HTLV-1-associated myelopathy:
Natural history and interventions

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MD(Res)
Mysteries of power

Who knows
Doesn’t talk.
Who talks
Doesn’t know.
Closing the openings,
shutting the doors,
Blunting edge,
Loosing bond,
Dimming light,
Be one with the dust of the way.
So you come to the deep sameness.

Then you can’t be controlled by love
Or by rejection.
You can’t be controlled by profit
Or by loss.
You can’t be controlled by praise
Or by humiliation.
Then you have honour and heaven.

LAO TZU
500 B.C.
I dedicate this work to Axel.
Abstract

Background: The human T-lymphotropic virus type 1 (HTLV-1) is associated with HTLV-1-associated myelopathy (HAM)/Tropical Spastic Paraparesis (TSP) and inflammation at other sites.

Objectives: of the thesis were to prospectively describe the clinical progression of HAM, document the incidence of all new inflammatory events (IE) in HTLV-1 asymptomatic carriers (AC) and in patients already diagnosed with an HTLV-1 associated inflammatory disease (HAID), explore the clinical and laboratory effects of two therapeutic approaches (anti-inflammatory and HTLV-1 reverse transcription inhibitor) and to detect and quantify extra-chromosomal (EC) HTLV-1 DNA circles as markers of HTLV-1 reverse transcriptase (RT) activity in vitro and in vivo.

Methods: Prospective clinical study of a cohort of patients with HAID and initially asymptomatic carriers. Treatment of patients with HAM with tenofovir and with intermittent, high dose, intravenous methylpredinosolone. Detection of EC HTLV-1 DNA in peripheral blood mononuclear cells (PBMCs) and MT-2 cell lines through polymerase chain reaction. Treatment of MT-2 cells with tenofovir and the peptide entry inhibitor Pcr-400.

Results: The incidence of IE historically associated with HTLV-1 infection was 3.4/100 person years (py) for ACs and 5.9/100 py for patients with HAID (Relative Risk: AC/HAID= 0.58). Idiopathic uveitis was most common but hepatic transaminitis was also commonly observed and described for the first time in association with HAM. Tax expression in conjunction with serum soluble TNF-α-receptor I predicted 97% of patients without IE correctly. Median time from onset of HAM to unilateral walking stick use was 11 years and to wheelchair dependence 18 years. During a median follow up of 3.8 years timed walk (tw) deteriorated in 77% at a mean rate of 2sec/10m/year and 33% of patients needed additional aid. HTLV-1 viral load was stable but higher in those who deteriorated. Age of onset <50 years predicted progression. Treatment with tenofovir was not associated with clinical improvement nor change in viral load. Methylprednisolone improved pain considerably and has been incorporated in routine management of chronic pain in patients with HAM. EC 1LTR DNA circles were detected but did not correlate with HTLV-1 disease status or viral load and levels did not change significantly with in vivo or in vitro treatment.

Conclusion: The progression of HAM, even in patients with chronic disease, the increased incidence of other IE and the response to pulsed methylprednisolone implies persistent inflammation that may respond to long-term anti-inflammatory therapy. The low concentration of, and lack of TDF effect in vivo and RT and entry inhibition in vitro on, EC 1LTR DNA circles argues against a significant role of viral replication in HTLV-1 infection.
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<table>
<thead>
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<th>Abbreviations</th>
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<tr>
<td>AC</td>
<td>asymptomatic carrier(s)</td>
</tr>
<tr>
<td>ALT</td>
<td>alanine transaminase</td>
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<td>ANA</td>
<td>antinuclear antibodies</td>
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<td>ATLL</td>
<td>adult T cell leukaemia/lymphoma</td>
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<tr>
<td>CD</td>
<td>cell differentiation</td>
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<td>CK</td>
<td>creatine kinase</td>
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<td>cm</td>
<td>centimeter</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>CT</td>
<td>computer tomography</td>
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<tr>
<td>CTL</td>
<td>cytotoxic T lymphocyte</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>EDSS</td>
<td>Expanded Disability Status Scale</td>
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<tr>
<td>ELISA</td>
<td>enzyme linked immunosorbent assay</td>
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<td>EMG</td>
<td>electromyography</td>
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<td>Env</td>
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<td>γGT</td>
<td>gamma-glutamyl transpeptidase</td>
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<td>group antigen</td>
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<td>GLUT-1</td>
<td>glucose transporter-1</td>
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<td>HBV</td>
<td>Hepatitis B virus</td>
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<td>HBZ</td>
<td>HTLV-1 B zip factor</td>
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<td>HCV</td>
<td>Hepatitis C virus</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>HLA</td>
<td>human leukocyte antigen</td>
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<td>HRCT</td>
<td>high resolution computer tomography</td>
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<td>HTLV-1/2</td>
<td>Human T-lymphotropic virus type 1/2</td>
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<tr>
<td>IC50</td>
<td>Inhibitory concentration 50%</td>
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<td>ICAM</td>
<td>intracellular adhesion molecule</td>
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<td>IE</td>
<td>inflammatory event</td>
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<td>IgA/G/IgM</td>
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<td>INF</td>
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<tr>
<td>IR</td>
<td>incidence ratio</td>
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<td>LTR</td>
<td>long terminal repeat</td>
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<td>macrophage chemoattractant protein-1</td>
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<tr>
<td>m</td>
<td>minutes</td>
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<td>MIP-1α</td>
<td>macrophage inflammatory peptide 1- alpha</td>
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<td>messenger RNA</td>
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<td>MS</td>
<td>multiple sclerosis</td>
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<td>NCHR</td>
<td>National Centre for Human Retrovirology</td>
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<td>NSAID</td>
<td>non-steroidal anti-inflammatory drug</td>
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<td>OMDS</td>
<td>Osame’s motor disability score</td>
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<td>ORF</td>
<td>open reading frame</td>
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<td>PBMC</td>
<td>peripheral blood mononuclear cell(s)</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PIC</td>
<td>pre-integration complex</td>
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<tr>
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<td>polymerase</td>
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<td>person years</td>
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<td>reverse transcriptase</td>
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<td>reverse transcription complexes</td>
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<td>Rex</td>
<td>regulatory gene of the pX region</td>
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<td>sec</td>
<td>second(s)</td>
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<td>SELDI-TOF</td>
<td>surface-enhanced laser desorption ionisation time-of-flight</td>
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<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
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<td>Sjögren’s syndrome</td>
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<td>sTNF αRI</td>
<td>soluble Tumour Necrosis Factor α-receptor I</td>
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<td>Tax</td>
<td>transactivating gene of the pX region</td>
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<td>tw</td>
<td>timed walk(s)</td>
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<td>TSP</td>
<td>tropical spastic paraparesis</td>
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<td>VAS</td>
<td>visual analogue score</td>
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<td>VCAM</td>
<td>vascular cell adhesion molecule</td>
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Chapter 1

1 Introduction

1.1 Human T lymphotropic virus type – 1

1.1.1 Structure

HTLV-1 is a strain of the Primate T-lymphotropic virus type 1, a complex retrovirus, in the genus *Deltaretrovirus* of the subfamily *Orthoretrovirinae* of the retroviridae family of viruses (2). The core particle of HTLV-1 carries a diploid plus-strand RNA genome comprising 9032 nucleotides (3) as well as viral enzymes reverse transcriptase, integrase, protease and RNAse H. Immediately after cell entry the viral core undergoes a partial and progressive disassembly, known as un-coating, that leads to the generation of sub-viral particles called reverse-transcription complexes (RTCs) and pre-integration complexes (PICs). PICs are usually defined as integration-competent complexes, whereas reverse-transcription is incomplete in RTCs. Both contain viral DNA and proteins such as reverse transcriptase and integrase (4). Early after the release of the viral core into the cytoplasm of the target cell the viral RNA is reverse transcribed into linear DNA which is then integrated into the host cell genome as a provirus. The genome of HTLV-1 contains three major open reading frames (ORF) that encode structural genes (Figure 1): the *gag* region encoding for structural proteins, the *pol* region encoding the viral enzymes, integrase and protease and the *env* region encoding the envelope proteins flanked by the 5’ and 3’ long terminal repeats (LTR) (5). Between *env* and the 3’ LTR lies a regulatory region known as *pX* which encodes HTLV-1 specific regulatory proteins: *Tax* (p40) and *Rex* (p21). *Tax* activates transcription of HTLV-1 and *in vitro* has been shown to increase transcription of a variety of host genes (6;7). *Rex* regulates the intracellular transport of unspliced and singly spliced HTLV-1 mRNAs (1;8). Also encoded by *pX* are the accessory proteins p12, p13 and p30 that are important for infectivity and cell signaling. HTLV-1 bZIP factor (*HBZ*) is a protein encoded on the negative strand of the viral genome which is reported to be expressed in late stages of ATLL and is associated with cell proliferation (9).
HTLV-1 varies very little in its sequence, which does not allow molecular typing to be reliably used to establish the source of transmission. HTLV-1 subtypes varying in the p21e trans-membrane protein gene could not be linked to HAM or ATLL (10), but Tax sub-type A was associated with HAM in the Japanese population (odds ratio, 2.46; 95% confidence interval, 1.26-4.80) (11).

Figure 1: HTLV-1 gene map (9032 nucleotide). Adapted from Franchini (1), Macnab (gsbs.utmb.edu/microbook/ch047.htm) and Youshya 2007.

Figure1: Gag, pol, env = structural proteins, pX = regulatory region coding for Tax, Rex, p12, p13 and p30 proteins. U3/R/U5= long terminal repeats (LTR).
1.1.2 Tropism

*In vivo*, the vast majority of HTLV-1 is found in CD4⁺ CD45RO T lymphocytes however CD8⁺ T lymphocytes *in vivo* (12;13) and a broad range of cells *in vitro* can also be infected [B lymphocytes (14), dendritic cells (15), monocytes/macrophages and glial cells (16), endothelial cells (17), natural killer cells (18), pro-myelocytic HL-60 cells (19) and a human osteo-sarcoma cell line (20)].

Cell free virions have extremely low infectivity so that only 1 in $10^5$ to $10^6$ virus are infectious *in vitro* (21;22). Therefore transmission of HTLV-1 usually requires a virus-containing T-lymphocyte and direct cell-to-cell contact (23). A proposed mechanism is the increased presence of intracellular adhesion molecules, ICAM-1 and 3, as well as vascular cell adhesion molecule, VCAM-1, stimulating HTLV-1 envelope mediated syncytia formation, heparan sulfate proteoglycans as part of extra-cellular matrix enabling virus attachment to the non-infected cell and GLUT-1, an ubiquitous glucose receptor, as a receptor for viral cell entry (24-29). How exactly the early cell to cell transmission of HTLV-1 occurs still remains unclear. The favoured mechanism is through the formation of a virological synapse between the infected and uninfected CD4⁺ T lymphocyte. Electron microscopy shows the release of an enveloped virus into a pocket within this synapse (30). Confocal microscopy visualises the synapse formation and the detection of Gag protein in the originally un-infected lymphocyte in the vicinity of synapse (18).

