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Neurophysiological Abnormalities in HIV-infected Long Term Survivors

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Abstract
Background: HIV is a neurotropic virus causing neuronal damage independent of opportunistic infections. A subgroup of patients suffer from long-term infection without developing significant disease symptoms requiring antiretroviral therapy (long-term survivors, LTS). We investigated the prevalence and severity of neurophysiological abnormalities in LTS.
Methods: The outpatient database of the Dept. of Neurology, University of Münster, was searched for HIV-infection LTS (infection for more than 9 years, no antiretroviral therapy since infection, stable CD4-positive lymphocyte count of more than 400/ul). Their neurophysiological test results (nerve conduction studies, event-related potentials, EEG) were compared to a control group of patients with similar disease duration not fulfilling the criteria for LTS.
Results: Sixteen LTS patients and 22 control patients were investigated. Median age at examination was 35 years. There were no significant differences between the groups regarding age, sex, duration of the disease and way of infection. By definition, CD4+-lymphocyte counts differed between LTS and both control groups. Standard nerve conduction studies of the peroneal or the sural nerve were abnormal in 1 LTS patient and 3 control patients. Sural nerve paired stimulation amplitude showed abnormal findings in 4 LTS patients and 4 control patients. P300 latency was prolonged in 4 LTS patients and 4 control patients. EEG background frequency was normal in all but one patient (LTS). There were no differences between groups regarding any of the parameters.
Conclusion: Sensitive methods showed subtle affection of the nervous system in HIV-infected outpatients infected for more than 9 years. However, there was no difference between patients fulfilling accepted criteria of LTS, and those who did not. LTS most likely form the extreme end of a continuum of disease severity.

Key words: HIV infection, long-term survivor, neurophysiology

Introduction

The infection with the human immunodeficiency virus subtype 1 (HIV-1) seems to induce symptoms of immunodeficiency or the acquired immunodeficiency syndrome (AIDS) within 10 years in up to 75% of cases (Munoz et al. 1995). Only a small group of individuals will remain free of symptoms and with a stable count of CD4-positive lymphocytes without taking antiretroviral medication (Barker et al. 1998). This subgroup, the long term survivors of HIV-Infection (LTS), has been studied predominantly to reveal possible immunological or virological factors that might protect against disease progress. Barker et al. (Barker et al. 1998) found a lower CD38-subset of CD8-positive lymphocytes, others discovered a higher rate of T-suppressor lymphocytes (Buchbinder et al. 1994, Cao et al. 1995) or more specific antibodies against HIV (Cao et al. 1995), or both. Mutations of HLA-Allels have been reported (Greenough et al. 1999). The influence of deletions in the nef-gene of the virus is discussed controversely (Carl et al. 2000,Greenough et al. 1999,Mourich et al. 1999). Low age at time of infection seems to be a protective factor (Bulterys et al. 1993, Collaborative Group on AIDS Incubation 2000). However, there seems to be consensus that the etiology of long term-survival is multifactorial (Cao et al. 1995, Greenough et al. 1999, Rowland-Jones 1999), and that LTS form the extreme of a continuum rather than a distinct subgroup (Altisent et al. 1996, Easterbrook 1999, Sheppard et al. 1993).

The estimated prevalence of LTS varies widely from 1% to 25% (Easterbrook 1999). This is due to the lack of an accepted and standardized case definition for LTS which markedly diminuishes the comparability of the studies. Most definitions consist of a minimum duration of infection without clinical symptoms, a minimum CD4-lymphocyte count and absence of antiretroviral therapy. Minimum duration of infection varies from 7 years (Pantaleo et al. 1995) to 14,5 years (Harrer et al. 1996) with most authors using an 8-year or 10-year duration. Most studies specify the CD4+-lymphocyte counts to be more than 400 to 500cells/ul (Altisent et al. 1996, Buchbinder et al. 1994, Harrer et al. 1996, Husstedt et al. 1997). Studies using stable CD4-lymphocyte counts regardless of absolute counts (Cao et al. 1995) tend to include a heterogenous group of patients, because the mechanism of stability may be quite different in patients with high or with low counts.

