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Detection of treponemal DNA in the CSF of patients with syphilis and HIV infection using the polymerase chain reaction

P E Hay, J R Clarke, D Taylor-Robinson, D Goldmeier

Abstract

The polymerase chain reaction (PCR) was used to detect *Treponema pallidum* DNA in the cerebrospinal fluid (CSF) of patients with and without syphilis. The CSF from 10 of 19 patients with positive serological tests for syphilis who were being investigated for late syphilis were treponemal DNA-positive. In contrast, the CSF from only one of 30 patients with no known history of syphilis was DNA-positive. CSF from 28 HIV-positive patients was also tested. Fourteen of these patients had central nervous system (CNS) disease and seven were DNA-positive, whereas none of the 14 without CNS disease were DNA-positive. Five of the seven DNA-positive patients had a history of syphilis. Such a history in an HIV-positive patient who had CNS disease was predictive of finding treponemal DNA in the CSF. The PCR had a sensitivity of 47% and a specificity of 93% for detecting a known history of syphilis and is a potentially useful tool in treponemal diagnosis.

Introduction

There is clinical evidence that the human immunodeficiency virus (HIV) alters the immune response to *Treponema pallidum*.[1–3] *T. pallidum* has been demonstrated in immunocompetent patients after treatment for syphilis,[4–9] but treponemes are more likely to persist in the cerebrospinal fluid (CSF) of patients infected with HIV than in that of patients who are uninfected.[10] Furthermore, patients infected with HIV who acquire syphilis may be more likely to progress to clinical neurosyphilis, and at an accelerated rate.[11–14] It is also possible that treponemes contribute to the neurological disease that is assumed to be caused directly by HIV (subacute encephalopathy) or by other opportunistic infections of the central nervous system (CNS).

We have determined already in the laboratory that the polymerase chain reaction (PCR) is a relatively sensitive and highly specific technique for detecting *T. pallidum* DNA.[15] In the current study we used the PCR in an attempt to detect treponemal DNA in CSF from patients with and without syphilis, and then to determine the frequency with which treponemal DNA was present in CSF from HIV-infected patients with and without disease of the CNS.

Clinical Research Centre, Harrow, Middlesex
P E Hay, D Taylor-Robinson
Department of Virology
J R Clarke
Department of Genitourinary Medicine, St. Mary's Hospital Medical School, London, UK
P E Hay, D Taylor-Robinson, D Goldmeier

Materials and methods

CSF was collected from the following groups of patients.

Group A—Patients with no known history of syphilis and at no obvious risk of contracting HIV infection. Lumbar puncture was performed to exclude non sexually transmitted pathogens.

Group B—Patients with treponemal disease undergoing lumbar puncture to look for evidence of neurosyphilis.

Group C—HIV-positive patients (CDC group 4) undergoing lumbar puncture for an indication other than syphilis. Half of these patients had CNS disease. Those without CNS disease underwent lumbar puncture as part of an investigation of fever or other non-specific symptoms.

The specimens of CSF were stored in liquid nitrogen until tested. The PCR was performed separately with two sets of 21 base primers to detect DNA sequences from the TMPA and 4D genes of *T. pallidum* as described previously.[15] Forty cycles of amplification were performed. Specimens were recorded as positive only if both DNA sequences were detected. Distilled water was included in every test as a negative control to exclude DNA contamination of the reagents, and all the results were confirmed by Southern blotting with ^{32}P-labelled probes. The sensitivity of the PCR was estimated by testing 500 μl aliquots of tenfold dilutions of a preparation of Nichols' strain *T. pallidum*, which contained $1{\cdot}3 \times 10^8$ organisms/ml. The threshold

for detection was the 10^{-6} dilution giving an estimated sensitivity of 65 organisms. In addition the PCR was 100% specific when used to test human DNA and a battery of organisms that could have caused false-positive results.[15]

Diagnosis of syphilis
This was confirmed for patients in group B by positive TPHA and FTA tests on serum, with or without a positive VDRL test, in the absence of a history of previous treatment for syphilis or yaws. Latency was implied by the absence of recent or current symptoms and signs of primary or secondary syphilis. Neurosyphilis was diagnosed in patients with signs compatible with the diagnosis and a positive CSF VDRL test in the absence of blood contamination. Possible neurosyphilis was diagnosed in patients in whom there were abnormalities of the CSF, but a negative VDRL test and no other cause for the abnormalities (such as HIV infection), or if the CSF TPHA or FTA test was positive making it difficult to exclude the diagnosis with certainty.

