serology: STS

T4 lymphocyte: T4

T8 lymphocyte: T8

Treponema pallidum haemagluttination antibody: TPHA

Trichomonas vaginalis: TV

Vaginal intra-epithelial neoplasia: VAIN

Venereal disease reference laboratory: VDRL

Vulval intra-epithelial neoplasia: VIN

Chapter 1.

General Introduction

I have worked in the fields of gynaecology and genitourinary medicine since General Medical Council registration. I undertook the elective year of RCOG training in genitourinary medicine in St Mary's Hospital, London W2, in 1984/1985. At this time the first substantial numbers of cases of HIV related disease in the UK were diagnosed in the department of Genitourinary medicine at St. Mary's and as a Senior House Officer in the unit I assisted in their management. Thereafter, I returned to a gynaecology post in Glasgow. At this time under the supervision of Dr J Davis in Stobhill Hospital, I developed an interest in colposcopy and undertook a study to determine the prevalence of sexually transmitted diseases in patients with normal and abnormal cervical cytology (1). Thereafter I was employed in Bellshill Hospital, where HIV, although not prevalent in the local population at that time stimulated much anxiety on the part of both medical and midwifery staff with regard to patient to staff transmission of the virus which, although extremely rare appeared to have occurred through needle stick injury. Whilst not investigating the incidence of needlestick injury directly, I undertook a study to determine the incidence of glove puncture at Caesarean section (2). After obtaining membership of the Royal College of Obstetricians and Gynaecologists I was keen to combine the above interests and was fortunate to obtain a research post which allowed

this. The research post was based jointly in the departments of Gynaecology and Genitourinary medicine at St Mary's Hospital. The project was funded by the AIDS virus education and research trust (AVERT), a charitable organisation aimed at promoting HIV research, primarily, in the often ignored area of HIV in women.

I was responsible for organising and coordinating the overall study and specifically for the clinical aspects of the study in collaboration with Mr Mark Botcherby much of the laboratory analysis.

The study was designed to test the following hypotheses:
(i) that HIV infection predisposed to cervical neoplasia,
(ii) that this cervical neoplasia would be as a direct result of HIV related immunosuppression,
and/or
(iii) that this cervical neoplasia might be as a result of immunosuppression causing increased expression of HPV and EBV in previously infected cervical epithelium,
and/or
(iv) as a result of decreased immune surveillance in the cervix.

Chapter 2.
Literature Review

(i) Introduction

An unusually high incidence of CIN has been reported in patients infected with HIV (3, 4, 5, 6, 7, 8, 11, 12). However, most of these studies were uncontrolled and may only have reflected an increased exposure of the subjects to recognised risk factors, such as age at first sexual intercourse, number of sexual partners and smoking. At the inception of the study an exhaustive literature search confirmed that a study with a control group had not been performed to determine which factors were predisposing HIV seropositive women to an apparent increase in CIN. The following review of the literature is divided into six sections each dealing with a different background component to the above hypotheses.

(ii) HIV and CIN

Uncontrolled observation has suggested that lower genital tract neoplasia is more common in HIV infected women than in those not infected (3, 4, 5, 6, 7, 8, 11, 12). Bradbeer (3) reported the first series of HIV positive women studied for CIN. In this study 11 patients were enrolled and studied by cytology, of these 9 were also studied by colposcopy and colposcopically directed biopsy. Of the 11 patients 2 had CDC stage 4 disease. The putative mode of HIV transmission in this group was that 3 patients were from sub-Saharan Africa, 5 were IVDUs. Nine of the 11 patients had changes suggestive of CIN on cytology and / or histology and 8 of these 9 patients had CIN confirmed on histology. No control group was recruited, the only comparison possible was with routine cytological examination performed within the same GUM clinic which showed an

8% prevalence of dyskaryosis. This report suffers from certain problems when it comes to interpreting why 82% of patients infected with HIV should have changes suggestive of CIN:

(a) Risk factors for CIN in the HIV seropositive patients were not established.

(b) Laboratory measurements of immunosuppression were not made.

(c) The control group was not specifically selected to match the HIV seropositive patients for CIN risk factors.

(d) The number of patients recruited to the study was small.

Following Bradbeer's report three further letters were published in the Lancet:

Spurrett et al (4) reported results on 10 patients who had contracted HIV infection by different means from those described by Bradbeer: 2 by heterosexual transmission, 6 by blood transfusion and 2 by artificial insemination. Of these patients, 5 had CIN on histological examination. Of these 5 patients with CIN, none had AIDS. T4 / T8 counts were performed which showed a possible trend towards an association between a low T4 count and the presence of CIN. This report whilst showing a lesser prevalence of CIN than Bradbeer still demonstrates a high prevalence of abnormality (50%). This study also has small patient numbers, lack of quantification of CIN risk factors and lack of any control group.

Crocchiolo et al (5) reported a larger series of 24 patients in whom 22 had acquired HIV by IVDU and 2 by heterosexual contact. In this study 2 patients had CIN on

histological examination and a further patient had CIN on cytology not confirmed by histology. Twenty two of their patients had evidence of koilocytosis which they took as evidence of HPV infection. No in-situ hybridisation techniques were used to confirm the diagnosis or subtype of HPV which may well have led to overdiagnosis of HPV infection. Sixteen of their patients were CDC stage 2 and 3 and 8 were CDC stage 4. They failed to show any association between immunosuppression as quantified clinically, or by laboratory measurement and CIN or HPV infection. Although the authors compared the prevalence of HPV infection with their general population (3% prevalence) and their colposcopy clinic population (32% prevalence) they had not quantified their HIV infected patients in terms of recognised CIN risk factors and had not enrolled an appropriate control group.

Byrne et al (6) reported a review of their 15 HIV seropositive patients attending the GUM clinic at St Mary's Hospital, London W2. Eleven patients had normal cervical cytology and 4 (36%) had cytology suggestive of CIN; this compared with a 12% prevalence of abnormal smears in the routine GUM clinic population. The 4 patients with abnormal cytology had histological examination performed confirming the presence of CIN. It was also noted that 2 had VIN and 1 had vulval papilloma virus infection. This study again suffered from the lack of an appropriately selected control group. The colposcopic examination of only those patients with abnormal cytology may have led to an underestimate of the prevalence of CIN, VIN and VAIN. Again no attempt was made to

quantify immunosuppression either by clinical features or laboratory measurement.

More recently Henry et al (7) described an association between HIV, HPV and CIN. This study was based on 4 patients attending their general practitioner. They showed that all 4 patients had histological evidence of koilocytosis and CIN. Their study however comprised inadequate patient numbers, no control group, no laboratory measurement of immunosuppression and histological evidence of HPV infection in the absence of laboratory confirmation .

Monfardini et al (8) reported 49 HIV infected patients with unusual malignant tumours of whom 9 had evidence of cervical dysplasia, invasive in one case. All were IVDUs and only one had evidence of immunosuppression clinically. This report is again small and uncontrolled with no laboratory measurement of immunosuppression.

Two studies (9, 10) of anogenital / rectal dysplasia (AIN) have been performed in HIV seropositive men. Whilst there are thought to be similarities between CIN and AIN, these are not fully understood. These studies again suffered from lack of appropriately selected control groups.

More recently Byrne et al (11) have reported a larger series of 19 HIV seropositive patients. Of these 4 had histologically proven CIN. This study had no HIV seronegative control group and HPV detection was performed colposcopically, cytologically and histologically, in the absence of DNA hybridisation techniques. No attempt was made to measure the degree of HIV related immunosuppres-

sion in these patients.

In 1991 Shafer et al (12) reported the first major study which included a control group. They enrolled 111 HIV seropositive women and studied them by cytology, colposcopy, and histology. The control group comprised 76 HIV seronegative intravenous drug users and 526 women (HIV untested) attending the colposcopy clinic. CIN was shown in 41% of the HIV infected women compared to 9% of the HIV seronegative drug users, and 4% of the HIV untested clinic attenders. Frequency and severity of cervical dysplasia appeared to increase with diminishing numbers of T4 cells. The investigators selected two control groups to maximise their chances of controlling for CIN risk factors, The HIV untested group, as the authors admit, is particularly unlikely to be comparable. It is also possible that some of this group may themselves be HIV seropositive. No attempt was made to detect other potentially oncogenic viruses apart from HPV which was detected by koilocytosis alone.

Conclusions

The above papers confirmed that no study with quantification of CIN risk factors, the enrolment of a suitable HIV seronegative control group, appropriate measurement of immunosuppression and sub-typing of HPV infection had been performed.

(iii) Tumours and immunosuppression

An increased incidence of neoplasia has long been noted in patients with primary and acquired immunosuppression

(13). This association will be discussed, however, HIV related tumours will be described only briefly in this section of the text. Many studies have shown an increased incidence of CIN (14, 15, 16, 17) in patients with immunosuppression, particularly in patients on immunosuppressive therapy following renal transplantation.

Inherited cellular immune defects are associated with an increased risk of neoplasia: in one study of the Wiskott-Aldrich syndrome the prevalence of malignancy was 15.4% and in ataxia-telangiectasia was 11.7% (18). Patients with acquired immunodeficiency secondary to cytotoxic or immunosuppressive therapy given for both malignant and non-malignant disease also have an increased risk of malignancy. Renal transplant recipients have a much greater incidence of neoplasia when compared to an age matched control group. These tumours comprise lymphomas, leukaemias and carcinomas. Cervical carcinoma has a 13 fold increase in incidence in organ transplant patients (19). Porreco et al (20) in renal allograft patients reported a 14 fold increase in the prevalence of CIN over the prevalence in women of the same age in the general population. HIV is associated with Kaposi's sarcoma, malignant non-Hodgkin's lymphoma, Hodgkin's disease and malignant oral carcinoma (21).

Cordiner et al (14) reported a series of 26 immunosuppressed patients post renal transplant on varying doses of prednisolone and azathioprine. The patients had been immunosuppressed for 6 months to 8 years. Five patients (19.2%) had histological evidence of CIN. At that time the detection rate of abnormal cervical cytology in the

West of Scotland was 0.3-0.5%, clearly much lower than that found in the immunosuppressed patients studied. MacLean et al (15) reported on 31 patients, 24 of whom were immunosuppressed and 5 (21%) had evidence of HPV infection on cytology and / or histology, although none of the patients had CIN. Schneider et al (16) studied 132 patients who had received renal transplants and found that 11 (8.5%) had cervical condylomata and 6 (4.5%) of these 11 had CIN.

In none of these studies was a comparable non immunosuppressed control group matched for CIN risk factors enrolled. Neither was laboratory quantification of immunosuppression undertaken, nor with the exception of McLean's study was the immunosuppressive therapy quantified. These factors make estimation of the exact level and influence of immunosuppression difficult. Diagnosis of HPV infection in all studies was made by the presence of koilocytosis on cytology and / or histology. This is associated with a potential false positive diagnosis and does not allow HPV type to be determined. It is known that in many cases cervical infection with HPV is not associated with histological evidence of koilocytosis.

Many of the defects in the studies so far discussed were resolved in the study reported by Alloub et al (17). In this study 49 patients with renal allografts and 69 non-immunosuppressed patients were enrolled and investigated by cytology, colposcopy, and histology for evidence of CIN. Cervical biopsies from these patients were also studied by DNA hybridisation for evidence of HPV types 6/11 and 16/18. Alloub et al showed that 26 (53%)

of patients with allografts had cervical abnormalities compared with 20 (29%) of the control group patients. CIN was found in 24 (49%) of the patients with allografts and 7 (10%) of the controls. Overall HPV DNA detection did not differ between the two groups but there was a significant difference in the rate of detection of HPV types 16/18 (27% in the patients with allografts and 6% in the controls). This study resolved many of the problems associated with those studies described above. It still however, left unquantified the degree of immunosuppression in the study group and the time span from commencing immunosuppressive therapy to enrollment in the study. These factors might be expected to influence the incidence and degree of CIN. The control group were well selected for those risk factors known to be associated with CIN, however a degree of bias in patient selection may have occurred, since for the controls to be eligible they must have had normal cervical cytology within the last two years. Unlike the immunosuppressed patients the controls did not have cervical cytology performed and this removed a further area of comparison between the two groups.

An association has also been noted between immunosuppression, HPV and other tumours. A number of case reports exist with reference to bladder neoplasia (19, 20, 21). Querci della Rovere et al (22) and Benoit et al (23) both reported single cases where bladder tumours had developed post renal transplantation and using DNA-DNA hybridisation had shown the presence of HPV 11. Neither case study states whether the presence of any other HPV

types was considered.

Kitamura et al (24) analysed 10 bladder tumours for the presence of HPV DNA. HPV 16 was found in one patient who was mildly immunosuppressed and also had VAIN 3, Bowen's disease of the vulva, common warts on the hand and a recent past history of bladder tumour. Immunosuppression in this patient was defined by a variety of laboratory tests, none of which were apparently performed on the remaining nine patients. Laboratory analysis was performed for HPV types 1, 2, 6, 11, 16, and 18 on all specimens. The failure to quantify immunosuppression in all patients however made it difficult in this small study to ascertain whether immunosuppression and/or HPV had contributed to the presence of neoplasia.

Following the report by Tay et al (25) that the T4 /T8 ratio in the cervical epithelium of women with CIN was abnormal, Turner et al (26) examined T lymphocytes in the peripheral blood of 16 patients with CIN. Nine of the 16 patients had less than 40% T4 lymphocyte counts and the T4 : T8 ratio was inverted in 7 (44%). The authors concluded that CIN was associated with abnormal T lymphocyte populations in peripheral blood. Their failure to enrol a control group made interpretation of the results difficult. Factors predisposing to the development of CIN may have caused these T lymphocyte abnormalities, eg. smoking (27), potentially oncogenic viruses or the immunosuppressive effects of seminal plasma (28).

Conclusion

The above studies all tended to suggest a correlation

between immunosuppression and tumours. The explanation as to why tumours are more likely to occur in immunosuppressed patients is not entirely clear but it would appear that the immunosuppressed patient may have a defect in immune surveillance (29) or may have increased susceptibility to infection with oncogenic viruses (13).

Quantification of immunosuppression is difficult and care needs to be taken in interpreting results of laboratory markers of immunosuppression.

(iv) HIV and immunosuppression.

In 1981, five cases of Pneumocystis carinii pneumonia (PCP) occurring in previously healthy homosexual men from the Los Angeles area were reported to the Centre for Communicable Diseases (CDC), Atlanta, Georgia, USA in their weekly report (30). Shortly thereafter, reports from New York detailed homosexuals with PCP and KS (31). These reports suggested an underlying defect in cell mediated immunity and the condition was termed Acquired Immune Deficiency Syndrome (AIDS). Initially the only apparent risk factor linking these cases was homosexual lifestyle. However, over the subsequent two years similar cases were recognised amongst intravenous drug addicts (32), blood transfusion recipients (33), haemophiliacs (34) , heterosexual contacts (35) and children of infected parents were shown to be at risk over the following two years. By 1986 it was apparent that the disease was epidemic in Central African countries (36). Epidemiologi-

cal studies in Africa and on a more limited basis in Western countries showed that AIDS was not confined to the above risk groups (36, 37). (For further details of the mode of HIV transmission see appendix 1.)

In 1983 the causative virus was first isolated in Paris from a patient with lymphadenopathy and was termed Lymphadenopathy Associated Virus (LAV) (38). These observations were confirmed and extended both in France and in the USA where the virus was named Human T-Lymphotrophic Virus Type 111 (HTLV3) (39). Compromise was reached on the name for the virus with Human Immunodeficiency Virus (HIV). There were few cases of AIDS in the UK before 1983 but by end of January 1989 there were 2049 cases diagnosed in the UK (40).

HIV is a retrovirus and as such possesses the enzyme reverse transcriptase which enables it to synthesise a DNA copy of its RNA genome within the infected host cell. This DNA is then "spliced" into host DNA as provirus, giving rise to viral latency. New virions are produced by budding from the cell surface, a process which occurs at low baseline levels during viral latency but which is greatly accelerated by activation of infected cells (41).

HIV bears a close genetic resemblance to animal lentiretroviruses such as visna virus (42) although important differences exist.

HIV is tropic for cells bearing CD4 antigen and demonstrates a cytopathic effect in cell culture (43). Cells which express CD4 antigen on their surface include "helper" (T4) lymphocytes , monocytes, macrophages and related (immunocompetent) cells including Langerhans'

cells. Glial and oligodendrititic cells within the CNS also express this antigen and are therefore susceptible to infection with HIV (44). Under certain circumstances, for instance following EBV infection, B cells may also express CD4 (45). Recently cell entry independent of CD4 has been demonstrated (46).

Cellular immunity

In HIV infected patients the characteristic immunological abnormality is a depletion of CD4 lymphocytes. This occurs as a result of the cytopathic effect of HIV either directly, by lysis, or indirectly by syncytial formation. In addition, HIV infected CD4 positive cells are targeted for elimination by the host immune system, which exhibits both cytotoxic and suppressive cellular immune responses (44). It has been demonstrated that T8 lymphocytes have cytotoxic activity against virus-infected cells in culture (47). This may have relevance in maintaining the antiviral state in the host. There is also evidence that T8 lymphocytes suppress viral replication without killing the infected cell (48).

Humoral immunity

The humoral response to HIV infection may lead to production of autoantibodies. This is compounded by HIV antigens exhibiting homology with portions of normal cellular components or products. A potential contributing factor to loss of T4 lymphocytes therefore, is the presence of antilymphocyte autoantibodies found in the serum of HIV infected patients. Infection of other CD4 positive cells

is enhanced by HIV stimulated non neutralising antibody formation and this may contribute to its pathogenicity (46).

In contrast to the above negative effects of the humoral response, HIV infection can lead to the production of neutralising antibodies which may help to prevent viral transfer within the host. Antibodies may also facilitate elimination of infected cells by antibody dependent cellular cytotoxicity (46).

Other immunological responses associated with HIV infection are shown in the table below (43, 46, 49):

Table A

Cellular immunity

T lymphocyte depletion (especially T4 cells)

Abnormal in vitro assays of T lymphocyte function

Defective natural killer cell function

Defective monocyte function

Antiviral suppressor cell response

Autoimmune cellular responses

Humoral immunity

Non specific B lymphocyte activation with polyclonal hypergammaglobulinaemia

Impaired response of B lymphocytes to new antigens

Enhancing antibodies

Neutralising antibodies

Autoantibodies

Others

Reduced alpha interferon levels and raised levels of an abnormal "acid labile" interferon

Raised interleukin 2 levels and elevated B 2 microglobulin

Many of these immunological abnormalities are secondary to the reduced number and impaired function of CD4 posi-

tive cells whilst others may be a direct result of HIV products, or of other concurrent infections.

HIV related disease is diagnosed on clinical grounds, but immunological factors may be of assistance in plotting disease progression.

The spectrum of HIV disease has been classified by various methods since the condition was first described. The classification advocated by the CDC originally in 1982, and later updated (50, 51) has proved broadly valid (52), is easily workable by large numbers of physicians working in diverse settings and has reasonable prognostic significance.

Table B shows the CDC classification (53).

Table B

Stage 1 Acute infection

Stage 2 Asymptomatic infection

Stage 3 Persistent generalised lymphadenopathy

Stage 4 Other disease

A Constitutional disease

B Neurological disease

C Secondary infectious disease

Category C1- Specified secondary infections listed in the CDC criteria for AIDS.

Category C2- Other specified secondary infections, diseases

D Secondary cancers

E Other conditions

Stage 1: acute infection

The majority of patients are symptom-free, however in approximately 10% of cases an acute glandular fever like illness may ensue, with tender lymphadenopathy, fever, arthropathy, macular-papular rash, headaches and occasionally aseptic meningitis. These symptoms and signs will occur in the first few weeks after infection and where present are probably features of viraemia. The HIV antibody test described below (pages 82-83) usually becomes positive within 1-3 months of infection or in the latter stages of the infectious mononucleosis like illness described above. After initial infection, patients either become asymptomatic or develop persistent generalised lymphadenopathy (PGL).

Stage 2: Asymptomatic infection

During the asymptomatic phase progressive subclinical damage is occurring to the immune system. This period is of variable length, ranging from months to years and is usually associated with HIV antibody seropositivity.

Stage 3; Persistent generalised lymphadenopathy

PGL is defined by the presence of enlarged lymph nodes (greater than 1 cm) in two or more extra-inguinal sites, persisting for more than 3 months, in the absence of any other recognised cause. Nodal size tends to fluctuate in response to intercurrent events such as infection and fatigue. The nodes are usually firm, non tender, mobile and symmetrical, and may include epitrochlear, axillary, supraclavicular, cervical, pre-auricular, intra-abdominal and inguinal nodes. PGL alone does not indicate poor prognosis and patients may remain well for long periods of time. Progression rates from CDC 2 and 3 to CDC 4 are 51% in 10 years (54).

Stage 4: (ARC and AIDS)

ARC: A prodromal syndrome for AIDS (Group IV A, C2 and E) consisting of greater than 10% weight loss, persistent diarrhoea, fevers, night sweats and oral candidiasis was first described in 1984 (55). Other prodromal features

include hairy oral leukoplakia, seborrhoeic dermatitis, shingles, recurrent pyogenic infection of the respiratory tract or skin, retinal cotton wool spots and moderately severe haematological abnormalities including thrombocytopenia. ARC progresses to AIDS in 25-70% of cases within 2 years.

AIDS: These patients have severe opportunistic infections (Group4 C 1) or opportunistic tumours (Group D) indicative of a profound defect in cellular immunity, where other causes of such immunosuppression have been excluded. The type of opportunistic infection a patient develops depends to an extent on his/her environment. In the USA the commonest opportunistic infection seen in AIDS patients is PCP, whereas in Sub-Saharan Africa, tuberculosis is the most significant pathogen in this group. The opportunistic tumours most commonly seen in AIDS are B cell non-Hodgkins lymphomas and Kaposi's sarcoma. Certain opportunistic infections and tumours carry a better prognosis than others. Patients with an AIDS diagnosis currently may survive for several years before succumbing usually to a variety of opportunistic infections and/or tumours. The range of tumours include B cell and Hodgkin's lymphomas, and Kaposi's sarcoma.

The psychiatric manifestations of HIV disease in women are discussed in appendix 3.

(v) Laboratory markers of HIV infection and immunosuppression

A number of immuno-assays are available which detect antibodies to virus proteins associated with the core and

envelope of the mature virion. These include the screening ELISA based systems, which may be direct (eg Abbott, Organon) or competitive (Wellcome) in type. Antibody is usually detected by 12 weeks following infection, although in some individuals detection may take longer. The presence of antibodies does not appear to play a major role in virus elimination or protection of the individual from disease, but merely serves as a marker of prior infection. Antibody detection is widely used as a surrogate test for the virus which is both difficult and expensive to detect directly by culture. HIV is a life long infection and antibody positive individuals must be assumed to be infectious by the known routes described above. Further details of the tests performed are described on pages 82-83.

As knowledge of HIV disease increased, it became clear that AIDS, as described above, was the end stage of a disease continuum which was largely asymptomatic. This stimulated interest in finding laboratory investigations which could be used to ascertain the stage of HIV disease, in order to facilitate appropriate patient counselling and to enable anti retroviral therapy to be commenced at a suitable juncture. To this end, the following 'surrogate markers' of HIV disease are now used.

T4 lymphocyte counts

The predictive value of T4 lymphocyte counts in HIV disease was calculated in American homosexual men over a 3 year study period (56). In this study, Moss et al showed that T4 counts decline in HIV seropositive men by a mean of 60-100 x 1000/litre a year from a normal value

of 800-900 x 1000/litre. In the first few years after infection most men are asymptomatic and have counts of T4 cells greater than 400 x 1000/ litre. In 3 years approximately 14% of men will progress to AIDS. Ninety percent of symptomatic men with fewer than 400 x 1000 lymphocytes /litre progress to AIDS. The critical number of CD4 cells that identify individuals at risk of developing an AIDS defining event is 200 x 1000 /litre (57). T4 counts are, however, variable and wide individual differences may occur making them, in isolation, potentially unreliable prognostic indicators for the individual patient.

The above figures are derived from men and are therefore not necessarily always applicable to women. It is known that pregnancy in the HIV seronegative patient is immunosuppressive and that cell mediated immunity, as measured by T4 counts is decreased in these individuals. However, this decrease is temporary and reverses in the postnatal period (58). Whilst pregnancy has not been shown to worsen clinical HIV disease in those patients who are CDC stages 2 and 3 at the time of becoming pregnant (59), T4 counts are reduced in pregnancy and this reduction is maintained post delivery in contrast to the recovery seen in an HIV seronegative control group (60). The long term significance of this remains unclear, but it is possible that increasing parity in the presence of HIV infection may have a cumulative adverse effect on cell mediated immunity.

Virological markers

The p 24 antigen of HIV is a virological marker of progressive disease. It is first detectable at the time of

acute infection and thereafter falls with the rise in p 24 antibody that ensues, usually remaining undetectable until late in the HIV disease process (61, 62, 63), (Figure 1). Persistent antigenaemia after primary infection, or the reappearance of antigenaemia later in the course of the disease is associated with a poor clinical outcome (62). Between 10 and 20% of asymptomatic homosexual men infected with HIV have detectable p 24 antigen on ELISA and are at high risk of rapidly progressing to AIDS (64). The above data were derived from studies in groups of male patients; De Wolf et al and Pederson et al in homosexual cohorts (62, 63) and Allain et al in a cohort of haemophiliacs (61). No similar data have been compiled in a female cohort of significant size. It may not be entirely appropriate to extrapolate these findings to the female population who have largely acquired their HIV infection through heterosexual contact and intravenous drug use. In addition pregnancy may influence the detection of p 24 antigen (65).

A more recent study has compared detection of p 24 antigen and antibody with frequency of isolation of the virus from peripheral blood mononuclear cells (PBMC), and cell free plasma in infected individuals (66). In this study Coombs et al showed that HIV could be isolated from 207 of 213 patients (97%) enrolled, regardless of the clinical stage of HIV disease. Plasma viraemia, in contrast, corresponded to the clinical stage of HIV infection. It was detected in 11 of 48 patients (23%) with asymptomatic infection, 32 of 71 patients (45%) with CDC stage 4A disease (ARC) and 75 of 92 patients (82%)

with CDC stage 4C disease (AIDS). Only 45% of patients with plasma viraemia had detectable p 24 antigen and no correlation was found between the plasma HIV titre and the amount of P 24 Ag in the plasma. These results suggest that the capture assay for p 24 antigen is a poor predictor of infectious virus. The inability to detect p 24 antigen in the presence of viraemia may represent the sequestration of HIV p 24 into complexes with antibody, core antigen, whole virions or each of these. Disease does not closely follow the production of, and release of HIV core antigen in plasma or serum (66). These authors also found that the absence of p 24 antibody was a better predictor of HIV disease progression than the presence of p 24 Ag. They concluded that plasma viraemia can be detected at all stages of HIV disease, a finding supported by Ho et al (67). In addition they found that among the four virological markers of HIV disease - namely HIV p 24 antigen, p 24 antibody, HIV isolation from PBMC and isolation of HIV from cell-free plasma - plasma viraemia was the best predictor of the clinical stage of HIV disease. However the assessment of plasma viraemia is both costly and labour intensive, and the results are operator dependent; these features make it an impractical investigation in many centres.

In conclusion, there appear to be differences in the patient populations studied and great heterogeneity in the virological course among patients with similar stages of HIV disease. Where HIV infected female populations are studied the effect of potential hormonal influences may need to be considered.

Beta-2 microglobulin

This small peptide consists of the light chain moiety of the major histocompatability complex (HLA) class 1 antigens. Increased production of beta-2 microglobulin (B2M) is seen with stimulation of lymphoid cells, particularly T cells. Raised serum levels are seen in a number of disease states other than HIV infection, including renal failure, hepatitis and myeloproliferative disorders. In HIV infection, B2M concentration follows a similar pattern to p 24 antigen level, spiking in acute infection, declining in the latent phase and rising with progressive disease (68). However, unlike p 24 Ag, the concentration of B2M is measureable throughout the disease process. Elevated levels of B2M have been shown to predict the development of AIDS both independently and in conjunction with T 4 counts in HIV infected homosexual men. Approximately 25% of infected men with T 4 counts greater than 400 x 1000/litre and serum concentrations of B2M greater than 3 mg/ml will progress to AIDS over 3 years, whereas as many as 75% of men with lower T 4 counts and equivalent concentrations of B2M will progress to AIDS over the same period of time (64). It has also been shown to be of predictive value in haemophiliacs (69) and female IVDUs (70). The upper limit of normal for B2M in blood donors and in non HIV infected homosexuals is 1.7 mg/l. This can be expected to rise to 5 mg/l or more with the development of AIDS (64).