HIV-1 is a neurotropic virus, and high virus load in cerebrospinal fluid as well as decreasing immunocom-

petence correlate positively with onset and worsening of neurologic symptoms of HIV-infection like HIV-related encephalopathy (Di Stefano et al. 1998) or polyneuropathy (Husstedt et al. 1993, Husstedt et al. 2000, Manji 2000). However, there is only one small series of five LTS patients focussing on neurological symptoms and neurophysiological data (Husstedt et al. 1997). Their data will be included here.

In this study we report neurophysiological data of a group of LTS and compare them with data of patients with the same disease duration but more advanced disease with and without antiretroviral therapy at the time of examination.

Patients and Methods

Group Definitions

In accordance with the literature we defined HIV infected longterm survivors (LTS) as patients with a minimal duration of HIV-1 infection of 9 years, no evidence for CDC stage C, normal CD4+ -lymphocyte counts (>400/ul), and no antiretroviral therapy.

As control group served patients with minimal duration of HIV-infection of 9 years but low CD+-lymphocyte counts (<400/ml) or evidence for CDC stage C, or both. This group was further divided in patients with and without antiretroviral therapy. Informed consent was obtained from all patients.

Data Acquisition and Analysis

The time of infection and way of infection was ascertained using all available data. All patients underwent a general physical and neurological examination. CD+4-lymphocyte count and viral load was documented. The patients underwent neurophysiological tests of the peripheral and central nervous system. Nerve conduction studies of the peroneal and sural nerves were performed in all patients. In addition, double stimulus neurography of the sural nerve was performed in 10 LTS and 17 control patients (10 without antiretroviral therapy). As a measure of cognitive adaptation, visual event-related potentials (P 300) were studied in 9 LTS and 9 control patients (3 without antiretroviral therapy). In addition, the frequency of EEG background activity was documented to study cognitive adaptation.

Nerve conduction studies were performed using surface electrodes in the conventional manner (Husstedt et al. 1993, Husstedt et al. 1998). For peroneal nerve conduction velocity (NCVP), amplitude and latency of nerve action potential after distal stimulation (MSAP, PDL) and F-wave technique (FWP), surface electrodes were placed on the extensor digitorum brevis muscle and a reference electrode on the tendon of the fifth toe. Distal stimulation was performed with a distance of 7.5cm to the recording electrode laterally of the tendon of the anterior tibial muscle. Proximal stimulation was applied below the head of the fibula. Temperature was kept at stable 37° C using an infrared lamp. Sural nerve stimulation was also performed using surface electrodes. The distance between stimulation and recording point on the calf was 15cm. Paired stimuli were applied in the sural nerve with a delay of 3ms. Latency prolongation of the second action after paired stimulation (LPSS) was calculated using the first positive peak of the second stimulus and determined as the difference between the first and the second latency, divided by the first latency. Nerve conduction below 40m/sec, peroneal nerve motor sum action potential (MSAP) below 3.5uv, peroneal distal latency below msec, peroneal f-wave latency above 60msec and sural nerve sensory nerve action potential (SNAP) after paired stimulation stimulus of more than 8% were considered abnormal.

Event-related potentials were performed using an oddball paradigm using 15% red and 85% white flashes of light. EEG was recorded at centroparietal according to the international 10/20 system, and linked to the mastoid as reference. The amplitude and latency of the P300 were analysed after 60 target stimuli. P300 latency above 420msec in patients 20-39 years old, above 430msec in patients 40-50 years old, and above 440msec in patients over 50 years of age were considered abnormal. EEG was performed using a Schwarzer Enzephaloscript 24 digital acquisition machine with 19 channels. Electrodes were placed according to the international 10-20 system. All studies included activation periods (hyperventilation, photic stimulation). Background activity was determined on a page with voluntary eye opening and eye closure. Background activity of lower than 8/sec was considered background slowing and thus abnormal.