However HTLV-1 viral load seems to be mainly maintained through mitotic host cell proliferation after HTLV-1 DNA integration into the host genome (31). The integrated HTLV-1 virus can be quantified as viral copies per 100 peripheral blood mononuclear cells (PBMCs) through the polymerase chain reaction. Shortly after infection the HTLV-1 viral load reaches a stable equilibrium ‘set point’ that fluctuates by only two to four fold over a period of years (32).
1.1.3 Transmission between individuals

HTLV-1 is transmitted between individuals by transfer of infected T lymphocytes (33) and therefore shares its routes of transmission with other blood-borne infections such as the human immunodeficiency viruses and hepatitis viruses B and C. In endemic areas transmission peaks at two time points - early in life through breastfeeding and late through unprotected sexual intercourse (34). The chance of transmission depends on the efficiency of the route of transmission, infectivity of the donor and the number of exposures. It is therefore highest with blood transfusions (40-60%) (35-37), organ transplantations and sharing needles and syringes (38) and less with breastfeeding (5% - 20% depending on whether fed for less or more than 6 months) (39) and unprotected sexual intercourse. In serodisconcordant couples HTLV-1 negative women were 3.9 times more likely to seroconvert compared to HTLV-1 negative men (40). Transmission of HTLV-1 to non-breast fed babies indicates that transmission may also occur through trans-placental mini blood-transfusions and/or exposure to maternal genital tract secretions (41). Transfusion with cell-free blood products appears to carry a negligible risk of HTLV-1 infection (42). Sero-positivity is associated with older age, female gender, lifetime number of sexual partners, commercial sex work and genital ulcers which point towards a more
effective transmission through an increase in lifetime unprotected sexual intercourse (43-45) encounters, from male to female (40) and through injured and inflamed mucosa.

1.1.4 Diagnosis of HTLV-1 infection

In daily clinical practice HTLV-1 infection is diagnosed through serological testing of peripheral venous blood for antibodies to the virus. Screening is performed through an enzyme-linked immunosorbent assay (ELISA) and HTLV-1 infection is confirmed by detection of antibodies against p19 and p24 as well as gp21 and rgp46-I by Western blot (46;47). Some individuals, particularly Africans, positive on screening ELISA may have an indeterminate Western blot result, with incomplete antibody reactivity to HTLV-1 antigens (48). So far there has been no report of a clearance of infection. Although it has been suggested that a sero-indeterminate HTLV-1/2 Western blot may suggest previous HTLV-1 exposure (49), this has also been attributed to cross-reactivity e.g. with malaria antigens (50). The immune response to HTLV-1 is strong and the serum antibody titer, which correlates with the virus load (51), may be as high as 1: 256, 000.

HTLV-1 viral DNA can be detected by PCR in PBMCs and is used to differentiate HTLV-1 from HTLV-2 infection and quantitative real-time PCR is used to quantify the viral load. In endemic areas the prevalence of HAM rises exponentially with log proviral load once the proviral load exceeds 1% (DNA copies per 100 PBMC), is generally higher in women than in men and higher in asymptomatic carriers related to patients with HAM (51). In our cohort in asymptomatic carriers (ACs) the viral load persists at a median low level of 1.55 (0.0003–28) copies/100 PBMCs and in patients with HAM the median viral load is 14 (0.002–112) copies/100 PBMCs (52) (Figure 3).

PCR for HTLV-1 detection can be used for rapid confirmation of HTLV-1 associated lymphomas by detecting viral DNA in tumor cells or other tissue samples (53).
1.1.5 Epidemiology of HTLV-1 infection

It has been estimated that 20 million people are infected with HTLV-1 world wide (54). The populations studied rarely represent the general population but are commonly groups of blood donors, pregnant women or injecting drug users (IDU). The areas of highest prevalence are in Japan (10% to 37% in selected groups) (55;56), Caribbean islands (1.3-8.4%) (57), parts of Africa (5%) (58), and South America (2%) (59-61). Clusters of high endemicity have been detected in Romania (0.065-0.1%) (62), Northern Iran (0.8-3%) (63;64) and Melanesia (0.2-5.8%) (65-68). HTLV-1 sero-prevalence is low in Europe (UK 0.035%, France 0.12%) (69) and North America (0.01-0.0.95%) (70).

There are six recognised subtypes of HTLV-1: subtype A, the cosmopolitan subtype, which is found in HTLV-1-endemic areas including Japan and Caribbean Islands (71), subtypes B, D, F are found in West and Central Africa, subtype C in Melanesia (72;73), and subtype E mainly in South and Central
Africa (74). Most reported human infections, and therefore HTLV-1-associated
diseases, are with the cosmopolitan subtype A.

1.1.6 Animal infections and animal models

Retrovirus-induced neurological disease has been recognized for many
years in animals. These include murine leukaemia virus myelopathy (75),
visna/maedi in sheep (76-78), caprine arthritis encephalitis in goats (79),
and the myelopathy seen in horses with equine infectious anaemia (79).
While ATLL has been extensively studied in Tax transgenic mice (Tax-
Tg) models (80), there are no published data on HTLV-1-associated
myelopathy in an animal model. The immune response in HTLV infected
macaques was similar to that found in HTLV-1 infected humans however
myelopathy has not been described in this animal model. This might be
due to the long clinical latency of HTLV-1 infection with HAM only
observed with longer follow up times or due to different species
susceptibilities.

1.2 HTLV-1- associated inflammatory diseases

HTLV-1 has been proven to be causative in the following conditions: adult T-cell
leukaemia/lymphoma (ATLL) (74;81;82), HTLV-associated myelopathy (HAM)
(83), HTLV-associated uveitis (HAU) (84;85) and infective dermatitis (86). In the
majority of cases HTLV-1 seroconversion remains asymptomatic, these patients
are referred to as “asymptomatic carriers” (ACs). However, 2-6% of infected
individuals develop ATLL (87) and a further 2-3% develop HAM which is the
most commonly reported inflammatory condition associated with HTLV-1
infection (83;88;89).
1.2.1 HTLV-1-associated myelopathy

1.2.1.1 History

A slow-onset spastic paraparesis in adults was first described by Cruikshank in the West Indian Medical Journal in 1956 (90) although some would say that Strachan included HAM in his Jamaican Neuropathies (91). An association between myelopathy and HTLV-1 seropositivity in serum and cerebrospinal fluid (CSF) was reported in 1985 for Tropical Spastic Paraparesis (TSP) in Jamaica (88) and independently in Japan, where the distinctive paraparesis was first named HTLV-1-associated myelopathy (HAM), in 1986 (83). The prevalence of HAM in HTLV-1 infected blood donors in the HTLV outcome study (HOST) in the USA, a low prevalence country, is 3.8% (38). A study comparing two high prevalence study groups, the Miyazaki cohort in Japan with the Food Handler cohort in Jamaica gave some insight into the regional differences of HAM. Although the mean HTLV-1 viral load was similarly high ($p=0.26$), female carriers were more commonly affected in the Jamaican cohort with higher mean HTLV-1 antibody titers ($p=0.03$) and higher mean anti-Tax antibodies ($p=0.002$). However, the most striking difference lay in the incidence of HAM which was 1/100 000 in the Japanese compared to 20/100 000 in the Jamaican population (92).

1.2.1.2 Clinical features

The natural history of HAM is chronic and variable and a slow deterioration is seen in most patients. Studies of HAM prior to 1996 described progression of the myelopathy during the first year followed by a plateau phase (35;36;93-95). However studies since 1996 describe a more insidious onset with delayed diagnosis and continuous progression in both Japanese and Afro-Caribbean cohorts (32;96). A much more rapid onset and progression of HAM has been reported in patients transfused with HTLV-1 infected blood (97).

A Japanese study of 64 patients with HAM followed up for 10 years, showed deterioration of gait, muscle power and bladder function in 56%, 41% remained
unchanged and 3% of the patients improved clinically. The rate of disease progression was associated with higher HTLV-1 viral load and older age of onset (>65 years) as well as blood transfusions. This study did not provide details of the presenting symptom, presence or absence of pain or bladder/bowel symptoms. Nor did it give information on walking aid usage at baseline, time to progression to additional aid or the mortality rate. The grade of Osame motor disability ranged from 0, normal gait and running, through 7, where the patient is unable to walk without aid, to 10 which describes a patient that is bedridden (32).

In 2006 a study group in Martinique published longitudinal data on 123 Afro-Caribbean patients with HAM followed up for a median of 9 years. Of these patients 81.3%, 56.9% and 36.6% patients reached the end point scores of one walking stick, two walking sticks or wheelchair dependence, with a median time from onset of HAM to the assignment of these aids of 6, 13 and 21 years respectively. In 15.4% of patients death was attributed to HAM. Patients took 25 years to become wheelchair bound if the age of onset was <50 years compared to 14 years if age at onset ≥ 50 years. The time from onset to wheelchair dependence was significantly shorter in those patients with HTLV-1 viral load of >10^6 HTLV-1 DNA copies/10^6 PBMCs. Gender, history of blood transfusion and initial symptom (gait impairment v urinary disturbance) did not affect the disease course. The authors concluded that motor disability worsens throughout the disease course and that faster early deterioration predicts the rate of progression in the long-term (96).

Two studies from Brazil and Peru reported an increased mortality risk in patients with a more rapid progression to immobility within two years of the onset of HAM (98;99).

Patients with HAM may present with back ache or falls. Patients typically complain of stiff legs, weakness and heaviness of the thighs, the inability to rise from a chair or to climb stairs, lower back ache and a wide range of sensory disturbances of lower limbs. Clinically there is evidence of lower limb proximal muscle weakness. Typically cranial nerves and upper limbs are not affected. Initially there is generalised hyperreflexia and up-going plantar (Babinski’s sign)
responses and later hyperreflexia in the arms but loss, mostly of the ankle, reflexes in the legs. Tone has been described as increased and clonus of one or both ankles may be present. Patients may present with or develop constipation and urinary frequency, urgency and incontinence together with an inability to fully empty the bladder. Treatment resistant neuropathic pain is a major feature which may be localised in the lower back or radiate down one or both legs (36;38;96;100). In 2006 a revision of the original WHO diagnostic criteria of HAM was published (Table 1) defining three levels of ascertainment for HAM diagnosis: definite, probable and possible (Table 2) excluding all conditions that could mimic HAM (Table 3) (101).
Table 1: WHO 1989 Guidelines for the diagnosis of HTLV-1 associated myelopathy (HAM).

| **Age and sex** | Mostly sporadic, sometimes familial. Adult females predominate, occasionally in childhood |
| **Onset**       | Usually insidious |
| **Main neurological manifestations** | Chronic spastic paraparesis, which usually progresses slowly, but may remain static after initial progression |
|                 | Weakness of both lower limbs, more marked proximally |
|                 | Bladder disturbance, usually an early feature; constipation usually occurs later; impotence and decreased libido are common |
|                 | Sensory symptoms are more common than sensory signs |
|                 | Low lumbar pain with radiation to the legs is common |
|                 | Vibration sense is frequently impaired |
|                 | Hyperreflexia of the lower limbs, often with clonus and Babinski’s sign |
|                 | Hyperreflexia of the upper limbs; positive Hoffman’s sign and Tromner’s sign are frequent; weakness may be absent |
|                 | Exaggerated jaw jerk in some patients |
| **Less frequent neurological findings** | Cerebellar signs, optic atrophy, deafness, nystagmus, other cranial nerve deficits, hand tremor, absent or depressed ankle jerk |
| **Other neurological manifestations that may be associated with HAM** | Muscular atrophy, fasciculations, polymyositis, peripheral neuropathy, polyradiculopathy, cranial neuropathy, meningitis, encephalopathy |
| **Systemic non-neurologic manifestations that may be associated with HAM** | Pulmonary alveolitis, uveitis, Sjögren’s syndrome, arthropathy, vasculitis, ichthyosis, cryoglobulinaemia, monoclonal gammopathy, adult T-cell leukaemia/lymphoma |
| **Laboratory criteria** | Presence of HTLV-1 antibodies or antigens in the blood or CSF may show mild lymphocytic pleocytosis. |
|                 | Lobulated lymphocytes may be present in blood or CSF or both. |
|                 | Mild to moderate increase of proteins may be present in the CSF. |
|                 | Viral isolation from blood and/or CSF when possible. |
Table 2: WHO diagnostic criteria for HTLV-1-associated Myelopathy (HAM).

