Specific syphilis serological tests may become negative in HIV infection

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The diagnosis of syphilis is frequently dependent upon the results of serological tests, but the reliability of syphilis serology in patients with HIV-1 infection has been questioned. We examined specific antibody to *Treponema pallidum* (TP) using the TP haemagglutination (TPHA) and fluorescent treponemal antibody-absorption (FTA-ABS) tests in AIDS patients and HIV-antibody-negative controls with a history of syphilis. Tests were carried out on two sera separated by an interval of at least 3 years from each patient. Twelve out of 29 AIDS patients compared with four out of 29 controls showed significant falls in titres of specific antibody as measured by the TPHA, FTA-ABS, or by both the TPHA and FTA-ABS ($P = 0.02$). Furthermore, in three out of 29 (10%) of the AIDS patients with past syphilis infections both the TPHA and FTA-ABS became non-reactive. We conclude that negative specific serology does not exclude a past syphilis infection in patients with AIDS.

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Introduction

Syphilis and AIDS are complex diseases which are interrelated. The AIDS epidemic in most Western countries was preceded by a high incidence of syphilis in those risk groups which later became the first AIDS victims [1]. There is evidence to suggest synergism between HIV-1 and *Treponema pallidum*: the presence of syphilitic mucosal lesions may allow easy access for HIV-1 to the host's general circulation [2–6], and the immune deficit produced by HIV-1 could in turn decrease host resistance to *T. pallidum*. Treatment failures have been reported when conventional regimens are used for early syphilis in patients also infected with HIV-1 [7], and it is now recommended that these patients receive higher doses of penicillin for a longer period to overcome this problem [8]. Because there is no reliable *in vitro* culture system for *T. pallidum*, diagnosis rests either upon identification of the organisms in tissue fluid from early lesions or the results of serological tests. In the pre-AIDS era, the reliability of both specific and non-specific serological tests for *T. pallidum* was well established. How reliable are these serological tests for syphilis in HIV-1-infected individuals? There have been reports of excessively high titres in non-treponemal syphilis serological tests [9], seronegative secondary syphilis [10], and falling levels of specific antibody in HIV-1-antibody-positive patients with syphilis [11]. Clearly, the specificity and sensitivity of serological testing for syphilis in patients also infected with HIV-1 needs to be re-established. Our study examines the effect of HIV-1 infection over time on levels of specific antibody to *T. pallidum* in patients previously treated for syphilis.

Subjects and methods

Fairfield Infectious Diseases Hospital is responsible for the care of the majority of AIDS patients in the Australian state of Victoria. The hospital's Department of Clinical Pathology has been the state's central reference laboratory for syphilis serology for many years. As serum from past syphilis testing was stored, we were able to obtain serum from a group of patients with AIDS who had earlier contracted syphilis. Strict confidentiality was maintained during this study; information that identified individuals was available only to staff who already had legitimate access to it in their routine work.

A check was made from case records of all past and current AIDS patients managed at Fairfield Hospital to the end of October 1988. Many of these patients had a prior

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history of syphilis, often from some years before they had developed AIDS. A substantial number had been treated for syphilis in Victoria and therefore had sera stored dating back to their initial syphilis diagnosis, treatment and follow-up. All such patients were included in this study provided that two serum samples were actually available and that at least 3 years had elapsed between samples in a pair (to allow for the detection of an effect). Earliest and latest available serum samples from these patients were batched and retested in parallel by the same technician who was blinded as to the origin of each sample. Specific antibodies against *T. pallidum* were assayed using the *T. pallidum* haemagglutination test (TPHA; Fujirebio TPHA test kit, FD 102, Fujirebio Inc., Shinjuku-ku, Tokyo, Japan) and fluorescent treponemal antibody-absorption (FTA-ABS) text (BioMérieux, Nichols strain *T. pallidum* used as antigen, BioMérieux Instruments et Réactifs de Laboratoire, Marcy - l'Etoile, France; sorbent based on extract of Reiter treponeme).