Statistical analysis was performed with a commercial software package (SPSS 12.0 for windows, SPSS inc, Chicago, Illinois, USA). Interval-and ordinal-scaled data were compared using the Kruskal-Wallis test, and the Mann-Whitney U-test for inter-group comparison. Adjustments for multiple comparisons were made using Dunn's test. Nominal-scaled data with the chi-square test or Fisher's exact test (2x2 tables). Significance level was set at $p<0.05$ for two-tailed tests.

Results

Sixteen LTS patients were identified. The control group consisted of 22 patients (11 patients under antiretroviral therapy. Most patients of both groups were male and were examined at a median age of approximately 35 years (Table 1). There were no significant differences between the groups regarding age, sex, duration of the disease and way of infection. By definition, CD4+-lymphocyte counts differed between LTS and both control groups, but not between the two control subgroups. All eleven patients receiving antiretroviral therapy were on highly active antiretroviral therapy (HAART).

Peripheral Nerves

Nerve conduction velocity of the peroneal or the sural nerve were abnormal in 1 LTS patient, no control patient without therapy and 3 control patients with therapy. Peroneal f-waves as well as amplitude of the peroneal neurography was also normal in the vast majority of the patients of all groups. There were no differences between groups regarding any of the parameters (Table 2).

Table 1. Patient characteristics.

variable		long term survivors (n=16)	control group without therapy (n=11)	control group with therapy (n=11)	significance Kruskal-Wallis test
age (years)	median quartiles (25/75)	34 30.5/39	34 27/38	35 30/50	p=0.68
age at seroconversion	median quartiles (25/75)	23.5 19.8/28	25 21/30	26 22.5	p=0.11
infected for…. years	median quartiles (25/75)	11 9/12	10 9/11	10 9/10	p=0.33
CD4+ lymphocytes count per ml	median quartiles (25/75)	560 481/625	291 26/350	197 86/240	p<0.001 p=0.35*
sex	female/male	1/15	3/8	3/8	p=0.26
way of infection	i.v.-drug use	5 (32%)	4 (36%)	1 (9%)	
	homosexual intercourse	4 (25%)	2 (18%)	2 (18%)	
	blood products - hemophilia	2 (13%)	3 (27%)	1 (9%)	
	blood products – other	1 (5%)	0	0	
	unknown	4 (25%)	2 (18%)	7 (64%)	

* = Mann-Whitney U-test for comparison between control subgroups

Table 2. Neurophysiological data.

variable		long term survivors (n=16)	control group without therapy (n=11)	control group with therapy (n=11)	significance Kruskal-Wallis test
peroneal nerve conduction velocity (m/sec)	median quartiles (25/75)	45.8 43.2/48.2	48.5 46.2/48.8	47 43/50	p=0.19 p=0.21*
	no. of patients with abnormal findings	1 (6%)	0	2 (18%)	p=0.99*
peroneal nerve distal latency (msec)	median quartiles (25/75)	4.35 3.7/4.7	4.2 3.7/4.7	4.5 3.6/6.3	p=0.6 p=0.74*
peroneal nerve F-wave (msec)	median quartiles (25/75)	52.6 50.1/54.2	52.5 50.5/56.4	51.1 46.7/57.5	p=0.72 p=0.9*
	no. of patients with abnormal findings	0	1(9%)	0	p=0.99*
sural nerve conduction velocity (m/sec)	median quartiles (25/75)	48 44/51.3	50 46.8/56.8	47.8 39.4/50	p=0.11 p=0.49*
	no. of patients with abnormal findings	1 (8%)	0	3 (27%)	p=0.99*
sural nerve paired stimulation amplitude (%)	median quartiles (25/75)	7.6 4.7/10.2 (n=10)	5.3 2.8/6.7 (n=10)	6.6 4.7/10.6 (n=7)	p=0.37 p=0.41*
	no. of patients with abnormal findings	4 (40%)	2 (20%)	2 (28%)	p=0.41*
P300 latency (msec)	median quartiles (25/75)	428 388/480 (n=9)	443 383/543 (n=3)	453 413/526 (n=6)	p=0.65 p=0.39*
	no. of patients with abnormal findings	4 (45%)	2 (66%)	4 (66%)	p=0.34*
EEG background activity per sec	median quartiles (25/75)	10 8.5/10.3 (n=9)	9 8/10 (n=7)	10 (8.5/11.5) (n=5)	p=0.57 p=0.9*