<table>
<thead>
<tr>
<th><strong>Definite:</strong></th>
<th><strong>Probable:</strong></th>
<th><strong>Possible:</strong></th>
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<tr>
<td>1. A non-remitting progressing spastic paraparesis with sufficiently impaired gait to be perceived by the patient himself, with or without sensory symptoms or signs, which, when present, remain subtle and without a clear cut sensory level. Sphincter signs or symptoms may or may not be present.</td>
<td>1. Monosymptomatic presentation: spasticity or hyperreflexia in the lower limbs or isolated Babinski sign, or spasticity in the lower limbs with subtle sensory signs, or pure neurogenic bladder confirmed by urodynamic tests.</td>
<td>1. Complete or incomplete clinical presentation.</td>
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<tr>
<td>2. Presence of HTLV-1 antibodies in serum and/or CSF confirmed by Western blot and/or HTLV-1 PCR positive in the blood.</td>
<td>2. Presence of HTLV-1 antibodies in serum and/or CSF confirmed by Western blot and/or HTLV-1 PCR positive in the blood.</td>
<td>2. Presence of HTLV-1 antibodies in serum and/or CSF confirmed by Western blot and/or HTLV-1 positive in the blood.</td>
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<tr>
<td>3. Exclusion of other disorders that can mimic HAM.</td>
<td>3. Exclusion of other disorders that can mimic HAM.</td>
<td>4. Disorders that can mimic HAM have not been excluded to a satisfactory degree (Table 3).</td>
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Table 3: WHO list of differential diagnosis, minimising misdiagnosis of other conditions for HAM: the following conditions should be excluded by appropriate laboratory and clinical evaluation.

<table>
<thead>
<tr>
<th>Condition</th>
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<tr>
<td>Multiple sclerosis</td>
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<td>Familial spastic paraparesis</td>
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<tr>
<td>Primary lateral sclerosis</td>
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<tr>
<td>Syringomyelia</td>
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<td>B12 and folate deficiency</td>
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<tr>
<td>Neurosyphilis</td>
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<td>Sarcoidosis</td>
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<td>Collagen vascular diseases</td>
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<td>Sjögren's syndrome</td>
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<td>Amyotrophic lateral sclerosis</td>
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<tr>
<td>Spinal cord compression (spinal tumour, cervical spondylosis, brain parasagittal tumour etc.)</td>
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<tr>
<td>Endemic regional myelopathies with similar clinical manifestations (including Schistosomiasis and Neurocysticercosis)</td>
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<tr>
<td>Carcinomatous meningitis</td>
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<tr>
<td>Transverse myelitis</td>
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<tr>
<td>Paraneoplastic syndromes</td>
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<tr>
<td>Lyme disease</td>
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<tr>
<td>Behçet's disease</td>
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<tr>
<td>Neurotuberculosis</td>
</tr>
<tr>
<td>HIV vacuolar myelopathy</td>
</tr>
<tr>
<td>Autoimmune myelopathies</td>
</tr>
<tr>
<td>Toxic myelopathies</td>
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<tr>
<td>Fungal myelopathy</td>
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</table>

Reported complications of HAM are urinary retention, recurrent and chronic urinary infections and hydronephrosis with renal failure (102) as well as increased risk of lower respiratory tract abnormalities and death from rapidly progressing HAM (103;104).

A number of other inflammatory conditions are associated with HTLV-1 infection and HAM (see Chapter 2). So far however no specific viral, bacterial or parasitic infection has been found to be specifically associated with HAM per se, except for common respiratory or urinary tract infections as part of complications of this condition.

1.2.1.3 Pathology

How exactly HTLV-1 causes damage to the central nervous system is not known.

Magnetic resonance studies of the central nervous system show non-specific findings of thoracic cord atrophy and increased signal in the peri-ventricular and
sub-cortical white matter on T2-weighted images in patients with HAM as well as ACs (105;106).

Figure 4: T2-hyperintense confluent lesions demonstrated in an axial T2W image (a) and fluid attenuated inversion recovery images (b). Adapted from Begnato F et al 2005 (105).

The MRI findings of brain and spinal cord of HTLV-1 positive patients are too non-specific to discriminate between patients with HAM and asymptomatic carriers; thoracic cord atrophy and an increased signal in the periventricular and subcortical white matter on T2-weighted images, asymmetrically distributed, have been described (107). However the specific CNS MRI lesion seen in patients with multiple sclerosis differentiate these patients from those with HAM (108).

Histopathology of new lesions in the central nervous system shows peri-vascular infiltration with CD4+ and CD8+ lymphocytes. In old lesions on CD8+ cells predominate but lesions become atrophic and a-cellular with disease progression (35;36;109). These changes have been described as active, chronic meningomyelitis mostly involving the spinal cord (36).

HTLV-1 viral load (VL) has been suggested to be a major risk factor for the development as well as progression of HAM (32;51;110). The VL does not differ in terms of mode of transmission and despite fluctuations from measurement to measurement each patient has his own set point. Although some asymptomatic carriers have high VL (111) it seems that reaching a threshold of > 1 % PBMCs makes the development of HAM more likely (51;110). HTLV-VL has been reported to be higher in those patients with a later age of onset of HAM, and is
associated with higher CSF HTLV-1 antibodies but not with CNS MRI abnormalities (36).

1.2.1.4 Pathogenesis

HTLV-1 is thought to cause systemic inflammatory disease. However the mechanism through which HTLV-1 causes these inflammatory conditions is not fully understood. Several factors have been associated with the development or presence of HAM:

- Female carriers of HTLV-1 are at higher risk of developing HAM (92;100).
- Patients most commonly develop HAM between the ages of 40 to 50 years (92;100).
- HAM is associated with higher HTLV-1 viral load, particularly when > 1% of peripheral blood mononuclear cells (PBMC) are infected (51;110;111).
- HTLV-1 viral load is independently but only partly determined by Tax protein expression, as measured ex vivo, and cytotoxic T lymphocyte (CTL) lysis (112).
- The integration of HTLV-1 into transcriptionally active sites and near to genes correlates with high ex vivo Tax expression and the presence of HAM (113).
- HTLV-1 viral load is under polymorphic control. Three host genetic factors: the promoter TNF -863A allele which predisposes to disease and SDF-1 +801A 3'UTR as well as IL-15 191C alleles which confer protection from HAM have been found to influence viral load (114).
- The class I gene HLA A*02 is associated with two fold reduction in viral load and 3-4 fold reduction in the risk of HAM. HLA A*02 restricted CTL are very efficient at eliminating HTLV-1 infected lymphocytes. HLA Cw*08 has protective effects similar to and independent from HLA-A02. HLA-B54 is associated with increased viral load and occurrence of disease. HLA-DRB1-0101 is associated with increased viral load and higher risk of HAM but only in the absence of HLA-A02 (115-117) .
• Expression of genes associated with CTL lysis: higher levels of granzyme A, granzyme B, granulysin and perforin were associated with low viral load (118).
• Cytokines have been found to be increased in HTLV-1 positive patients, with or without disease. Asymptomatic carriers were found to have higher frequency of pro-inflammatory monocytes. Anti-inflammatory IL-10⁺ CD4⁺ and IL-10⁺ CD8⁺ T lymphocytes have been found to counter balance the cytokine TNF-α derived from these monocytes. In the blood and CSF of patients with HAM increased levels of INF-γ, TNF-α, IL-2 and IL-6 can be found (119;120).

HTLV-1 associated inflammatory disease seems to be caused by damage to the infected and the non-infected cell for which two hypotheses exist:

• The first hypothesis assumes that the host immune system attacks self-cell because of antigen cross-reaction known as the “autoimmune antigen mimicry” (121).
• The second suggests that in the course of the response to T-cells expressing HTLV-1 HTLV-1 specific cytotoxic cells excrete cytokines that damage and/or destroy non-infected tissue known as the “innocent bystander” (121).

As yet there are no validated surrogate markers that predict the development of an HTLV-1 associated inflammatory disease. In a Japanese cohort, knowledge of age, sex, viral load, HTLV-1 Tax subgroup, genotypes at loci HLA-C, SDF-1 and TNF- α allowed for the correct identification of 88% of cases of HAM (114). A recent study, using logistic regression analysis examined predictors of developing HAM after genotyping 181 asymptomatic HTLV-1 carriers for HAM associated genes. However only brisk patellar deep tendon reflexes and the poly-lobulated lymphocytes known as ‘flower-cells’ were associated with the risk of developing HAM (122).

Although the viral env protein is expressed on the surface of infected T lymphocytes (18) the virus is able to persist at the same viral load set point despite a very active and aggressive immune response over many decades. The
explanation for this might be that, although HTLV-1 virus is able to go through full-cycle replication via reverse transcriptase, it is mostly spread through cell-to-cell contact and mitotic proliferation of the infected host cell, creating a pool of infected T lymphocytes that maintains infection and infectivity.

Asquith et al were able to shine some light on the relationship of viral load, proportion of patient CD4+ cells expressing Tax (Tax expression) ex vivo and high CTL frequency showing that virus-host immune interactions are important in determining disease development and viral load. High Tax expression exposes infected cells to the CTL response but is also able to up-regulate cellular proliferation and deregulate cell cycle checkpoints. When the host CTL response is weak and Tax expression strong a high viral load threshold is set and the virus-host balance is tipped towards disease development. In case of a strong CTL response but weak Tax expression the virus remains “silent”, with only little protein production, escaping the CTL response and maintaining a low viral load, and the patient remains asymptomatic (112).