With the exception of Wallace et al (70) the above studies are again based exclusively on men, and therefore

may not be directly applicable to women. Pregnancy is known to increase lymphocyte turnover (71) and therefore elevated values of B2M might be expected. Whether B2M might continue to be elevated post natally in HIV infected women is as yet unknown.

Neopterin concentrations

Neopterin is a low molecular weight compound derived biosynthetically from guanosine triphosphate. Neopterin concentration in urine and in serum has been described as a predictor of HIV disease progression independently of T4 counts in homosexual men, tending to rise with disease progression (72). Facilities were not available for its measurement in this study.

Biological properties of HIV variants

Isolates of HIV appear to differ in biological properties such as replication rate, host range and syncytium-inducing capacity. It has been shown that the most rapid progression to AIDS is observed in individuals with high replicating, syncitium-inducing isolates, whilst individuals with low replicating, non syncitium-forming isolates, have a more indolent pattern of disease (73). In addition, cross-sectional studies have shown that the prevalence of particular HIV variants differs between the early and late stages of infection (74). These findings indicate that differences in the biological properties of HIV isolates are related to the clinical course of HIV

disease and may in fact be causal.

Determination of HIV sub-type was not used in our study since it is technically difficult and expensive to perform, and its exact prognostic significance still requires further elucidation.

(vi) The effect of IVDU on HIV disease

Limited information is available regarding the potential effect of continuing intravenous drug use on HIV disease progression. However, in a study of significant size, Weber et al showed a relative lack of HIV disease progression in a cohort of HIV infected individuals who had discontinued their illicit drug use or who had complied with a methadone maintenance programme, in comparison with those individuals who had continued using parenteral drugs (75). This was a well designed study, which controlled for age, sex and initial T4 count, and used a readily quantifiable end point, namely development of CDC stage 4 disease. Their data suggests that stopping injecting drug use lessens the risk of HIV associated morbidity in infected subjects, and are consistent with those of Des Jarlais who found that continued drug injecting was associated with an increased loss of CD4 positive cells in a New York City cohort of HIV infected IVDUs (76). No association has been demonstrated between the use of non intravenous psychoactive drugs including opiates and accelerated immune deficiency in a study of a relatively small number of HIV seropositive individuals (77).

The deleterious effect of continued illicit parenteral

drug use is thought to stem from repeated antigenic stimulation of HIV infected CD4 cells, which may promote HIV expression and viral replication (78). There is however no evidence in the literature that IVDU in the absence of HIV infection predisposes to immunosuppression. The only studies suggesting this were performed prior to ready availability of HIV testing (79) and the results are therefore uninterpretable.

(vii) The effect of zidovudine on HIV disease and immunosuppression

Azidothymidine (Zidovudine, AZT) is a thymidine analogue that inhibits HIV replication. It is phosphorylated by cellular enzymes and in this active form, inhibits reverse transcriptase and terminates viral DNA production. Zidovudine, at a dose of 250 mgs four times daily was shown by Fischl et al (80) to decrease both the frequency and the severity of opportunistic infections and the mortality in those individuals with advanced ARC or AIDS, when compared to a similar group of patients given placebo. This benefit was shown to last well beyond the study period, but was not indefinite (81).

Since then, the use of zidovudine in prolonging the asymptomatic phase of HIV disease has been much studied both in the UK and France (Concorde trial) and in the USA where the first results were published by Volberding et al (82). In this study, 1338 male patients with CD4 counts of less than 500 x 1000 per litre were randomised fairly equally into three treatment arms: placebo, 500 mg zidovudine per day, and 1.5 G per day. After 55 weeks, 33 of the subjects assigned to placebo had developed AIDS,

as compared to 11 of those assigned to receive 500 mg of zidovudine and 14 of those assigned to receive 1.5 G zidovudine. In the 3 treatment groups the rates of progression (per 100 man-years) were 7.6, 3.6, and 4.3 respectively. The patients assigned to receive zidovudine had a significant increase in the number of T4 cells and a significant decrease in the levels of p 24 Ag. In those patients receiving 1.5 G zidovudine haematological (anaemia or neutopenia) toxicity was more frequent than in the other two groups. The authors concluded that zidovudine in a daily dose of 500 mg was a safe and effective treatment for those patients asymptomatic for HIV disease and with a T4 count less than 500 x 1000/l. The long term benefits of a strategy of early intervention to delay progression and to prolong survival are not yet known. The development of in vitro viral resistance to zidovudine, a feature related to duration of therapy also merits consideration, although its clinical relevance has not yet been established (83). However, the consensus in the USA was that these results merited the prescribing of zidovudine to asymptomatic patients with reduced T4 cell number (83).

The UK interpretation was a more cautious one, since these results showed that zidovudine therapy produced o nly a 5% improvement in outcome, as measured by progression to AIDS, over a period of 1 year. The long term toxicity of zidovudine is unknown, and the finacial cost of lifelong therapy is high. The effects of long term zidovudine in women are unknown, but Wellcome laboratories have described vaginal carcinoma occurring in rats

on long term therapy (personal communication: Wellcome laboratories); the mechanism for this is not understood. The potential effects of this drug on pregnancy have not been elucidated.

(viii) Local cervical immunity, CIN and HIV

Langerhans' cells are epithelial dendritic cells that originate from bone marrow (84) and are important in presenting antigen to T lymphocytes (85). Their numbers in cervical epithelium have been shown to be decreased in the presence of HPV infection and in those individuals who smoke (86, 87, 92).

Hawthorn et al (86) showed decreased Langerhans' cells associated with moderate to high copy numbers of HPV 16 and with low copy numbers of HPV 18. In this study, increased numbers of Langerhans' cells were seen in patients with CIN in the absence of HPV. However, T6 antigen alone was used to identify Langerhans' cells, and no attempt was made to control for smoking. HPV analysis was undertaken on colposcopically abnormal tissue, whereas biopsies taken from colposcopically normal tissue to act as an internal control for Langerhans' cells analysis were not analysed for HPV. In other words the same analyses were not performed in the study and control groups, thus calling into question the validity of the conclusions drawn.

Early studies suggested that in the presence of CIN, Langerhans' cells were increased (88, 89) but more recent work suggests the opposite (87). The more recent results were obtained using two Langerhans' cell markers S 100

(90) and common thymocyte antigen, T 6 (CD1) (91).

Smoking has also been shown to influence Langerhans' cell number and there was a failure to control for this in earlier studies. Barton et al showed that smoking produces a significant decrease in Langerhans' cells in both normal and neoplastic cervical epithelium, whilst cervical tissue from ex-smokers tends to have intermediate Langerhans' cell counts between that seen in smokers and non smokers (92). These authors also showed a significant decrease in Langerhans' cells in cervical tissue from patients with coexistent HPV and CIN. In this study colposcopically directed punch biopsies were taken from abnormal tissue and in the absence of abnormality, from the transformation zone. Their failure in the presence of abnormal epithelium to take biopsies from normal tissue meant the study lacked any form of internal control group. In addition, HPV infection was diagnosed on the basis of histological criteria alone, a technique known for its lack of sensitivity and specificity. These criticisms do not however, invalidate the main findings of the study which were related to the influence of smoking on Langerhans' cell number.

HIV infection also exerts an effect on Langerhans' cell number. The concentration of Langerhans' cells was found to be reduced in skin biopsies from patients with both asymptomatic infection and AIDS (93). This reduction was more pronounced with progression of disease from ARC to AIDS (94). These studies utilised only one monoclonal antibody to identify Langerhans' cells, namely the thymocyte marker T6 (CD1). In only one study was additional

staining of the S100 protein used, and these authors reported weaker staining of Langerhans' cells in skin biopsies from patients with Kaposis' sarcoma when compared with results seen in tissue from HIV seropositive controls not suffering from Kaposis' sarcoma (95).

Daniels et al (96) examined Langerhans' cell number in biopsies taken from areas of oral hairy leukoplakia, an EBV associated condition, seen in patients with advanced HIV disease. They found that in the 23 HIV infected homosexual men studied there was a significant reduction or absence of Langerhans' cells within the lesions in comparison to autologous non lesional mucosa.

Conclusion

These authors postulated that a reduction of Langerhans' cells in oral mucosa, occurring as a result of advancing HIV disease, may result in local immunosuppression permitting EBV replication to continue unchecked. A similar effect might be found in the cervical epithelium of HIV seropositive women and this could contribute to a higher incidence of CIN in these women. However, no data exists to support this hypothesis.

(ix) Lower genital tract neoplasia and HPV

(a) Lower genital tract neoplasia: cytology, colposcopy and biopsy.

The lower genital tract comprises the uterine cervix, vagina and vulva, all of which have the capacity to undergo neoplastic change. At each site, there is a well described premalignant stage, that of intra-epithelial

neoplasia.

Uterine cervix

Pre-malignancy in the cervix was first described by Sir John Williams in 1886 when 8 cases of cervical cancer were presented, one of which today would be described as CIN 3 (97). Rubin in 1910 (98) went on to summarise current feeling at that time by stating that pathological examination of the uterus could reveal evidence of latent carcinoma and that diagnosis of pre-invasive cervical carcinoma was possible. He went on to state that the ability to differentiate between metaplastic, non-malignant epithelial change and atypical epithelium with the capacity for future malignancy would determine the future ability to improve prophylaxis for invasive cervical carcinoma (98). At this time it was accepted that treatment in the pre-malignant phase was likely to produce improved results over treatment of overt malignancy. There was, however, no method except chance of detecting premalignancy until 1925, when Hinselmann described the earliest colposcope and with which he hoped to detect the cancer in its earliest stages, as either a small ulcer or a small exophytic lesion invisible to the naked eye (99).

In 1929, Schiller (100) described the iodine test subsequently named after him. He had noted that malignant squamous epithelium like columnar epithelium and unlike normal squamous epithelium, did not contain glycogen. He also noted that the presence of glycogen affected the ability of the epithelium to stain with a solution of iodine, and went on to recommend that those areas failing

to stain with iodine should be carefully scraped to allow detection of potentially abnormal epithelium by cytology (100). It was not until 1943, however, that large scale screening of the population for cervical premalignancy became feasible, with the development of the technique of exfoliative cytology by Papanicolaou and Traut (101).

Many countries now undertake routine screening of their female populations by cervical cytology. The purpose of the cervical screening programme is to reduce the incidence of invasive cervical cancer within a specified population (102). Judged on this criterium, the success of cervical screening programmes has varied considerably between geographical areas. Finland has one of the most successful programmes, as declining incidence of mortality from cervical carcinoma has been seen since the instigation of cervical cytological screening in the early sixties (103). Smear reports differ between cytology laboratories, however all cytological findings are graded in terms of normal, koilocytosis and/or dyskaryosis (mild, moderate or severe).

After obtaining an abnormal smear, routine management in the UK is to examine the patient colposcopically and take punch biopsies under direct vision for histological analysis.

The histological terminology for squamous intraepithelial lesions of the female genital tract:

CIN 1 = mild to moderate dysplasia

CIN 2 = moderate dysplasia

CIN 3 = severe dysplasia and carcinoma in situ

Gradation of dysplasia is according to the degree of

cellular atypia and change in epithelial architecture:

Mild dysplasia - loss of polarity and of regular stratification are minimal. The nuclei are always enlarged, are often irregular, and are darkly stained. Mitoses are often found and are occasionally abnormal; they are confined to the lower third of the epithelium. The cytoplasm is generally well preserved and keratinisation of single cells of the epithelial surface is a common feature.

Moderate dysplasia- the degree of epithelial abnormality is intermediate between mild and severe dysplasia.

Severe dysplasia- atypia is very pronounced. There is loss of polarity and the crowded cells have large, darkly stained nuclei. Mitoses, occasionally including atypical forms, are seen. The abnormal cells tend to be present in the upper third, as well as the middle and lower thirds, of the epithelium. The superficial cells show a degree of maturation. A layer of flattened cells may form the surface. At its most severe the whole lesion shows the cellular features of carcinoma, but with no invasion of the underlying stroma. This used to be referred to as carcinoma in situ.

Invasive cervical carcinoma- stromal invasion has occurred (104).

Histological grading of CIN lesions is subjective, as demonstrated by Ismail et al (105) who showed a poor level of concordance between histopathologists in diagnosing CIN 1 and 2. The diagnosis of CIN 3 and invasion was, however, much more reliable.

HPV and CIN

General

Papilloma viruses are DNA viruses which belong to subgroup A of the family of papovaviruses. There are still many gaps in our knowledge about the life cycle of the HPV. This virus only fully replicates within terminally differentiated keratinocytes of the host epithelium, and there is no standard tissue culture system available to study the virus *in vitro*. Restriction endonuclease analysis has been used to map genomically distinct types of HPV. More than 60 have currently been identified,and certain types may correlate with infection at different clinical sites and different oncogenic potential (103, 106, 107, 108, 109). HPV types 6 and 11 have been associated with genital warts (103), type 10 with flat lesions of the uterine cervix (103), and types 16 and 18 have been found in association with cancers of the cervix, vulva, vagina and penis, and in cell lines derived from cervical cancers. HPV 16 and 18 DNA sequences are usually detected as non-integrated episomal DNA in non-malignant lesions, however they have been shown to be integrated within the host cell genome of malignant tumours (107).

Epidemiological evidence:

It is known that, independently of her age, a woman's risk of cervical cancer is strongly associated with indices of sexual activity such as age at first sexual intercourse, and number of sexual partners (110, 111). There also appears to be an increased risk if the woman's

partner has himself had multiple sexual partners. In addition, it is also known from epidemiological studies that smoking predisposes towards CIN (112) and there has been much speculation that the oral contraceptive pill might be implicated (113). Viruses have also been implicated. HSV has been studied extensively and its role has never been conclusively confirmed (114) or refuted (115). The potential role EBV is discussed on pages 64-66.

In 1974 the hypothesis arose that certain HPV subtypes may play a key role in the development of CIN, and since then, much experimental work has emerged to substantiate this view. In the 1980's, HPV emerged as the prime candidate amongst sexually transmitted agents in the aetiology of cervical cancer. The favourite current hypothesis is that certain types of HPV play a key aetiological role (108). This hypothesis emerged in the 1970's (109). Since then much experimental work has emerged to substantiate it (108, 109). However the epidemiological evidence is more difficult to assess due to problems in determining prior HPV exposure. No reliable serological test for its antigens exists, nor is it possible to culture HPV in vitro.

Assessment of HPV infection has therefore been based on different criteria in different studies. Clinical examination, colposcopy, cytology, and histopathology have all been used as diagnostic methods for HPV. Cloning of HPV-DNA in bacteria and the application of DNA hybridisation methods revealed the large variety of HPV types and is useful as a means of assessing type specific infection. The DNA amplification technique of PCR pro-

vides the most sensitive method of viral detection.

Doubt has been cast on the role of HPV 16 and 18 since these viruses have been commonly found in normal cervical tissue (116, 117). In the case of HPV 16, Meanwell et al (118) showed that after age adjustment the relationship between HPV 16 and cervical neoplasia disappeared. Further doubt exists since the prevalence of HPV 16 has varied from 18-92% in studies of cervical cancer biopsy specimens and 0-34.6% in studies of normal cervical epithelium (119). These prevalence rates became even higher when studies using PCR to detect cervical infection with HPV 16 and 18 were referenced. This will be discussed below.

Epidemiological studies fall into two categories, prevalence and cohort studies:

(c) Prevalence studies

A large number of studies have been performed in which the prevalence of cervical infection with HPV 6, 11, 16, and 18 have been determined by DNA hybridisation techniques. The patients were classified into 2 categories: normal, and invasive cervical cancer (118), and in 3 of the studies referenced, CIN including gradation was differentiated (117, 120, 121). The initial impression on studying these reports is that HPV 16 and 18 were more prevalent in CIN lesions than they were in normal cervical epithelium and that HPV 6 and 11 were uncommon in invasive cervical lesions but more common in CIN lesions than in normal cervical epithelium.

In all the reports cited above control groups were used, however, the selection of these may have introduced an element of bias. In every case the control groups were much smaller than the study groups. Ideally the controls should have been selected to match the study group for risk factors for CIN. Controls should have been taken from a population representative of that from which the cases were derived, preferably by case matching the controls for CIN risk factors. In a case control study, a control should be defined by the absence of the disease which defines the case, regardless of the presence or absence of the exposure under study, in this case HPV (122).

Problems also exist as described previously in cytological and histological grading of CIN (105). This was particularly marked in low grade lesions, where the difference between CIN 1 and evidence of HPV infection as detected by koilocytosis may have been small, thus causing some HPV lesions to be classified as CIN. This could have led to an increase in the reported incidence of HPV types detected in CIN lesions. The selection of controls could have been biased by the exclusion of those individuals with abnormal cytology, since this inevitably would have excluded many of those with HPV infection.

Cervical sampling methods were also different between patients and controls. Patients with cervical neoplasia had biopsies performed whilst the controls underwent cervical cytology alone. Biopsies were taken from colposcopically abnormal areas, whereas cytology potentially sampled the entire cervix. The problem of false negative

smears is also well recognised and is related to the proportion of the transformation zone which is abnormal. In those women with small areas of abnormality the chance of a false negative smear is increased (123). No comparison has been made between the efficacy of DNA hybridisation using the 2 sampling methods and, therefore the effects of the above discrepancies remain unknown.

Crum et al (124) studied two groups, one with CIN and the other with condylomata. They used Southern blot hybridisation and DNA in situ hybridisation to detect HPV sequences and compare the epithelial distribution of HPV 16, and 6/11 in CIN and selected condylomata. HPV 16 was found primarily in areas of CIN which contained either maturation or koilocytotic atypia. Some differences in patterns of hybridisation for HPV between CIN and condylomata may be explained by morphologic differences in the 2 classes of lesions. This study is flawed by the lack of a control group with normal histology.

DNA hybridisation was performed by three techniques in the above studies (117, 118, 120, 121, 124): dot-blot, Southern blot, and in situ hybridisation. Dot-blot analysis is performed by extraction of cellular DNA followed by the application of hybridisation probes to the unseparated DNA samples. This technique does not allow assessment of HPV integration. Southern blotting involves hybridisation of radiolabelled cloned HPV DNA to cellular DNA extracts, which have been cut and separated by gel electrophoresis; this is more specific than dot-blot analysis. In in situ hybridisation, DNA is not extracted, but the probes are applied directly onto

fixed tissue (in situ) or to cells which have been filtered onto nitrocellulose gel (filter in situ). None of these 3 methods had been evaluated against each other, making direct comparison between studies difficult. It is also apparent that the sensitivity and specificity varies depending on whether the tissue is neoplastic or normal. Southern blot and dot-blot analysis, which involve DNA extraction, are more sensitive in the presence of the low viral copy number seen in malignant tissue. The in situ methods are more sensitive when the copy number is high in relatively few cells, as is found in condylomata or normal cervices (117, 118, 120, 121, 124)

Statistical analysis was confounded by failure to select appropriate control groups. Of the studies cited, the only one which controlled for age was that by Meanwell et al (118). McCance et al (120) documented the ages of their study and control groups, however, the others did not.

More recently the polymerase chain reaction has been used to determine the prevalence of HPV (125, 126, 127). Young et al (125), reported a study of 38 women with cytological abnormality all of whom were positive for cervical HPV infection using PCR analysis. In thirty two of these HPV 16 was detected and in 22 of these, HPV 11 was also found. In seven of the 10 women enrolled with no cytological abnormality, cervical infection with HPV 16 or 11 was detected using this technique. The PCR method employs a primer directed, enzymatic amplification of specific target DNA sequences (128), and the technique has the ability to amplify a specific target sequence present

only once in a sample of 10,000-100,000 cells. The sensitivity of the technique is many times that of Southern blotting (119), in which the detection of less than one copy of viral DNA per haploid cell genome is difficult (125). However owing to its extreme sensitivity PCR analysis may produce false positive results with DNA contamination of either the samples or the primers (129). The authors point out that careful handling and the inclusion of negative control DNA avoids this possibility.

Subsequently, Tidy et al (126) reported a substantially larger study in which they corroborated the high percentage prevalence of HPV 16 in cervical tissue from women with normal cervical cytology (84%), normal cytology post laser cone biopsy (100%), dyskaryosis (70%) and cervical carcinoma (100%).

Syrjanen (103) stated "extreme scepticism should be exercised in interpreting the reports of an 80% detection rate of HPV 16 DNA in "normal women" (ie those with normal cytology)". This scepticism proved to have been justified when Tidy and Farrell published a retraction (130) of their findings in both "normal" and "abnormal" women (131). Reanalysis of 50 of the same specimens with normal cytology, again using PCR, revealed that 7 (14%) had HPV 16 (personal communication, D Wrede). In the light of the above retraction the data of Young et al should be treated with caution. Pao et al (127) using PCR showed that 43 (42.2%) of 102 patients with normal cytology had evidence of HPV DNA in the cervix. No attempt was made to determine HPV type and their findings

suggest a feasible prevalence rate.

(d) Cohort studies:

In these studies a group of women with cervical abnormalities are enrolled and are then followed up to assess the occurrence of advancing cervical lesions and HPV infection. However, such studies have lacked a control group since they do not involve the follow up of women with initially normal cytology.

Three studies have assessed HPV exposure on the basis of type-specific DNA hybridisation (117, 132, 133). Campion et al (132) carried out filter DNA hybridisation on cytological specimens from women with CIN 1, both at initial diagnosis and during follow-up. In 22/26 (85%) of cases with progressive disease infection with HPV 16 was detected. This study seemed to provide good evidence for the involvement of HPV 16. However all the patients enrolled were young (under 30) and there was no control group of women with normal cervical cytology.

Schneider et al (117) followed a group of 36 women with a cytological diagnosis of CIN or HPV. Five of the 24 women in whom only HPV 16/18 was detected progressed to CIN 3, as compared with none of the 12 women positive for HPV 6/11 alone. In this study, the control group was poorly selected.

Syrjanen et al (133) has shown that the progression rate of cervical abnormality in his cohort, was higher in those women infected with HPV 16/18, than in those with HPV 6/11 infection alone. In this study both smears and biopsies were taken. It is not known what the effects of

multiple biopsy are on the natural history of cervical HPV infection or neoplasia, however it has been suggested that in some cases, cervical biopsy may be therapeutic (132).

In conclusion, on the basis of the existing epidemiological studies, the evidence implicating HPV as the primary aetiological agent of cervical neoplasia is still not conclusive.

(e) Experimental evidence:

In 1933 Shope found evidence of warts in the cottontail rabbit. He demonstrated the infectious nature of these warts by infecting domestic rabbits with papilloma material from their wild cousins (134). The cottontail rabbit papillomavirus is now known as the Shope papilloma virus (135). In the same institution, Rous and Beard observed that some of the cottontail rabbits infected with papilloma virus developed squamous carcinoma at the same site. They also noted that certain chemicals including tar and methyl cholanthrene when applied to the papillomata seemed to encourage the development of squamous carcinoma (136).

In humans there were isolated reports of malignant transformation of condylomata accuminata. However, the best evidence of malignant potential in condylomata accuminata came from a rare condition, epidermodysplasia verruciformis (EV), which was described in 1922 by Lewandowski and Lutz. In this condition, a defect in cell mediated immunity renders the affected patient incapable of mounting an adequate response to infection with HPV. As a consequence of this, patients develop multiple skin

warts, in which malignant transformation occurs, particularly in those areas exposed to sunlight. This suggests that ultraviolet light may act synergistically with HPV in the development of malignancy. More recently it was noted that bovine papillomavirus (BVP) which is capable of transforming cells in vitro appeared to induce fibroblastic tumours in horses and hamsters (135). BPV also induces alimentary tract papillomata in cows. These had a much higher chance of undergoing malignant transformation if the cow grazed on bracken fern, possibly by a direct effect on the hosts' immune system (137).

HPV viral DNA is commonly found within biopsy specimens from malignant epithelial tissue. This however, does not necessarily imply a causative role, but may merely signify a "high affinity" of particular HPV types to transformed cells. There is however reasonable evidence that the role of HPV is causative rather than coincidental in established cancer cell lines.

HPV 16 unlike HPV 6 has transforming properties and the ability to immortalise primary human keratinocytes in vitro (138). The early viral proteins E6 and E7, play a major role in the immortalisation of these cells (139, 140). HPV 6 (141, 142) and 11 (142, 143) are mostly found in genital warts, whereas HPV 16 and 18 are associated with 70% of cervical cancers (143). If other virus types thought to have malignant potential are included (HPV types 31, 33, 35 and 39) there is an association in over 90% of cases (143). The papillomavirus genome is commonly found in primary tumours and metastases, and it is also found in cell lines derived from biopsies of malignant

tissue eg HeLa cells (144). These cell lines show a persistence of HPV genome over many years even in the presence of multiple chromosomal alterations. Under these circumstances genes encoding for E6 and E7 regions of the HPV genome were consistently conserved.

HPV viral DNA has been shown to be actively transcribed within the cells of malignant epithelial tissue (144, 145). Viral DNA replicates and is found in an episomal form in benign lesions, but within cancer cells, it is covalently linked to cellular DNA sequences (146). Integration appears to occur at random and both episomal and integrated forms of the complete HPV 16 genome have been implicated in cervical tumours (147). It has also been demonstrated that the growth efficiency of cancer cell lines in vitro is correlated with the amount of early viral protein expressed within the cells (143). Whilst HPV appears to play a role in the aetiology of cervical cancer, alone it is insufficient to cause the malignant transformation of a normal epithelial cell. The epidemiological studies described above demonstrate that the association is not direct. In addition, it is known that malignant tumours are monoclonal and, therefore, only one or a few of the many HPV infected cells escape the normal cellular control mechanisms resulting in malignancy. It appears therefore that cervical oncogenesis is largely dependent on cofactors. The mode of action of these cofactors is currently poorly understood.

Cofactors have been discussed in detail under the various sub-headings within the introduction. Their relation to HPV will be discussed here with cross refer-

encing to other parts of the introduction in brackets. Smoking is known to be a cofactor and it has been speculated that mutagens in cigarette smoke induce the cellular repair mechanisms including cleavage and rejoining of DNA. It is likely that at the same time recombinational events are triggered favouring the integration of foreign DNA into the host genome (143, 148). Mutagens may also act to inhibit factors which have a down-regulatory influence on HPV gene expression (149).

It is known that pregnancy increases the incidence and severity of genital warts, probably as result of a temporary reduction in cell mediated immunity (as discussed on page 36). In addition, long term oral contraceptive usage may be a risk factor for cervical cancer, by altering hormone status and thus potentially influencing HPV expression (150, 151). Corticosteroids increase expression of HPV 16 and 18 and enhance growth in cell lines infected with these HPV types (143).

Conclusion

The outcome of genital HPV infection appears to depend on the site of the infected cell, virus type, and immunological status of the host, as well as the genetic background of the host and exposure to cofactors.

There is ample evidence to suggest that latent HPV infection occurs although its role in cervical malignancy is unknown. The HPV genome often appears to undergo transition from free episome in cervical premalignancy to an integrated state in invasive disease. It therefore seems likely that integration usually precedes malignant

transformation.

In conclusion the experimental evidence is compelling that certain HPV types have oncogenic potential in cervical epithelium, but conclusive experimental and epidemiological evidence is still lacking.

(x) EBV, CIN, and HIV

EBV is a member of the herpesvirus family, and as such once infection has occurred, there is lifelong persistence of the viral genome, resulting in latent infection (152). Its role in oncogenesis was first described by Burkitt, who demonstrated its close association with a lymphoma seen in central African children (153). It was later found to be the aetiological agent in the development of nasopharyngeal carcinoma, in the Chinese male populations of South East Asia (152, 154). Herpesviruses, including EBV were also previously implicated in the development of AIDS (155, 156). In-vitro data suggests that herpesviruses may act as cofactors in HIV pathogenesis by transactivation of the HIV genome (157). Attempts to investigate this association have been hampered, since more than 90% of HIV seronegative men and women worldwide (158) and approaching 100% of the HIV seropositive population have previously been infected with EBV. Amongst HIV seropositive men, investigators have attempted to determine whether significant rises in EBV antibody titre occur with progression from CDC stages 2 and 3 to CDC stage 4 disease (159). However, the polyclonal gam-

mopathy associated with HIV infection renders these results very difficult to interpret (160).

Holmberg et al in his study of 34 patients with 34 matched controls failed to demonstrate a rise in EBV antibody titre with HIV disease progression (159).

EBV has recently been isolated from cervical smears and washings (161, 162). Sixbey et al (161) detected EBV infection in 5 of 28 (18%) women studied by cytohybridisation and viral culture techniques. In four of the women, cell free EBV was detected and in 2 EBV DNA was detected within cervical epithelial cells. Two of the women were recovering from infectious mononucleosis and therefore cervical excretion might be expected. In the remaining patients however, there was no serological evidence of acute EBV infection. The detection of EBV within the cervix suggests that cervical epithelium is a site for viral replication. No cytological or colposcopic examination of the cervix was carried out in this study, the authors did, however, speculate on the oncogenic potential of EBV at this site.