A control group was selected from other stored syphilis sera. This was done by random sampling of 100 serum pairs with subsequent reduction of the sample by specific exclusion of known migrants from South East Asia (because of the problem of false-positive syphilis serology due to yaws), sources associated with HIV-1 infection (samples from clinics treating mainly male homosexuals) or if less than 3 years had elapsed between available serum samples.

A significant change in the level of specific antibody was prospectively defined as a fourfold (two doubling dilution) or greater change in the TPHA titre (for example, 1280 to 320) and a '2+' or greater change in the FTA-ABS result (for example, 3 + to 1 + fluorescence). Lesser changes were not considered significant and were ignored. To ensure that the control group did not contain unrecognized HIV-1-antibody-positive patients, we screened the 29 control pairs using enzyme-linked immunosorbent assay (ELISA).

HIV-1-antibody testing was carried out in a 'de-linked' manner using randomized unmarked test aliquots under supervision from an independent observer. HIV-1-antibody testing was also performed on the first serum in each pair from the AIDS patients to see whether antibody positivity at the time of the diagnosis of syphilis influenced later serological changes. As all the sera from both AIDS patients and controls were originally sent to Fairfield Hospital from either private physicians or sexually transmitted disease clinics, the case records and information regarding the stage and treatment of syphilis infections were not available to us. In no patient from either group was reinfection with syphilis observable by a rise in previously performed non-treponemal tests over time. All patients were treated with antibiotics as indicated by repeated testing by their doctors and rapidly falling non-treponemal tests. Recommended antibiotic regimens at this time consisted of either intramuscular benzathine penicillin, intramuscular procaine penicillin with probenicid or intravenous penicillin (or suitable alternatives in allergic patients) depending on the stage of the disease.

Results were analysed using the two-tailed unpaired Student's t-test for continuous data and Fisher's exact test for discontinuous data.

Non-treponemal serological tests (rapid plasma reagin) were not repeated. Because all patients in both groups had been treated for syphilis and followed-up, non-treponemal serological test titres had already been shown to fall to low or undetectable levels.

Results

To October 1988, 203 AIDS patients were managed at Fairfield hospital. Of these, 59 (29%) had a past history of syphilis documented at the central reference laboratory. From this group, 29 paired sera satisfying the previously stated selection criteria were tested. Serum pairs from 29 controls were also tested. The occurrence of the same final number in each group was fortuitous; exclusions were made only according to the stated criteria, all samples qualifying were tested and included. The main reason for the 71 exclusions from the initial 100 control serum pairs was that 3 or more years had not elapsed between samples.

The final two groups had similar mean ages [AIDS: 34.6 ± 9.3 (mean ± 1 s.d.), controls: 39 ± 1.44 years; $P = 0.16$], mean intervals between serum samples in each pair (AIDS: 84.9 ± 35.2; controls: 77.5 ± 33.3 months; $P = 0.4$) and geometric means of the TPHA titre on the first serum samples (AIDS: $\log^{10}$ 2.7 ± 0.5; controls: $\log^{10}$ 2.6 ± 0.5; $P = 0.2$). The initial serum samples were collected for both groups between 1972 and 1985. The mean time of collection of the first sample was 2.5 ± 3.5 years earlier for the controls than the AIDS patients ($P < 0.01$). If longer storage time was responsible for declining antibody titres this would be biased towards the control group, not the AIDS group. The male : female ratio in the control group was 15 : 14, but only one of the patients in the AIDS group was female. All control-group patients were HIV-1-antibody-negative. It was assumed that the different numbers of men and women in the two groups would not confuse the interpretation of results (see below).

Twelve out of 29 (41%) of the AIDS patients showed a significant fall in levels of specific antibody as measured by either the TPHA (nine out of 29), the FTA-ABS (eight out of 29) or by both the TPHA and FTA-ABS tests (five out of 29).

Falls in specific antibody were seen in four (14%) of the control-group patients: three out of 29 TPHA only, both TPHA and FTA-ABS one out of 29. This difference is statistically significant (12 out of 29 versus four out of 29; $P = 0.02$).