* = LTS vs. control group total, Mann-Whitney U-test

Event Related Potentials (P300)

Event related potential studies were performed in 9 LTS patients and 9 control patients (3 without therapy). In 4 LTS patients, 2 control patients without therapy and 4 control patients with therapy, the P300 latency was significantly prolonged. There were no significant inter-group differences (Table 2).

EEG

EEG data were available for 9 LTS patients and 12 control patients (7 without therapy). Only one LTS patient and no control patient had background slowing with an average background frequency of below 8/sec. There was no difference between groups regarding the background frequency.

Discussion

Among patients with HIV-infection for 9 years or more who fulfilled the criteria of non-progression, only a small percentage showed abnormal findings in standard neurophysiological tests. More sensitive studies like the sural nerve paired stimulation paradigm and visual event-related potentials indicated that the HIV infection already had subtly affected a significant proportion of the patients. However, there was no significant difference between LTS patients and control patients with similar disease duration but less stable CD4+-lymphocyte counts, more advanced clinical symptoms, or patients receiving antiretroviral therapy.

The low incidence of abnormal findings in neurophysiological tests is surprising. Other studies reported a higher percentage of alterations of NCV even in the early stage of the infection in asymptomatic patients (Aznar-Bueno et al. 2000, Sinha et al. 2003). Although there most likely is direct correlation between the amount of abnormalities and the viral load (Polich et al. 2000), at least event-related potentials may be prolonged even when viral replication is suppressed by antiretroviral therapy (Chao et al. 2004). Perhaps viral replication persists in central as well as peripheral neural structures and thus contributes to abnormal functioning. This may explain the relatively high percentage of abnormal ERP in all groups.

In earlier studies we reported that comparison of CDC-classification goups revealed that less than 10% of the patients with CD4-count of 200/ul or more had abnormalities of the NCV of the sural nerve or prolonged f-waves (Husstedt et al. 1997). However, sural nerve paired stimulation was abnormal in more than a quarter of these patients. In contrast to that, almost half of the patients with CD4-lymphocyte count of less than 200/ul showed abnormal NCV and f-wave latencies (Husstedt et al. 1997). Other studies revealed that other factors like age, weight loss and serum albumin and hemoglobin levels also correlated with deterioration of NCV and other neurophysiological parameters indicating polyneuropathy (Tagliati et al. 1999). However, these studies did not focus on long-term survivors but included patients at very different stages of disease.

It is surprising that there were no differences between LTS and patients with more severe disease in our cohort. However, even the control patients had a long duration of disease with a relatively stable health and most of them without significant opportunistic infections. Thus, the viral load in all patients most likely was rather low. Since our patients were recruited in an specialized neurological outpatient-clinic, patients with a more rapid course of disease or more severe disease symptoms might not have been able to attend. In addition to the small sample size and the recruitment bias, differences might have been obscured by antretroviral therapy. On the one hand, HAART significantly reduces viral load and thus contributes to maintaining of neurological functioning (Evers et al. 2004) . In addition, antiretroviral agents by themselves can cause neuropathy (Manji 2000). The lack of differences between groups supports the hypothesis that LTS form the end of a continuum of disease severity rather than a discrete group reflecting distinct disease mechanisms (Altisent et al. 1996, Easterbrook 1999, Sheppard et al. 1993). Our study has several limitations: First, the study sample was small, and the patients were recruited in one hospital only. Therefore, the results can not be generalized easily. Second, the retrospective design precluded a more detailed analysis of the cilinical correlates of the neurophysiological findings. Although all patients underwent a neurological examination, subtle abnormalities may have been overlooked or not documented. Neuropsychological testing was not performed.

More studies with larger sample sizes are needed to further elucidate neurological and neurophysiological characteristics of long-term survivors of HIV-infection.

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