1.2.1.5 Treatment

Attempts at chemotherapy for HAM have been reported from HTLV-1 endemic and non-endemic countries since 1990. From study to study the aims as well as the therapies differ. Even the cohorts with a definite diagnosis of HAM (some have included HTLV-1 negative Tropical spastic paraparesis) have different genetic backgrounds and this may be important in treatment response. Thus HAM is more common than ATLL in HTLV-1 positive patients of African descent (3%) than in Japanese patients (0.3%) and HTLV-1 carriers from Jamaica have higher anti-HTLV-1 antibody titres and higher anti-Tax antibody than Japanese carriers despite having the same range of HTLV-1 viral load (VL)(92). Moreover the studies are usually small and, although this is not usually commented on, include patients at different stages of disease.

Outcome measures are most commonly clinical improvement and a reduction of HTLV-1 VL since the latter is associated with the diagnosis of HAM and might predict the development of HAM. Other measures have examined inhibition of
lymphocyte migration and cytotoxic activity of CTLs in order to reduce tissue inflammation. None of the studies aimed to prevent HAM from developing in asymptomatic carriers at risk, namely those with high HTLV-1 VL and high titre of antibodies to HTLV-1 and specifically high levels of ex vivo Tax expression.

The mode of action of the therapies trialled varies greatly. Agents include the anti-inflammatory corticosteroids and steroid sparing drugs such as azathioprine, sulfasalazine, cyclosporin and methotrexate (123), interferons α and β1A (124-127), the anabolic steroid danazol (128;129), lymphocyte migration inhibitor heparin (130) and pentoxyphylline which inhibits lymphocyte activity and reduces TNF-α release (131;132). However these studies are all small and open studies with a tendency to report a favourable outcome including placebo bias. Unfortunately there is a lack of subsequent or comparative data or randomised controlled trials to support the reported findings.

Nucleoside analogue reverse transcriptase inhibitors (NRTI) have been of great interest with the aim to reduce the HTLV-1 VL as one of the main biological markers associated with HAM.

Zidovudine, a thymidine analogue, was the first NRTI found to inhibit HTLV-1 reverse transcriptase (RT) in vitro (21;133-135). No clinical improvement was documented in six patients with chronic HAM receiving zidovudine in 1991 (136). However high dose zidovudine (2 g/day for 4 weeks followed by 1g/day for 20 weeks) given to 10 patients with HAM (one patient was walking without aid, three with one walking stick, three used a walker and three were wheelchair bound), was associated with 50% improvement of timed gait (time required to walk 50 feet) and an improvement of mean Expanded Disability Status Scale (EDSS) from 5.5 to 4.0 in seven ambulant patients with HAM in 1993 (137). There are no details given on concomitant medication (muscle relaxants, analgesics) or adjuvant physiotherapy.

There are conflicting data on HTLV-1 RT inhibitory activity of lamivudine, a cytosine analogue, in vitro, some demonstrating good RT inhibition and others high primary resistance (138-140). In 1999, in an observational study at St Mary’s Hospital, one patient with early HAM (onset of symptoms < 2 years) and high HTLV-1 VL showed only limited response to zidovudine but a 2 log
reduction in VL followed the introduction of treatment with lamivudine and this was associated with clinical improvement. Treatment with lamivudine was associated with HTLV-1 VL reduction but without clinical improvement in four further patients with chronic stable disease. The decrease in HTLV-1 VL in peripheral blood was associated with a reduction in HTLV-1 specific CTL activity which was presumed to be due to a decrease in viral antigen burden consequent upon therapy with lamivudine (141).

Another group reported a 2.2 log reduction in HTLV VL but no clinical improvement in a patient with HAM after three months treatment with zidovudine 250mg and lamivudine 150mg twice daily. Thereafter the HTLV-1 VL stayed at the new lower set point and new clinical symptoms did not develop during one year of therapy. However, in the same publication clinical improvement was documented in a male patient with HAM receiving zidovudine 250mg with lamivudine 150mg twice daily despite a 1 log increase of HTLV-1 VL. However this treatment was prescribed after one month of subcutaneous treatment with INF-α that had not led to any reduction in HTLV-1 VL or clinical improvement. The rise in HTLV-1 VL might have reflected an increase in lymphocyte numbers after INF-α therapy. Both patients received these NRTIs within the first year of symptoms. Despite ongoing viral production neither patients developed mutations at the conserved YMDD domain of the RT gene that would have suggested the development of lamivudine resistance (142). The reduction in VL and clinical improvement might be due to the suppression of unrecognised RT activity and/or a decrease in clonal expansion of infected cells caused by zidovudine’s cytostatic effects.

The first randomised, double blind, placebo controlled trial of NRTI treatment of patients with HAM was completed in 2002 by the St Mary’s group. In the 16 study patients no clinical, viral or immunological response to the combination of zidovudine 300mg and lamivudine 150mg twice daily could be demonstrated after 24 – 48 weeks therapy. This lack of response was attributed to the long duration of HAM, potentially inadequate intracellular concentrations of active triphosphate NRTI metabolites and/or the possibility that HTLV-1 RT might not play a major role in maintaining viral load (143). Subsequent ex vivo analysis showed pre-treatment sensitivity of HTLV-1 RT to zidovudine but resistance to
lamivudine which remained unchanged during 48 weeks of treatment (144). This means that the patients, despite the study design, were effectively receiving zidovudine monotherapy.

Two studies have indicated that tenofovir disoproxil fumerate (TDF), an adenosine nucleotide analogue, is the most potent HTLV-1 RT inhibitor tested in vitro (145;146) but so far there are no in vivo data.

Oral corticosteroids have been found to be of short term benefit in observational studies (123;147). A poster presented at a conference in Guatemala in 2005 reported oral prednisolone 1mg/kg /day for two weeks followed by dose tapering to 0 mg by day 60 to be of clinical benefit in 25 patients with HAM compared to eight patients in the placebo arm in a cross-over double blind control study in Santiago, Chile. However high dose oral steroids are known to cause early and late side effects and are therefore not recommended long term.

A more recent publication from São Paulo, Brazil concerning the treatment of 39 patients with methylprednisolone 1g / day for three days every 3-4 months reported a significant reduction of one of the three disability scores from baseline at first and second visit (148). However those patients who benefited most also received physiotherapy and the significance of the improvement was lost by the third round of infusion. This study does not comment on the patients’ experience of change in life quality or pain score.

To date there is no known therapy to prevent the development of HTLV-1 associated disease in asymptomatic carriers or cure HAM or any other HTLV-1-associated inflammatory disease.
1.2.2 Other HTLV-1 associated inflammatory conditions

1.2.2.1 HTLV-1 associated uveitis

Uveitis is an intraocular inflammatory condition that is idiopathic in 40% of cases. An association between idiopathic uveitis and HTLV-1 infection was first made in Kyushu, an HTLV-1 endemic region of Japan (149). In this region, 35.4% of patients with idiopathic uveitis were found to be HTLV-1 seropositive compared to 10.3% of patients with uveitis with defined aetiologies. This association was confirmed in Martinique, French West Indies, where of 200 HTLV-1 infected patients (38.5% asymptomatic carriers and 61.5% had HAM) 14.5% had idiopathic uveitis (150). HTLV-1-associated uveitis (HAU) is unilateral in 60% of the affected, more common in females (60%) and below the age of 50 years but children can also be affected (151). HAU has been seen in patients with other HTLV-1 associated inflammatory conditions such as HAM (152) and Graves’ disease (153-155). The patient typically presents with sudden onset of a painful and red eye with blurred vision and floaters obscuring the vision. The clinical examination reveals iritis (97%), vitreous opacity (92%), retinal vasculitis (62%) and retinal exudates and haemorrhages (20%). The HTLV-1 viral load is increased in patients with HAU (3.84 copies per 100 PBMCs [\%]) compared to asymptomatic carriers (ACs) (<1%) (156). HTLV-1 is detected in infiltrating mononuclear cells in the vitreous humour which have also been found to express the inflammatory cytokine interleukin-6 (157). Topical corticosteroids and mydriatics usually improve the symptoms rapidly but relapse is common (150).

1.2.2.2 HTLV-1 associated polymyositis

Polymyositis, dermatomyositis and sporadic inclusion body myositis are uncommon but have been reported in HTLV-1 infected patients (158;159) and HTLV-1 has been found to be myotoxic in vitro (160). The first case was reported in 1989 in a Haitian woman with myelopathy, myositis and Sjögren’s syndrome living in Clichy, France (161). In a small Jamaican cohort 85% (11/13) of patients with polymyositis were found to be HTLV-1 sero-positive (162). In a Brazilian cohort 36% (4/11) of patients with HAM were found to have evidence of
polymyositis on muscle biopsy (163). In Martinique 50% (7/14) of patients diagnosed with polymyositis (5 cases) or dermatomyositis (2 cases) were HTLV-1 positive (159). These patients were followed up for 8 years. Initially patients report proximal upper and lower limb weakness and muscle tenderness. Muscle bulk and reflexes are preserved. Creatine kinase (CK) is markedly raised, but the autoantibody screen is often negative and electromyography (EMG) is normal. Over the ensuing decade there is severe loss of muscle bulk, reduction in CK levels and the EMG shows myopathic and neurogenic changes. At this stage there might be evidence of type II (hypercapnic) respiratory failure with reduced vital capacity and evidence of global respiratory muscle weakness (104). In the muscle biopsies of three patients with HAM, CD8+ cells, CD4+ cells and macrophages were the predominant cells surrounding healthy muscle fibres. HTLV-1 sequences were amplified from the whole muscle biopsy specimens but the cells harbouring viral antigens were rare endomysial macrophages and not myocytes. Although HTLV-1 DNA was amplified from all patients' PBMCs, these cells did not exert myotoxicity and viral replication could not be detected in co-cultures with their homologous myotubules (164). Another study showed predominantly HTLV-1 infected CD4+ cells in the muscle biopsies of HTLV-1 positive patients with polymyositis. The disease is resistant to corticosteroids and immuno-modulatory therapies such as ciclosporin showing only mild improvement of symptoms and no delay in progression (104;158).