The geometric mean titres for the TPHA also showed significant changes. In the AIDS group, but not in the control group, there was a significant fall in the geometric mean titres between the first and second serum samples (results expressed as mean ± 1 s.d. of the logs of the

titres: AIDS first serum 2.7 ±0.5, second serum 2.1 ±1.0; $P = 0.002$; controls first serum 2.6 ±0.5, controls second serum 2.5 ±0.5; $P = 0.3$).

Significant rises (two doubling dilutions only) were observed in two out of 29 of the AIDS patients and one out of 29 of the controls with the TPHA (two out of 29 versus one out of 29; P = not significant). No patient in either group showed a significant increase in the FTA-ABS.

The most important finding overall was that all serological evidence of past syphilis infection may disappear. In three (10%) of the AIDS patients, but in none of the control patients, specific antibody became undetectable by both the TPHA and FTA-ABS (three out of 29 versus none out of 29; $P = 0.12$).

The above results are displayed diagrammatically in Figs 1 and 2; for clarity only patients demonstrating significant changes in antibody titres are depicted.

When the sera interval of the patients in the AIDS group who showed significant falls was compared with the sera interval of those with stable results, no significant difference was observed (AIDS patients showing falling levels: 81.7 ±36.6 months; AIDS patients with stable levels: 87.2 ±35.0 months).

All the AIDS patients showing significant falls in antibody levels were men. In the control group, three of the four patients showing significant falls were women, including the single control patient to show a fall with both the TPHA and FTA-ABS. When male subjects only were considered, 12 out of 28 male AIDS patients compared with one out of 15 male controls showed significant falls in antibody titre ($P = 0.02$).

Of the 29 AIDS patients, 25 had sufficient remaining sera to undergo retrospective HIV-1-antibody testing (ELISA) on the initial sample. Seven patients were positive at the time of their first syphilis serology. Of these seven, four had stable treponemal antibody levels and three showed falls in levels of specific antibody. In the 18 who were initially HIV-negative, 12 remained stable and six showed significant falls (three out of seven versus six out of 18; $P = 0.5$).

The defining illness for the 29 AIDS patients were as follows: *Pneumocystis carinii* pneumonia 14 out of 29, Kaposi's sarcoma (KS) six out of 29, oesophageal candidiasis three out of 29, cerebral toxoplasmosis two out of 29, disseminated *Mycobacterium avium-intracellulare* infection two out of 29, chronic cryptosporidiosis one out of 29, and chronic herpes simplex infection one out of 29. Three of the six patients presenting with KS alone compared with eight of the remaining 23 showed significant falls in antibody by one or other test (three out of six versus eight out of 23; $P = 0.4$).

Discussion

The strong epidemiological association between syphilis and AIDS is well recognized. We have shown that, of AIDS patients in the state of Victoria to late 1988, 29% had a clear past history of syphilis. This is likely to be an underestimate of the true prevalence as some syphilis cases may have been diagnosed and treated elsewhere. Other investigators have reported similar or higher prevalences of past syphilis in HIV-1-infected populations [1,12]. Although hypergammaglobulinaemia and the appearance of autoantibodies can be a hallmark of HIV-1 infection [13], there is clearly a reduction in levels of specific antibody to *T. pallidum* in some HIV-1-infected individuals as they progress through their illness. This finding has been reported recently in patients with AIDS, patients