More recently Bevan et al (162) used PCR to determine the incidence of HPV, EBV and HSV viruses in 36 women with histologically proven cervical epithelial abnormality. They demonstrated EBV DNA in 12 women (33%) and suggest that further study is merited. This study failed to have a control group with normal cervical epithelium and no definition was given as to what categorises cervical epithelial abnormality. A literature search has revealed no study comparing rates of EBV DNA detection in patients with normal and abnormal cervical epithelium.

Langerhans' cells in the human nasopharyngeal epithelium may be infected by EBV. It was postulated that the infection of Langerhans' cells by cytolytic viruses removed the cellular defences in the epithelium and allowed the epithelial cells to undergo malignant transformation in response to infection with other viruses (163). This process may also occur in the cervix.

The association of EBV with malignancy at other sites has been noted, particularly in immunosuppressed patients (96). Oral "hairy" leukoplakia (OHL) (as discussed on page 33) is a lesion of the intraoral mucosa associated with EBV proliferation in immunosuppressed individuals (96, 164, 165). EBV has also been demonstrated in epidermal skin lesions in a patient immunocompromised as a result of chronic lymphocytic leukaemia (166).

A literature search revealed that no studies had been performed observing for potential correlation between HIV related immunosupression, the permissive replication of EBV in the cervix and a potential oncogenic effect.

Chapter 3

Methods

(i) Study Design and Patient Recruitment

Prior to commencing the study calculations were performed to ascertain the number of patients that were required to be enrolled to give the study a power of 89%. These were based on there being a 45% incidence of CIN in HIV seropositive women, as derived from the studies which had already been published (3, 4, 6). An incidence of 10% was assumed for the HIV seronegative patients, as derived from a review of the incidence of CIN in women attending the Genitourinary medicine department at St. Mary's Hospital. Using the above criteria, to obtain a power of 89% 40 subjects were required in each group, and it was intended to enrol 50.

Forty HIV seropositive patients were enrolled from the Department of Genitourinary medicine and the Drug Dependency Unit at St. Mary's Hospital, London W2. The author over the two years that the study was undertaken provided a gynaecological and obstetric service for HIV seropositive patients in the Parkside Health Authority. This was under the supervision of Messrs. P. Mason and A. Fraser. In addition, a general gynaecological service, a pre and post pregnancy counselling service were provided. Facilities were available to offer pregnant patients termination of pregnancy or antenatal care depending upon patient preference. Patients were recruited from the GUM clinic by referral to the author of known HIV seropositive women for gynaecological and obstetric advice.

Thereafter patients were with informed consent enrolled into the study. Psychologists at the DDU also referred patients to the author for medical and gynaecological advice which allowed further recruits to the trial. Referral of HIV seropositive women was also made by Dr C Smith of the Raymede Family Planning Clinic.

A further 10 HIV seropositive patients were enrolled from the Unit of Infectious Diseases, Ruchill Hospital, Glasgow via the Claremont Terrace family planning clinic. These known HIV seropositive patients were referred to Dr. M. Hepburn for general gynaecological advice particularly related to contraception, pre-pregnancy counselling, and termination of pregnancy. Thereafter, again with informed consent, patients were enrolled onto the study. All patients enrolled in the study had been tested for HIV by enzyme linked immunosorbent assays (ELISA) to detect envelope antibodies to HIV 1 and 2 and p 24 antibody to HIV 1. In London all the HIV seropositive women regularly attending St Mary's Hospital between October 1988 and November 1990 were enrolled. In Glasgow all the HIV seropositive women who attended the Family Planning Cinic at Claremont Terrace between February 1989 and August 1990 were enrolled, representing approximately 30% of the HIV seropositive patients attending Ruchill at that time.

The HIV seronegative case matched control group were all enrolled via the GUM department at St. Mary's Hospital. The method of recruitment is described below. The HIV seronegative patients had all been tested by the method described above for HIV 1 & 2 within the previous

12 months. They gave no current epidemiological risk factors for HIV infection. For ethical reasons HIV seronegative control group patients were not retested for HIV after enrollment in the study.

The HIV seronegative patients were case matched against the HIV seropositive patients for CIN risk factors, namely age (banded by decade), age of first sexual intercourse (banded <16, or >=16), total number of sexual partners in a lifetime (banded 1-10, 11-40, >40), and a current history of smoking (banded yes/no) (168, 169). It had originally been intended to control for nationality, drug-use and prostitution as an occupation; these parameters, however proved too difficult to match for.

More than 2500 HIV seronegative patients were interviewed in the search for suitable controls. They were taken through the following questionnaire by either the author, Ms. C. Wells or Ms. M. James:

date of birth

Have you ever had an HIV test? yes/ no

If the answer to this question was "no", then no further questions were asked and the patient was excluded from the trial since it would have been unethical to undertake HIV testing purely to allow patient recruitment. If the answer to the above was "yes", as it was in approximately 1800 of the questionnaires undertaken, then the following questions were asked:

Are you a cigarette smoker? yes/ no. If "yes" how many per day?

Age of first sexual intercourse?

Approximate number of sexual partners in your lifetime?

The patients were then matched individually against their HIV seropositive counterpart.

(ii) Data Collection

After the patients had agreed to take part in the study they attended the colposcopy clinic at the GUM Department. Both HIV seropositive and seronegative patients were firstly taken through the questionnaire in appendix 4 which comprised both clinical history and examination. In addition to the questions in the questionnaire, the date of each patient's first positive HIV test was also documented, as was their putative mode of transmission and the year this was thought to have occurred.

(iii) Clinical Examination

Patients had a full physical examination performed to allow staging of HIV disease. This comprised a general examination of the mouth for oral hairy leukoplakia, oral candidiasis and other pathology. Examination of the skin for evidence of rashes: Kaposi's sarcoma and fungal or parasitic infection. Lymph nodes were then looked for: pre and post auricular, cervical, supra-clavicular, axillary, and inguinal. This was followed by a full respiratory and cardiovascular system examination. Thereafter, abdominal examination was performed and the presence of hepatosplenomegaly or abdominal masses noted. The central nervous system was examined by assessment of

the cranial nerves, reflexes, tone, power and sensation. Finally if clinically merited the optic fundi were examined. The results of the above examination were documented in the questionnaire as shown in appendix 4.

(iv) Gynaecological and STD examination : Colposcopy

Following inspection of the vulva, a urethral swab was taken and smeared onto a slide for Gram staining for NG, the swab was then directly plated onto Thayer's medium. Thereafter a speculum was passed and cervical cytology performed using an Aylesbury spatula. The spatula after being smeared on a slide was placed into phosphate buffered saline. A second spatula was used to obtain a further exfoliative cytological specimen from the cervix and this was placed directly into the same container. Swabs were then taken from the endocervix for Gram stain and culture for NG, a microtrak and ELISA for CT and culture for HSV. This was followed by a high vaginal wet preparation for CA, TV and GV. Thereafter colposcopic examination using 3% acetic acid and Lugol's iodine were undertaken. If an abnormality was observed, a biopsy was taken and sent for histological analysis.

In the final fourteen HIV seropositive patients enrolled a cervical biopsy was taken regardless of perceived abnormality and sent for Langerhan's cells estimation. These biopsies were taken from colposcopically abnormal epithelium if any remained after biopsy for histology; if not a biopsy was taken from epithelium with normal appearance. Colposcopic assessment of the vagina, vulva and perianal area was then performed and any abnormal areas visualised were biopsied. If at perianal exami-

nation the anal margin was
involved proctoscopy was performed. If biopsy was required from the vagina, vulva or perianal area, it was performed under local anaesthetic. Finally a bimanual pelvic examination was undertaken to ascertain uterine size and position and the presence or absence of adnexal pathology.

(v) Cytology and Histology

Cervical cytology specimens obtained in London were analysed in the Cytology Department at St. Mary's Hospital by the same senior cytologist. In Glasgow all specimens were examined by the same senior cytologist in the laboratory at Stobhill Hospital. Both centres reported in terms of mild dyskaryosis suggestive of CIN 1, moderate dyskaryosis suggestive of CIN 2, and severe dyskaryosis suggestive of CIN 3. Koilocytotic change was also reported.

Histopathology was performed in the histopathology departments of the Samaritan Hospital, London and Stobhill Hospital, Glasgow. Specimens were reported in terms of CIN1, 2, and 3 respectively. The presence or absence of invasion was always documented.

(vi) Haematological Investigations

HIV seropositive patients had venous blood drawn for full blood count estimation with a differential white count. T4 and T8 counts were also performed and their relative ratios calculated. T4/ T8 counts were performed by flow cytometry. Flow cytometry is a method of studying

cells which can be induced to show fluorescence. The commonest method of inducing fluorescence in cells is by the use of antibodies to specific cell antigens which have been conjugated to fluorescent compounds eg. fluorescine isothionate (FITC) = green and Phycoerythrin (PE) = orange. Using such labelled antibodies it is possible to study the percentage of particular lymphocyte subsets which are immunologically important. The use of dual antibody reagents allows the rapid enumeration of two subsets at one time, in this case T4 and T8 cells. Analysis was done on a histogram.

Direct labelling of lymphocytes was performed using the following reagents: 1 "Cytostat" antibodies: MsIgG1 negative control, T11-RD1 B4-FITC, T8-RD1 T4-FITC.

Methods

1. Three tubes were labelled for each patient negative, T11/B4 and T4/T8.

2. One hundred ul of well mixed blood was added to each tube.

3. Ten ul of appropriate antibody were added to each of the 3 tubes.

4. The tubes were then incubated at room temperature for 10 minutes.

5. Each tube was then in turn run through the "Q-prep" lyse/fix system on a 35 second cycle. The Q-prep is a work station that lyses the red blood cells and fixes lymphocytes automatically. The active ingredients of the Q-prep are: Solution A - Formic acids (lyses red cells).

Solution B - Stabilisers to buffer the system (sodium bicarbonate, sodium chloride and sodium

sulphate).

Solution C - Paraformaldehyde which fixed the lymphocytes.

6. Test protocols were then selected on profile in the following order: Q-prep (neg), T11/B4, then T4/T8.

7. The specimens were then analysed on the profile in the order specified above.

Haematological investigations were not performed in HIV seronegative control patients.

(vii) Virological Investigation

HPV

Cervical epithelial DNA was extracted from the PBS solution into which the Aylesbury spatulae had been placed.

DNA isolation from cervical specimens:

(a) Each sample was vortexed and their respective Aylesbury spatulae discarded into a Chloros pot using forceps. The forceps were then steeped in glutaraldehyde for at least 24 hours after use.

(b) Each sample was then spun down at 3000 rpm for 5 minutes and the supernatant discarded.

(c) The pellet was resuspended in 160 ul of twice concentration Summer's buffer, and transfered to a sterile screw-cap Eppendorf centrifuge containing 100ul sterile water, 28 ul 2.5% sds, 32 ul 10 mg/ ml Pronase E and 4 ul 0.8 mg/ ml t-RNA.

(d) This was then incubated overnight at 37 degrees C.

(e) Phenol/ Chloroform extract was produced by adding

320 mls of TE equilibrated phenol, vortexing it and spinning the solution at maximum speed for 10 seconds in the Eppendorf centrifuge. The top aqueous phase was transferred to a sterile Eppendorf and the process repeated. Three hundred and twenty ul 1: 24 isoamyl alcohol in chloroform was then added, vortexed and spun for 10 seconds at top speed in an Eppendorf centrifuge and then transferred to a sterile screw top Eppendorf.

(f) Ethanol precipitate was then produced by adding 640 ul ethanol and 32 ul 5M NaCl. This was then frozen at -70 degrees C for 1 hour or at -20 degrees C overnight. This was then spun down for 10 minutes and the supernatant taken off, 200 ul of 70% ethanol was then added, vortexed and spun for 5 minutes. The supernatant was then removed and the resulting pellet dried in the vacuum dessicator for 1 hour. This was then resuspended in 40 ul sterile double distilled water.

Electrophoresis

Twenty ul of the sample was digested with 20 units (2 uL) of BamH1 for 1 hour at 37 degrees C. It was then run overnight on a 1% agarose gel at 14 mAmps (7 mAmps if 2 rows of wells were used). The gel was then stained for 10 minutes with 160 ul 1mg/ml Ethidium Bromide in TAE. It was then destained in TAE for 60 minutes. The DNA was then broken up in 0.25 M HCl for 15 minutes. The DNA was then denatured in 1.5 M NaCl/ 0.5 M NaOH for 30 minutes. This was thereafter neutralised in 1.5 M NaCl / 0.5 M TRIS pH 7.5/ 1 mM EDTA for 60 minutes.

Southern Blotting

The gel former was then upturned in a tray to create a

bridge. A length of blotting paper was cut as wide as the gel and long enough to reach the bottom of the tray at both ends in order to act as a wick. Two further pieces of blotting paper were also cut to the size of the gel. A piece of HyBond membrane was cut to the size of the gel. The tray was filled with six times SSC.

The wick was placed on the bridge and soaked in solution in the tray. Any resultant air bubbles were removed with a pipette used in the fashion of a rolling pin. The gel was placed on top of the wick and the SSC was pipetted under it to get rid of any air. The membrane was carefully positioned and SCC again soaked in. A pipette was again used to remove air bubbles. This process was repeated with the 2 pieces of blotting paper. Two packets of medical wipes were placed on top of the blotting paper, and topped with a plastic lid. This was compressed with a weight of 1-1.5 kg and left overnight. In the morning the medical wipes and the blotting paper were remove and discarded. The corner of the filter paper was cut with a scalpel to allow orientation, washed briefly in 2 x SSC to remove traces of agarose, blotted dry and placed in an incubator for 30 minutes. When dry it was wrapped in cling film and irradiated with ultra violet light for 5 minutes to bind the DNA to the membrane.

DNA hybridisation

Hybridisation mixture: 6 x SSC (4.5ml)
5 x Denhard (3.75ml)
0.5% SDS (0.75ml)
1 mM EDTA (30ul)
Standard double distilled water

Salmon sperm DNA 50 ug/ml (75ul)

(total=15ml)

Stringent washes: 2 x 10ml 20 x SSC

1ml 10% SDS

89ml water

0.1ml x 0.5 ml 20x SSC

1 ml 10% SDS

98.5ml water

The incubator was then warmed to 65 degrees C, as were the hybridisation tubes and mixture and the stringent washes.

Prehybridisation: The membrane was rolled up and inserted into the hybridisation tube, DNA side inwards. The hybridisation mixture was added and any large air bubbles trapped between the membrane and the glass wall of the tube were chased out. The tube was placed in a roller inside an incubator preheated to 65 degrees C, and was set to rotate for 2 hours. During this time the probe was labelled.

Hybridisation: the probe was denatured by adding one fifth volume 1 M NaOH, mixing it and leaving it to react for 10 minutes. This was followed by neutralisation which involved adding one fifth volume 1M Tris at pH 7.5 and one fifth volume 1M HCl. The hybridisation mixture was then removed and the probe mixed into it and returned to the hybridisation tube. The tube was then placed into the roller and set to rotate overnight at 65 degrees C.

Stringent washes: The hybridisation mix was then poured off and replaced by 100mls of 2 x wash. It was rotated for 60 minutes in a temperature of 65 degrees C.

It was then replaced by 0.1 x wash and rotated for a further hour again in a temperature of 65 degrees C. The membrane was removed, patted dry with 3 mm filter paper, avoiding complete drying and wrapped in cling film and placed in an autoradiography cassette with radiographic film (Kodak x-OMAT S) and exposed overnight at -70 degrees C. The film was developed and the membrane was thereafter exposed for a further five days at -70 degrees C.

Washing probe off: The membrane was unwrapped and incubated in 0.4M NaOH at 45 degrees C for 30 minutes and neutralised with 0.1% SDS, 0.1 x SSC, 0.2M Tris pH 7.5 at 45C for 15 minutes. After checking that the membrane was no longer radioactive it was then deemed ready for re-probing.

Probe labelling: the Amersham Multiprime DNA labelling system was used with 100ng of probe per membrane. It was made up to 12 ul in water and denatured by heating at 97 degrees C for 2 minutes. Thereafter 10 ul buffer, 5 ul primer/dNTP mix, 3 ul [alpha-32P]dCTP (30uCi), and 2 ul (2 units) DNA Polymerase I (Klenow fragment) were incubated at 37 degrees C for at least 30 minutes.

The labelled probe then required to be separated from the unreacted labelled dCTP. This was done by running the reaction mixture through a Sephadex G50 column. The Sephadex was autoclaved in two volumes of TE buffer prior to use and then kept in the fridge.The column was prepared by taking a long form glass Pasteur pipette, and scoring it with a diamond pen about 1" from the end. The cotton wool plug was then removed and a wad of silico-

nised glass pushed towards the bottom using another pipette. The Pasteur pipette was then clamped in a vertical position on a retort stand. Sephadex was then added to the column and washed down with TE until the settled Sephadex fills the column upto the dimple in the pipette. The column was prevented from drying out by washing with TE. Probe mixture was added to the top of the column and topped up with TE. Using a minimonitor,as soon as activity was detected moving down the column fractions of 2 drops were collected into Eppendorf tubes. Twenty fractions were collected and universal solution replaced in the column. Each tube was checked for activity by holding them against the probe of the monitor. On each occasion there should have been two peaks of activity, the first representing the labelled probe. The two or three fractions of maximum activity were pooled and the volume roughly estimated by using a pipettor set at 20ul; they were kept aside in a lead pot. A 1ml aliquot was taken for estimation of the activity of the probe on a Beta counter. The HPV probes were full length genomic probes type 6/11, 16 and 18 obtained from Dr H Zur Hausen (Deutches Krebsforschungzentrum, Heidelberg).

PCR

Target sequences on the L1 open reading frame of HPV types 6, 11, 16 and 18 were amplified using general primers GP5 and GP6. Each cycle consisted of denaturation at 94 degrees C for 20 seconds, annealing at 40 degrees C for 60 seconds and chain elongation at 72 degrees C for 20 seconds. Full length genome of HPV types 6, 11, 16 and

18 were used as positive controls. Negative controls were a fibroblast line (MRC5) and ddH2O.

To avoid cross contamination, preparation of reagents, processing of patient material, the PCR and Southern blotting took place in different rooms. Positive displacement pipettes or single use Pasteur pipettes were used throughout. Subsequent confirmation and typing by Southern blotting was carried out by the method described above.

EBV

EBV was also detected by Southern blot on the same samples. The EBV probe was a construct of the BAM H 1 fragments of viral DNA for genes L, W, and d kindly provided by Dr PJ Farrell of the Ludwig Institute for Cancer Research, St. Mary's Hospital, London. Serology for EBV was performed using IgG antibody to viral capsid antigen. In those patients with a high titre (640 or greater) an early antigen titre was also performed to elucidate whether the patient was suffering an acute infection or a high variant of normal. The laboratory performing the titres use < 10 = no evidence of past infection, < 640 > or = 10 as evidence of past infection and > 640 as suspicious of acute infection. A four fold difference in titre was within the normal limits of the laboratory.

HSV

Endocervical swabs were placed into viral transport medium and transported to the laboratory on the same day

for tissue culture. If overnight storage was required specimens were stored at 4 C. The swab was innoculated into cell lines of VERO and HEP 2 with Eagle's minimum essential medium (GIBCO) with Earle's salts. This medium contained freshly added Nystatin and gentamycin and was incubated at 37 C overnight. It was then changed the following day to a maintenance medium with streptomycin and penicillin. The following day examination for cytopathic effect was performed. Tubes were read daily for 7 days and if no virus was isolated they were discarded. If a positive cytopathic effect was noted then the solution was innoculated into fresh VERO and fibroblast cell lines. If a further cytopathic effect was noted HSV isolation was reported.

HIV

All patients had HIV testing by the ABBOTT RECOMBINANT HIV 1 / HIV 2 EIA prior to enrollment in the study. Those patients who were HIV seropositive had this test repeated upon enrollment into the study. The ABBOTT RECOMBINANT HIV 1 / HIV 2 EIA is a solid phase enzyme linked immunoassay utilising polystyrene beads coated with recombinant proteins (E. coli) representing HIV 1 core and envelope and HIV 2 envelope antigens.

All patients with a positive test had a confirmatory ENVACOR HIV 1 EIA performed prior to being given their diagnosis as to HIV seropositivity and upon enrollment into the study, this test, as well as the ABBOTT RECOMBINANT HIV 1 / HIV 2 EIA was routinely repeated. The ENVACOR HIV 1 EIA detects antibodies directed against the HIV

1 envelope and core proteins. The ENV bead detects antibodies directed against gp160 and gp41. The CORE bead detects antibodies directed against p24 and p55.

HIV 1 antigen was also tested for to aid in diagnosis and as a measure of immunosuppression. ABBOTT HIVAG-1 test was used to detect HIV 1 antigen. Specimens which tested positive with the HIVAG-1 test were repeated and those with repeatedly reactive specimens were tested using the ABBOTT HIVAG-1 BLOCKING ANTIBODY. This uses a specific antibody neutralisation step followed by testing with ABBOTT HIVAG-1 to indicate the presence of HIV 1.

(viii) Bacteriological Investigations

Full microbiological investigation was performed for Syphilis, NG, CT, TV, CA, and GV. Specimens were taken as described above (page 67.). Those slides which had been Gram stained were examined by direct microscopy. The swabs taken from the urethra and endocervix were as plated onto Thayer's medium for NG culture and the plates incubated at 37 degrees C in CO2 enriched atmosphere for 48 hours. Tests for CT comprised an enzyme linked immunosorbent assay (ELISA) and a direct immunofluorescent antibody technique (Microtrak, SYVA) on samples obtained from the endocervix as described above (page 71). A high vaginal wet preparation in saline was read immediately for TV, and CA. Clue cells were taken as evidence of BV.

Serum was obtained and tested for Syphilis Treponemal Serology (VDRL, TPHA and if indicated an FTA).

(ix) Immunological investigations

General

These comprise clinical staging as described in the introduction (pages 31-34) and categorised into the CDC classification for HIV disease. T4/ T8 subsets were per formed, as were p 24 antigen estimations: these are described above. Beta 2 microglobulin estimations were performed using the Pharmacia B2-micro RIA. Assays were performed on serum. The Pharmacia B2M RIA test used is a double antibody radioimmunoassay. B2M in the sample competes with a fixed amount of I 125 labelled B2M for the binding sites of the specific antibodies. Bound and free B2M are separated by the addition of a second antibody immunoadsorbent followed by centrifugation and decanting. The radioactivity in the pellet is then measured. The radioactivity is inversely proportional to the quantity of B2M in the sample.

Local cervical immunity

As stated in the declaration, this part of the study was performed in collaboration with Dr S Barton, of St Stephen's Hospital, London and Mr P Maddox of the Whittington Hospital, London. This part of the study was commenced at a time when the main study was already well advanced, and therefore only 14 of the 50 HIV seropositive patients were enrolled. The remaining 13 patients HIV seropositive patients who partook in this part of the

study were enrolled by Dr S Barton at St Stephen's Hospital and these patients are not used in any other part of this thesis.

Colposcopically directed punch biopsies were obtained from the cervices of 27 HIV seropositive women whose disease ranged from CDC stages 2 - 4. In the presence of colposcopically abnormal epithelium a biopsy was taken from the most abnormal area; if the cervix was colposcopically normal the biopsy was taken from the transformation zone. The biopsies were cut and stained using 2 monoclonal antibodies for Langerhans' cell markers, S100 protein and T6 (CD1). Detailed description of this technique is described by Barton et al (92).

The concentration of Langerhans' cells was measured both per unit area and per length of cervical epithelium and association sought with stage of HIV disease, CIN, HPV infection, smoking, p24 Ag, and T4 count.

(x) Statistical Methods

The study was originally designed in collaboration with Dr Jane Wadsworth of the Academic Department of Community Medicine at St. Mary's Hospital and she has supervised the statistical analyses involved. In the original planning of the trial an incidence of lower genital tract neoplasia of 45% was assumed in the HIV seropositive patients and 10% in the HIV seronegative patients. With

these incidences 40 subjects in the HIV seropositive study group would have been required, as would 40 subjects in the HIV seronegative control group to give the study a power of 89%.

The questionnaire (appendix 4) was entered onto the St Mary's Medical School main frame computer to facilitate analysis. Statistical analyses have been performed using the McNemar test, the Chi Square with Yates correction, the Fisher Exact test with 95% confidence intervals or the Mann Whitney U test as appropriate. These tests were performed using the SSPS/PC statistical package (Version 2). The test used is indicated alongside the results.

Chapter 4

Results

(i) Introduction

Presumed mode of HIV transmission is described, followed by risk factors for CIN. The 7 unmatched HIV seropositive patients are then described.

All comparisons between HIV infected and noninfected patients use only the 43 case matched HIV seropositive patients. Where comparison of CIN, cervical viral detection, and immunosuppression was made, the entire group of 50 HIV seropositive patients was analysed.

Cytology, colposcopy and histology results are compared for the cervix, vagina and vulva. The results of virological testing for HPV by Southern blot and PCR, and the results for EBV as detected by Southern blot are then compared. Investigations performed to measure immunosuppression are then presented and associations demonstrated. Finally results of studies of Langerhans' cells are presented.

None of the patients enrolled in the study were either pregnant or on zidovudine therapy at the time of enrollment.

The tables, bar charts and scattergrams are shown on pages 96-122.

(ii) Transmission of HIV

In table 1 HIV infection "risk activity" is shown in the 43 HIV seropositive women and their case matched controls. All the control patients had had a negative

HIV test performed in the last year, many of them were the "worried well" who had no high risk activities, however many had placed themselves at risk of HIV infection in the past. As was to be expected many more of the HIV seropositive patients had placed themselves at risk of HIV infection, thus becoming infected, than had the HIV seronegative controls. None of the control group patients admitted to having placed themselves at increased risk of HIV infection since testing. None of the patients enrolled were haemophiliacs or the partners of haemophiliacs.

Thus from Table 1 the following ascribed mode of transmission for those patients who were HIV seropositive was derived: thirty one (62%) of the 50 patients were thought to have acquired their HIV infection by intravenous drug use and 19 (38%) by heterosexual contact. Amongst the 43 matched HIV seropositive patients 27 (63%) were thought to have acquired their infection by intravenous venous drug use and 16 (37%) by heterosexual contact.

(iii) Matching for CIN risk factors

As described above, the variables used for matching the control group were age (bar chart 1), age at first sexual intercourse (bar chart 2), total number of sexual partners in lifetime (bar chart 3) and smoking; as these were individually matched the number of controls and cases in each band are by definition, the same. With respect to age no patients were less than 20 years, 20 (46.5%) were between 20 and 29 years, 19 (44%) were between 30 and 39 years and 4 (9.5%) were 40 years or

over. Ten (23%) of the patients had first had sexual intercourse at less than 16 years and 33 (77%) at 16 years or greater. Eight (19%) had had 1-10 partners, 21 (49%) had had 11-40 partners and 14 (32%) had had > 40 partners.

Twenty eight (65%) of both the study and control groups gave a current history of smoking. Thirty three (66%) of the 50 HIV seropositive patients gave a current history of smoking. A current history of smoking included those who had given up within the previous 6 months. A further 5 patients were previous smokers who had stopped 6 months or more before enrollment into the study and were therefore considered non smokers.

The bar charts also show the matching parameters for the entire HIV seropositive group (n=50). They demonstrate that the matched 43 patients are not significantly different from the entire group.

Analysis was performed to detect if there were any potentially significant differences between the study and control groups with respect to current oral contraceptive pill use and past cervical cytological findings. Five (12%) of the 43 HIV seropositive patients and 9 (21%) of the HIV seronegative patients were currently taking the oral contraceptive pill; this is not a statistically significant difference.

Table 2 shows the comparison between the study and control groups with respect to past cervical cytology. No statistically significant difference was demonstrated.

Table 3 demonstrates the matching parameters in the 7 unmatched patients.

(iv) Cytology, colposcopy and histology.

Comparison and analysis were performed for the 43 HIV seropositive and seronegative patients and the 7 non-matched patients were not considered. Fifteen (35% (CI: 21%-51%)) of the 43 case matched HIV seropositive patients and 8 (19% (CI: 8%-33%)) of the 43 HIV seronegative controls had evidence of CIN on cytology, whereas 6 (14% (CI: 5%-28%)) and 4 (9% (CI: 3%-22%)) respectively had histologically confirmed CIN. In addition, 4 (9%) of the 43 HIV seropositive patients and 1 (2%) of the HIV seronegative patients had intra epithelial neoplasia elsewhere in the lower genital tract (VAIN & VIN). These differences were not statistically significant. 95% Confidence Intervals were also performed for the difference between the two groups with respect to CIN as detected on histology and this was -8% - 18%.

The results described above of cytology, colposcopic findings, and histology of the cervix, vagina and vulva for the HIV seropositive and negative patients are shown in the Tables 4-7. The p value for each analysis and the test used are shown at the foot of each table.