There were four patients in group C who gave a history of syphilis but had negative serological tests (table 2). In three cases the history was confirmed by reviewing their records. In the remaining case the patient gave a reliable history of treatment with daily injections of penicillin for 15 days.

Definition of CNS disease
Patients in Group C were defined as having CNS disease if there were confirmed symptoms or signs referable to the CNS, as recorded in table 2. Non-specific headache, although frequently the indication for lumbar puncture was not interpreted as indicating CNS disease in the absence of other abnormalities.

Results

Group A patients
CSF from one of 30 patients was positive in the PCR with both sets of primers. The sample was from a neonate born at 27 weeks gestation, who had developed an intraventricular haemorrhage. Serological tests for syphilis in both the infant and mother were negative.

Group B patients
The results of tests on 19 patients who had undergone lumbar puncture as part of an investigation of late syphilis are shown in table 1. All patients had a positive serum TPHA and FTA test but only three had a serum VDRL titre greater than 1 : >2. Two patients were found to have neurosyphilis. One of these (patient M) presented with a headache and had positive serum TPHA and FTA tests, with a serum VDRL titre of 1/128, but no focal neurological signs. A VDRL test proved positive on undiluted CSF which contained 92 white cells/mm^3, and 1·0 g protein/l. The headache resolved following treatment with procaine penicillin which was given at a dose of 1·2 MU daily for 15 days. The other patient had a diagnosis of neurosyphilis established on the basis of a positive VDRL test on CSF. Treponemal DNA was detected by the PCR in the CSF of both of these patients.

Two patients had a diagnosis of possible asymptomatic neurosyphilis. The TPHA test on the CSF of one of these was strongly positive and treponemal DNA was detected in the CSF. The other patient had an increased concentration of protein in the CSF with a pleocytosis. However, FTA and VDRL tests on the CSF were negative and treponemal DNA was not detected. It subsequently emerged that he had been treated for asymptomatic neurosyphilis at another hospital nine years previously.

Fourteen of the remaining 15 patients had a diagnosis of latent treponemal infection, seven had CSF that reacted positively in the PCR for treponemal DNA. The possibility of yaws existed in only one of these patients and the PCR proved negative.

Two patients were HIV antibody-positive, both with CDC stage 2 disease. One of these was patient M with neurosyphilis who was treponemal DNA positive. The other patient had been treated for latent syphilis with erythromycin five years previously and had requested reinvestigation for syphilis. His CSF was normal and treponemal DNA was not detected.

Many of the patients in whose CSF treponemal DNA was detected had serum VDRL titres of 1: <2. Only one patient with latent syphilis had a serum VDRL of 1: >4. She had been treated for syphilis in 1947 and eventually had been discharged with apparently negative serological tests. However, a serum VDRL titre of 1 : 32 was found following a right cerebral infarct in 1988. Treponemal DNA was not detected in CSF, which was normal except for a slightly elevated protein concentration of 0·5 g/l. After treatment with penicillin the VDRL remained unchanged and the patient is being investigated for a paraproteinaemia. It is likely that this was a biological false-positive VDRL.

Table 1 Presence of treponemal DNA detected by the PCR in CSF from 19 patients undergoing lumbar puncture to look for evidence of neurosyphilis

Diagnosis	*PCR-positive*	*PCR-negative*
Neurosyphilis	2*	0
Possible asymptomatic neurosyphilis	1	1
Latent syphilis	7	7†
Latent syphilis or yaws	0	1

*Includes one HIV-positive patient.
†Includes 1 HIV-positive patient, previously treated with erythromycin.