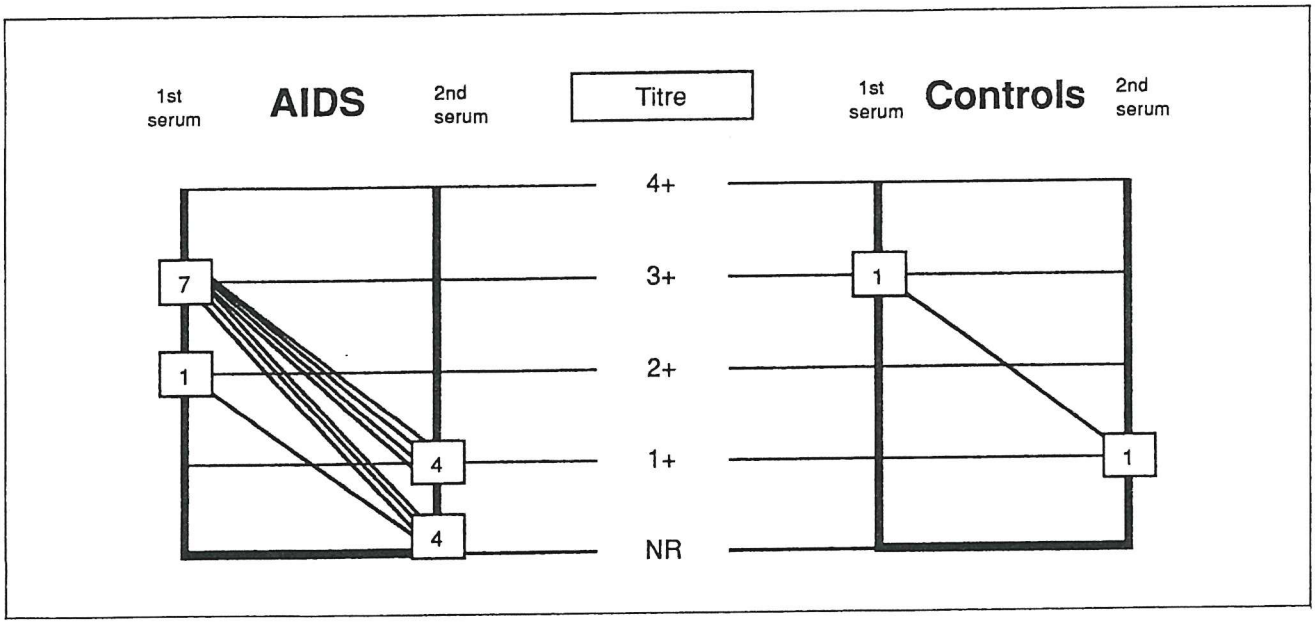

Fig. 1. Changes in serology: fluorescent treponemal antibody-absorption (FTA-ABS) test. n = 29 in each group. Each oblique line represents one patient who showed a significant change in titre. Numbers in squares show the number of patients with result that is indicated. Patients showing no significant change between tests are not shown. NR, non-reactive.