(v) Virological Investigations

Table 8 shows the number and percentage of patients in whom cervical infection with HPV and EBV was detected by Southern blot. Results of HPV detection by PCR are shown in Table 9. HSV as detected by tissue culture was

present in the cervices of none of the patients in the study. It was found to be present in the vulvae of one HIV seropositive and one HIV seronegative patient. An association was sought between HPV and CIN, and EBV and CIN, but none was demonstrated.

EBV serology shown in Table 10 demonstrated evidence of past infection in all cases and controls. Two patients from each group had reciprocal IgG titres of 1280, a result which in the absence of HIV infection is highly suggestive of primary infection.

Investigation was also performed for hepatitis B surface antibody serologically, for herpes simplex virus by tissue culture, and for molluscum contagiosum clinically. Statistical analysis was performed for hepatitis B using the Fisher Exact test. The results are presented in Table 11.

(vi) Bacteriological investigations

The results of bacteriological investigations are presented in Table 12. Culture and microscopy results have been combined for N. gonorrhoeae. Microtrak and ELISA testing for C. trachomatis are also combined. None of the patients studied had lymphogranuloma venereum, chancroid or granuloma inguinale. Since numbers were small no statistical analysis was performed.

(vii) Immunological investigations:

Clinical

Of the 50 HIV seropositive patients 18 (36%) were CDC

stage 2 and 27 (54%) were CDC stage 3. Five (10%) of the patients showed evidence of clinical immunosuppression (CDC stage 4) (see pie chart 1). Significant association between cytological and histological findings and CDC staging is shown in Tables 13 and 14. Cytological and histological evidence of koilocytosis in the presence of CIN was not additionally considered and koilocytosis in the absence of CIN was grouped with normal cytological and histological results for the purposes of analysis.

Table 15 shows the comparison of CDC stage of HIV disease and virology results on the 50 HIV seropositive patients analysed. Statistical analysis was made using the FE test and no associations were demonstrated.

Laboratory

Significant association between CIN and laboratory markers of advancing HIV disease was found and is shown in Scattergrams 1, 2 & 3 and tables 16 & 17 (T4 count - scattergram 1, beta 2 microglobulin - scattergram 2 & 3 and p 24 antigenaemia - tables 16 & 17). No association was found between cervical viral detection and clinical (Table 15) or laboratory evidence of HIV related immunosuppression.

Scattergrams 4 & 5 show T4 count and beta 2 microglobulin plotted against CDC stage of HIV disease. As described in the introduction (pages 33-34 and 37-38 respectively) these tests are known to correlate with increasing immunosuppression and thus advancing HIV

disease. Scattergrams 4 & 5 demonstrate that as expected T4 counts fell and beta 2 microglobulin levels rose with advancing HIV disease.

(viii) Local immunology in the cervix (n=27)

The cervical epithelium of immunocompromised women with HIV infection contains significantly fewer Langerhans' cells per unit area as measured by S100 compared to non immunocompromised HIV seropositive patients. This is demonstrated in Table 18, $p = 0.04$.

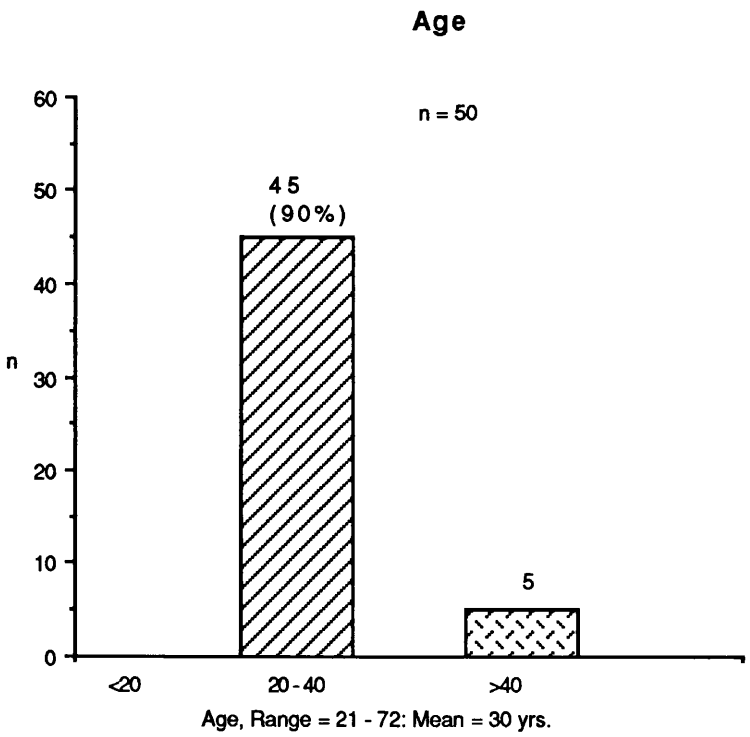
Analysis of these T6 and S100 markers with respect to area and length of biopsy was performed to seek an association with CIN, HPV infection, smoking, T4 count and p24 Ag. This showed no association between any of these parameters. Thus, it can be concluded that the reduction in Langerhans' cells per unit area in immunocompromised patients would appear to be independent of the other parameters studied.

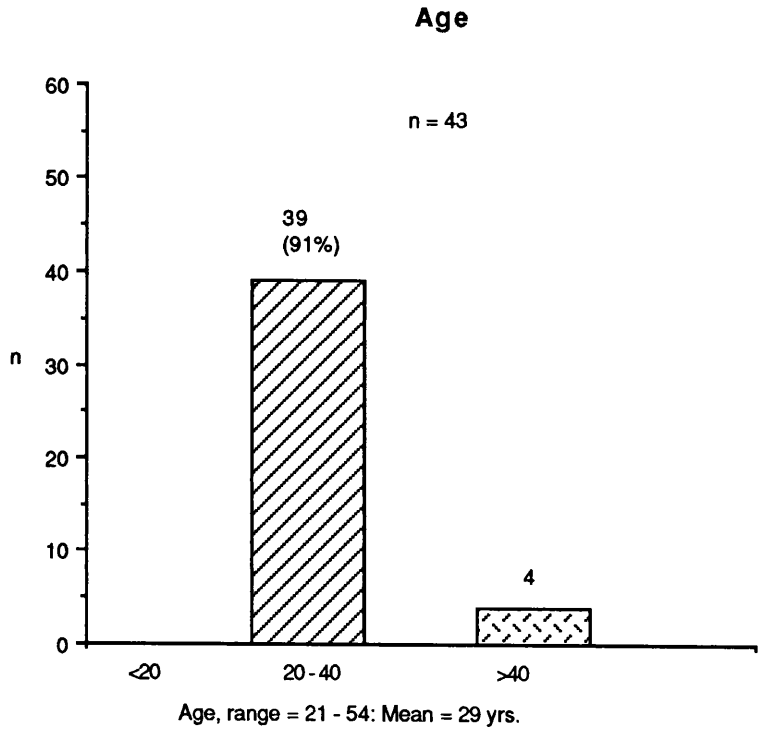
Table 1

"Risk" factors for HIV infection

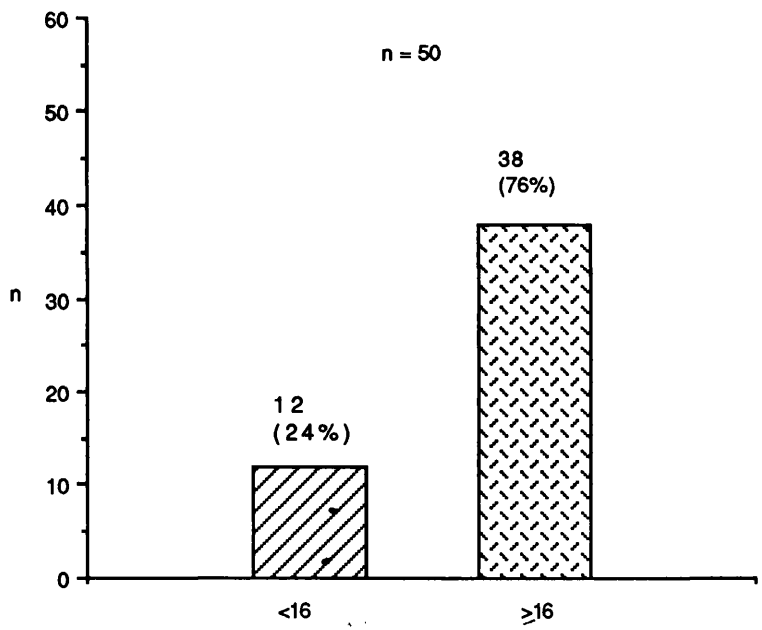
	HIV seropositive (n=43)	HIV seronegative (n=43)
HIV partner	19(44%)	1(2%)
Homo.partner	9(21%)	13(30%)
Blood trans.	4(9%)	4(9%)
"African"	14(33%)	15(35%)
"USA"	8(19%)	20(47%)
IVDU part.	22(51%)	8(19%)
Cur.IVDU	7(16%)	0
Past IVDU	24(56%)	6(14%)

HIV partner=sexual contact with known HIV infected partner, Homo. partner=homosexual or bisexual partner, Blood trans.=patient was the recipient of a blood transfusion, "African"=African in origin and or lived for 1 year in Africa, "USA"=partner from the USA, IVDU part.=IVDU partner, Cur.IVDU=current IVDU, Past IVDU=>6 months since last IVDU.

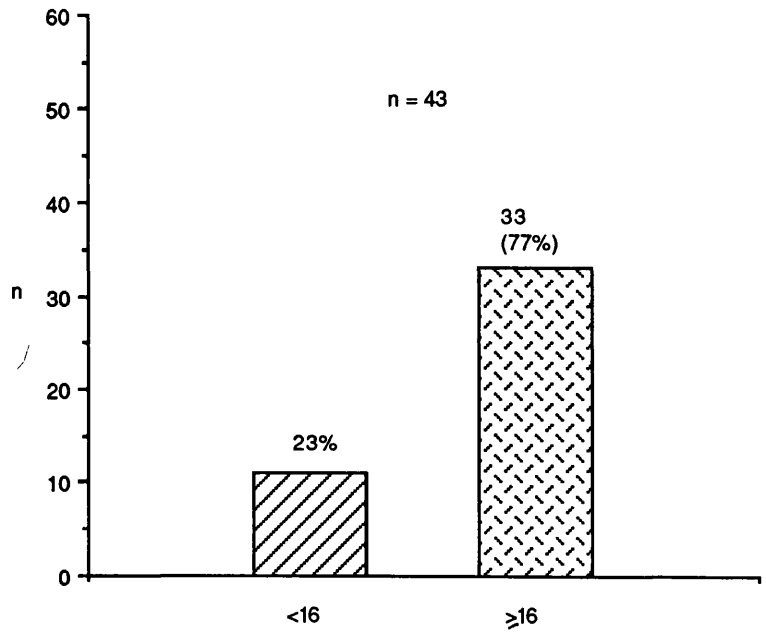

Age
n = 50
45
(90%)
5
n
60
50
40
30
20
10
0
<20
20-40
>40
Age, Range = 21 - 72: Mean = 30 yrs.


Age
n = 43
39
(91%)
4
n
60
50
40
30
20
10
0
<20
20-40
>40
Age, range = 21 - 54: Mean = 29 yrs.

Age of first sexual intercourse.

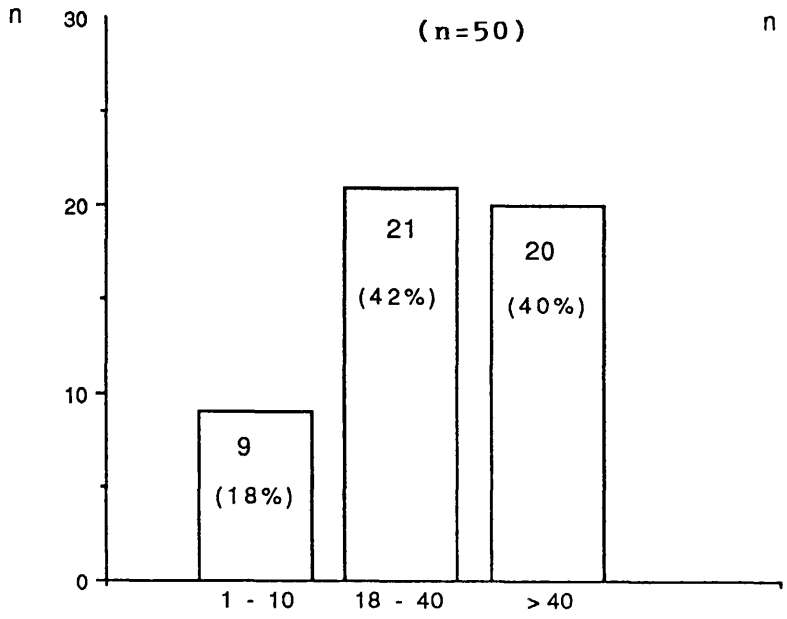


Range, 13 - 28 yrs : Mean 17 yrs.



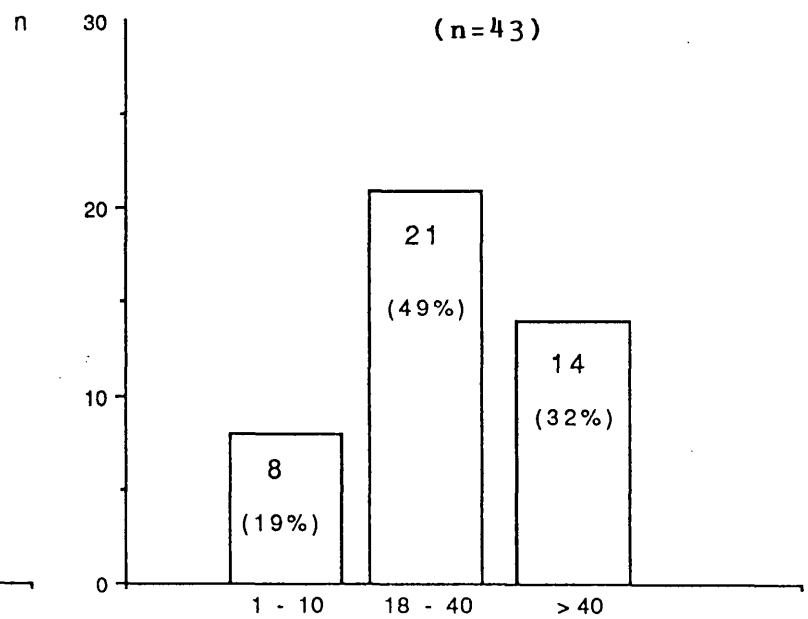
Range = 13 - 28 yrs: Mean = 17 yrs.

Total number of sexual partners



No. of partners

Range = 1 - many thousands
Mean = incalculable



No. of partners

Range = 1 - many thousands
Mean = incalculable

Table 2

Past Cervical Cytology

	HIV seropositive (n=43)	HIV seronegative (n=43)
Normal	24(55%)	30(70%)
Koilo.	0	1(2%)
CIN	8(19%)	9(21%)
Not done	5(12%)	3(7%)
Not avail.	6(14%)	0

(Chi Square with Yates correction = 0.00096; $p>0.05$)

(Koilo. = koilocytosis, N. avail. = not available; ie. cytology performed in other centre and patient did not know the result)

Table 3

Matching parameters in the 7 unmatched patients.

	Age	Coitarche	Smoking	No. of partners
Patient 1	22	15	yes	>40
Patient 2	25	15	yes	>40
Patient 3	40	20	no	5
Patient 4	28	16	yes	>40
Patient 5	72	17	no	>40
Patient 6	27	16	yes	>40
Patient 7	28	17	yes	>40

(coitarche=age of first sexual intercourse)

Table 4

Cytology

	HIV seropositive (n=43)	HIV seronegative (n=43)
Normal	25(58%)	33(77%)
Koilo.	3(7%)	2(5%)
CIN 1	9(21%)	7(16%)
CIN 2	4(9%)	0
CIN 3	2(5%)	1(2%)
Inv.	0	0
Unsuit.	0	0

Normal/koilo. was compared with CIN: no significant difference was found, $p>0.05$, X^2 with Yates correction $=2.14$.

(koilo. = koilocytotic atypia, in the presence of no other abnormality, CIN = changes suggestive of cervical intraepithelial neoplasia, Inv. = cytological changes suggestive of invasive carcinoma, unsuit. = unsuitable)

Table 5

Colposcopy of the cervix

	HIV seropositive (n=43)	HIV seronegative (n=43)
Normal	24(56%)	31(71%)
A-W	18(42%)	8(19%)
Warts	1(2%)	2(5%)
Malig.	0	0
Inad.	0	2(5%)

Analysis was not performed due to the subjectivity of colposcopy.

(A-W = aceto white areas seen, Malig. = appearance suggestive of invasive malignancy, Inad. = inadequate ie squamo-columnar junction not visualised)

Table 6

Histology of the cervix

	HIV seropositive (n=43)	HIV seronegative (n=43)
Normal	3(7%	3(7%)
Not done	24(55%)	31(72%)
Koilo.	10(23%)	5(12%)
CIN 1	2(5%)	2(5%)
CIN 2	2(5%)	1(2%)
CIN 3	2(5%)	1(2%)
Inv.	0	0

Normal, not done and koilocytosis were compared with CIN and no significant difference was found, $p>0.05$, X^2 with Yates correction = 0.113.

(koilo. = koilocytotic atypia, CIN = cervical intraepithelial neoplasia, Inv. = invasion, Not done = since colposcopy was normal)

Colposcopy of the vagina, vulva and perianal area

	HIV seropositive (n=43)	HIV seronegative (n=43)
VAIN	1(2%)	1(2%)
VIN	3(7%)	0
PAIN	0	0
Wart vag.	2(5%)	1(2%)
Wart vul.	5(12%)	6(14%)
Wart p.an.	3(7%)	2(5%)

Numbers were too small to allow any meaningful statistical analysis.

VAIN, VIN and PAIN were diagnosed histologically.

(VAIN = vaginal intraepithelial neoplasia, VIN = vulval intraepithelial neoplasia, PAIN = perianal intraepithelial neoplasia, Wart vag. = appearance suggestive of vaginal HPV infection, Wart vul. = appearance suggestive of vulval HPV infection, Wart p.an.= appearance suggestive of perianal HPV infection.)

Table 8

HPV and EBV detection by Southern blot.

Southern blot

	HIV seropositive (n=43)		HIV seronegative (n=43)			
	No.	(%)	No.	(%)	p value	X^2
HPV 6/11	5	(12%)	1	(2%)	<0.25	1.5
HPV 16	5	(12%)	2	(5%)	<0.5	0.57
HPV 18	3	(7%)	6	(14%)	<0.75	0.44
Total HPV	12	(28%)	9	(21%)	<0.75	0.24
EBV	9	(21%)	5	(12%)	<0.5	0.64

(McNemar test)

One HIV seropositive patient was not tested for HPV, two patients had both HPV 6/11 and 16/18. Three of the HIV seropositive patients who had HPV also had EBV.

Table 9

HPV detection by PCR

PCR

	HIV seropositive No. (%) (n=43)	HIV seronegative No. (%) (n=43)	p value	X^2
HPV 6/11	3 (7%)	1 (2%)	<0.75	0.25
HPV16	5 (12%)	5 (12%)	-	0
HPV18	2 (5%)	8 (19%)	<0.10	3.12
Unid. HPV	5 (12%)	3 (7%)	<0.75	0.25
Total HPV	15 (35%)	17 (40%)	<0.9	0.06

(No. = number of patients, % = percentage of patients, unid. = unidentified)

(McNemar test)

Table 10

Results of EBV serology

Titre	HIV seropositive (n=36)	HIV seronegative (n=37)
0-9	0	0
10-640	34	35
>640	2	2

A titre of 0-9 is interpreted as indicating no past infection, 10-640 as evidence of past infection and >640 as possible recent infection.

Table 11

Virological Investigations

	HIV seropositive (n=43)	HIV seronegative (n=43)	
HB	12(28%)	0	p=0.0001
HSV	1(2%)	1(2%)	
MC	2(5%)	1(2%)	
Warts	4(9%)	7(16%)	

(HB = hepatitis B surface antibody, HSV = herpes simplex virus, MC = molluscum contagiosum, Warts = macroscopic warts and do not include those identified with the colposcope)

(Fisher Exact test)

Table 12

Bacteriological investigations.

	HIV seropositive (n=43)	HIV seronegative (n=43)
GC	0	1(2%)
TV	3(7%)	0
CT	0	0
CA	7(16%)	8(19%)
BV	3(7%)	5(12%)
STS	1(2%)	0

(GC = N. gonorrhoea, TV = Trichomonas vaginalis, CT = Chlamydia trachomatis, STS = syphilis treponemal serology, CA = Candida albicans, BV = bacterial vaginosis.)

CDC stage of HIV Disease (n = 50)

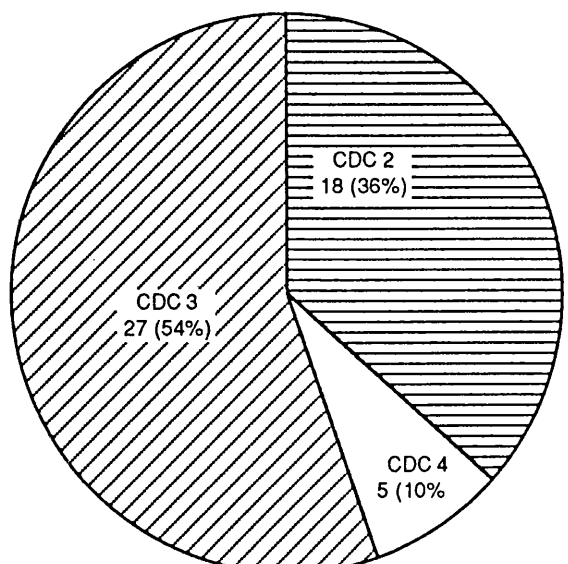


Table 13

CDC stage of HIV disease and cervical cytology (n=50)

	Non immunocompromised CDC 2&3 (n=45)	Immunocompromised CDC4 (n=5)
Normal	31	0
Koilo.	3	0
CIN	11	5

Comparison was made between normal/koilo. and CIN by the Fisher Exact test, p = 0.0014

Difference = 78% (95% CI: 66% - 90%)

(CIN may or may not include koilocytosis, Koilo. = koilocytosis alone with no evidence of CIN)

Table 14

CDC stage of HIV disease and cervical histology

	Non immunocompromised	Immunocompromised
	CDC 2&3	CDC 4
	(n=45)	(n=5)
Normal	32	0
Koilo.	11	1
CIN	2	4

Comparison was made between normal/koilo. and CIN by the Fisher Exact test, p = 0.0003

Difference = 76% (95% CI: 40% - 100%)

(CIN does not include koilocytosis, Koilo. = koilocytosis alone with no evidence of CIN)

Table 15

CDC stage of HIV disease and virology results

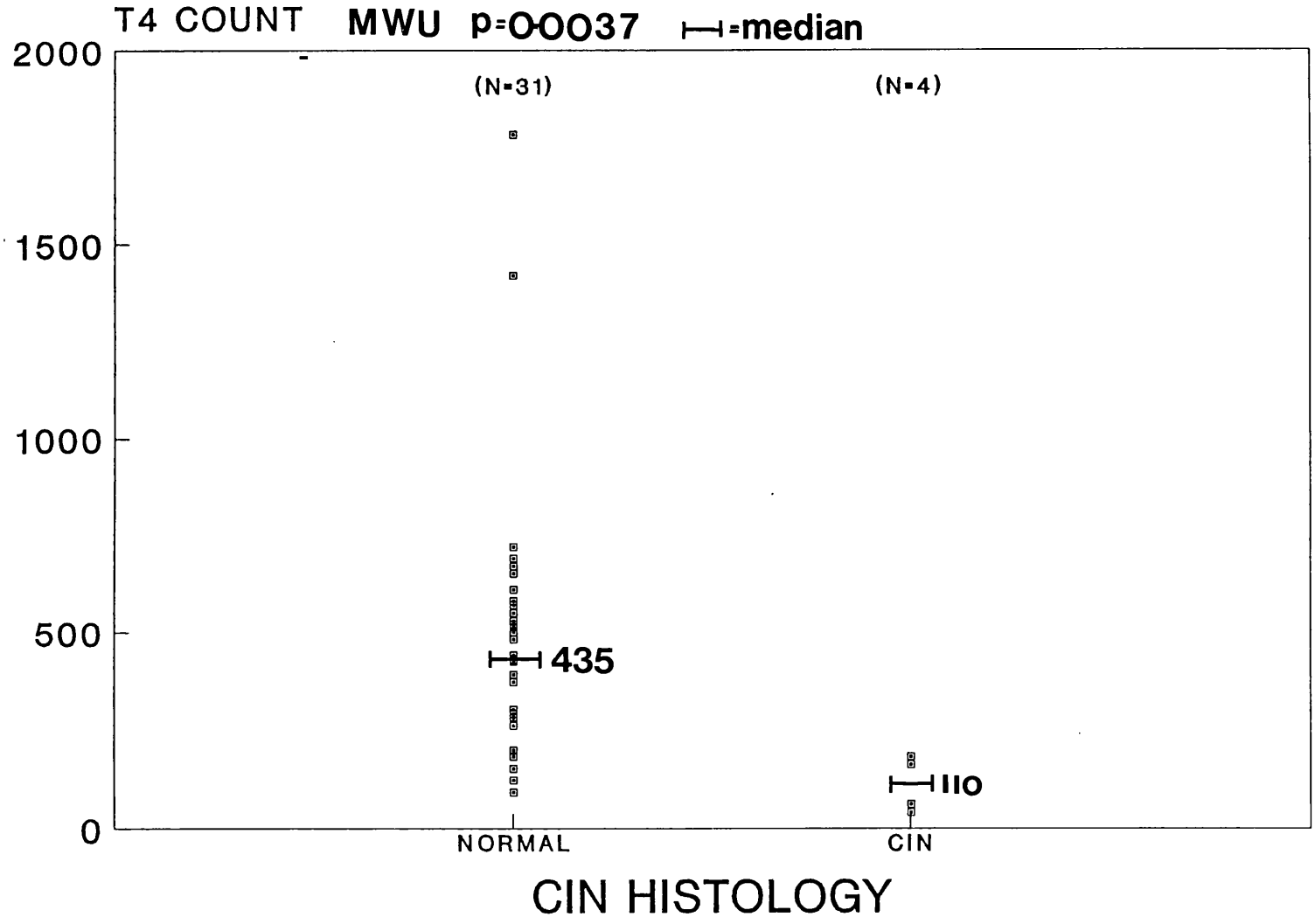
	Non immunocompromised CDC 2&3 (n=45)	Immunocompromised CDC4 (n=5)	p value
HPV 6/11	5	0	0.57
HPV16/18	9	2	0.94
Total HPV	12	2	0.87
EBV	9	0	0.35

Two patients had HPV 6/11 and 16/18 concurrently. One of the immunocompromised patients did not have analysis performed for HPV.