Sporadic inclusion body myositis has been reported in HTLV-1 positive patients (165;166). Muscle biopsies show primary endomysial inflammation, red-rimmed vacuoles, amyloid deposits, eosinophilic inclusions and small round fibres in groups, without direct viral infiltration of the muscle (165;167). HTLV-1-infected CD4+ T cells and CD8+ T cells specific for HTLV-1 tax antigen were isolated from muscle cell cultures of patients with HAM and sporadic inclusion body myositis (166) confirming the inflammatory nature of this condition.
1.2.2.3 Hashimoto’s thyroiditis and Graves’ disease

In the Tokushima and Kochi prefectures in Japan 6.3% of patients with Hashimoto’s thyroiditis were reported to be HTLV-1 infected. This is significantly higher than the prevalence of HTLV-1 infection in the general population of this region (2.2%, \( p=0.01 \)) (168). A similar association was also observed in the Fukuoka prefecture (169). In blood donors 7.9% of patients infected with HTLV-1 infection also had anti-thyroid antibodies (170). Hashimoto’s thyroiditis is associated with HAM (168) and Graves’ disease with uveitis (154). The clinical picture ranges from no symptoms and only a positive thyroid auto-antibody screen and/or an abnormal thyroid on ultrasound to full-blown hyperthyroidism. The titres of anti-microsomal antibodies may be elevated and anti-T4, anti-T3 and anti-thyroglobulin antibodies can be found. Ultrasonography of the thyroid can show enlargement and decreased echogenicity. Thyroid scintigram may show non-homogeneous uptake of labelled iodine with more discreet areas of decreased uptake. Histological examination of the thyroid may demonstrate lymphocytic infiltration, germinal centres, hypertrophy of follicular epithelium and interstitial fibrosis (171). HTLV-1 envelope protein and mRNA but no viral particles were found in follicular epithelial cells of HTLV-1 infected patients with Hashimoto’s thyroiditis and HTLV-1 viral DNA was found in thyroid tissue of patients with Hashimoto’s thyroiditis and Graves’ disease (172;173). In patients with Hashimoto’s thyroiditis and Graves’ disease the HTLV-1 viral loads were five times higher than in ACs but did not correlate with the thyroid peroxidase antibody or thyroglobulin antibody titres in the peripheral blood (174). Patients may be treated with partial or complete thyroidectomy and hormone replacement therapy.

1.2.2.4 HTLV-1 associated arthritis

An association between arthritis/polyarthritis and HTLV-1 infection has been observed in patients with ATLL (175;176), and HAM (177) as well as in ACs (178). The prevalence of rheumatoid arthritis (RA) was significantly higher in HTLV-1 carriers (0.56%) compared to sero-negative (0.31%) patients in
Tsushima, Japan (179). A study from Nagasaki suggested that 13% of cases of RA were associated with HTLV-1 infection (180). An increased incidence of arthritis was found in HTLV-1 (incidence ratio [IR] 2.84) and HTLV-2 (IR 2.66) infected patients in a prospective blood donor study in the USA (181). However, an association between HTLV-1 seropositivity and arthritis could not be established in a South African study (182). The clinical picture of HTLV-1 positive patients with polyarthritis does not differ from HTLV-1 negative patients with RA matched by gender, ethnic origin and disease duration (183;184). Patients display symptoms and signs of RA indistinguishable from idiopathic RA with or without neurological signs (185). In a retrospective study in Martinique out of 17 HTLV-1 positive patients with polyarthritis five (29%) also had HAM and one (6%) ATLL. Fever, myalgia and/or skin lesions were present at onset of the polyarthritis in seven (41%) cases and all 17 patients had peripheral, bilateral, symmetric polyarthritis. The most commonly involved sites were the hands (17/17) and knees (14/17). Three patients had a positive rheumatoid factor (18%) and five (29%) had antinuclear antibodies (183). Possibly the migration of T lymphocytes into the articular space (186;187), attracted by viral antigens such as HTLV-1, which is synovial cell tropic (188), leads to the development and progression of arthritis and arthropathy. The synovial fluid and tissue of HTLV-1 positive patients with arthritis have been shown to contain atypical T lymphocytes (184;189;190), high titres of anti-HTLV-1 IgM antibodies, integrated HTLV-1 viral DNA (189) and tax mRNA and protein (191). Transfection of a tax-expressing plasmid into synovial cell clones resulted in the same phenotype of increased proliferation and cytokine expression as exhibited by HTLV-1 provirus-carrying and tax-expressing synovial cell clones (191). Finally HTLV-1 transgenic mice developed a chronic arthritis at two to three months of age, resembling RA. Synovial and peri-articular inflammation with articular erosion caused by invasion of granulation tissues was also observed (192;193).
1.2.2.5 HTLV-1 associated pulmonary disease

A broad spectrum of pulmonary involvement of HTLV-1 carriers has been described. This includes alveolitis (194), bulla formation, diffuse panbronchiolitis, lymphocytic interstitial pneumonia and bronchiectasis (103;195). A recent report from central Australia found 72% of patients admitted with bronchiectasis were HTLV-1 antibody positive (196). A retrospective review of high resolution computed tomography of the chest in 320 HTLV-1-infected Japanese patients showed abnormal findings in 98 (30.1%) patients with a peripheral parenchymal predominance in 71% of these. The abnormalities comprised centrilobular nodules (97%), thickening of bronchovascular bundles (56%), ground-glass opacity (52%), bronchiectasis (51%), interlobular septal thickening (29%) and consolidation (5%). 59% of patients who underwent surgical biopsy or transbronchial biopsy, which showed lymphocytic infiltration along respiratory bronchioles and bronchovascular bundles (103). In most cases patients remain asymptomatic but can present with chronic and productive cough unresponsive to long-term steroid therapy (197).

Bronchoalveolar lavage shows T-lymphocytosis, high HTLV-1 viral DNA (198), increased IL-2 receptor α (IL-2Rα/CD25) positive T-lymphocytes and marked elevation of soluble IL-2Rα (199) together with an increase in chemokines (200;201), such as macrophage inflammatory peptide-1 α (MIP-1 α) and macrophage chemo-attractant protein-1 (MCP-1). HTLV-1 Tax protein is known to induce the expression of IL-2 (202;203). A correlation between lymphocytosis and increased CD3+CD25+ cells with tax mRNA expression in bronchoalveolar fluid was demonstrated in HTLV-1 carriers (204). The presence of HTLV-1 infected cells in the alveoli seems to induce T-lymphocyte migration and clonal expansion leading to inflammation of pulmonary tissue through secretion of cytokines and chemokines.
Primary and secondary Sjögren’s syndrome (SS) is an autoimmune exocrinopathy causing keratoconjunctivitis sicca and/or xerostomia. Characteristic histological findings are sialadenitis with lymphocytic infiltration of the salivary and lacrimal glands and proliferating nests of epithelial cells. Both lacrimal and salivary glands may be enlarged. Rheumatoid factor is usually positive, antinuclear antibodies (ANA) and anti-Ro (SSA) are positive in 60-70% of cases (205). Retroviruses have been implicated in the aetiology of SS (206;207) and in 1989 an exocrinopathy resembling SS was reported in HTLV-1 tax transgenic mice (208). In an ophthalmology clinic in Nagasaki, an HTLV-1 endemic area (10-15% sero-prevalence), 13 (36%) of the mostly female patients with primary SS tested positive for HTLV-1 antibody. Some patients also had extraglandular manifestations including recurrent uveitis, arthropathy, interstitial pneumonitis, Raynaud’s phenomenon and inflammatory bowel disease but none had RA or mixed connective tissue disease (209). Another study from the Nagasaki Prefecture reported a 23% seroprevalence of HTLV-1 in patients with primary SS compared to blood donors (3%). Salivary IgA class antibodies to HTLV-1 were common among HTLV-1 seropositive patients with SS (5/7). Ocular and oral manifestations of SS were more frequently detected in HAM patients compared to HTLV-1 ACs and HTLV-1 negative patients.

Low volume of saliva and frequency of ANA correlated with the density of mononuclear cell infiltration in labial salivary glands which itself was higher in HTLV-1 seropositive than in seronegative patients with SS (210).

The HTLV-1 seroprevalence in 135 patients with primary SS and 97 patients with secondary SS was 25% and 29.2% respectively. There was no significant demographic or immunological, such as the prevalence of other auto-antibodies [rheumatoid factor, ANA, anti-SS-A (Ro), anti-SS-B (La)] difference between the infected and uninfected SS patients, apart from a significantly higher anti-centromere antibody (ACA) prevalence in HTLV-1 negative SS patients (211).

Treatments options are artificial tears and saliva replacement solutions as well as systemic pharmacotherapy with pilocarpine hydrochloride.
1.2.2.7 HTLV-1 associated conjunctivitis sicca syndrome and interstitial keratitis

The most comprehensive report on ocular disease is a prospective case series of 200 HTLV-1 infected patients (39% ACs and 62% HAM patients) in Martinique. Apart from uveitis, keratoconjunctivitis sicca was found in 74 patients (37%), accompanied by lympho-plasmocytoid infiltration of the secondary salivary glands rated 3 or 4 on the Chisholm scale in nearly 50% of cases (155). The sicca syndrome related to HTLV-1 differs from primary or secondary Sjögren’s syndrome in that auto-antibodies can not be demonstrated. This is similar to the sicca syndromes seen in infections with HIV or hepatitis C virus (212;213). Symptoms were exceptional, with ocular pruritus being the most commonly reported complaint. No filamentous keratitis, ulceration, or corneal neo-vascularisation was observed. Interstitial keratitis was observed in 20 cases (10%) (155). The essential characteristics were rounded, cloudy, anterior stromal opacities that were whitish, bilateral, painless and without ulceration or neo-vascularisation, which did not respond to local corticosteroid therapy. Both keratoconjunctivitis sicca (46% v 23 %) and interstitial keratitis (15% v 3%) were more common in patients with HAM than in ACs (155).

1.2.2.8 HTLV-1 associated dermatitis (Infective dermatitis)

Infective dermatitis (214) is a unique clinical entity described in HTLV-1 infected, mostly female (60%), children with the mean age of onset at two years (86) which has been rarely described outside the tropics. Familial clustering (215) and development of ATLL have been observed and in a recent study 12/17 children with HAM also had infective dermatitis (216). The patients present with severe exudative dermatitis of the scalp, ears, eyelids, nose, axillae and groins accompanied by chronic watery nasal discharge and crusting of the para-nasal skin. Skin super-infection with Staphylococcus aureus and/or β-haemolytic streptococcus is commonly diagnosed. CD4+ and CD8+ T-lymphocyte counts and the CD4+/CD8+ T cell ratio are raised. Histologically infective dermatitis may represent a benign form of mycosis fungoides with a predominance of CD8+ T-
lymphocytes and a low percentage of cells with cytotoxic granules, indicating that most CD8+ T-lymphocytes are not activated (217). Treatment consists of antibiotics and corticosteroids but relapse is common when treatment is stopped. The condition becomes less severe as the children get older.

1.2.2.9 Inflammatory conditions less commonly associated with HTLV-1

Until recently mixed connective tissue disease (218) but not systemic lupus erythematosus (SLE) (219) had been associated with HTLV carrier status. However a publication in 2007 suggests that SLE in HTLV-1 positive patients might behave differently than in seronegative patients. Seropositive patients had an older age of onset of SLE (median 45.5 v 30 y; p < 0.0005) and the maintenance dose of prednisolone was significantly lower than in seronegative patients (median 5 v 9 mg/day; p = 0.012) (220). Diabetes mellitus type I and endogenous asthma, both idiopathic conditions with a high prevalence, have not been associated with HTLV-1 infection.