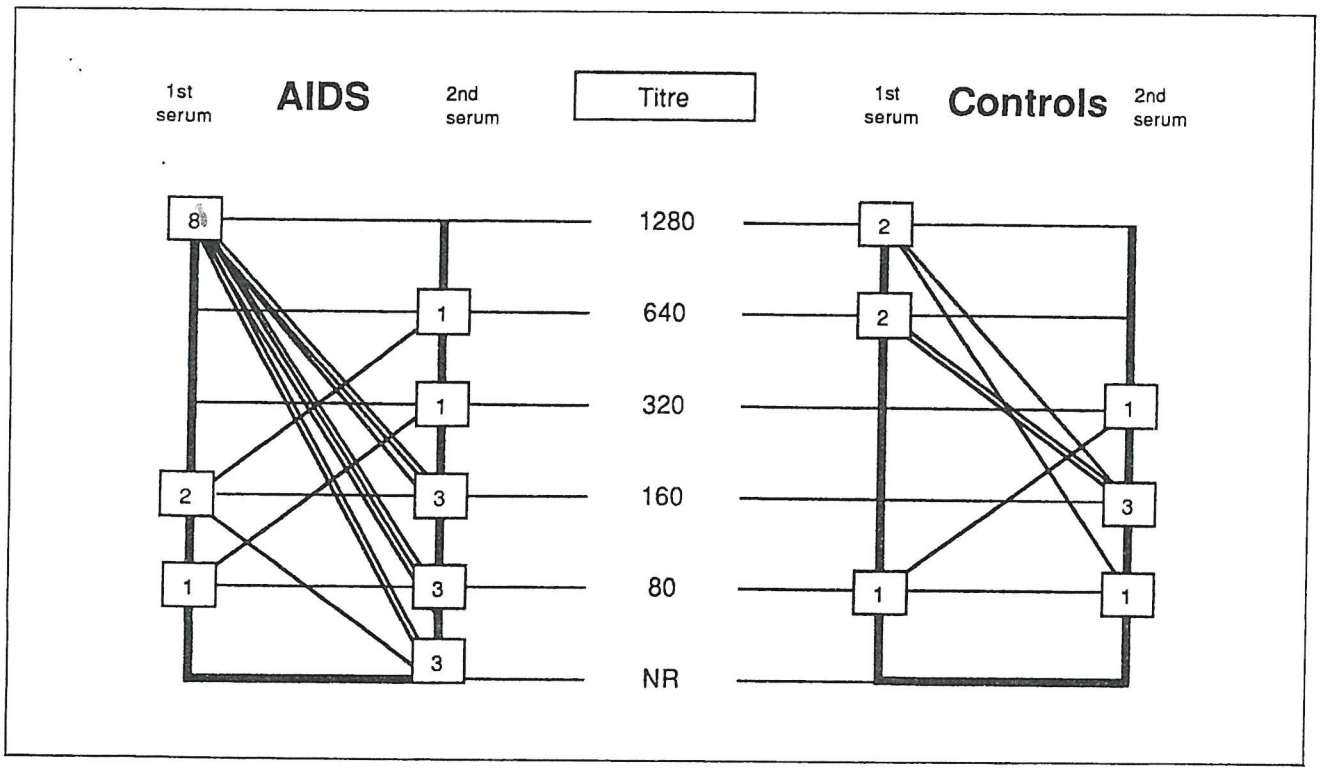

Fig. 2. Changes in serology: treponemal haemagglutination (TPHA) test. n = 29 in each group. Each oblique line represents one patient who showed a significant change in titre. Numbers in squares show the number of patients with result that is indicated. Patients showing no significant change between tests are not shown. NR, non-reactive.

with AIDS-related complex, people who are HIV-1-antibody positive [10] and occasionally in non-HIV-infected individuals with syphilis [14]. Apart from one study by Haas *et al.* [16], this is the only study comparing changes in syphilis serology in AIDS patients with HIV-1 antibody-negative controls. We only became aware of the paper by Haas *et al.* which draws the same conclusions as this paper, during revision of the manuscript. Most importantly, we have shown that specific antibody may become undetectable in up to 10% of AIDS patients who previously had positive tests. Other serological tests apart from those for syphilis have also been reported as becoming non-reactive late in the course of AIDS [13].

There was no observable relationship between HIV-antibody positivity at the time of initial syphilis diagnosis and the later disappearance of antibody.

Although patients presenting with KS alone may be less immunosuppressed than other AIDS patients, we did not establish any difference in the likelihood of having falling antibody levels in these patients, but our numbers were small.

What is the clinical relevance of our findings? Clinicians managing AIDS patients are familiar with syndromes of fever and acute neurological disturbance that defy diagnosis. Is it possible that some of these cases may be due to reactivation of insufficiently treated syphilis infections? Non-treponemal tests for syphilis have been shown to be falsely negative in at least one HIV-1 antibody-positive patient [10], and we have shown that specific antibody against *T. pallidum* became undetectable by standard tests in 10% of the AIDS patients we studied. As there is no *in vitro* culture system for *T. pallidum* that can exclude the reactivation of syphilis in a previously infected AIDS patient, it is clearly not possible to completely rule out syphilis in this situation.

Despite these uncertainties, it would appear that in most HIV-1-infected patients, including those with AIDS, syphilis serological tests behave as expected [9] but their specificity and sensitivity are not the same as they are in normal hosts. In view of the frequency of the association between AIDS and syphilis, empirical intravenous penicillin treatment (as others have recommended [15]) should perhaps be considered when the presence of HIV infection makes the diagnosis of syphilis problematic.

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