(Fisher Exact test)

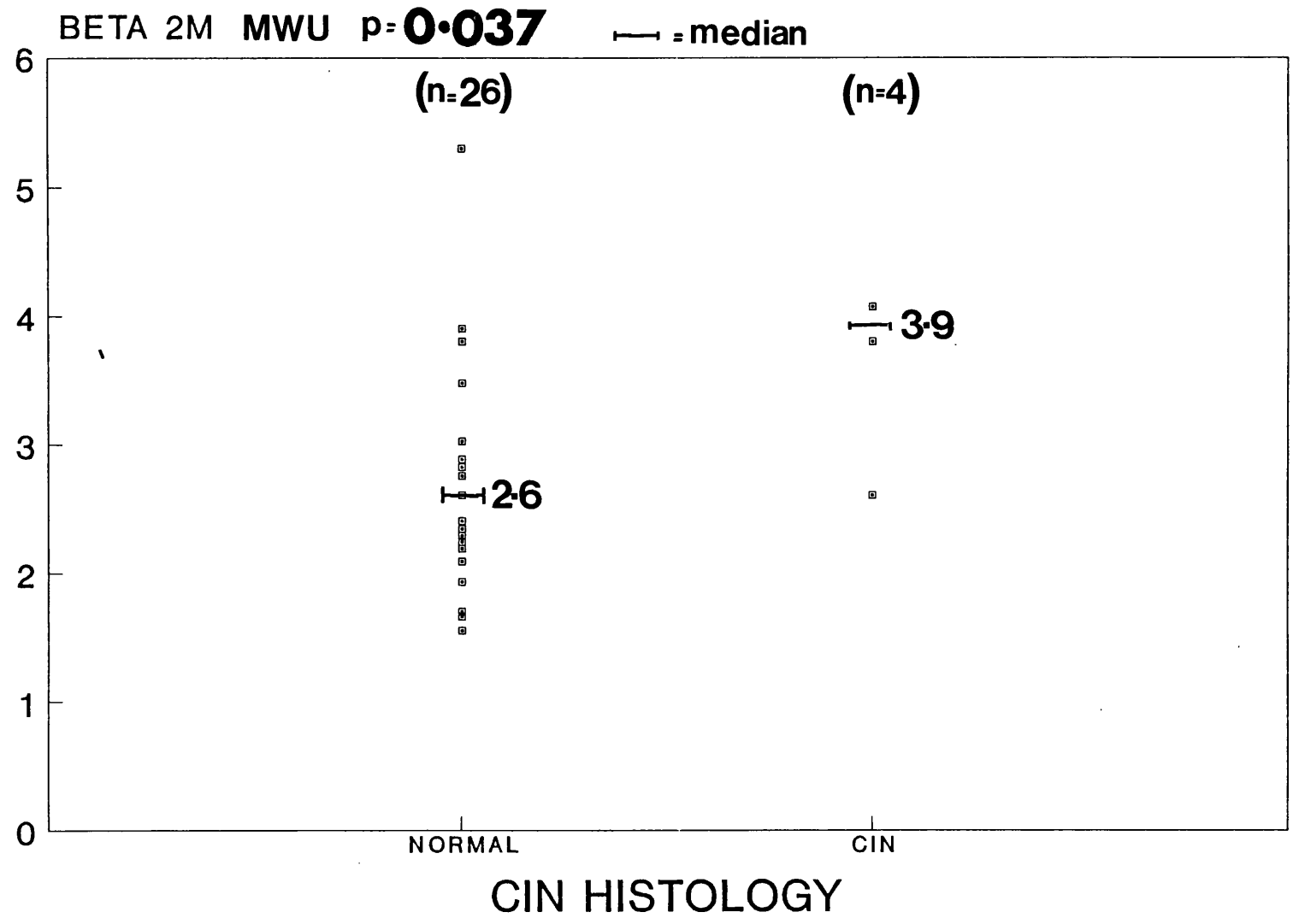
Scattergram 1

T4 COUNT AND CIN - HISTOLOGY



Scattergram 2

BETA 2 MICROGLOBULIN AND CIN - HISTOLOGY



Scattergram 3

BETA 2 MICROGLOBULIN AND CIN - CYTOLOGY

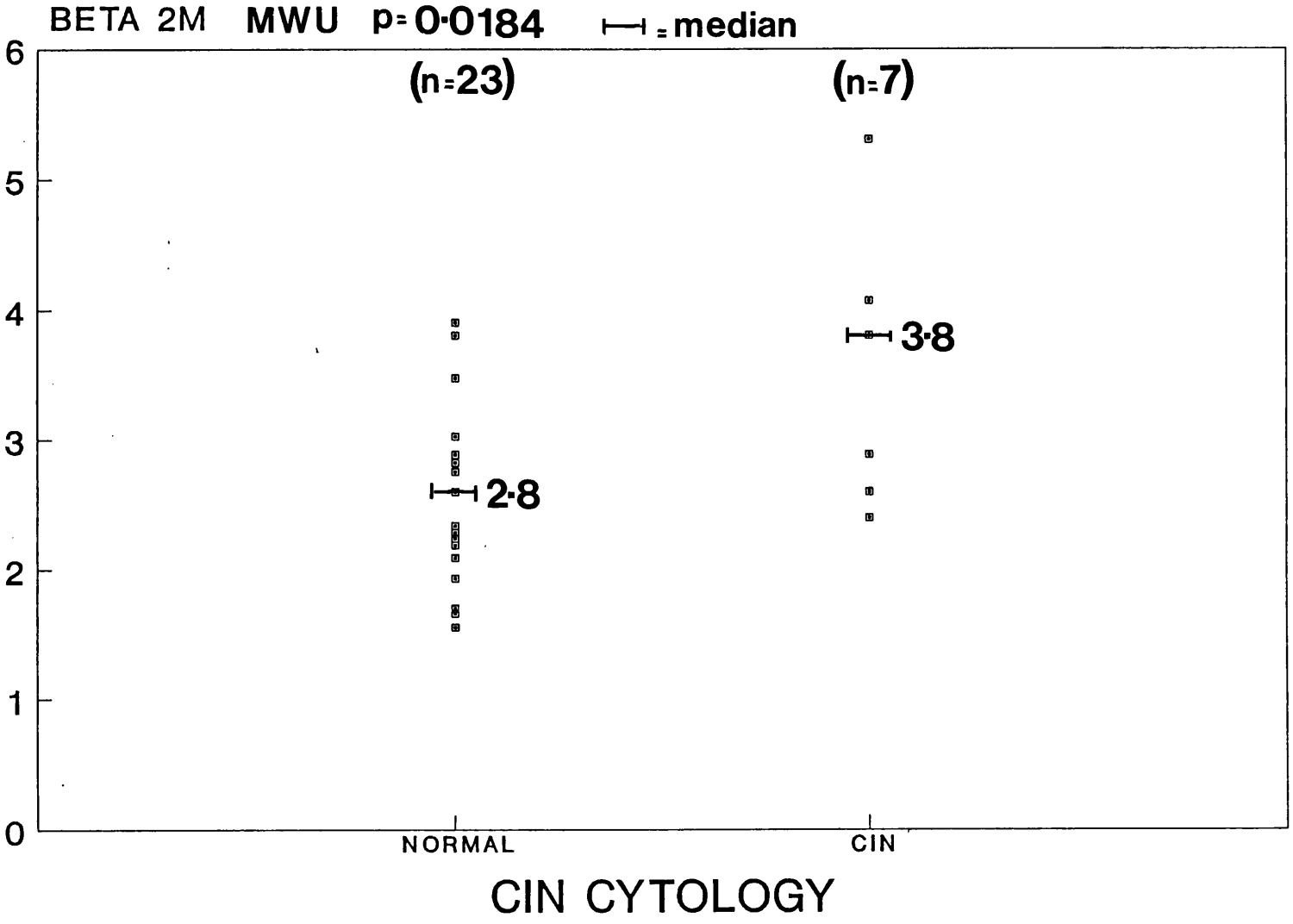


Table 16

P 24 antigen and cervical cytology

	p 24 antigen positive (n=7)	p 24 antigen negative (n=36)
Normal	4	21
Koilo.	0	3
CIN	3 (43%)	12 (33%)

Fisher Exact test , p = 0.47

Difference = 9.5% (CI: -30% - 40%)

(CIN and normal/koilocytosis were compared)

Table 17

P 24 antigen and cervical histology

	p 24 antigen positive (n=7)	p 24 antigen negative (n=36)
Normal)	4	22
Koilo.)	0	11
CIN	3 (42.9)	3 (8.3%)

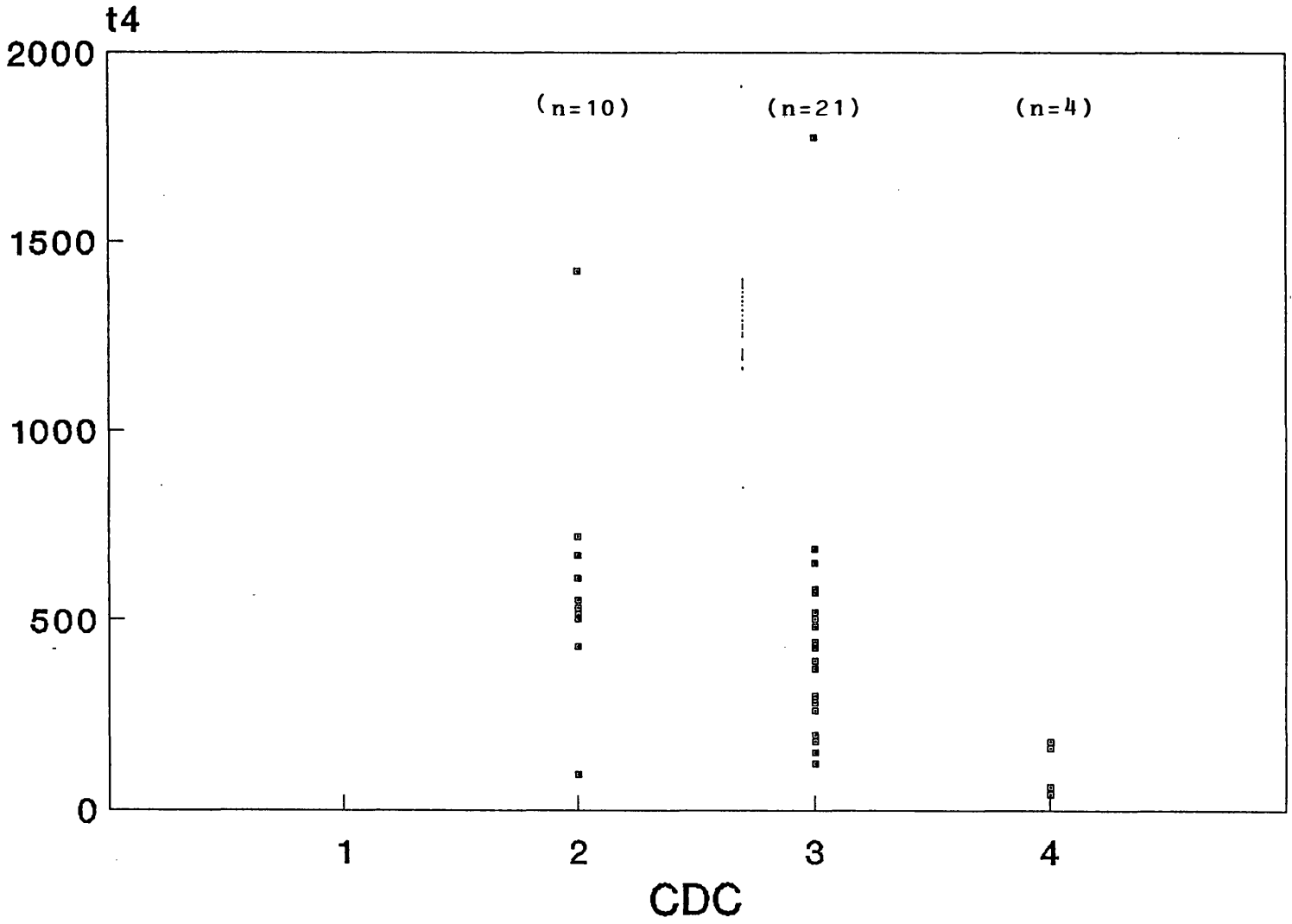
Fisher Exact test, p = 0.0447

Difference = 34% (CI: -3% - 72%)

(CIN and normal/koilocytosis were compared)

SCATTERGRAM 4

T4 CELL NUMBER AND CDC STAGE



SCATTERGRAM 5

BETA 2 MICROGLOBULIN AND CDC STAGE

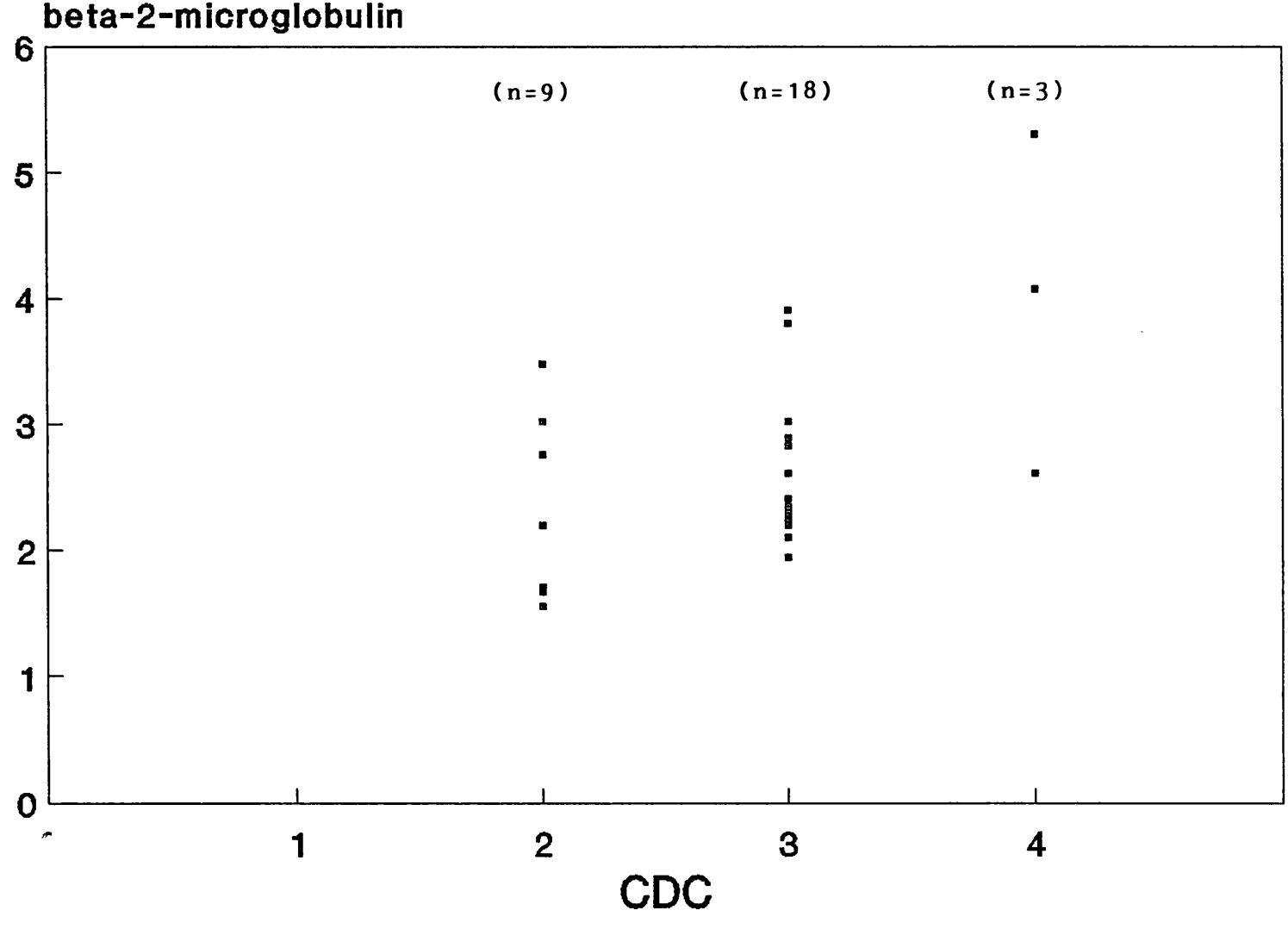


Table 18

Comparison of S100/unit area with Stage of HIV disease

Disease Stage	No.	median	rank	z value
Asymptomatic	5	18	14.9	0.28
PGL	15	7	14.3	0.22
ARC	1	8	14.0	0.00
AIDS	6	4	12.5	-0.52
Total	27			

No. = number of patients, median = median value of S100/unit area.

Wilcoxon Rank Sum test p = 0.04

Chapter 5

Discussion

(i) Mode of HIV transmission and controlled parameters

The HIV seropositive patients fell largely into 2 categories, namely indigenous patients who acquired the infection by intravenous drug usage and African patients with sexually acquired infection. However, it can often prove difficult to assign a single mode of transmission to HIV infected women, particularly IVDUs who also have HIV infected sexual partners.

The study group was enrolled in London and Glasgow, whereas the control group were enrolled exclusively in London. However, the racial characteristics of patients in the control group were similar to that of the study group. Oral contraceptive pill use and past cervical cytology were not controlled for, however both the study and the control group were similar in these respects. An immunosuppressive effect of intravenous drug use in the absence of HIV infection has not been described. However, in those patients infected with HIV, intravenous drug use has been shown to increase HIV disease progression (75). It was therefore felt invalid to control for intravenous drug use.

In 7 of the HIV seropositive patients, a history of smoking, large numbers of sexual partners and early age of first intercourse precluded the enrolment of matched controls.

The HIV seronegative control group patients were selected from those women attending the genitourinary clinic with negative HIV tests and were matched for age,

cigarette smoking, total number of sexual partners, and age of first sexual intercourse. The match made for smoking was based on a history of current smoking; to be classified as "non smoking" the patient had to have stopped smoking for more than 6 months. This period was selected arbitrarily. Ex-smokers tend to have Langerhans' cell counts between those of smokers and non-smokers (92). It was not possible to match for a history of being an "ex-smoker" or to make allowance for specific numbers of cigarettes smoked.

None of the HIV seropositive patients in the study were either pregnant or taking zidovudine at the time of enrolment, and these factors are therefore not confounding variables.

(ii) Cytology and histology

Those patients with normal colposcopic findings, but cytology suggestive of CIN 2 or 3 on 2 consecutive occasions, had a diagnostic and therapeutic cone biopsy performed. The discrepancy between cytological and histological findings occurred because patients with cervical cytology suggestive of CIN 1 in the presence of normal colposcopic findings did not necessarily undergo cervical cone biopsy. These patients were either reviewed colposcopically at 6 monthly intervals or if they preferred, a cone biopsy was performed after 2 consecutive smears showing mild dyskaryosis. For this reason, in several cases where smears showed mild dyskaryosis, histological confirmation of CIN was not available.

A lower prevalence of CIN in HIV seropositive women

(14%) has been shown than that found in previously published studies (3-7, 11, 12). The earlier sudies may have been biased towards patients with symptomatic HIV disease. It is also possible that there were a lower number of IVDUs amongst the HIV seropositive group in this study than those previously reported (3-7, 11, 12), this was certainly the case with the work of Shafer et al (12). In this study we actively sought out and recalled HIV positive women for colposcopic assessment, rather than recruiting patients routinely attending the genitourinary medicine clinic. We may therefore have selected a group of patients who are currently less sexually active and arguably less symptomatic with regard to their HIV disease (90% CDC stages 2&3).

(iii) Bacteriological findings

The incidence of STDs is surprisingly low (0% GC, 0% CT) in those infected with HIV and (2% GC, 0% CT) in the control group, particularly for a group of women many of whom had had multiple sexual partners. This, however may reflect their close medical supervision in the department of genitourinary medicine. Candida albicans is a common opportunistic infection in symptomatic HIV infected women. The majority (45/50) of the women in this study were, however not clinically immunocompromised and therefore, as would be expected there is no difference between the HIV seropositive and seronegative groups.

(iv) Virological findings

Twelve (33%) of the HIV seropositive patients and 9

(21%) of the HIV seronegative controls had cervical infection with HPV 6/11, 16, and 18 detectable by Southern blot. There was no statistically significant difference between the HIV seropositive and seronegative groups with regard to the detection of HPV types on testing by Southern blotting. PCR showed a higher prevalence of HPV 18 in the HIV seronegative women, this did not however reach statistical significance. By controlling closely for CIN risk factors, it was not expected that there would be a difference in the prevalence rates of HPV infection as detected by PCR. Higher detection rates for HPV and EBV by Southern blotting might have been expected in the HIV seropositive group since HIV related immunosuppression could be expected to enhance viral replication once infection has occurred. This was not seen, perhaps because of the low number of significantly immunosuppressed patients in this study.

In the past koilocytosis, as detected by cytological and histological analysis, was presumed to be evidence of HPV infection, however its lack of sensitivity and specificity are well known (171). In this study the much more specific and sensitive techniques of Southern blotting and PCR have been used. Analysis of the data on koilocytosis has not been performed.

Nine (21%) of the HIV seropositive and 5 (12%) of the HIV seronegative patients had EBV detected in the cervix by Southern blot and all the cases and controls had serological evidence of past infection. HIV infection is associated with a polyclonal gammopathy (160) and this may explain the high reciprocal EBV IgG titre of 1280

obtained in 2 of the HIV seropositive patients. However, a similar titre in 2 of the HIV seronegative patients may represent acute EBV infection, a phenomenon previously associated with viral shedding from the cervix (161).

No statistical association was found between cervical infection with HPV or EBV and CIN in either the HIV seropositive or negative groups. The number of patients enrolled in this study was however small and these results have no statistical power to refute an association of these viruses to CIN.

Testing for other viral infections revealed a small number of patients to have molluscum contagiosum, warts and herpes simplex virus. No difference was found between the HIV seropositive and negative patients. Testing however for hepatitis B surface antibody, a marker of past infection with hepatitis B, showed a highly significant difference between the two groups of patients (12 (28%) in the HIV seropositive patients versus 0 in the controls). This was to be expected since the mode of transmission of HIV and hepatitis B are similar and hepatitis B is known to be the much more infectious agent (168); The lack of hepatitis B in the control group reflects not having recruited HIV seronegative IVDUs.

(v) Immunosuppression

An association was sought between abnormalities detected on cervical cytology and histology and the stage of HIV disease (CDC stages 2 and 3 were considered

together). Cytology and histology results were classified into 3 categories: normal, koilocytosis and CIN (CIN 1, 2 and 3 combined). For analytical purposes cytologically and histologically diagnosed koilocytosis in the absence of CIN was grouped with normal. This was felt to be valid since, although koilocytosis is not normal per se it is not a precursor of CIN.

A significant association was found between clinical immunosuppression and CIN as detected both cytologically ($p=0.0014$, CI=66-90%) and histologically ($p=0.0003$, CI=40-100%). This finding is in accordance with those of previous studies which have shown an increased incidence of CIN and invasive cancer in iatrogenically immunosuppressed patients (13-24) and in those patients with advanced HIV disease (12).

The laboratory markers of HIV related immunosuppression used in this study are known to correlate with each other and with worsening HIV disease (61-64, 68-70); the results in this study concurred with these findings. Although this study was not designed to investigate this, confirmation is useful since previous studies have been hampered by innacurate laboratory results. Scattergrams 4 & 5 show the expected trends in T4 count and beta 2 microglobulin with advancing HIV disease. The lack of association of p 24 antigenaemia with advancing HIV disease is most likely due not to laboratory error, but to the small numbers of patients involved.

In this study reducing T4 cell numbers ($p=0.0037$), rising beta 2 microglobulin levels ($p=0.037$) and the presence of p 24 Ag ($p=0.0447$) were independently asso-

ciated with an increasing incidence of CIN as detected histologically, and for beta 2 microglobulin this was also the case cytologically ($p=0.0184$). There was no association between immunosuppression as measured in this study and the detection of EBV or HPV in the cervix.

The lack of association demonstrated may be due to there being small numbers of significantly immunocompromised patients in this study.

(vi) Langerhans' cells

Progressive HIV disease as measured clinically resulted in a reduction in the marker S100 of Langerhans' cells per unit area of cervical epithelium. No other associations were shown. This suggests that this association is independent of the known effect of smoking and HPV infection on Langerhans' cells. Failure to show an association with CIN, HPV, smoking, and falling T4 count is probably a reflection of the small number of patients enrolled in this part of the study.

Chapter 6

Conclusions

(i) General

In conclusion, it has been shown that 14% of the HIV infected women had CIN detectable by histological analysis and that immunosuppression predisposed towards cervical intraepithelial neoplasia as detected both cytologically and histologically. There was an increased detection of HPV 6, 11, 16, and EBV from the cervix in HIV infected women; this difference did not reach statistical significance. In this study HIV infection per se did not appear to significantly increase the risk of CIN developing, but immunosuppression as a result of HIV infection did increase this risk. Cervical Langerhans' cells have been shown to be reduced with advancing HIV disease and although it has not been demonstrated in this study, it may contribute to the association of neoplasia with advancing immunosuppression.

Prospective follow up of these patients is currently in progress and an increasing incidence of CIN and possibly increasing cervical detection of HPV and EBV may be expected, as these patients become progressively more immunocompromised.

Originally four hypotheses were to be tested:

(i) That HIV infection predisposed to cervical neoplasia.

(ii) That the cervical neoplasia would be as a direct result of HIV related immunosuppression

and/or

(iii) That this cervical neoplasia might be as a result of immunosuppression causing increased expression of HPV and EBV in previously infected cervical epithelium.

and/or

(iv) as a result of decreased immune surveillance in the cervix.

This study suggests that hypothesis (i) is only true when patients are immunocompromised which confirms hypothesis (ii). These data do not, however, have the power to refute hypothesis (i), however the 95% CI of the difference between the respective incidences of CIN of -8% - 18% suggests that, if there is a difference between the two groups it is less than 20% and therefore much less than the difference originally postulated (45% versus 10%). Although it has failed to answer hypothesis (iii), a study of this size was unlikely to succeed in this respect. It is not possible to draw from this study the opposite conclusion "that these viruses do not predispose to CIN" since the data carries no statistical power in this respect. With respect to hypothesis (iv) a reduction in Langerhans' cells has been shown, but this has not been shown to associated with cervical neoplasia in this study.

(ii) Management

In the light of these results annual cervical cytology and colposcopy for HIV seropositive women who are not immunosuppressed either clinically or by laboratory investigation is advised; this is in contradistinction to previous guidelines (3). In those patients who are immunocompromised, more frequent assessment may be desirable.

Appendices

(i) HIV transmission

HIV has been shown to be transmitted by three routes: sexual, blood-to-blood, and materno-foetal contact. This has been derived from epidemiological studies and well documented anecdote. The mode of HIV transmission of the St Mary's cohort is demonstrated in the enclosed publication (172). HIV is an organism which survives very poorly outside the body, making very close contact necessary for person to person transmission. Its poor survival is due to its very fragile outer lipid membrane derived during budding from the infected host cell. Various properties of HIV make it less infectious than Hepatitis B infection (See appendix 2, (170)).

Sexual transmission of HIV can occur amongst heterosexuals through vaginal intercourse or anal intercourse and in homosexuals by anal intercourse. Although a single exposure to an infected individual is all that is required for transmission, risk rises with a rising number of sexual partners. This has been reflected geographically whereby homosexual transmission is most common in the USA, Europe and Australasia whilst heterosexual transmission is most common in Africa, the Caribbean, and South-East Asia. Where spread occurs by other means eg. intravenous drug abuse, this can form a bridge for further sexual spread, particularly since many IVDUs fund their drug habit by prostitution. Previously there was much emphasis placed on specific aspects of sexual contact in the transmission of HIV, this does not tend

to be borne out by the evidence. Mucosal trauma, genital ulceration, practices such as "fisting" amongst homosexuals and anal intercourse were thought to represent special risk since trauma occurs more readily. It would appear, that rather than the act being causal, it reflects that these practices are most prevalent in the most sexually active persons. The virus is present in blood and genital secretions and trauma and genital ulceration therefore, although facilitating spread, are not essential for transmission.

Early on in the HIV epidemic attention focussed on spread through blood products particularly amongst haemophiliacs receiving factor concentrate, and the potential for spread to health care workers by needle stick injury. Screening of donors by the blood transfusion service has virtually eliminated the risk from blood products. The risk to health care workers appears to be slight (see appendix 2 (170)).

The use of intravenous injections by drug users sharing needles and syringes is the most frequent means of blood to blood transmission, particularly if the syringes are flushed with blood to prevent drug wastage. Local sharing practices and relative immobility on the part of IVDUs have produced marked regional variation. Currently, areas such as New York, New Jersey and in Scotland, Edinburgh and Dundee have high incidences of HIV infection in contrast to Glasgow, which whilst having many IVDUs, has a relatively low incidence of infection.

Materno-foetal transmission occurs mostly transplacentally, although transmission may occur at birth or rarely

by breast feeding. Infectivity may be related to the stage of maternal HIV disease and is approximately 13% (173).

(ii) HIV and health care workers

The risks of HIV and hepatitis B infection for health care workers are discussed in the enclosed editorial (170).

(iii) Psychiatric manifestations

The psychiatric manifestations of 25 of this cohort of HIV positive women and a number of the HIV seronegative controls have been studied. A reprint of these results is enclosed (174).

(iv) Questionnaire

The questionnaire used for all patients enrolled in the study is found on the following pages.

(v) HIV and fertility/infertility

These issues are addressed in the two enclosed publications (175, 176).

References:

1. Smith JR, Murdoch J, Carrington D, et al. Prevalence of Chlamydia trachomatis infection in women having cervical smear tests. BMJ 1991; 302: 82-4.

2. Smith JR, Grant JM. The incidence of glove puncture during caesarean section. J Obstet Gynaecol 1990; 10: 317-8.

3. Bradbeer C. Is infection with HIV a risk factor for cervical intraepithelial neoplasia? Lancet 1987; ii: 1277-8.

4. Spurrett B,Jones DS, Stewart G. Cervical dysplasia and HIV infection. Lancet 1988; i:237-8.

5. Crocchiolo P, Lizioi A, Goisis F, et al. Cervical dysplasia and HIV infection. Lancet 1988; i: 238-9.

6. Byrne M, Taylor-Robinson D, Harris JRW. Cervical dysplasia and HIV infection. Lancet 1988; i: 239.

7. Henry MJ, Stanley MW, Cruickshank S, Carson L. Association of human immunodeficiency virus-induced immunosuppression with human papillomavirus infection and cervical intrepithelial neoplasia. Am J Obstet Gynecol 1989; 160: 352-3.