Table 2 Presentation, CSF findings and syphilis history in 28 HIV-positive individuals undergoing lumbar puncture for an indication other than syphilis. FTA and VDRL tests on CSF were negative in all cases

Presentation	CSF	Serum		Comment	Syphilis
		TPHA	VDRL		
	CNS disease and treponemal DNA detected				
Focal seizure	Ptn* 0·8	–	–	Attributed to HIV	None
Encephalopathy	Ptn 0·5	–	–	Attributed to HIV	Primary 1986
Encephalopathy	Normal	+	–	Attributed to HIV	Secondary 1985
Myelopathy	Ptn 0·6 WCC† 15	+	–	Died 5 days later	Latent 1972
Cerebellar lesion	Normal	+	–	? PML§	Latent ? date
Ataxia	Normal	+	–	Attributed to HIV	Latent 1983
Generalised seizure	Ptn 0·5	–	–	Attributed to HIV	None
	CNS disease but treponemal DNA not detected				
Myelopathy	Normal	–	–	Attributed to HIV	None
Right sided weakness	Normal	+	–	? PML or Lymphoma	? Type 1984
Ataxia	Ptn 0·7	–	–	Attributed to HIV	Primary 1982
Truncal ataxia	Ptn 0·5	–	–	Attributed to HIV	None
Acute psychosis	Normal	–	–	No cause established	None
Falls, confusion	Ptn 0·6	–	–	Died 2 days later	? Type 1979
Left sided weakness	Normal	–	–	PML on brain biopsy	None
	No CNS disease and treponemal DNA not detected				
Headaches, depression	Normal	+	–	Resolved	? Type ? date
Peripheral neuropathy	Ptn 0·6	+	1/2	Attributed to HIV	Latent 1977
Peripheral neuropathy	Ptn 0·9	–	–	Attributed to HIV	None
Fever	Ptn 0·6	–	–	Fever resolved	None
Fever	Ptn 0·9	–	–	PCP‖	Latent 1976
Fever	Normal	–	–	Oesophageal candida	None
Fever	Normal	+	–	PCP	Latent 1981
Fever, oral candida	Normal	–	–	Fever resolved	None
Fever, headache	Normal	+	–	PCP	Secondary 1984
Fever, headache	Normal	–	–	Acute leukemia	None
Headache	Ptn 0·6	–	–	Headache resolved	None
Headache	Normal	–	–	Headache resolved	None
Headache, depression	Normal	–	–	Resolved	None
Headache, depression	Normal	–	–	Resolved	None

*Ptn : protein concentration (g/l)
†WCC : white cell count (/mm^3)
§PML : progressive multifocal leucoencephalopathy
‖PCP : *Pneumocystis carinii* pneumonia

Group C patients

Details of these patients and the results of their investigations are shown in tables 2 and 3. There were 14 patients with CNS disease, of whom seven were treponemal DNA positive. In contrast, of 14 patients without CNS disease, none were treponemal DNA positive (Fishers exact test $p < 0{\cdot}005$). Furthermore, five of eight patients who had a history of syphilis and current CNS disease were treponemal DNA positive, while the five patients with a history of syphilis but without CNS disease were all treponemal DNA negative (Fishers exact test $p < 0{\cdot}05$). Four patients gave a history of syphilis but had negative FTA, TPHA and VDRL tests of the serum. One of these patients had been treated for primary syphilis with benzathine penicillin 3 years earlier and was treponemal DNA positive. Another of the four had been treated abroad and it was not possible to confirm the prior diagnosis of syphilis, but he gave a good description of a chancre and treatment with daily injections of penicillin for 15 days. There were two men who did not give a history of syphilis and who had negative serological tests for it, in whom treponemal DNA was detected. These two men had

Table 3 Summary of results for history of syphilis and detection of treponemal DNA in CSF

Treponemal DNA	Patients in groups A/B		Patients in group C	
	Syphilis		Syphilis	
	Untreated	None known	Treated	None known
Positive	10	1	5	2
Negative	7*	29	8	13

*Two patients previously treated for syphilis who were treponemal DNA negative have been excluded from this table.
It is possible that 1 treponemal DNA negative patient had yaws rather than syphilis.

had many casual homosexual partners and had been treated on several occasions for gonorrhoea and non-gonococcal urethritis with antibiotics which are active against *T. pallidum*.