1.3 HTLV-1 associated mortality

There are surprisingly few data on HTLV-1 associated mortality. HTLV-1 associated death is most commonly due to ATLL, but it has been suggested that there is a higher risk of mortality, excluding ATLL, with HTLV-1 infection per se compared to uninfected subjects (relative risk: 1.3, 95% CI=1.0-1.7) (221). The relation between mortality and HTLV-1 infection was also examined in a HIV-2 and HTLV-1 co-infected cohort in Guinea-Bissau. Adjusted for age, sex and HIV status the HTLV-1 associated mortality was 2-fold higher (222) and the risk of death increased with HTLV-1 provirus load (mortality hazard ratio was 1.59 for each log10 increase in HTLV-1 provirus copies, p= 0.038) (223). In Bahia, Brazil, co-infection with HTLV-1 (20%) was associated with shorter survival time in a
retrospective case-control study involving 63 HIV-1 infected patients. Dually infected patients had a shorter mean survival (1849 days) than HTLV-1 negative/HIV-1 positive controls (2430 days, \( p = 0.001 \)), regardless of sex or baseline CD4 cell count (224). In an unpublished UK study of 190 HTLV-1 infected individuals <60 years of age, the median survival time from diagnosis of HTLV-1 infection to death was 389 days and mortality rate was 40.84 deaths per 100 person-years. 77% of the cohort died of ATLL (225).
Chapter 2

2 A 15-year prospective longitudinal study of disease progression in patients with HAM in the UK.

2.1 Background

It is estimated that 20,000 persons are currently infected with HTLV-1 in the UK and that 3% of these will develop HAM at some point during their lives (226). Worldwide female dominance (female: male = 8:1) is recognised, with the first symptoms developing mostly between 40 to 50 years of age (38;96). However, children have also been diagnosed with HAM and in contrast to adults their course is described as more rapid and progressive (216).

The natural history and long term clinical outcome of HAM in the UK has not been described and may differ from the Japanese experience because of genetic, transmission and environmental factors. Conversely, since the majority of patients with HAM in the UK are of Afro-Caribbean origin, genetic differences with the Afro-Caribbean cohorts should be less important but environmental factors may result in different patterns of progression.

2.2 Objectives

To describe the natural history of HAM in 48 patients attending a single health care centre in the UK with the aim of identifying predictors of disease progression.
2.3 Material and Methods

Data have been collected prospectively on all patients with HAM attending the HTLV clinic at St Mary’s Hospital in London, from July 1993 to April 2007. All patient clinical notes were also hand searched retrospectively to answer specific questions. Not all data were available for all patients and the denominator for each analysis is given at the beginning of each result section.

The descriptive data collected were gender, ethnicity, perceived risk factor for HTLV-1 infection, the first ever complaint noticed by the patient as the presenting symptom, age of onset of first symptom based on patient recall, age at diagnosis of HTLV-1 infection as a surrogate for diagnosis of HAM, time between first and last visit to the HTLV clinic as years of follow up and deaths during follow up.

2.3.1 Clinical variables

The clinical variables were timed walk, usage of walking aids, ankle clonus, localisation and severity of pain, cramps of lower limbs and sphincter function.

Timed gait was reported in patients with HAM treated with high dose zidovudine in 1993 (137) and was introduced as timed walk (tw) at self selected speed into the NCHR in 2003 to identify more subtle changes than was possible with the existing disability scales, such as Kurtzke’s Expanded Disability Status Scale (EDSS) (227;228) designed for multiple sclerosis and Osame’s Motor Disability Scale (OMDS) for HAM, in which motor dysfunction is graded on a scale from 0 (normal walking and running) to 13 (completely bedridden) (32).

Timed walk is the time a patient takes to walk straight for 10 meters (m) with his/her usual walking aid in seconds (sec). The baseline 10m tw, which was the average of the first two tw within the first 3 months of attendance, was compared with last tw, being the mean of the tw documented during the last 3 months of
follow up. The rate of change was calculated by dividing the difference between baseline and last tw in seconds/10m by the duration of follow up in years (sec/10m/y). However for those patients who had been followed up for less than a year the rate of change in tw is only a projected value and the rate of change would possibly be less dramatic with a higher denominator. Tw is not available for those patients who did not attend the clinic after 2003. There are no published data for HTLV-1 positive patients defining a normal or abnormal TW. TW was available for 60 ACs during the same time period. Their mean tw was 9.1 (5.7-22) sec/10m and median 7.6 sec/10m.

Patients were categorised as using no aid (AID0), one walking stick (AID1), two walking sticks (AID2), a tripod (AID3) or being wheelchair (AID4) dependent, as documented at sequential clinic visits.

The presence (inducible/ spontaneous) or absence of ankle clonus and cramps of lower limbs were recorded.

Pain was categorised as absent or present, localised in the lower back, lower limb or both, and the severity was scored by the patient using a 10 cm visual analogue scale, 0 being pain free and 10 being the worst possible pain.

The recorded measures of bladder function were urinary frequency and urgency whilst retention was the need for intermittent or persistent urinary catheters. Bowel function was recorded as mild (>3 times weekly), moderate (1-3 times weekly) and severe (< once weekly) constipation.

Data were collected on the number of patients whose disability progressed to having to use a wheelchair in ≤ 2 years of onset, which we used to define rapidly progressing HAM. The number of patients who died during follow up was recorded and the HTLV-1 associated mortality rate calculated.
2.3.2 Treatment

No effective therapy for HAM has so far been reported however some patients were receiving potential disease modifying drugs at first and last follow up. A summary of symptomatic treatment received for pain, spasticity and bladder and bowel problems is outlined in the treatment paragraph.

2.3.3 HTLV-1 viral load

The major laboratory variable was HTLV-1 viral load. HTLV-1 DNA was quantified by ‘in house’ real-time PCR using the Roche LightCycler (Roche, Mannheim, Germany) as described in Chapter 3 and routinely performed. All data were compared between the baseline and last assessment. HTLV-1 viral loads were also analysed longitudinally to detect fluctuation.

2.3.4 Statistical methods

The data were originally recorded in an Excel spreadsheet. Data were analysed in SPSS-14 through parametric (t-test) and non-parametric tests (Mann-Whitney Test and Wilcoxon Signed Ranks Test) for continuous variables and chi-square (Pearson Chi-Square Test for large and Fisher’s Exact test for small sample size) and odds ratio/ relative risk for categorical variables. Kaplan Mayer survival analysis was used for survival data (Tarone –Ware Test was used if the survival curve for the two groups crossed over time). A multivariate logistic regression model was used to identify independent predictors of disease progression. Backward Wald was used for the best-fit model. Cox regression was used to create a predictive model for time-to-event data. Results were assumed statistically significant and the null hypothesis was rejected if a p value < 0.05 was achieved.
2.4 Results

2.4.1 Description of the cohort

Until 2007 55 patients diagnosed with HAM have attended the clinic. Seven (12.7%) patients were excluded: four attended only once; for one patient all medical records were missing; one patient also had polymyositis and one was co-infected with HIV-1. Forty-eight patients who were followed up at least twice were included in the study.

Demographic data are outlined in Table 1. Patients were mostly female and of Afro-Caribbean origin. One patient from West Africa was co-infected with hepatitis B virus and one Brazilian patient with hepatitis C virus. The most common risk factors for HTLV-1 acquisition were having been breast-fed and having had unprotected sexual intercourse in an endemic area or with a partner from an endemic area. Four patients (8.3%), none of whom were of Afro-Caribbean origin, identified blood transfusion before 2002, i.e. before blood donations were screened for HTLV-1/2 antibodies in the UK, as their sole risk factor for HTLV-1 acquisition.

Table 1: Demographics.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number (%)</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>38 (79.2)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>38 (79.2)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>6 (12.5)</td>
</tr>
<tr>
<td>African/Brazilian/Iranian</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>Risk factor for HTLV-1 infection</td>
<td></td>
</tr>
<tr>
<td>Sexual intercourse alone</td>
<td>3 (6.3)</td>
</tr>
<tr>
<td>Breast-fed + Sexual intercourse</td>
<td>40 (83.3)</td>
</tr>
<tr>
<td>Blood product recipient before 2002</td>
<td>4 (8.3)</td>
</tr>
<tr>
<td>Breast-fed + Sexual intercourse + Blood product recipient</td>
<td>1 (2.1)</td>
</tr>
</tbody>
</table>

The mean age at first recalled symptom was 46 years; in 28 (58%) patients the age of onset was ≤ 50 years. Median time from onset of symptoms to diagnosis
of HAM or HTLV-1 infection was 2 (1-19) years and the median duration of disease from onset to last follow up was 11.6 (1.2-31) years (31[65%] patients). Five (10.4%) patients were diagnosed with HTLV-1 infection prior to developing HAM, one of whom developed HAM during follow up at the NCHR. In this sub-group the mean time from diagnosis of HTLV-1 infection to onset of disease was 1.2 years.

Table 2: Age and Follow up.

<table>
<thead>
<tr>
<th></th>
<th>Mean (range)</th>
<th>95%CI</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at time of first recalled symptom</td>
<td>46 (10-72)</td>
<td>42.5-50</td>
<td>48</td>
</tr>
<tr>
<td>Age at time of diagnosis</td>
<td>50.5 (26-73)</td>
<td>46-55</td>
<td>51</td>
</tr>
<tr>
<td>Age at 1st visit to HTLV clinic</td>
<td>54 (19-75)</td>
<td>51-58</td>
<td>56</td>
</tr>
<tr>
<td>Time: onset - diagnosis (years)</td>
<td>3.8 (1-19)</td>
<td>2.2-5.5</td>
<td>2</td>
</tr>
<tr>
<td>Time: onset - 1st HTLV clinic visit (years)</td>
<td>8 (1-28)</td>
<td>0.6-10</td>
<td>8</td>
</tr>
<tr>
<td>Duration of follow up (years)</td>
<td>4.4 (0.5-13)</td>
<td>2.9-5</td>
<td>3.8</td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td>13 (1.2-31)</td>
<td>10.6-15</td>
<td>11.6</td>
</tr>
</tbody>
</table>

The most common first recalled symptom associated with HAM was unilateral leg weakness (Table 3).

Table 3: First recalled symptom associated with HAM.

<table>
<thead>
<tr>
<th>First recalled symptom</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral leg weakness</td>
<td>16 (36)</td>
</tr>
<tr>
<td>Static pain of lower back/leg</td>
<td>13 (30)</td>
</tr>
<tr>
<td>Unexplained fall</td>
<td>8 (18)</td>
</tr>
<tr>
<td>Bladder dysfunction</td>
<td>7 (16)</td>
</tr>
</tbody>
</table>

At last follow up 31 (65%) of the cohort had had HAM for more than 10 years. Six (13%) of the patients were followed up at the HTLV clinic for > 10 years (10.6-12.9), 13 (27%) for 5-10 years (5-9.5), 19 (40%) for 1-5 years (1-4.7) and 10 (20%) for < 1 year (0.1-0.9), (Figure 1). This equates to 212 person-years of follow up.
Figure 1: Patients categorised by length of follow up.