8. Monfardini S, Vaccher E, Pizzocaro G, et al. Unusual malignant tumours in 49 patients with HIV infection. AIDS 1989; 3: 449-52.

9. Rudlinger R, Grob R, Buchmann R, Christen D, Steiner R. Anogenital Warts of the Condyloma acuminatum Type in HIV-Positive Patients. Dermatologica 1988; 176: 277-81.

10. Frazer IH, Melday G, Crapper RM, Brown TC, Mackay IR. Association between anorectal dysplasia, human papillomavirus, and human immunodeficiency virus infection in homosexual men. Lancet 1986; ii: 657-60.

11. Byrne MA, Taylor-Robinson D, Munday PE, Harris JRW. The common occurrence of human papilloma virus infection and intraepithelial neoplasia in women infected by HIV. AIDS 1989; 3: 379-82.

12. Shafer A, Friedman W, Mielke M, Schwartlander B, Koch MA. The increased frequency of cervical dysplasia-neoplasia in women infected with the human immunodeficiency virus is related to the degree of imunosuppression. Am j Obstet Gynecol 1991; 164: 593-99.

13. Penn I. Cancer is a complication of severe immunosuppression. Surg Gynecol Obstet 1986; 162: 603-10.

14. Cordiner JW, Sharp F, Briggs JD. Cervical intrepithelial neoplasia in immunosuppressed women after renal transplantation. Scott Med J 1980; 25: 275-7.

15. MacLean AB, Lynn KL, Bailey RR, Swainson CP, Walker RJ. Colposcopic assessment of the lower genital tract in female renal transplant recipients. Clin Nephrol 1986; 26: 45-7.

16. Schneider V, Kay S, Lee HM. Immunosuppression as a high-risk factor in the development of condyloma accuminatum and squamous neoplasia of the cervix. Acta Cytologica 1982; ?: 220-224.

17. Alloub MI, Barr BBB, McLaren KM, Smith IW, Bunney MH, Smart GE. Human papillomavirus infection and cervical intraepithelial neoplasia in women with renal allografts. BMJ 1989; 298: 153-6.

18. Spector BD, Perry GS, Kersey JH. Genetically determined immunodeficiency disease (GDID) and malignancy: report from the immunodeficiency cancer registry. Clin Immunol Immunopathol 1978; 11: 12-29.

19. Penn I. Depressed immunity and the development of cancer. Clin Exp Immunol 1981; 46: 459-74.

20. Safai B, Lowenthal DA, Koziner B. Malignant neoplasms associated with the HTLV-III/LAV infection. Antibiot Chemother 1987; 38: 80-98.

21. Porreco R, Penn I, Droegemueller W, Greer B, Makowski E. Gynecologic malignancies in immunosuppressed organ homograft recipients. Obstet Gynecol 1975; 45: 359-64.

22. Querci della Rovere G, Oliver RTD, McCance DJ, Castro JE. Development of bladder tumour containing HPV type 11 DNA after renal transplantation. Brit J Urol 1988; 62: 36-38.

23. Benoit G, Charpentier B, Orth G et al. Presence of papilloma virus type 11 in condyloma accuminatum of bladder in female renal transplant recipient. Urol 1988; 32: 343-4.

24. Kitamura T, Yogo Y, Ueki T, Murakami S, Aso Y. Presence of human papillomavirus type 16 genome in blad der carcinoma in situ of patients with mild immunodeficiency. Canc Res 1988; 48: 7207-11.

25. Tay SK, Jenkins D, Maddox P, Singer A. Lymphocyte phenotypes in cervical intraepithelial neoplasia and human papillomavirus infection. Br J Osbstet Gynaecol 1987; 94: 16-21.

26. Turner MJ, Ford MR, Barrett M, White JO, Soutter WP. T lymphocytes and cervical intraepithelial neoplasia. Ir J Med Sci; 157: 184.

27. Miller LG, Goldstein G, Murphy M, Ginns LC. Reversible alterations in immunoregulatory T cells in smoking. Chest 1982; 5: 527-9.

28. Turner MJ, White JO, Soutter WP. Seminal plasma and AIDS. Immunol Today 1987; 8: 258.

29. Sheil AGR. Cancer in organ transplant recipients: part of an induced immune deficiency syndrome. BMJ 1984; 288: 659-61.

30. Centre for Disease Control. Pneumocystis pneumonia-Los Angeles. Morbidity and Mortality Weekly Report 1981; 30: 250-2.

31. Centre for Disease Control. Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men in New York and California. Morbidity and Mortality Weekly Report 1981; 30: 305-8.

32. Masur H, Michelis MA, Greene JB. 1981; An outbreak of community acquired Pneumocystis pneumonia. Initial manifestation of cellular immune dysfunction. New Engl Med J 1981; 305: 1431-38.

33. Centre for Disease Control. Possible transfusion associated AIDS-California. Morbidity and Mortality Weekly Report 1982; 31: 652-4.

34. Centre for Disease Control. Pneumocystis Carinii pneumonia among persons with Haemophilia A. Morbidity and Mortality Weekly Report 1982; 31: 365-7.

35. Harris C, Small CB, Klein RS, et al. Immunodeficiency in female sexual partners of men with the Acquired Immune Deficiency Syndrome. New Engl J Med 1983; 308: 1181-4.

36. Pinching AJ. AIDS and Africa: lessons for us all. J Roy Soc Med 1986; 79: 501-3.

37. Redfield RR, Markham PD, Salahuddin SZ, Wright DC, Sarngadharan MG, Gallo RC. Heterosexually acquired HTLV III/LAV disease (ARC and AIDS): epidemiological evidence for female to male transmission. JAMA 1985; 254: 2094-6.

38. Barre-Sinoussi F, Chermann J , Rey F. Isolation of a T-lymphotrophic retrovirus from a patient at risk for AIDS. Science 1983; 220: 868-71.

39. Gallo RC, Salahuddin S, Popovic M, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV III) from patients with AIDS and at risk for AIDS. Science 1984; 224: 500-3.

40. Anonymous. AIDS in the UK and world wide. Lancet 1989; i: 397.

41. Margolick JB,Volkman DJ, Folks TM, Fauci AS. Amplification of HTLV3/LAV infection by antigen-induced activation of T cells and direct suppression by virus of lymphocyte blastogenic responses. J Immunol 1987; 138: 1719-23.

42. Forster SM, Pinching AJ. AIDS and HIV infection. In: Recent Advances in Medicine; 20. Edinburgh: Churchill Livingstone 1987; 85-108.

43. Stephens RM, Casey JW, Rice NR. Equine infectious anaemia virus gag and pol genes: relatedness to visna and AIDS virus. Science USA 1986; 231: 589-94.

44. Weber JN, Weiss RA. HIV infection: The cellular picture. Sci Amer 1988; ??: 81-87.

45. Montagnier L, Gruest J, Chamaret S. Adaptation of the Lymphadenopathy Associated Virus (LAV) to replication in Epstein Barr Virus transformed B lymphocytes and cell lines. Science 1984; 225: 63-6.

46. Levy JA. Human Immunodeficiency Viruses and the pathogenesis of AIDS. JAMA 1989; 261: 2997-3006.

47. Walker BD, Chakrabati S, Moss B, et al. HIV-specific cytotoxic T lymphocytes in seropositive individuals. Nature 1987; 328: 345-8.

48. Walker CM, Moody DJ, Stites DP, Levy JA. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science 1986; 234: 1563-6.

49. Ho D, Rota TR, Hirsch MS. Infection of monocytes and macrophages by the human T-lymphotrophic virus type III. J Clin Investig 1986; 77: 1712-5.

50. Centres for Disease Control. Revision of the case definition of AIDS for national reporting- US. Morbidity and Mortality Weekly Report 1985 ; 34: 373-5.

51. Centres for Disease Control. Revision of the CDC Surveillance Case Definition for Acquired Immunodeficiency Syndrome. Morbidity and Mortality Weekly Report 1987; 36 (suppl no. 1S): 1-15.

52. Redfield RR, Tramont EC. Toward a better classification system for HIV infection. N Engl J Med 1989; 320: 1414-6.

53. Adler MW: ABC of AIDS, pp13 (BMJ publications 1987)

54. Rutherford GW, Lifson AR, Hessol NA. Course of HIV-1 infection in a cohort of homosexual and bisexual men: an 11 year follow up study. BMJ 1990; 301: 1183-8.

55. Centres for Disease Control. AIDS Update. Morbidity and Mortality Weekly Report 1984; 32: 688-91.

56. Moss AR, Bacchetti P, Osmond D, et al. Seropositivity for HIV and the development of AIDS or AIDS related condition: three year follow up of the San Francisco General Hospital cohort. BMJ 1988; 296: 745-50.

57. Taylor JM, Fahey JL, Detels R, Giorgi JV. CD4 pecentage, CD4 number, and CD 4/8 ratio in HIV infection: Which to choose and how to use. J AIDS 1989; 2: 114-24.

58. Sridama V, Pacini F, Yang SL, Moawad A, Reilly M, De Groot LJ. Decreased levels of helper T cells. N Engl J Med 1982; 307: 352-6.

59. Johnson MA, Webster A. Human immunodeficiency virus infection in women. Br J Obstet Gynaecol 1989; 96: 129-34.

60. Biggar RJ, Pahwa S, Minkoff H, et al. Immunosuppression in pregnant women infected with human immunodeficiency virus. Am J Obstet Gynecol 1989; 161: 1239-44.

61. Allain JP, Laurian Y, Paul DA, et al. Long-term evaluation of HIV antigen and antibodies to p 24 and gp 41 in patients with haemophilia. N Engl J Med 1987; 317:1114-21.

62. De Wolf F, Goudsmit J, Paul DA, et al. Risk of AIDS related complex and AIDS in homosexual men with persistent HIV antigenaemia. BMJ 1987; 295: 569-72.

63. Pederson C, Nielsen CM, Vestergaard BF, Gerstoft J, Krogsgaard K, Nielsen JO. Temporal relation of antigenaemia and loss of antibodies to core antigens to development of clinical disease in HIV infection. BMJ 1987;295: 567-9.

64. Moss AR. Predicting who will progress to AIDS. BMJ 1988; 297: 1067-8.

65. Coombs RW, Collier AC, Allain JP, et al. Plasma viraemia in human immunodeficiency virus infection. N Engl J Med 1989; 321:1626-31.

66. Ho DD, Moudgil T, Masud Alum MS. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 1989; 321: 1621-5.

67. Delfraisy JF, Sereni D, Pons JC, et al. Antigenaemia p24 and CD4 cells counts in HIV pregnant women. Fifth International Conference on AIDS, Montreal 1989. Abstract Number MBP 2.

68. Lacey CJN, Forbes MA, Waugh ME, Cooper EH, Hambling MH. Serum B-2 microglobulin and human immunodeficiency virus infection. AIDS 1987; 1: 123-7.

69. Cooper EH, Forbes MA, McVerry BA, Hall L, Howard M, Helbert M. Beta-2-microglobulin in the follow up of haemophilia with HIV infection. Fourth International AIDS Conference, Stockholm 1988. Abstract number 7792.

70. Wallace JI, Beatrice S, Bekesi JG. Prognostic tests for detection of AIDS disease development in heterosexually active women. Fourth International AIDS Conference, Stockholm 1988. Abstract Number 7833.

71. Siegal I, Gleicher N. Changes in peripheral mononuclear cells in pregnancy. Am J Reprod Immunol 1981; 1: 154-5.

72. Fuchs D, Haussen A, Reibnegger G, et al . Neopterin as a marker for activated cell-mediated immunity. Immunology Today 1988; 9 : 150-5.

73. Tersmette M, Lange JMA, DeGoede REY, et al. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet 1989; i: 983-5.

74. Cheng-Mayers C, Seto D, Tateno M, Levy J. Biologic features of HIV-1 that correlate with virulence in the host. Science 1988; 240: 80-2.

75. Weber R, Ledergerber B, Opravil M, Siegenthaler W, Luthy R. Progression of HIV infection in misusers of injected drugs who stop injecting or follow a programme of maintenance treatment with methadone. BMJ 1990; 301: 1362-5.

76. Des Jarlais DC, Friedman SR, Novic DM, et al. HIV 1 infection among intra venous drug users in Manhattan, New York City, from 1977 through 1987. JAMA 1989; 261: 1008-12.

77. Kaslow AR, Blackwelder WC, Ostrow DG, et al. no evidence for a role of alcohol or other psychoactive drugs in accelerating immune deficiency in HIV-1 positive individuals. JAMA 1989; 261: 3424-9.

78. Zagury D, Bernard J, Leonard R, et al. Long term cultures of HTLV 3 infected cells: a model of cytopathology of T cell depletion in AIDS. Science 1986; 231: 850-3.

79. Orangio GR, Della-Latta P, Marino C, Guarneri JJ, Giron JA, Palmer J, Margolis IB. Infections in parenteral drug abusers. Further immunologic studies. Am J Surg 1983; 146: 738-41.

80. Fischl MA, Richman DD, Grieco MH, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: a double-blind, placebo-controlled trial. N Engl J Med 1987; 317: 185-91.

81. Creagh-Kirk T, Doi P, Andrews E, et al. Survival experience amongst patients with AIDS receiving zidovudine: follow-up of patients in a compassionate plea program. JAMA 1988; 260: 3009-15.

82. Volberding PA, Lagokos SW, Koch MA, et al. Zidovudine in asymptomatic human immunodefiency virus infection. N Engl J Med 1990; 322: 941-9.

83. Friedland GH. Early treatment for HIV. The time has come. N Engl J Med; 322: 1000-2.

84. Tamaki K, Stingl G, Katz S. The origin of Langerhans' cells. J Investig Dermatol 1980; 74: 309-311.

85. Stingl G, Katz SI, Clement L, Green I, Shevach EM.Immunological function of Ia bearing epidermal Langerhans' cells. J Immunol 1978; 121: 2005-8.

86. Hawthorn RJS, Murdoch JB, McLean AB, MacKie RM. Langerhans' cells and subtypes of human papillomavirus in cervical intraepithelial neoplasia. BMJ 1988; 297: 643-6.

87. Tay SK, Jenkins D, Maddox P, Campion M, Singer A. Subpopulations of Langerhans' cells in cervical neoplasia.Br J Obstet Gynaecol 1987; 94: 10-15.

88. Morris HHB, Gatter KC, Sykes G, Casemore V, Mason DY. Langerhans' cells in human cervical epithelium: effects of wart virus infection and intaepithelial neoplasia. Br J Obstet Gynaecol 1983; 90: 412-20.

89. McCardle JP, Muller HK. Quantitative assessment of Langerhans' cells in human cervical intraepithelial neoplasia and wart virus infection. Am J Obstet Gynecol 1986; 154: 509-15.

90. Nakajima T, Watanabe S, Sato Y, Kameya T, Hirota T, Shimosato Y. An immunoperoxidase study of S100 protein distribution in normal and neoplastic tissues. Am J Surg Pathol 1982; 6: 715-27.

91. Dubertret L, Picard O, Bagot M, et al. Specificity of monoclonal antibody anti-T 6 for Langerhans' cells in normal human skin. Br J Dermatol 1982; 106: 287-89.

92. Barton SE, Maddox PH, Jenkins D, Edwards R, Cuzick J, Singer A. Effect of cigarette smoking in cervical intraepithelial immunity: a mechanism for neoplastic change.Lancet 1988; ii: 652-4.

93. Dreno B, Milpied B, Bignon JD, Stalder JF, Litoux P. Prognostic value of Langerhans' cells in the epidermis of HIV patients. Br J Dermatol 1988; 118: 481-6.

94. Dreno B, Milpied B, Dutartre H, Litoux P. Modification de la secretion d'interlekine 1 par l'epiderme sain au cours de l'affection VIH. Fifth World AIDS Conference, Montreal 1989. Abstract Number C 549.

95. Ramsauer J, Racz P, Tenner-Racz K, Dietrich M, Kern P, Meigel W. Langerhans cells (LC) in the pathogenesis of HIV infection. Fourth World AIDS Conference, Stockholm 1988. Abstract Number 2090.

96. Daniels TE, Greenspan D, Greenspan JS, et al. Absence of Langerhans' cells in oral hairy leukoplakia, an AIDS-associated lesion. J Investig Dermatol 1987; 89: 178-82.

97. Williams Sir John 1886; Cancer of the uterus. Harveian Lectures for 1886. Lewis, London.

98. Rubin IC. The pathological diagnosis of incipient carcinoma of the uterus. Am J Obstet Gynecol 1910; 62: 668-76.

99. Hinselmann H. Verbesserung der Inspektionmoglichkeit von Vulva, Vagina and Portio. Munchener Medizinische Wochenschrift 1965; 77: 1733.

100. Schiller W. Jodpinselung und Abschabung des Portioepithels. Zentrablatt fur Gynakologie 1929; 53: 1056-64.

101. Papanicolaou G, Traut HF. The diagnosis of uterine cancer by the vaginal smear. Commonwealth Fund, New York 1943.

102. Boyes DA, Worth AJ. The value of cervical cytology for screening for carcinoma of the cervix. In: Jordan JA, Singer A, eds. The Cervix. London: Saunders, 1976; 404-11.

103. Syrjanen KJ. Epidemiology of Human papillomavirus (HPV) infections and their associations with genital squamous cancer. APMIS 1989; 97: 957-70.

104. Jordan JA. The management of pre-malignant conditions of the cervix. In Studd J, ed. Progress in Obstetrics and Gynaecology, Vol 2. London: Churchill Livingstone, 1982; 151-163.

105. Ismail SM, Colclough AB, Dinnen JS, et al. Observer variation in histopathological diagnosis and grading of cervical intrepithelial neoplasia. BMJ 1989; 298: 707-710.

106. Oriel JD. Sex and cervical cancer. Genitourin Med 1988; 64: 81-9.

107. Zur Hausen H. Papillomaviruses in human cancers. Molec Carcinogen 1988; 1: 147-150.

108. Crawford L.Papilloma viruses and cervical tumours. Nature 1984; 310: 16.

109. Howley PM. On human papillomaviruses. N Engl J Med 1986; 315: 1089.

110. Brinton LA, Fraumeni Jr JF. Epidemiology of uterine cervical cancer. J Chron Dis 1986; 39: 1051.

111. Rotkin ID. A comparison review of key epidemiological studies in cervical cancer related to current searches for transmissible agents. Cancer Res 1973; 33: 1353.

112. Slattery ML, Robison LM, Schuman KL, et al. Cigarette smoking and exposure to passive smoke are risk factors for cervical cancer. JAMA 1989; 261: 1593-8.

113. Owen-Drife J. The benefits of combined oral contraceptives. Br J Obstet Gynaecol 1989; 96: 1255-57.

114. Armstrong BK, Allen OV, Brennan BA, et al. Time trends in prevalence of cervical abnormality in women attending a sexually diseases clinic and their relationship to trends in sexual activity and specific infections. Br J Cancer 1986; 54: 669.

115. Vonka V, Kanka J, Hirsch I, et al. Prospective study on the relationship between cervical neoplasia and herpes simplex type-2 virus. II. Herpes simplex type-2 antibody presence in sera taken at enrollment. Int J Cancer 1984; 33: 61.

116. Macnab JCM, Stephen A, Walkinshaw MB, Cordiner JW, Clements JB. Human papillomavirus in clinically and histologically normal tissue of patients with genital cancer. N Engl J Med 1986; 315: 1052.

117. Schneider A, Sawada E, Gissmann L, Shah K. Human papillomaviruses in women with a history of abnormal papanicolaou smears and in their male partners. Obstet Gynecol 1987; 69: 554.

118. Meanwell CA, Cox MF, Blackledge G, Maitland NJ. HPV 16 DNA in normal and malignant cervical epithelium: implications for the aetiology and behaviour of cervical neoplasia. Lancet; i: 703-7.

119. Anonomyous. Human papillomaviruses and the polymerase chain reaction. Lancet 1989; i: 1051-2.

120. McCance DJ, Campion MJ, Clarkson PK, Chesters PM, Jenkins D, Singer A. Prevalence of human papillomavirus type 16 DNA sequences in cervical intraepithelial neoplasia and invasive cancer of the cervix. Br J Obstet Gynaecol 1985; 92: 1101.

121. Wagner D, Ikenberg H, Boehm N, Gissmann L. Identification of human papillomavirus in cervical swabs by deoxyribonucleic acid in situ hybridisation. Obstet Gynecol 1984; 64: 767.

122. Munoz N, Bosch X, Kaldor JM. Does human papillomavirus cause cervical cancer? The state of the epidemiological evidence. Br J Cancer 1988; 57: 1-5.

123. Barton SE, Jenkins D, Hollingworth A, Cuzick J, Singer A. An explanation for the problem of false-negative cervical smears. Br J Obstet Gynaecol 1989; 96: 482-5.

124. Crum CP, Nagai N, Levine R, Silverstein S. In situ hybridisation analysis of HPV 16 DNA sequences in early cervical neoplasia. Am J Pathol 1986; 123: 174.

125. Young LS, Bevan IS, Johnson MA, et al. The polymerase chain reaction: a new epidemiological tool for investigating cervical human papillomavirus infection. BMJ 1989; 298: 14-8.

126. Tidy JA, Parry GCN, Ward P, et al. High rate of human papillomavirus type 16 infection in cytologically normal cervices. Lancet 1989; i: 434.

127. Pao CC, Lin C-Y, Maa J-S, Lai C-H, Wu S-Y, Soong Y-K. Detection of human papillomaviruses in cervicovaginal cells using the polymerase chain reaction. J Infect Dis 1990; 161: 113-5.

128. Saiki PK, Grelfand DM, Stoffel S, et al. Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988; 239: 487-91.

129. Lo Y-MD, Mehal WZ, Fleming KA. False positive re sults and the polymerase chain reaction. Lancet 1988; ii: 679.

130. Tidy JA, Farrell PJ. Retraction: human papilloma virus subtype 16b. Lancet 1989; ii: 1535.

131. Tidy JA, Vousden KH, Farrell PJ. Relation between infection with a subtype of HPV 16 and cervical neoplasia. Lancet 1989; i: 1225-7.

132. Campion MJ, McCance DJ, Cuzick J, Singer A. Progressive potential of mild cervical atypia: Prospective cytological, colposcopic and virological study. Lancet 1986; ii: 237.

133. Syrjanen K, Mantyjarvi R, Saariskoski S, et al. Factors associated with progression of cervical Human papillomavirus (HPV) infections into carcinoma in situ during a long term prospective follow-up. Br J Obstet Gynaecol 1988; 95: 1096-1102.

134. Shope RE. Infectious papillomatosis of rabbits . J Exp Med 1933; 58: 607.

135. Krebs H-B. Milestones in HPV research. Clinical Obstetrics and Gynaecology 1989; 32: 109-11.

136. Rous P, Beard JW. The progression to carcinoma of virus induced rabbit papillomas (Shope). J Exp Med 1935; 62: 523.

137. Jarrett WFH, Mcneil PE, Grimshaw WTR, Selman IE, McIntyre WM. High incidence area of cattle cancer with a possible interaction between an environmental carcinogen and papilloma virus. Nature 1978; 274: 215.

138. Zur Hausen H. In: Salzman NP, Howley PM, eds. The papoviridae 2. The papillomaviruses. New York: Plenum 1987: 245-63.

139. Bedall MA, Jones KH, Laimins LA. The E6-E7 region of human papilloma virus type 18 is sufficient for transformation of NIH 3T3 and rat cells. J Virol 1987; 61: 3635.

140. Kanda T, Furona A, Yoshiike K. Human papillomavirus type 16 open reading frame E7 encodes a transforming gene for rat 3Y1 cells. J Virol 1988; 62: 610.

141. Gissman L, De Villiers EM, zur Hausen H. Analysis of human genital warts (condylomata acuminata) and other genital tumors for human papillomavirus type 6 DNA. Int J Cancer 1982; 29: 143-6.

142. Wilson RW, Chenggis ML, Unger ER. Longitudinal study of human papillomavirus infection of the female urogenital tract by in situ hybridisation. Arch Pathol Lab Med 1990; 114: 155-9.

143. Gissmann L. Linking HPV to cancer. Clinical Obstetrics and Gynecology 1989; 32: 141-7.

144. Schwarz E, Freese UK, Gissmann L, et al. Structure and transcription of human papillomavirus sequences in cervical cancer cells. Nature 1985; 314: 111.

145. Scheider-Gaedcke A, Schwartz E. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. EMBO J 1986; 5: 2285.

146. Durst M. Kleinheinz A, Hotz M, Gissmann L. The physical state of human papillomavirus type 16 DNA in benign and malignant tumours. J Gen Virol 1985; 66: 1515.

147. Matsukura T, Koi S, Sugase M. Both episomal and integrated forms of human papillomavirus type 16 are involved in invasive cervical cancers. Virology 1989; 172: 63-72.

148. Sarasin A. SOS response in mamalian cells. Cancer Invest 1985; 3: 163.

149. Zur Hausen H. Intracellular surveillance of persisting viral infections. Lancet 1986; ii: 489.

150. Doll R. Implications of epidemiological evidence for future progress. In: Peto R, zur Hausen H. eds. Viral etiology of cervical cancer. Banbury Rep 1986; 21: 321.

151. Harris RWC, Brinton LA, Cowdell RH, et al. Characteristics of women with dysplasia or carcinoma in situ of the cervix uteri. Br J Cancer 1980; 42: 359.

152. Epstein MA, Achong BG Eds. The Epstein-Barr Virus. Sringer-Verlag. Berlin-Heidelberg-New York. 1979.

153. Burkitt D. A sarcoma involving the jaws in African children. Brit J Surg 1958; 46: 218-23.

154. Melnick JL. Human studies following models of tumorigenes DNA tumor viruses in animals. Bull Pan Am Health Organ 1978; 12: 28-33.

155. Hirsch MS, Schooley RT, Ho DD, Kaplan JC. Possible viral interactions in the acquired immunodeficiency syndrome (AIDS). Rev Infect Diseases 1984; 6: 726-731.

156. Quinnan GV Jr, Masur H, Rood AH, et al. Herpesvirus infections in the acquired immunodeficiency syndrome. JAMA 1984; 252: 72-77.

157. Gendelman He, Phelps W, Feigenbaum ? , et al. Trans-activation of the human immunodeficiency virus long repeat sequence by DNA viruses. Proc Natl Acad Sci USA 1986; 83: 9759-63.

158. Allday MJ, Crawford DH. Role of epithelium in EBV persistence and pathogenesis of B-cell tumours. Lancet 1988; i:855-7.

159. Holmberg SD, Gerber AR, Stewart JA, Lee FK, O'Malley PM, Nahmias AJ. Herpesviruses as co-factors in AIDS. Lancet 1988; ii: 746-7.

160. Pinching AJ. AIDS and HIV infection. Clinics in Immunology and Allergy 1986; 6: 650.

161. Sixbey JW, Lemon SM, Pagano JS. A second site for Epstein-Barr virus shedding: the uterine cervix. Lancet 1986; ii: 1122-24.

162. Bevan IS, Blomfield PI, Johnson MA, Woodman CBJ, Young LS. Oncogenic viruses and cervical cancer. Lancet 1989; i: 907-8.

163. Becker Y. Does neoplasia in-situ develop due to the interaction of Epstein-Barr virus or Herpes simplex virus-2 with Langerhans' cells in the epithelium? Virus Genes 1988; 1: 385-97.

164. Greenspan D, Greenspan JS, Conant M, Peterson V, Silverman S, De Souza Y. Oral "hairy leukoplakia" in male homosexuals; evidence of association with both papillomavirus and a herpes-group virus. Lancet 1984; ii: 831-4.

165. Greenspan JS, Greenspan D, Lennette ET, et al. Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, an AIDS-associated lesion. N Engl J Med 1985; 313: 1564-71.

166. Fermand JP, Gozlan J, Bendelac A, Delauche-Cavallier MC, Brouet J, Morinet F. Detection of Epstein-Barr virus in epidermal skin lesions of an immunocompromised patient. Annals of Internal Medicine 1990; 112: 511-15.

167. Nuovo GJ. Correlation of histology with human papilloma virus DNA detection in the female genital tract. Gynecologic Oncology 1988; 31: 176-83.

168. Alberico S, Facca MC, DiBonita L, Millo R, Casaccia R, Mandruzzato GP. Frequency of cervico-vaginal infections in cervical intraepithelial neoplasia. Eur J Gynaec Oncol 1988; 9: 252-7.

169. La Vecchia C, Franchesca S, Decarli A, Fasoli M, Gentile P, Tognoni G. Cigarette smoking and risk of cervical neoplasia. Am J Epidemiol 1986;123:22-7.