Discussion

In total, the CSF from 77 patients in groups A, B and C were tested. Fifteen of 26 patients with a history of syphilis were treponemal DNA-positive compared to 3 of 45 patients without a history of syphilis. Treponemal DNA was detected in CSF from 10 of 17 patients with untreated syphilis in group B (two patients had been treated but were being re-evaluated), and in CSF from one of 30 patients not known to have syphilis in group A. Thus, detection of treponemal DNA had a sensitivity of 59%, a specificity of 97%, a positive predictive value (PPV) of 91%, and a negative predictive value (NPV) of 83% in patients not previously treated for syphilis. In group C all the patients were HIV-positive and those known to have syphilis had all been treated. Nevertheless, treponemal DNA was detected in five of 13 patients with a history of syphilis, giving a sensitivity of 38%, a specificity of 87%, a PPV of 87%, and a NPV of 62% for predicting a history of syphilis.

We estimated that DNA equivalent to no less than 65 organisms in 0·5 ml of CSF could be detected by the PCR, which could account for the relatively low sensitivity of the procedure in tests on clinical specimens. However, since this figure was based on multiple dilutions it is possible that the sensitivity is greater than we have estimated. We detected treponemal DNA in the CSF from more than half of the patients with a history of untreated latent syphilis. Previous attempts to detect treponemes in the CSF from patients with late latent syphilis by animal inoculation have failed[10] or have been successful in only a small proportion of patients.[16] Our failure to detect treponemal DNA in specimens from more than 40% of patients with late latent syphilis may be because in a proportion of the patients the infection had been eliminated with or without the assistance of antibiotics prescribed for other conditions. The organisms may not be freely present in CSF, but exist possibly in other tissues or even intracellularly.[17] Our method does not distinguish between live and dead organisms. It is possible that the differences between our results and those obtained with rabbit inoculation are because the PCR is able to detect non-viable organisms.

The specificity of the PCR was not 100% because CSF from three patients without a history of syphilis reacted positively in the test. Two of these patients were HIV antibody-positive and had negative serological tests for syphilis. It is possible that they had incubating or early syphilis that had been treated fortuitously, resulting in the inability to detect treponemal or reaginic antibody subsequently. Alternatively, they may have failed to produce antibodies as a consequence of their HIV infection.[3] Although these two positive results could represent true false positive ones, the overall specificity of the PCR procedure would suggest otherwise. The third patient was in group A and the result must be assumed to be a genuine false positive. Since this work was completed the PCR technique has been modified with the addition of a second amplification cycle for the 4D gene using internal primers ("nested" PCR). Initial results suggest that this provides increased sensitivity and specificity. Although it has not been possible to retest all the samples with this modification, the CSF from the patient in group A that was positive has been retested on three occasions and has been negative each time, while the fluids from the two patients in group C with no prior history of syphilis have been confirmed positive. The initial result for all the other samples that have been retested has been confirmed. While this points to a high specificity of the original tests it also indicates that the probes and primers used initially were not 100% specific and, furthermore, suggests that contamination is not the explanation for these three "false-positives".

In this study CNS disease and a history of syphilis predicted the finding of treponemal DNA in the CSF of patients with HIV infection. Immune deficiency is associated with proliferation of *T. pallidum* in the rabbit model,[5,18] and it is possible that the patients with CNS disease were more immunocompromised than the others. Whether the detection of treponemal DNA means that treponemes contribute actively to the CNS pathology or merely replicate benignly is open to conjecture. None of the patients exhibited signs such as abnormal pupils that are commonly found in patients with parenchymatous neurosyphilis. Given the sensitivity of treponemes to a rise in temperature it is possible that an intercurrent pyrexial illness in an HIV-positive individual might affect the availability of treponemal DNA in the CSF. A review of the literature has not revealed any reports of treponemes being detected in the brains of patients dying from AIDS with neurological disease. However, only small numbers of treponemes might be needed to cause neurological disease through hypersensitivity or toxic mechanisms, and the techniques used for detecting them may not have been sufficiently sensitive.

The results we have recorded suggest several further lines of investigation. Clearly, examination of further CSF samples from HIV-positive patients to establish the relationship with treponemal disease is warranted. Furthermore, histological examination of tissue in which treponemal DNA can be detected by the PCR may yield information on how treponemes

could produce disease in immunosuppressed patients. Ultimately, a clinical study, in which treponemal DNA-positive patients are retreated for syphilis with a high dose penicillin regime will show whether our findings are of clinical importance. The PCR would be useful in monitoring the efficacy of treatment in eliminating treponemes in such a study.

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Address for correspondence: Dr Phillip Hay, The Jefferiss Wing, St Mary's Hospital, London W2 1NY, UK.

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