2.4.2 Time to first use of a walking aid

Mobility was described for all patients. Thirty-eight (79%) patients were ambulant at first and 32 (68%) at last clinic visit ($p = 0.25$). At first clinic visit 13 (27%) patients were walking unaided (group AID0), 25 (52%) were using walking aids (groups AID1, 2 and 3), eight (17%) were wheelchair dependent (group AID4) and two (4%) patients were bed bound. At last follow up six (13%) patients were in group AID0, 26 (55%) in AID 1 – 3, 15 (31%) were in AID4 and one (2%) patient was bed bound. At last follow up 31/38 (82%) ambulant patients were using a walking aid and 19/38 (50%) patients needed additional aid compare to first visit: seven patients progressed into group AID1, three into AID2, three into AID3 and six into AID4. The mobility of two patients (4%) had improved: one could transfer from bed to wheelchair and one needed only one walking stick as opposed to two (Figure 2).
Figure 2: Walking aid at 1st and last clinic visit.

![Walking Aid at 1st and last clinic visit](image)

Figure 2: 100% stacked bar chart showing the walking aids documented at 1st and last clinic visit. None = no walking aid used. Bed = bed bound.

The median times from onset of HAM to AID1, AID2, AID3 and AID4 were 11 years (range 0.8-24.5, 95% CI 2.8-17.30 [CI]), 11.2 years (range 6.4-16.5, 95% CI -1.2 – 16), 11.3 years (range 6.9-14, 95% CI 8.3-14) and 18 years (range 14-22.1, 95% CI 14-22) respectively (Figure 3).
2.4.3 Correlation between walking aid and pain

Data were available for 42 patients at first and 43 patients at last clinic visit. At first visit 7/11 (64%) patients not using an aid and 19/31 (61%) aid dependent patients were complaining of pain ($p=1$), a total of 26/42 (62%). At last clinic visit 2/5 (40%) walking without aid and 21/38 (55%) with walking aid were in pain ($p=0.7$), a total of 23/43 (53%). At 1st visit the pain score was 3.8 cm (range: 0-10, 95%CI: 1-6.6; median 2) for patients without aid and 3.5 cm (range 0-10, 95%CI: 2.2-4.8; median 3) for those in need of aid ($p=0.9$). Similarly there was no significant difference between the mean pain score of patients without aid (2.8 cm [0-9], 95%CI: -2.3-7.9, median 0) compared to patients with aid (3 cm [0-10], 95%CI: 1.8-4.2, median 0) at last visit ($p=0.9$). A correlation between aid and presence/absence, duration or location of pain could not be established at the two time points.
2.4.4 Progression in timed walk

First and last timed walk (tw) was documented for those patients who remained ambulant at last follow up. At first clinic visit the mean tw was 21.15 sec/10m (8.2-80, 95%CI: 15.8-26.5, median 17.8) and at last follow up 28.7 sec/10m (8.5-95, 95%CI: 21.6-35.8, median 21.3). An overall average deterioration in tw of 1.98 sec/10m/y.

Tw deteriorated in 25 (73%) patients, improved in seven (24%) and was not documented in one (3%). This patient was not using any aid at any time point. In those whose tw deteriorated 10 (30%) patients needed additional aid and one patient used less (3%). None of the patients whose tw had improved had changed aid at last follow up.

2.4.4.1 Change in timed walk for those patients whose need for walking aid did not change between first and last follow up

In 21 (66%) patients with documented tw the need for aid did not change. The tw deteriorated in 14 patients (67%): four patients in group AID0, six in AID1 and four in AID2 with a mean rate of +2.32, +1.95 and +0.48 sec/10m/year within each aid group respectively.

Tw improved in 7 patients (33%): one patient in AID0, two in AID1 and three in AID2 and one in AID3 by a mean trend of -0.92, -9.66, -130 and -34 sec/10m/year. However the patients in AID1 and AID2 were followed up less than one year and therefore their improvement rates are overestimated.

In group AID0 four patients’ tw deteriorated (TAT, TBI, TBP, TCB) between the 1st and last clinic visit (mean 10.6 sec/10m, range 7.7-12.2, 95% CI: 8.3-14.8, median 12.1 v mean 14 sec/10m, range 6.4-18.7, 95% CI: 8.8-17.4, median 13.54). This gives an average trend of deterioration of +2.32 sec/10m/y (range 0.047-8.12). One patient’s tw (TBX) had improved by a rate of -0.92 sec/10m/y (first visit: 8.80 sec/10 m; last visit: 8.45 sec/10m) (Figure 2).
One patient (TAT), who was followed up 6.8 years, was identified as a long term non-progressor with a tw of 12 sec/10 at first and 12.41 sec/10m at last follow up.

In the AID1 group six patients (TAU, TBW, TBG, TBK, TBS, TCO) had a deterioration of tw between the first and last clinic visit (mean: 18.9 sec/10m, range 13.6-26.7, 95% CI: 11.6-25.5, median 18.5 v 24.4 sec/10m, range 16-37, 95% CI: 14.3-34.5, median 24.6) was documented. The mean rate of deterioration was +1.95 sec/10m/y. Two patients’ tw (TCQ, TCK) had improved over time (mean 20.5 sec/10m, range 18.7-22.3, 95%CI: 17-28.5, median 20.5 v mean 17.84 sec/10m, range 15.78-19.9, 95%CI: 1.4-35.7, median 17.8). This is an improvement by a mean trend of -9.66 sec/10m/y (range 6.52-12.8), (Figure 3).

In the AID2 group four patients’ tw deteriorated (TAD, TAG, TCF, TCL) between the first visit and last follow up (mean 28.3 sec/10m, range 16-55.4, 95%CI: -1-58, median 21.5 v mean 34.7sec/10m, range 21-65.4, 95%CI: 1.8-68, median 26.7). This gives a trend of deterioration of +0.48 sec/10m/y with two patients being followed up for less than one year. Two patients’ tw (TCM, TCC) improved between the first and last clinic visit (mean 74 sec/10m, range 68.2-80, 95%CI:-0.9-149, median 56.5 v mean 38 sec/10m, range 38.8-37.5, 95%CI: 30.2-46, median 35.5). The trend of improvement was -130 sec/10m/y at last follow up (Figure 4).
Figure 4: Change in tw in group AID0

Figure 4: Change in tw between 1st and last clinic visit in those patients who did not use a walking aid (AID0). One patient’s tw improved, as indicated by a negative slope.

Figure 5: Change in tw in group AID1

Figure 5: Change in tw between 1st and last clinic visit in those using one walking stick. Two patients’ tw improved as indicated by a negative slope.
2.4.4.2 Change in timed walk for those patients whose need for walking aid changed and who remained ambulant at last visit

Altogether ten patients needed more and one needed less aid at last clinic visit. In all cases the tw had deteriorated. The mean rate of deterioration in tw for seven in AID0, one in AID1 and three in AID2 was +2.4, +7.4, +3.7 sec/10m/y respectively.

Five patients initially in group AID0 (TCJ, TAS, TAE, TAQ, TCG) were using one walking stick, one patient (TAN) two walking sticks and another (TBJ) a tripod at last visit. Their tw had increased significantly (first visit: mean 13.8 sec/10m, range 8.2-23.1, 95%CI: 8.5-19.1, median 12.4; last visit: mean 22.8, range 17-38.4, 95%CI: 13.4-32.3, median 17.4; p= 0.03) with mean deterioration rate of +2.4 sec/10m/y.
The patient (TAW) initially, in group AID1, was using a tripod at last visit and her tw had increased considerably (First visit: 16 sec/10m, last visit: 75.5 sec/10m) with a rate of deterioration +7.4 sec/10m/y.

Two patients (TBO, TW) initially, in group AID2, were using a tripod at last follow up. Their gait had slowed considerably between the two time points (first visit: mean 27 sec/10m, range 21-33, 95%CI: - 0.49-103, median 27; last visit: mean 56 sec/10m, range 41-71, 95%CI -132-245, median 56; p=0.2) with a mean rate of deterioration of +3.7 sec/10m/y.

Although one patient (TCD) in AID2 needed only one walking stick at last follow up her tw had deteriorated (first visit: 17.59 sec/10m; last visit: 20.75 sec/10m) with a minimal rate of deterioration of + 2 sec/10m/y.

2.4.5 Effect of pain on timed walk

The mean timed walk of patients who were pain free at first visit did not differ from those who were in pain (pain absent: mean 28 sec/10m, range 8.2-70, 95% CI 18-38, median 19; pain present: mean 23 sec/10m, range 8.3-80, 95% CI 18-38, median 19; p=0.4). Similarly the mean timed walk at last visit did not differ significantly whether the patients were in pain or not (pain present: mean 27 sec/10m, range 8.5-71, 95% CI 14-39, median 17.4; pain absent: mean 30 sec/10m, range 9.5-95, 95% CI 20.1-40, median 21.3; p=0.7).

Timed walk did not correlate with the severity of pain at either time point (first visit: p=0.8, last visit: p= 0.9) as demonstrated in Figure 7.
2.4.6 Ankle clonus and lower limb cramps

Ankle clonus was documented in 11/48 (23%) patients, cramps in 12/48 (25%) patients and both in 9/48 (19%) patients at both first and last time points. Ankle clonus was absent in two patients (T, TAC) and cramps were absent in three patients (T, TAA, TAC) at both time points, two of these patients had already been wheelchair bound for a long time at their first clinic appointment (TAC, TAA). In five patients the ankle clonus was inducible while one patient displayed spontaneous ankle clonus at both time points. Lower limb cramps were present in seven patients. One patient who was asymptomatic at first clinic visit developed HAM with spontaneous ankle clonus and lower limb cramps. One patient presented with rapid progressive HAM and had flaccid paraparesis and therefore no clonus at first visit but had inducible clonus at the last visit. She complained of leg cramps on both occasions.
2.4.7 Description of pain

Location (pain in the lower back, lower limbs or both), duration throughout the day (permanent or intermittent) and severity of chronic pain (visual analogue score 0-10/10) were documented. Thirteen (30%) patients reported pain as their first persistent symptom in association with the onset of HAM. In 43/48 (90%) patients data on presence of pain was collected at both time points. 27/43 (63%) were in pain at first and at 23/43 (53%) at last clinic visit (\(p=0.3\)). At last visit 8/27 (30%) patients, who previously had reported pain, were pain free and 4/16 (25%) previously pain free patients, reported pain (1 lower back, 2 legs, 1 both sites).

At first visit more patients had lower back pain (14/27 [52%]) compared to leg pain (5/27 [19%]) and pain at both sites (7/27 [26%]). At last visit four patients who had presented with lower back ache were now pain free, two patients had leg pain instead of back ache and one had developed additional leg pain. Two of the patients who had leg pain at first visit, had developed additional lower back pain and two became pain free. Only two of those patients who were pain free at last visit were in need of more aid (tripod).

Information on duration of pain throughout the day was available on 16/27 (59%) patients. At both time points more patients had permanent compared to intermittent pain (first clinic visit 11 [69%] v 5 [31%], last visit 13 [81%] v 3 [19%]). Over a mean follow up of 3.9 years five (31%) patients reported improvement in the duration of their pain: four patients were pain free, one had intermittent instead of permanent pain. Five (31%) patients had progressed over a mean period of 3.4 years: four previously pain free patients had developed intermittent \((n=2)\) and permanent \((n=2)\) pain and one had progressed from intermittent to permanent pain.