170. Smith JR, Kitchen VS. Reducing the risk of infection for obstetricians. Br J Obstet Gynaecol 1991; 98: 124-6.

171. Singer A, Jenkins D. Viruses and cervical cancer. BMJ 1991; 302: 251-2.

172. Smith JR, Murphy S, Mellors J et al. Clinical parameters of HIV seropositive women attending the department of genitourinary medicine at St Mary's Hospital, London W2. International Journal of STD and AIDS 1990; 5: 328-9.

173. European Collaborative Study. Children born to women with HIV-1 infection: natural history and risk of transmission. Lancet 1991; 337: 253-60.

174. Mellors J, Smith JR, Harris JRW, King M. Psychiatric manifestations of HIV disease. Seventh International AIDS Conference, Florence 1991. Abstract No. MB2100.

175. Smith JR, Reginal PW, Forster SM. Safe sex and conception: a dilemma. Lancet 1990; 335: 359.

176. Smith JR, Forster GE, Kitchen VS et al. Infertility management in HIV positive couples: a dilemma. BMJ 1991; 302:1447-50.

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Reducing the risk of infection for obstetricians

The exposure of surgeons to needlestick injury and contamination with blood places them at risk of infection with hepatitis B virus (HBV) and more recently with human immunodeficiency virus (HIV). HBV has the lower mortality rate but is highly infectious and HIV, although much less infectious, carries a fatal prognosis. HBV would appear to be the most important occupational infection in health care workers in the developed world whilst HIV probably stimulates the most anxiety.

In addition to the greater infectivity of HBV, it is a much more resilient virus than HIV, surviving air drying and disinfection to a greater degree; this may facilitate transmission by environmental contamination of fomites.

Following needlestick injury from a patient with HBV, the seroconversion rate is 25% if the patient is HBe antigen positive and 5% if he is HB surface antigen positive in the absence of e antigen (Hadler 1990). Where the patient is HIV seropositive, needlestick injury carries a seroconversion rate of approximately 0·2% (Ippolito *et al.* 1990). Needlestick injury involving the injection of an inoculum understandably carries the greatest risk.

Rates of needlestick injury amongst surgeons are difficult to ascertain due to a low level of reporting, whereas glove puncture is readily measureable. Glove puncture represents potential needlestick injury and facilitates contamination of the surgeon's skin with infected material. The degree of contamination will depend on the size and length of time the perforation is present. Smith & Grant (1990) have shown that glove puncture in obstetricians occurs in 54% of caesarean sections and, where such perforations were recognized by the surgeon, 60% of them occurred during closure of the lower segment of the uterus. The frequency of glove puncture has been shown to increase with increasing length of the surgical procedure but, surprisingly, there is a lack of correlation with emergency surgery.

The seroconversion rate for HBV and HIV amongst health care workers is unknown following skin or mucous membrane contamination but is thought to be much lower than that following needlestick injury. There are only three recorded cases of HIV seroconversion following such exposure and each of these had unusual features, including delayed seroconversion and disseminated exposure in association with significant skin pathology.

In the USA it is currently estimated that 15 health care workers die annually from fulminant hepatitis, 1000 become HBV carriers and a further 200–300 will eventually die due to the chronic consequences of HBV infection, namely cirrhosis and primary hepatocellular carcinoma (Hadler 1990). Despite this, the current uptake of HBV vaccine amongst health service personnel in the UK is still inadequate (87% uptake in orthopaedic surgeons (Williams & Flowerdew 1990)). When HBV vaccine became generally available in 1982, uptake was initially poor, related perhaps to anxiety over its derivation. However, since 1987 genetically engineered vaccine has been available that is free from any potential pathogens and no adverse effects have been proven. More than 90% of infections with HBV in health care workers can be prevented by vaccination. In approximately 10%, an adequate course of vaccination will fail to mount a protective immune response, and presumably these individuals will remain at risk from infection (Westmoreland 1990). In view of its availability, reasonable cost, safety and effectiveness, we feel that HBV vaccination should be actively encouraged in all health care workers. Currently the medical profession are notoriously poor attenders at occupational health departments, but we believe this should become a requirement of their employment.

The standard HBV vaccination regimen comprises three injections with a 1-month interval between the first and second injection and a third injection 6 months after the first. This regimen results in peak antibody levels 1 month after the third dose, and therefore cannot provide rapid protection to individuals at high risk. It has been suggested that an accelerated vaccination schedule (0, 4 weeks and 8 weeks) with a booster vaccination at one year, if required, may result in more rapid protection. Antibody levels should be measured at approximately 3 months after

the initial visit and if the result shows a non-protective response, a booster vaccination should be given. Thereafter antibody levels should probably be measured every 3–5 years and if they fall below a protective level, a further booster is required. If these measures fail to stimulate an adequate antibody response the person should be informed that he is not fully protected against HBV and should be advised to undergo post exposure prophylaxis with hyper-immune gammaglobulin as the need arises.

Reduction of risk for surgeons requires a one tier approach to infection control. Such a policy must include the wearing of gloves, waterproof gowns and goggles or protective spectacles during any procedure that may involve possible contamination with patients' body fluids. Additionally, any patient known to be seropositive should be placed last on the theatre list to facilitate thorough disinfection of the operating theatre following the procedure. A two tier infection control policy is likely to induce appropriate caution in the surgeon when he is aware of the patient's seropositivity, but also encourages a less careful approach in the management of those patients who are perceived to be at low risk of infection. Most patients have not been tested for either virus and of course many do not disclose 'at risk' behaviour. Furthermore, patients who have been recently infected may have negative antibody tests thus giving falsely reassuring results.

For an infection control policy to be effective, it must be universally implemented. A recent study conducted in an accident and emergency department demonstrated poor compliance with such a policy. Health personnel cited as reasons for lack of compliance, the perceived loss of manual dexterity and lack of time to implement the appropriate techniques. These excuses suggest complacency in the face of serious potential infection.

Double gloving reduces by a factor of six the frequency of inner glove puncture, with little loss in operator sensitivity (Matta *et al.* 1989). Glove manufacturers are currently developing gloves strengthened in the digits since this is the area most prone to puncture. Blunt tipped needles appear to reduce the frequency of glove puncture whilst being capable of perforating uterine muscle (J. M. Grant, personal communication) and cervical tissue (C. Hauxwell, personal communication). Special forceps for suturing the uterine wound during caesarean section are being developed and may reduce the risk of glove puncture. The use of staples during closure of the skin and internal wounds will further reduce the amount of suturing required, thus diminishing the puncture rate.

Nonoxynol-9 has been shown to have anti HIV properties *in vitro*. This has caused some surgeons, particularly in the USA, to smear nonoxynol-9 on their hands before donning surgical gloves. This is a practice with far from proven benefit. Nonoxynol-9 may increase skin permeability and thus, theoretically, increase the potential for infection with either HIV or HBV (Jeffries 1990).

Following exposure to potential infection by a needlestick injury a number of steps should be taken: the person should immediately decontaminate the wound by encouraging free flow of blood and by washing the area thoroughly with disinfectant. Expert counselling should be available to discuss the risk of infection by HBV and HIV. Serum should be saved for possible future HIV testing. In the event of subsequent seroconversion this allows prior seronegativity to be confirmed.

In those not previously vaccinated for HBV, a course should be instituted as soon as possible and hyperimmune globulin administered within 72 h. The fast induction regimen for HBV vaccination is also appropriate for post-exposure prophylaxis. In view of the fact that HBV infection usually has an incubation period of 2 or more months, post-exposure vaccination alone may be sufficient in most cases.

Where a needlestick injury involves a known HIV seropositive patient, post exposure prophylaxis with zidovudine can be given, although this is of unproven benefit. To maximize the potential benefit, zidovudine should be given as soon as possible, preferably within 1 hour and not later than 24 hours after injury. It is currently prescribed at high dosage (1200 mg/day) for 6 weeks and as yet no serious side effects have been reported (Gerberding 1990). No data are available on the long term toxicity of this regimen. Due to the low HIV seroconversion rate following needlestick injury and the lack of enthusiasm shown by health care workers for placebo treatment, it is unlikely that the efficacy of zidovudine in post-exposure prophylaxis will ever be proved.

Reducing the risk of HBV infection in surgeons requires a substantially increased uptake of HBV vaccination. Current surgical practice

also requires modification to reduce the risk of inoculation injury and skin and mucous membrane contamination. A high grade one tier infection control policy, coupled with the use of a combination of the measures described above, would greatly reduce the risk to obstetricians and gynaecologists of HBV and HIV infection.

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References

Gerberding J., Wugofski L., Chambers H., Dilley J. & Rinaldi J. (1990) Azidothymidine chemoprophylaxis for health care workers occupationally exposed to HIV. *Sixth International Conference on AIDS, San Francisco, USA,* Abstract No. FC 35.

Hadler S. C. (1990) Hepatitis B virus infection and health care workers. *Vaccine* **8,** 24-28.

Ippolito G., Puro V. & Italian Collaborative Study Group on Occupational Risk of HIV Infection (1990). Rate of sero conversions after occupational exposures to HIV in health care settings: the Italian multicentric study. *Sixth International Conference on AIDS, San Francisco, USA,* Abstract No. FC 34.

Jeffries D. (1990) Nonoxonyl 9 and HIV infection. *Br Med J* **296,** 1798.

Matta H., Thompson A. M., Rainey J. B. (1989) Does wearing two pairs of gloves protect operating theatre staff from skin contamination? *Br Med J* **297,** 597-598.

Smith J. R. & Grant J. M. (1990) The incidence of glove puncture during Caesarean section. *J Obstet Gynaecol* **10,** 317-318.

Westmoreland D., Player V., Heap D. C. & Hammond A. (1990) Immunization against hepatitis B—what can we expect? *Epidemiol Infect* **104,** 499-509.

Williams J. R. & Flowerdew A. D. S. (1990) Uptake of immunization against hepatitis B among surgeons in Wessex Regional Health Authority. *Br Med J* **301,** 154.

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MIDDLES

Infertility management in HIV positive couples: a dilemma

Mr J R Smith and colleagues present the case of a couple, both HIV positive, who presented for investigation and management of infertility. Legal, ethical, and medical experts consider the issues and say what they would have done. The concluding section presents the outcome of the case.

Case summary

J R Smith, G E Forster, V S Kitchen, Y S Hooi, P E Munday, D B Paintin

A 31 year old woman and her 36 year old husband wished to be investigated and managed for infertility. In 1989 both had tested positive for antibodies to HIV-I. It was not possible from the clinical history to estimate when HIV seroconversion had occurred, but infection most probably occurred through heterosexual transmission. The woman had conceived one pregnancy by a previous partner, and the husband had fathered three children by a previous partner. Before knowing their HIV antibody state they had had some investigations for secondary infertility and the woman had been treated with clomiphene. This was discontinued after her HIV state was known. The couple had been having unprotected sexual intercourse for two years without conception.

Before discussing infertility the couple was given prepregnancy counselling with respect to HIV to explain the pertinent facts about the natural history of HIV disease and the risk of vertical transmission. They were told that the vertical transmission rate in well HIV positive women is unknown but is probably 13-30%,[1,2] at least in developed countries, and that it may be greater if the woman has symptoms of HIV infection,[3] and that detecting HIV infection in utero is currently impossible and excluding HIV infection in the first 18 months of life is difficult. Furthermore, the risks to the infected mother are unknown, although theoretically the immunosuppressive effects of pregnancy may precipitate a deterioration in maternal condition.[4,5]

The couple were investigated after this initial counselling to determine the stage of HIV infection in each and to quantify their respective prognoses and that of their potential offspring. Clinical examination showed Centers for Disease Control stage IV-C2 disease in the man and stage II disease in his wife.[6] Serum P24 antigen was not found in either patient and T4 lymphocyte numbers were low in the man and at the lower limit of normal in the woman. It is not possible to predict the long term prognosis for either prospective parent, but on average 39% of people will develop AIDS within 9·2 years of infection with HIV.[7] With time, it is likely that most people infected with HIV will develop AIDS. Survival thereafter depends on the particular manifestation of HIV related disease. This suggests that any potential offspring if not infected will be orphaned, at best as teenagers. This was explained to the couple.

After counselling, the couple seemed to be fully aware of the consequences of pregnancy for them and their potential offspring. Furthermore, they wished to have their infertility actively managed. They were informed that although we were able to perform simple endocrinological and seminal investigations for infertility, active management could not be promised as we were uncertain of the ethical implications. The couple felt that knowing the cause of their infertility would be helpful, and investigations were therefore performed. These suggested polycystic ovarian disease and oligospermia.

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Legal analysis

Kathy Doyle, Linda Delany

DOCTORS' LIABILITY TO PARENTS

Decisions concerning access to infertility treatment have received little legal attention. In the only relevant reported case the treatment at issue was in vitro fertilisation (IVF).[8] The consultant refused to treat the (married) woman who sought it because of her history of prostitution and because Manchester City Social Services Department had rejected her applications to foster or adopt. The High Court judge assumed, but did not find as a matter of law, that the consultant's decision was open to judicial review and should have been reached fairly; he also appeared to accept the relevance of criteria used by adoption agencies. Had the assumptions been formulated as legal principles, useful legal guidance for decisions on eligibility for infertility treatment would be available; as it is, the legal significance of the case is unclear.

When the Human Fertilisation and Embryology Act 1990 comes fully into force infertility treatment involving IVF and gamete donation will require a licence from the Human Fertilisation and Embryology Authority. It will be a condition of every treatment licence that a woman shall not be treated unless account has been taken of the welfare of any child who may result, including the need of such a child for a father (section 13(5)). Guidance concerning this provision should appear in the code of practice to be devised by the authority (section 25(2)).

If IVF succeeds the doctors could incur liability to the HIV positive mother in negligence if they fail to inform her of the risk that the pregnancy may accelerate the progress of the disease.[9] If she wishes to undergo the treatment and fully understands and accepts the risk then the defence of volenti (consent) would be available to the medical team.

DOCTORS' LIABILITY TO CHILD

According to the Congenital Disabilities (Civil Liability) Act 1976 as amended by the Human Fertilisation and Embryology Act 1990, a child born disabled can sue whoever has negligently caused the disability. The act was introduced in response to the general disquiet in the aftermath of the thalidomide case. The

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problems facing those plaintiffs were, firstly, the doubt as to whether a child could sue in respect of injuries that had occurred before birth and, secondly, the difficulties in proving causation—that is, proving that the negligence of the pharmaceutical company had caused the disability in question. The 1976 act provides that a child can sue for a disability where the negligent act causing the disability occurred before birth or even before conception, and section 44 of the Human Fertilisation and Embryology Act 1990 extends liability to cover negligent acts done in the course of infertility treatments such as IVF, gamete intrafallopian transfer (GIFT), or artificial insemination.

It is necessary, however, to distinguish between acts by the doctor that have caused disability—for example, negligent forceps delivery causing brain damage—and those that have caused the child to be born at all—for example, negligently failing to diagnose that the child is likely to be born with serious handicaps and failing to advise on termination of pregnancy. A child would be able to succeed against the doctors in the first case but not in the second, where the child's action is known as one for "wrongful life" and is not sustainable as a matter of public policy.[10]

PARENTS' LIABILITY TO CHILD

Finally, the HIV infected child may have a remedy against its own parents. Under the 1976 act a father can be sued for negligently causing the child's disability but normally the mother cannot. If the father only was infected by HIV then provided there is proof of paternity it would follow that the child's infection had come from the same source. If, however, both parents are infected it may be impossible to show which of them had caused the HIV infection in the child, and as indicated earlier the 1976 act has established the right of a child to claim for prenatal injuries but has not provided any means of overcoming the often overwhelming obstacle of proving causation. In practical terms the child is unlikely to bring an action against the father because he will not have the means to satisfy a successful claim.

Medical ethics

David Seedhouse

Although itself uncommon, this dilemma reflects a widespread quandary in medical decision making. Whatever the protagonists decide there is every possibility that others elsewhere will arrive at contrary solutions in similar situations. The problem is that no one has yet asked fully the purpose of infertility treatment. Until this fundamental question is addressed, apparently fiendish dilemmas will continue. For some the solutions may lie in legal or ethical rights, for others in reducing harm to society, and for others in egalitarianism. But without overt understanding of rationale only ad hoc answers can be expected.

The obvious purpose of treatment is to allow people of low fertility to produce children, but there are deeper puzzles. Which people: everybody, or only those with good chances of success? Which children: any child, or only normal children? There is a range of possible strategies that might be adopted. For example, doctors might choose:

- To treat people on request
- To treat all people who might benefit clinically
- To treat only those people for whom there is a probability above a given value that treatment will succeed
- To treat according to any of these criteria but to exclude from treatment those people who are themselves ill in a way that might directly affect the health of the baby
- To treat according to one of the first three criteria but to exclude those people who already have children
- To treat according to the first three criteria but to exclude those not in stable relationships, heterosexual relationships, or marriages.

If a methodology for ranking alternatives could be agreed then a national system of indicators for treatment could be generated. Many decisions not to treat might then cause little controversy (for example, where there is no chance of pregnancy, whatever is done, or where there is high probability of severe handicap) but those based in value (the last three criteria given above) will remain factious.

By insisting on the consideration of the welfare of possible children the law seems to have put the responsibility on clinicians to impose personal judgments on potential clients. This is an unacceptable burden from the point of view of both doctors (who are not trained in such decision making) and patients (who are likely to resent unfavourable verdicts). In my opinion, the basic principle of infertility treatment should be to treat all people who might benefit clinically. If resources are scarce then the standard ought to be to treat those people for whom there is a probability that the treatment will succeed, where the value of the probability should fluctuate according to availability of treatment (the more restricted the service, the higher the probability).

The level of resources in the present case is unclear. If they are sufficient then treatment should be offered to the couple, who are competent and aware of the implications. Where enough resources exist, "best interest" judgments made against the wishes of competent people must be avoided.

If resources are limited then account should be taken of negative factors, which would include the likelihood of success of treatment, the possibility of a diseased baby, and the three existing children. If treating this couple means that other couples with fewer negative factors will be denied help then treatment should be refused—clinical considerations should always take precedence and all judgments of value justified thoroughly.

View from the London Fertility Centre

Ian Craft

The second report of the Voluntary Licensing Authority (1987) recommended in its guidelines that "all patients entering an IVF programme should be tested for hepatitis B and HIV antibodies." The reaction to this recommendation provoked such an adverse response that subsequent reports have suggested this need be mandatory only when donated gametes are used, screening both potential donors and recipients.

The London Fertility Centre has introduced a policy whereby all new and existing patients undergoing assisted conception treatment—including IVF, GIFT, and artificial insemination, with and without donated gametes—should be tested for both hepatitis B and HIV, and this recommendation has the support of its ethics committee. This committee is constituted of eight lay members and three medical or scientific members. Close collaboration exists between the centre and the consultant medical staff of the referral medical diagnostics laboratory, who themselves have in depth knowledge of the HIV state and disease.

We consider that the policy is prudent with the increase in HIV prevalence even in the heterosexual community. In addition, recent reports have indicated

that it is not possible to predict accurately who is at risk of HIV infection at the best of times.[11] Since the policy was introduced no patients have been found to be HIV positive, and far from suggesting that the policy should be discontinued we think that it should be maintained because of the changing trends in scientific reporting.

The dilemma as to whether infertility management should be undertaken in couples positive for HIV concerns assessing the rights of such infertile couples to be treated; the rights of any resultant offspring to be free of disease and to have a supportive and longlasting family relationship; and the right of the attendant medical and laboratory staff to maintain their own health, which has particular relevance when IVF, GIFT, or artificial insemination is being considered.

Our centre would decline to treat the couple concerned as a matter of policy because we believe that it would be irresponsible to put staff members at risk, even if this risk is minimal. In addition, we are concerned that the incidence of vertical transmission to offspring may well turn out to be much higher than is currently quoted. Is it really considerate to want to have a child in these circumstances?

Our only qualms concerning our decision is whether we should be as stringent with the management of a couple positive for hepatitis B virus as there is a degree of flexibility of treatment in these circumstances. Our main reasoning is in part based on the likelihood of the child infected with HIV either dying or being orphaned by its parents' early death; with regard to hepatitis B, vaccination and passive immunity can be provided to the infant.

We also do not consider that couples positive for HIV are being treated any differently from others with severe life threatening disease in whom fertility treatment would not be advised—for example, those with unstable diabetes, severe hypertension, non-Hodgkin's lymphoma, etc.

In any event, we feel that the pregnancy potential of the couple reported is likely to be markedly reduced by contributory factors—the polycystic ovarian condition and suboptimal sperm—despite both partners having previously proved their fecundity. Counselling by members of the fertility team concerning the contributory relevance of these factors, together with supportive counselling of the negative decision, is indeed as essential as independent HIV counselling.

Medical viewpoint

Danielle Mercey, Susan Bewley

Such cases are not rare and will increase. Fertile or infertile, HIV infected patients need careful counselling, within the limits of current knowledge, about infectivity, pregnancy, breast feeding, parental illness, and death, and ideally this should start before initial testing.

HIV may decrease fertility: oligomenorrhoea and testicular atrophy have been reported. The rate of vertical transmission, from the latest European data, is <15% (but it may be different in late stages of HIV infection and in Africans). Seventeen per cent of infected babies become ill and die under one year, and there are insufficient data to predict the fate of the remainder. Although it has been postulated that pregnancy worsens the mother's prognosis, no evidence has emerged. Drugs used in pregnancy have potential adverse effects. Present advice is not to breast feed.

Even if the date of infection were known, the prognoses for the parents in this case are difficult to assess. The woman has "good" prognostic markers but this does not preclude sudden, rapid deterioration. The results of large cohort survival studies, mainly including men, may not apply. The man has an uncertain, but probably worse, prognosis as his T4 lymphocyte count is low and he is stage IV-C2. The exact T4 count, its trend, and the defining diagnosis are not given. At best, with current survival, a child would have an evens chance of losing two sick parents by late childhood. Childcare arrangements during illness and after death must be discussed. The risks to health care staff are of subordinate concern.

Many couples choose not to have children, and this terrible loss compounding the grief of being HIV positive should not be underestimated. Genitourinary physicians manage most patients with HIV, but they are not gynaecologists; if this couple still wants a pregnancy they deserve an opinion for information about investigations and treatment. Account will then be taken of current medical standards and the estimated chances of creating a medical catastrophe. We would refer them to an infertility specialist but would warn this couple that this is not with an expectation of receiving treatment.

It might be argued that doctors should simply help patients become pregnant if they wish. Although we cannot interfere and should not condemn a fertile couple who choose to become pregnant, we have no positive duty to accede to an infertile couple's request for help. If procedures to make this couple pregnant cannot be justified as being in their or the future child's best medical interests then it is wrong to administer them.

Caring for patients demands unprejudiced listening, empathy, flexibility, and careful negotiations. However, the duty of care is not "trumped" by autonomy or a notion of the right to reproduce. We respect our patients' autonomy within the duty of care, and not for its own sake. We are not merely technicians and do make hard moral decisions involving a complex weighing of considerations, recognising that each course of action has personal and ethical costs. Every case must be judged on its individual merits and subtle nuances, and all the medical factors are relative—not absolute—contraindications to aiding conception. Whenever we withhold infertility treatment this must be a source of painful regret.

Outcome

Mr Smith and colleagues continue: Fertility is a basic human desire and it was thus inevitable that an HIV infected couple would present for management of infertility, particularly when the female partner is well. We were unable to draw parallels with any other condition because HIV infection is incurable and has a long asymptomatic incubation period followed by a high probability of death: this is coupled with a variable but nevertheless appreciable vertical transmission rate.

We have treated a couple in which the woman was HIV positive and the man negative, but here the only cause of infertility was the use of barrier contraception for safer sex. Treatment was artificial insemination by husband performed by the husband himself, thus removing the gynaecologist from active management. This also had the advantage of protecting the HIV seronegative husband from potential infection.[12]

The management of infertility with either medical or surgical treatment seemed to us indivisible ethically, and although the prescribing of clomiphene is undoubtedly technically much easier than, for example, performing in vitro fertilisation, the end result is essentially the same: the gynaecologist shares the responsibility for the potential birth of an infant infected with HIV or, if the child is not infected, for its

likely status as an orphan in the future. It might be interesting to consider whether the child could bring an action against the gynaecologist for "wrongful life." Consideration should also be given to any risk, however slight, of HIV transmission to health care staff involved in the care of such patients.[13]

The obverse viewpoint is that aware, consenting patients have the right to determine the course of their own child bearing, in much the same fashion as HIV infected women can elect to continue with their pregnancy rather than have a termination. It is also possible that after being refused treatment for infertility the couple could present themselves elsewhere and fail to disclose their HIV state. For the asymptomatic couple it is unlikely that their HIV state would be detected.

After careful consideration we did not feel justified in managing their infertility. We informed the couple of our decision not to undertake active management and they accepted this. The couple were also told that it was their right to seek a second opinion. We do, however, feel that patients deserve sympathetic counselling and that an explanation of their infertility in selected cases may assist this. We explained to the couple that in the future, developments may occur which will reduce the vertical transmission rate and increase life expectancy, necessitating a change in this view. We were careful to make it clear that this was unlikely, particularly in the short term.

1 European Collaborative Study. Mother to child transmission of HIV infection. *Lancet* 1988;ii:1039-42.
2 European Collaborative Study. Children born to women with HIV-1 infection: natural history and risk of transmission. *Lancet* 1991;i:253-9.
3 Moq JQ, Giaquinto C, De Rossi A, Grosh-Wormer I, Ades AD, Peckham CS. Infants born to mothers seropositive for human immunodeficiency virus; preliminary findings from a multicentre European study. *Lancet* 1987;i: 1164-7.
4 Letsky E. The haematological system. In: Hytten F, Chamberlain G, eds. *Clinical physiology in obstetrics*. Oxford: Blackwell Scientific, 1980:49-51.
5 Biggar RJ, Pahwa S, Minkoff H, *et al.* Immunosuppression in pregnant women infected with human immunodeficiency virus. *Am J Obstet Gynecol* 1989;**161**:1239-44.
6 Adler MW, ed. *ABC of AIDS*. London: BMJ Publieations, 1987:13.
7 Hessol NA, Lifson AR, O'Malley PM, Doll LS, Jaffe HW, Rutherford GW. Prevalence, incidence, and progression of human immunodeficiency virus infection in homosexual and bisexual men in hepatitis B vaccine trials, 1978-1988. *Am J Epidemiol* 1989;**130**:1167-75.
8 *R v Ethical Committee of St Mary's Hospital (Manchester) Ex parte Harriott* [1988] 1 FLR 512.
9 *Sidaway v Board of Governors of the Bethlem Royal Hospital and the Maudsley Hospital* [1985] 1 All ER 643.
10 *McKay v Essex Area Health Authority* [1982] 2 AER 771.
11 Barbacci M, Repke JT, Chaisson RE. Routine prenatal screening for HIV infection. *Lancet* 1991;**337**:709-11.
12 Smith JR, Reginald PW, Forster SM. Safe sex and conception: a dilemma. *Lancet* 1990;**335**:359.
13 Smith JR, Kitchen VS. Reducing the risk of infection for obstetricians. *Br J Obstet Gynaecol* 1991;**98**:124-6.

(Accepted 20 March 1991)

M.B.2100

Title: CASE CONTROL STUDY OF PSYCHOSOCIAL STATUS IN HIV SEROPOSITIVE WOMEN

Authors: Mellers John+*, Smith JR*, Harris JRW*, King MB+.
Centre: Academic Department of Psychiatry, Royal Free Hospital+;Department of Genitourinary Medicine, St Mary's Hospital*, London, UK.

Objective: This study was designed to assess the prevalence of psychiatric morbidity in a group of HIV seropositive women.
Methods: Using the Clinical Interview Schedule, psychiatric morbidity was assessed in 25 HIV seropositive women and 14 HIV seronegative controls, randomly selected from women enrolled in a colposcopic study of cervical neoplasia. Demographic data and details of psychiatric history, HIV test circumstances, mode of transmission, sexual and drug related behaviour, attitudes towards fertility and pregnancy, counselling and CDC stage were recorded.
Results: Six patients and 1 control demonstrated psychiatric morbidity,this difference was not significant. Psychiatric morbidity was not significantly related to demographi or clinical variables. Mode of transmission: Intravenous drug user (IVDU)-13 (2 curren IVDUs); heterosexual-12. CDC stage 2&3 -23; CDC 4 -2. 15 patients had regular sexual partners (6 practised safer sex) 4 had casual sexual partners (2 practised safer sex). 4 patients were pregnant, 2 wished to become so, and 7 were undecided.
Discussion and conclusions: The low rate of psychiatric morbidity (24%), which was restricted to minor depression and anxiety, is similar to that found in studies of HIV seropositive men. The high proportion of patients with sexually acquired infection reflects current epidemiological trends. Despite extensive counselling, the number of women who continued to have unsafe sex, and the number who were pregnant or wished to become so, raises important ethical and practical considerations.
THIS STUDY WAS SUPPORTED BY A GRANT FROM AVERT.

NOTES

M.B.2101

THE WHO CROSS-CULTURAL STUDY ON THE NEUROPSYCHIATRIC ASPECTS OF HIV-1 INFECTION: A PROGRESS REPORT

Maj, Mario; Janssen, R.; Satz, P.; Zaudig, M.; Starace, F.; Bhirom, S.; Bing, E.; Luabeya, M.; Ndetei, D.; Riedel, R.; Schulte, G.; Sartorius, N.; WHO, Geneva, Switzerland.

Objective: The study aims to explore the nature and prevalence of HIV-1-associated neurological and psychiatric disorders and neuropsychological abnormalities in persons living in different geographic and socio-cultural contexts.
Methods: The study is being performed in Bangkok, Thailand; Kinshasa, Zaire; Los Angeles, CA, USA; Munich, Germany; Nairobi, Kenya; and Sao Paulo, Brazil. A comprehensive instrument for the collection of neuropsychiatric data (including a neuropsychological test battery suitable for cross-cultural use) is administered to HIV-1-seropositive symptomatic and asymptomatic subjects and to age-, sex- and education-matched HIV-1-seronegative individuals, consecutively recruited in outpatient units. All investigators were formally trained in the use of the evaluation instrument. The inter- and intra-centre reliability in the use of each module of the instrument was found to be satisfactory. A pilot study was carried out in order to test the feasibility of the recruitment and assessment procedure. The main study is ongoing and the relevant results will be available by the end of 1991.
Results of the pilot study: The pilot study, involving 175 subjects, demonstrated the feasibility of the recruitment and assessment procedure. The percentage of drop-outs throughout the procedure was 6.8%. The time for administering the evaluation instrument was on average about two hours. The instrument was well accepted by all subjects.

NOTES

M.B.2102

HIV ISOLATION AND p24-ANTIGEN DETECTION FROM BLOOD AND CEREBROSPINAL FLUID OF PATIENTS IN DIFFERENT CLINICAL CONDITIONS

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Objective: to relate HIV-1 isolation efficiency and p24 antigen detection in paired specimens of blood and cerebrospinal fluid (CSF) with patients clinical staging according to Walter-Reed (W.R.) criteria.
Methods: 40 paired specimens of CSF and blood from seropositive subjects were examined for HIV virus isolation in peripheral blood lymphocytes (PBL) coltures; p24 antigen detection in blood and CSF was carried out in ELISA test, reactive specimens were confirmed by an antigen neutralization test. Clinical staging of infection was estabilished according to the W.R. criteria.
Results: frequency of HIV isolation from blood increased with progress of clinical staging: 90% at W.R. 5-6 and 60% at lower stages.
HIV was isolated from CSF in 20% of subjects at W.R. 5-6 and in 25% of both W.R. 1-2 and W.R. 3-4 patients; p24 antigen detection in CSF yielded a positivity only in advanced stages of infection (35%). In three paired specimens HIV-1 was isolated from CSF but not from blood.
Conclusions: our data show that HIV-1 can be recovered from CSF at all stages of systemic infections, while p24 antigen detection is related with advanced stages only. However the efficiency of HIV-1 isolation from PBL and CSF were not equally related to the progress of systemic infection.

M.B.2103

CRYPTOCOCCAL MENINGITIS EM BRAZILIAN AIDS PATIENTS.

Lima, Josué Nazareno; Souza-Dias, M.; Trabasso, P.; Aoki, F.; Abreu, W.; Oliveira, A.; Pedro, R. UNICAMP Medical School, Campinas, São Paulo - Brasil.

Between January 1989 and december 1990, 24 patients with Cryptococcal Meningitis were seen at a 400 bed acute care UNIVERSITY HOSPITAL in CAMPINAS, a 1,2 million inhabitants town in southeastern Brazil. Twenty patients had serologic evidence of HIV infection. They represented 9% of the newly diagnosed AIDS cases in the period. Their mean age was 28.9 y.o.(range 9-5 y.o.) and 90% were male. Risky behavior was identified in 18 patients: 6 were bissexual men, 4 IVDU, 3 haemophiliacs, 2 homossexual men, 2 transfusion recipients,and 1 IVDU bissexual man. The mean time of seropositivity for HIV was 8,1 months; of note, 44% had no knowledge of HIV status at presentation. Only 6% denied previous HIV related symptoms. Cerebrospinal fluid values were: white cell count>4/ mm³ in 11/16(69%); lympho-monocyte predominance in 7/10(70%); glucose less than 40 mg/dl 9/15(60%); protein over 45 mg/dl in 11/15(73%); positive India-ink test (IIT) 18/20(90%); growth of Cryptococcus 18/18(100%). In two patients diagnosis was based only on a positive IIT; 2 patients had negative IIT and positive CSF cultures (sensitivity of 89%). On physical examination findings were temperature over 38.5°C (80%) meningismus(55%); alteration of consciousness(50%) and focal neurological deficitis(16%). Therapy was based in IV Amphotericin B(100%); 7(35%) patients also received Ketoconazole(K); 3(15%) 5-Fluorcytosine (5FC) and one each combination with: K and Fluconazole(F); K, F and 5FC; K and 5FC. Lethality up to January/91 was 85%. Mean survival time was 66,8d for the 17 patients who died and 56,7 days for those still on maintenance therapy. We conclude that Cryptococcal meningitis often presents without classical meningeal signs in AIDS patients who frequently ignore their status in Brazil We suggest that the IIT be done routinely on all CSF drawn for diagnostic purposes.

International Journal of STD & AIDS 1990; **1**: 328–329

ORIGINAL ARTICLE

Risk factors of female HIV-seropositive patients attending the clinic for sexually transmitted diseases at St Mary's Hospital, London

J R Smith MRCOG, **S Murphy** MRCPI, **J Mellers** MB BS, **M James** SEN, **L E M Osborne** MD, **M Byrne** MRCOG, **P Munday** MD FRCOG, **J R W Harris** FRCP and **S M Forster** BSc MRCP

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Summary: Of 3450 women tested for antibodies to human immunodeficiency virus HIV-1 and HIV-2 between September 1985 and July 1989, 61 were positive (1.8%). Twenty-seven of these (44%) were presumed to have acquired their HIV infection by heterosexual contact and 23 (38%) were intravenous drug addicts. In geographical origin, 23 (38%) of the patients were from the UK and 19 (31%) from Africa. Amongst these 61 women, 2 (3%) have since died, one committed suicide and one was suspected of committing suicide.

Keywords: Human immunodeficiency virus, woman, risk factors, incidence

INTRODUCTION

Between September 1985 and July 1989, 3450 women who had attending the Department of Genito-urinary Medicine at St Mary's Hospital were tested for antibody to the human immunodeficiency virus (HIV). Of these, 61 (1.8%) were seropositive. Although the geographical origin of HIV seropositive male patients, their probable mode of HIV acquisition and the annual incidence of infection are well documented[1], these data were not available for the HIV-seropositive female patients. A retrospective study was therefore undertaken.

METHODS

The notes were examined of all HIV-positive women who had attended the Department of Genito-urinary Medicine at St Mary's Hospital since September 1985 when testing for HIV began. Patients were selected for testing by offering at booking a leaflet explaining the HIV test which they could then request if they so wished. In addition to this self selection method, 'at risk' patients were offered the test. All patients requesting an HIV test were counselled by our medical staff and health advisors.

Tests for HIV comprised enzyme-linked immunosorbent assays (ELISA) to detect envelope antibodies to HIV-1 and HIV-2 and p 24 antibody to HIV-1.

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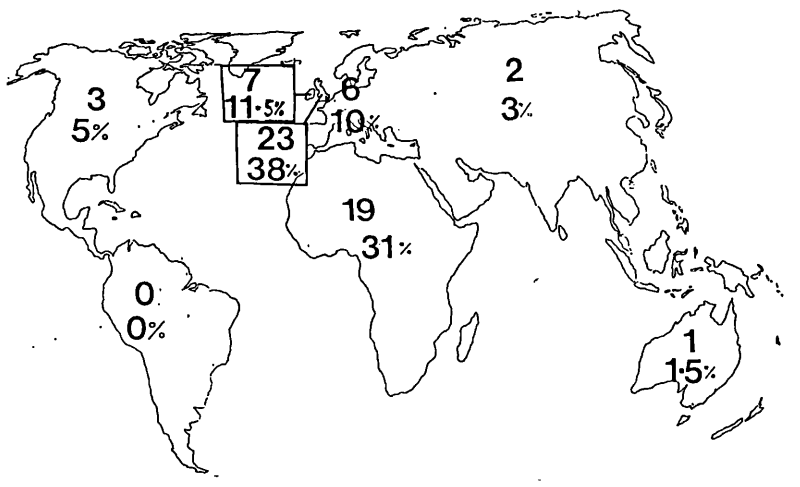

Figure 1. Geographical distribution of the origin of HIV-positive women

RESULTS

Between 1 September 1985 and 31 July 1989, 3450 women were tested for HIV antibody, of whom 61 (1.8%) were positive. In 1989, 172 women were tested for HIV-2 antibody but none were positive. The ages of the HIV-1 antibody-positive patients ranged between 17 and 51 years with a mean age of 30 years. Two patients have died since the diagnosis was made.

Twenty-seven (44%) of the patients were presumed to have acquired their HIV infection by heterosexual contact, 23 (38%) were intravenous drug users (IVDUs), 4 (7%) had had blood transfusions and 7 (11%) had no identifiable risk factors. Twenty-three (38%) of the patients were from the United Kingdom and 19 (31%) were African (Figure 1). Of the African patients, 15 (79%) were presumed to have contracted their infection

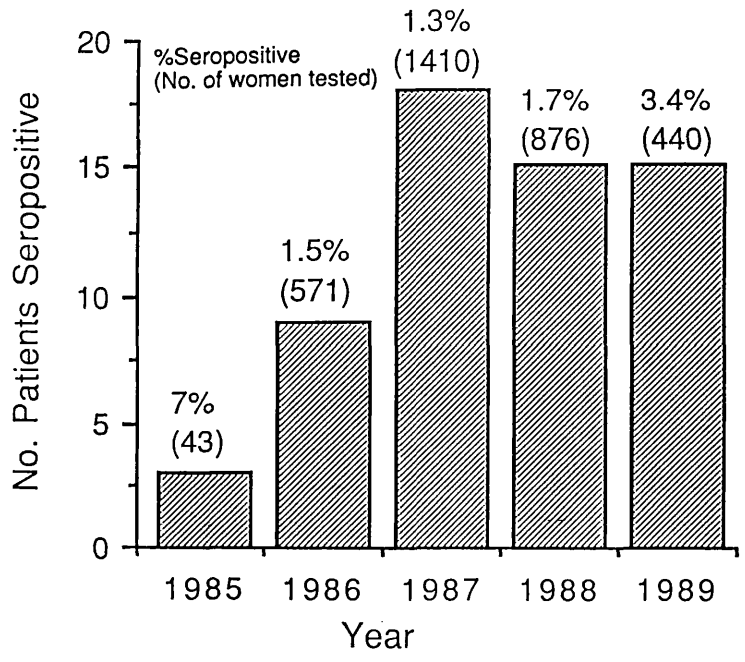

Figure 2. Number of women tested for HIV each year and number and percentage found positive (1985 September to December inclusive only; 1989 January to July inclusive only)

heterosexually, 3 (16%) had had blood transfusions and 1 (5%) was an IVDU. All other IVDUs were European. Four IVDUs also worked as prostitutes and a small number of IVDUs had had sexual contact with HIV-seropositive men.

Figure 2 shows that the number of HIV-positive women has increased since 1985. This, however, is probably due to the larger number of women tested because, apart from 1985, the proportion of HIV-seropositive women has remained about the same.

DISCUSSION

At the Department of Genito-urinary Medicine, 1.8% of women tested for HIV-1 antibody were positive. This compares with 11.2% of male patients tested over a similar period of time[1]. This percentage, although lower than that for men, is larger than the female seroprevalence rates of 0.2% to 0.5% reported previously in London[2,3]. These women, however, were attending antenatal clinics and, therefore, may be at low risk for HIV. The larger proportion of tests which were positive in 1985 probably reflects a policy of testing only those patients who had symptoms of HIV disease. It can often prove difficult to assign a single mode of transmission to HIV-seropositive women; this is especially the case in IVDUs some of whom also have HIV-infected heterosexual partners.

Our patients fell largely into two categories, namely African patients who acquired the infection heterosexually and indigenous patients who acquired the infection by intravenous drug usage. Thus, with the exception of one drug abuser who was an African of European origin, all IVDUs were European. Many of the African patients had received injections or vaccines in Africa, although this is not considered to be a major mode of transmission[4]. None of the prostitutes were HIV seropositive in the absence of other risk factors although in this series of patients the number of prostitutes was small.

Two of the 61 patients (3%) have died since the diagnosis, neither directly of HIV infection. One committed suicide within one month of diagnosis, and the other died within 2 years of diagnosis, due to a drug overdose, which may have been suicide. A 3% (or even a lesser) suicide rate calls into question the efficacy of counselling and psychological follow-up after the diagnosis of HIV infection, when psychiatric morbidity is high in this group of women. Suicidal tendency in association with AIDS has been reported[5] and is a component of intravenous drug addiction.

In conclusion, a significant proportion of our HIV-seropositive women are African and special attention needs to be directed at this population. The high rate of suicide amongst HIV-seropositive women makes the provision of counselling facilities and follow-up essential in all clinic settings where HIV testing is offered.

References

1 Beck EJ, Donnegan C, Cohen CS, *et al.* Risk factors for HIV-1 infection in a British population: lessons from a London sexually transmitted diseases clinic. *AIDS* 1989;**3**:533–8
2 Howard LC, Hawkins DA, Marwood R, Shanson DC, Gazzard BG. Transmission of HIV by heterosexual contact with reference to antenatal screening. *Br J Obstet Gynaecol* 1989;**96**:135–9
3 Heath RB, Grint PCA, Hardiman AF. Anonymous testing of women attending antenatal clinics for evidence of infection with HIV. *Lancet* 1988;**i**:1394
4 Hyrdy DB. Cultural practises contributing to the transmission of human immunodeficiency virus in Africa. *Rev Infect Dis* 1987;**9**:1109–19
5 Marzuk PM, Tierrey M, Tardiff K, *et al.* Increased risk of suicide in persons with AIDS. *JAMA* 1988;**259**:1333–7

(Accepted 25 April 1990)

TABLE II—END-POINT TITRE OF HIV-SPECIFIC ANTIBODY IN MATCHED PLASMA AND URINE SPECIMENS

Sample	Optical density (410 nm) end-point dilution*	
	Plasma	Urine
1	14 580	16
2	4860	4
3	>43 740	64
4	4860	4
5	1620	1
6	4860	16
7	14 580	1

*Reciprocal of highest dilution yielding optical densities over cut-off.

would indicate renal dysfunction. There was an excellent correlation between the ELISA result in serum and in urine; even so, the low urine antibody titres create the potential for a false-negative result (eg, in seroconverters or in individuals with low serum antibody titres). Furthermore, urinary pH and specific gravity, the presence of preservatives or bacterial contaminants, and storage conditions may compromise antibody stability or recovery and lead to a false-negative result. Although these findings are promising, additional studies are required before urine testing can be a practical screening method.

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1. Cao Y, Friedman-Kien AE, Chuba JV, Mirabile M, Hosein B. IgG antibodies to HIV-1 in urine of HIV-1 seropositive individuals. *Lancet* 1988; ii: 831–32.
2. Cao Y, Hosein B, Borkowsky W, et al. Antibodies to human immunodeficiency virus type 1 in the urine specimens of HIV-1 seropositive individuals. *AIDS Res Hum Retroviruses* 1989; 5: 311–19.

Safe sex and conception: a dilemma

SIR,—We were consulted by a 26-year-old woman who first tested seropositive for HIV-1 in 1988 and her 48-year-old husband. She is seronegative for HIV-2. Transmission of HIV-1 was thought to have occurred in 1987 heterosexually. The husband has tested seronegative for HIV-1 and HIV-2 twice since 1988. Since they started sexual relations, the couple had consistently practised safe sex to protect the husband. They sought advice about pregnancy and over six counselling sessions spread over 6 weeks the facts about HIV, vertical transmission, and risks to the mother were provided.

We told the couple that the vertical transmission rate in well-HIV-positive women is unknown but is probably 25–30%,[1] at least in developed in countries; it may be greater if the woman has symptoms of HIV infection.[2] They were told that it is impossible to detect before delivery which babies will have HIV infection and that HIV infection in a baby born to an infected mother is difficult to diagnose. Maternal antibodies may persist for up to 18 months, producing serological confusion. For the present we are assuming that these babies are not infected, even though no data are available yet on the long-term clinical outcome of this group. Definite HIV infection can only be confirmed by the appearance of symptoms or positive HIV culture. The risks to the HIV-infected mother are unknown, though theoretically pregnancy may precipitate a deterioration in her disease.[3]

After this counselling T4 counts and p24 antigen tests were done; the T4 count was normal ($0{\cdot}72 \times 10^9$/l) and the p24 antigen was negative. Having been given every opportunity to ask questions they decided they wanted to have a child. They then pointed out that they had been practising safe sex and that they would be unable to have children without placing the husband at risk.

After discussion between the genitourinary medicine and obstetric departments it was decided to offer artificial insemination by husband (AIH), done by the husband himself. The couple were advised to keep a temperature chart to time ovulation and to have safe sex at times other than ovulation. However, at ovulation time the husband was requested to produce a semen specimen into a sterile pot and to inject this into the high vaginal area via a 5 ml syringe and a dextrose quill. Pregnancy is still awaited.

We were reluctant to encourage the pregnancy in view of the risk to the child and, possibly, the mother. The couple, however, had decided to proceed with pregnancy. Respecting their wishes and taking into account the need to protect the husband we felt obliged to offer AIH. It seems to us that the proper course for doctors in this situation lies in thorough counselling, allowing the couple to make an informed decision. Once the couple decides to proceed, doctors should do what is required to protect the seronegative male partner. We also felt that the husband should carry out the AIH procedure, removing the doctor from the act of conception. AIH may have a part to play in a small number of HIV seropositive patients, but only after extensive counselling.

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1. European Collaborative Study. Mother to child transmission of HIV infection. *Lancet* 1988; ii: 1039–42.
2. Mok JQ, Giaquinto C, De Rossi A, Grosh-Worner I, Ades AE, Peckham CS. Infants born to mothers seropositive for human immunodeficiency virus: preliminary findings from a multicentre European study. *Lancet* 1987; i: 1164–67.
3. Letsky E. In: Hytten F, Chamberlain G, eds. Clinical physiology in obstetrics. Oxford: Blackwell Scientific, 1980: 49–51.

Mother-to-infant transmission of HIV

SIR,—Dr Goedert and colleagues (Dec 9, p 1351) report that analysis of antibody reactivity in sera from HIV-1 transmitting and non-transmitting mothers shows a correlation between lack of high affinity antibodies to gp120 HIV envelope protein and infection of the offspring. They could not confirm our observation[1-3] of a positive association between the presence of antibodies to epitopes of the hypervariable loop of gp120 and lack of HIV transmission from mother to child.

There are significant differences between our studies and Goedert and colleagues' investigations. They use one 24 aminoacid peptide for each of five HIV-1 strains covering only part of the hypervariable loop. We had recorded that the size and position of the peptides used would be of great importance.

We analysed sera from 30 HIV infected mothers who gave birth to 19 uninfected and 11 infected infants. Criteria for infection/non-infection were those of the Centers for Disease Control. In addition, several DNA samples from infected and non-infected children were assayed for HIV viral sequences by the polymerase chain reaction with three primer pairs, and results were confirmed by Southern blot and fragment restriction analysis. Antibody reactivity was assayed in a peptide ELISA with fifteen mer peptides overlapping by 10 aminoacids describing most of the structural protein of HIV (*gag*, *env*). Sera were diluted from 1 in 100 to 1 in 10 000 and tested against each peptide in duplicate. Although no significant difference was found in reactivity to most of the epitopes tested, sera of non-transmitting mothers consistently reacted with the cysteine-containing peptides of the hypervariable loop. Only 2 of the transmitting mothers showed measurable reactivity to the conserved sequences C51, C57, C58 (table).

REACTIVITY TO CONSERVED SEQUENCES C51, C53, C57, AND C58 IN TRANSMITTING AND NON-TRANSMITTING MOTHERS

Synthetic peptides*	Transmitting mothers (n=11)	Non-transmitting mothers (n=19)	Fisher's exact test
gp120/C51-INCTRPNNNTRKSIR	2	11	<0·05
gp120/C53-RKSIRIQRGPGRAFV	4	10	NS
gp120/C57-GNMRQAHCNISRAKW	1	11	<0·01
gp120/C58-AHCNISRAKWNNTLK	1	7	NS

*Aminoacid sequences according to Ratner et al.[4] NS = not significant.

* TRIAL NO. ___ ___ ___

A = study gp.
C = control gp.

* Date of Visit ___ ___ / ___ ___ / ___ ___

Name .....

Address .....

.....

.....

Tel.No. .....

Date of Birth .....

Marital Status .....

Occupation .....

Country of Origin .....

* Age [ ] [ ]

* Sampling Frame [ ]

1 = PSC
2 = Obstetric
3 = Gynaecology
4 = DDU
5 = Glasgow
6 = Others
9 = Not available

* Country of Origin [ ]

1 = UK
2 = Other European
3 = North Africa
4 = Sub-Saharan Africa
5 = Asia
6 = North America
7 = South America
8 = Australasia
9 = Unavailable

Sexual contact in/with someone from above

Details .....

.....

Past Medical History .....

.....

.....

.....

* Past STD

Syphilis ☐

Gonorrhoea ☐

Chlamydia ☐

T Vaginalis ☐

Warts ☐

Candidiasis ☐

BV (Gardnerella) ☐

HSV ☐

Hepatitis B ☐

Chancroid/LGV/G/ ☐

Others ☐

1 = yes
2 = no
9 = unavailable

* IV Drug Use

1 = yes, now
2 = yes, past
3 = no
9 = unavailable ☐

Details of IV drug use .....

.....

Oral drug abuse .....

.....

Past & Current Prescribed drugs .....

* History of immunosupressive illness or therapy

1 = yes
2 = no
9 = unavailable

Details .....

.....

* Family History - Cancer

1 = Genital tract
2 = Elsewhere
3 = Both
4 = None
9 = Unavailable

Family History (Other) .....

.....

* Smoking

Number per day

Ex-smoker

1 = yes
2 = no
9 = unavailable

* Alcohol __ __ __ (no. units per week)

1 unit = 1 measure spirits
½ pint beer
1 glass wine

Obs. & Gynae. History

LMP .....

Menstrual History .....

IMB .....

PCB .....

Date of last smear .....

Past gynaecological history & obstetric history .....

.....

.....

* PARA

Live Births		
Still Births		
Spontaneous Abortion >12/52		
T.O.P.'s		
Spontaneous Abortion ≤12/52		

Details .....

.....

* Current Contraception

1 = Pill	10 = Rhythm
2 = Coil	11 = Vaginal sponge
3 = Condom	12 = Sponge + condom
4 = Cap/Diaphragm + jelly	13 = Sterilization
5 = Condom + jelly	14 = Vasectomy
6 = Jelly alone	15 = Vaginal condom
7 = Pill + condom	16 = Other
8 = Cap or Diaphragm + condom	17 = None
9 = Withdrawal	99 = Unavailable

Details (Include type of IUCD and OC) .....

.....

* Have you ever used this form of contraception?

1 = yes
2 = no
9 = Unavailable

Pill	
Coil	
Condom	
Cap/Diaphragm	
Jelly	
Sponge	
Vaginal Condom	

Details .....

X

* Past Cervical Smears

1 = CIN1
2 = CIN2
3 = CIN3
4 = Warty Change
5 = Malignancy
6 = Other
7 = Never done
9 = Unavailable

Details .....

.....

* Sexual History

Age first intercourse ☐☐

Date last sexual intercourse __ __ / __ __ / __ __

Details .....

.....

.....

Total No. of Partners ---

♂ ♀

* No. of regular partners

ie. > 2 months etc.

1 = None
2 = 1
3 = 2-5
4 = 5-10
5 = > 10
9 = unavailable

♂ ♀

* No. of casual partners

for payment (money/drugs)

1 = None
2 = < 10
3 = 11-20
4 = 21-100
5 = 101-500
6 = > 500
9 = unavailable

♂ ♀ X

* No. of casual partners

NOT for payment

1 = None

2 = < 10

3 = 11-20

4 = 21-100

5 = 101-500

6 = > 500

9 = unavailable

* Sexual Practices

1 = yes

2 = no

9 = unavailable

Oral Sex

Anal Sex

Sex Toys

Details .....

.....

* HIV Risk Partners

1 = yes

2 = no

9 = unavailable

Known positive

Partner of homosexual

Partner of haemophiliac

Partner of IVI user

Partner of transfusion recip.

Partner of African

Partner of USA/Haiti

YEAR OF SERCONVERSION

C> _ _

DATE OF 1ST (+ve) TEST

_ _ - _ _ - _ _

CLINICAL HISTORY AND EXAMINATION

History (include history of STD)

P.V. dis. itch sores

G.U.

C.V.S. Resp.

G.I.

On Examination

glands

abdo.

- mouth

C.V.S

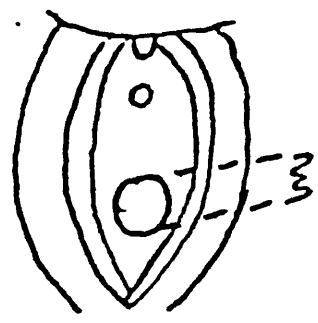
resp.

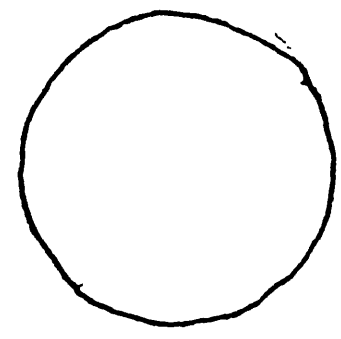
- loins

	GC	PUS		WET	DRY
Ur			MON		
Cx			TV		
Vag			BV		

P.V.

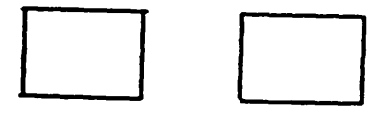
Ext. genit.
Vag.
Cx.
Ut.
Adnex.





* Stage of HIV disease

1 = gp 1
2 = 2
3 = 3
4 = 4A
5 = 4B
6 = 4C1
7 = 4C2
8 = 4D
9 = 4E
99 = unavailable



Details .....

.....

Colposcopy

* vulva 1 = None

2 = Aceto-white

3 = warts

4 = suspicious of malignancy

5 = other

9 = unavailable

Details .....

.....

* perianal area 1 = None

2 = Aceto-white

3 = warts

4 = suspicious of malignancy

5 = other

9 = unavailable

Details .....

.....

* vagina 1 = None

2 = Aceto-white

3 = warts

4 = suspicious of malignancy

5 = other

9 = unavailable

Details .....

.....

* cervix 1 = None

2 = Aceto-white

3 = warts

4 = suspicious of malignancy

5 = other

9 = unavailable

Details .....

.....

* Biopsy Taken

1 = Taken
2 = Not taken
9 = Unavailable

Vulva ☐
Cervix ☐
Vagina ☐
Other ☐

* STD present on this examination

1 = yes
2 = no
9 = unavailable

Syphilis ☐
Gonorrhoea ☐
Chlamydia ☐
T.V. ☐
Warts ☐
Candidiasis ☐
B.V. ☐
H.S.V. ☐
Hepatitis B ☐
Chancroid/ ☐
L.G.V. ☐
G.I. ☐
Others ☐

Details of treatment .....

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* Smear cytology

1 = Normal
2 = Warty change
3 = CIN1 - mild dysk.
4 = CIN2 - mod. dysk.
5 = CIN3 - severe dysk.
6 = malignant cells
7 = other
9 = unavailable

☐

* In Situ
1 = HPV not present
2 = HPV present
9 = not available

* Biopsy histology
1 = Normal
2 = Warty change
3 = CIN1
4 = CIN2
5 = CIN3
6 = Microinvasion
7 = Macroinvasion
9 = not available

* Biopsy In Situ HYB
1 = HPV not present
2 = HPV present
9 = not available

* Abnormal Microscopy 1 = yes 2 = no 9 = unavailable

GC cultures
TV
BV
HSV culutres
Chlamydia IF
SB
Hepatitis B
HSV ab

* Chlamydia serology
1 = pos.
2 = neg.
9 = unavailable

IgG+
IgM+

HIV ab

PH = __.__

P24 ag = __ __ __ __

P24 ab = __ __ __ __

T4 = __ __ __ __

T8 = __ __ __ __