Pain score was recorded in 41/48 (85%) patients (Figure 6, 7). In those who had pain the pain score did not differ significantly at first and last clinic visit (first visit: 6 cm [2-10] 95%CI: 4.9-7.1, median 5.9; last visit: 6.4 cm [1.5-10] 95%CI: 5.2-7.6, median 5.5, \(p=0.2\)).
In 10 patients the pain score worsened significantly from a mean of 3 cm (range: 0-8, 95CI: 0.8-5.2, median 2.5) at first visit to a mean of 7.3 cm (range: 4-10, 95%CI: 5.7-8.8, median 8, \( p = 0.05 \)) at last clinic visit. In 15 patients it improved significantly between the two time points (first visit: mean 6.5 cm [2-10], 95%CI: 5-8, median 7; last visit: mean 2.6 cm [0-9.5], 95%CI: 0.8-4.4, median 0, \( p = 0.001 \)) of whom 8 had become pain free.

Figure 8: Patients in whom the pain score (visual analogue score) had worsened at last clinic visit.
2.4.8 Bladder function

Seven/48 (15%) patients reported bladder problems as their first symptom of HAM. Data were available on 41/48 (85%) patients at both time points. At first visit 37/41 (90%) patients compared to 35/41 (85%) at last visit were complaining of bladder symptoms \( (p = 0.5) \). 32/37 (86%) patients had symptoms associated with spastic bladder and five/37 (14%) patients had flaccid bladder dysfunction. Comparing first with last visit: Four (10%) v seven (17%) patients were asymptomatic \( (p=0.5) \), six (15%) v five (12%) patients complained of urgency \( (p=0.8) \), 19 (46%) v seven (17%) complained of incontinence \( (p=0.04) \), 11 (27%) v 20 (49%) patients were using a urinary catheter \( (p=0.04) \) and one (2%) v two (5%) had obstructive nephropathy \( (p = 0.6) \). All patients with flaccid bladders were using intermittent or indwelling catheters. During follow up 10 (24%) progressed: 9 (22%) needed an intermittent (3) or indwelling (6) catheter for urinary retention (1) or incontinence (8), and one (2%) patient developed obstructive nephropathy.
2.4.9 Bowel function

Data were available for 40/48 (83%) patients at both time points. 25/40 (63%) were constipated at first and 24/40 (60%) at last visit. This apparent lack of change in the population masks considerable individual variation. Seven (18%) patients worsened between first and last visit whilst ten (25%) improved. Of those with symptoms at first visit 17 (68%) were moderately and 8 (32%) severely constipated. Six/15 (40%) initially asymptomatic patients had become moderately constipated and 1/17 (6%) with initial moderate constipation was severely constipated at last visit. Whilst 6/17 (35%) initially reporting moderate constipation and 1/8 (12.5%) with severe constipation had become asymptomatic at last visit and a further 3/8 (38%) patients with severe constipation had improved to moderate constipation.

2.4.10 Rapid progression

Three/48 (6%) patients progressed within two years of the onset of HAM to being wheelchair or bed-bound. All were female, two of Afro-Caribbean origin and one Caucasian. The mean age of onset for this small group was 49 years (range: 38-57, median=51). One patient died at the age of 40 years of respiratory failure. Rapid progression was independent of blood transfusion, age of onset or HTLV-1 viral load at first visit.
Mortality

Five/ 48 (10.6 %) patients infected with HTLV-1 died during a follow up period of 212 person-years (py), (Figure 10). Four deaths were associated with HTLV-1 infection. This gives a mortality rate of 2.4/ 100 py follow up (5 cases/ 212 py) including one death due to ATLL and 1.9/100 py follow up (4 cases/209 py) without it. Three of the patients were male and the median age at time of death of all patients was 57 years (mean 54 [36-78]).

At first clinic visit one patient was not using any walking aid (1/13 without walking stick), one patient one walking stick (1/25 ambulant with AID), two patients wheelchairs (2/8 using wheelchair) and one patient was bed bound (1/2 bed bound).

The causes of death were lymphomatous ATLL (n=1), respiratory failure due to pneumonia (n=1) and HAM itself with disseminated inflammation (n=2). The cause of death of a 78-year -old wheelchair dependent patient with HAM was not reported (n=1). Death in these five patients occurred after a mean of 4.8 years of follow up (range: 1.34-10.7, median 4.4) and 9.7 years from the onset (range: 2.42-20.3, median 5.8).
Figure 10: Cumulative survival (%) of all patients with HAM from onset of first symptom to death or last follow up.

Figure 10: Censored = patient reaching their last follow up. % Cumulative Survival: 0.0-1.0= 0-100%.

2.4.12 HTLV-1 viral load

HTLV-1 viral loads (copies/100 PBMCs) are described and compared between first and last clinic visit (Table 4). The HTLV-1 viral load of the cohort remained essentially unchanged over time (Figure 11).

Table 4: HTLV-1 viral load of all patients at 1st and last clinic visit.

<table>
<thead>
<tr>
<th>HTLV-1 (copies/100 PBMC)</th>
<th>Number</th>
<th>Mean (range)</th>
<th>Median</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st visit</td>
<td>48</td>
<td>20.5 (0.002-140)</td>
<td>14</td>
<td>13-28</td>
<td>0.6</td>
</tr>
<tr>
<td>Last visit</td>
<td>47</td>
<td>18.5 (0.01-70)</td>
<td>13</td>
<td>13.3-24</td>
<td></td>
</tr>
</tbody>
</table>
First HTLV-1 viral load was not associated with progression in aid over time ($p=0.8$) in the whole cohort. The rate of deterioration of tw (sec/10m/y) seemed to be faster with a higher HTLV-1 viral load at first clinic visit (Figure 12) although this was not found to be statistically significant ($p=0.2$).

Figure 12: Scatter plot of patients whose rate of timed walk/year worsened correlated with their HTLV-1 viral load at first visit.
Interestingly the first and last HTLV-1 viral loads were higher in patients who were <50 years old at onset of HAM and whose need for aid had increased over time (Table 6). One patient (TAN) developed HAM during the first eight months of follow up in the HTLV clinic (= index case). She had been diagnosed with HTLV-1 three years prior to the onset of myelopathy. Compared to other asymptomatic HTLV-1 carriers she had a higher mean baseline HTLV-1 viral load that essentially remained unchanged over time (mean 10.7 [1.6-22.5], median 9.8 copies/ 100 PBMCs).

Presence or absence of pain at first and last visit did not correlate with first or last HTLV-1 viral load (first clinic visit: \( p = 0.7 \); last clinic visit: \( p = 0.6 \)).

### 2.4.13 Age of onset of HAM and progression

Timed walk, rate of progression and HTLV-1 VL were analysed by age of onset as per previous publications (32;96). All data are shown in Tables 5 and 6. The sub-group of patients who did and did not need additional aid over time were analysed separately. Patients of younger age of onset had a longer duration of disease at first visit.

The sub-group of HAM patients, whose need for aid did not change over time, was composed of significantly more patients of older age of onset (<50 years 9/22 [41%] \( v \) >50 years in 13/22 [59%], \( p=0.0001 \)) and patients of younger age of onset had a longer duration of disease at last visit (\( p=0.03 \)).

In contrast patients who needed additional aid at last follow up recalled a younger age of onset of HAM (<50 years in 12/16 [75%] patients and >50 years in four/16 [25%] patients, \( p=0.005 \)). There was no significant difference between the duration of disease between the younger and older age of onset at last visit (\( p=0.8 \)). As previously mentioned tw deteriorated in all of these patients. Equally there was no significant difference in tw or rate of progression between these two groups at either time points. Interestingly in this sub-group of patients where tw and need for aid deteriorated the HTLV-1 viral load of patients of younger
onset was higher than those with later age of onset at both visit (first visit: \( p= 0.09 \), last visit: \( p= 0.05 \)).

Overall there was neither a significant difference in both time walk and the HTLV-1 viral load at the first and last clinic visit nor a significant difference in the rate of change of tw between the two time points when comparing these age groups.

More patients of younger age of onset, < 50 years, had pain at first clinic visit than patients of older age, > 50 years (18 v 8, \( p=0.03 \)), but this difference was lost at last follow up (12 v 11 \( p=0.4 \)).

### 2.4.14 Blood transfusions and progression

Four patients (TBJ, TAZ, T, TBP) recalled blood transfusions as their only risk for HTLV-1 acquisition. One patient, who gave blood transfusion, being breast-fed and heterosexual intercourse as risk factors of HTLV-1 acquisition, was not included in this sub-analysis. Time from transfusion to onset could not be accurately established however none of these patients progressed to a wheelchair within 2 years of onset. Two patients were female, one from Pakistan and one from Iran. In three patients the age of onset of HAM was < 50 years. The first presenting symptom was leg weakness in two patients, a fall in the third and bladder dysfunction in the fourth patient. There were no deaths during follow up (mean 3.5 years [0.25-5.3], median 4.2) but in half of these patients the need for aid progressed; from no walking aid to a tripod (TBJ) and from two walking sticks to a wheelchair (TAZ). At first and last clinic visit timed walk was longer in patients who had received a blood transfusion compared to those who had not but did not reach statistical significance (Table 7). It had improved in one and worsened in two ambulant patients at last follow up.
Table 5: Timed walk (tw), rate of progression, HTLV-1 viral load (VL) by age of onset in those patients whose need for aid did not change during follow up.

<table>
<thead>
<tr>
<th></th>
<th>&lt;50 years</th>
<th>95% CI</th>
<th>≥50</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age of onset</strong></td>
<td>n=9</td>
<td></td>
<td>n=13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) years</td>
<td>34 (10- 49)</td>
<td>26- 43</td>
<td>58 (51-67)</td>
<td>55- 61</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>37</td>
<td></td>
<td>59</td>
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<td></td>
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<tr>
<td><strong>Follow up</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) years</td>
<td>1.8 (0.3- 5.3)</td>
<td>0.7- 3</td>
<td>3.2 (0.2-7.3)</td>
<td>1.6- 4.8</td>
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</tr>
<tr>
<td>Median</td>
<td>1.6</td>
<td></td>
<td>2.7</td>
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<tr>
<td><strong>Duration: onset-first visit</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean (range) years</td>
<td>11.6 (0.8- 28.3)</td>
<td>7- 16</td>
<td>5.6 (0.8-14)</td>
<td>3.2- 7.9</td>
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<tr>
<td>Median</td>
<td>10.6</td>
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<td>4</td>
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<tr>
<td><strong>Duration: onset-last visit</strong></td>
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<td></td>
</tr>
<tr>
<td>Mean (range) years</td>
<td>14.8 (2.4- 31)</td>
<td>10- 19.5</td>
<td>9 (2.5-21)</td>
<td>6- 12</td>
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<td><strong>Timed walk at first visit (n= 32)</strong></td>
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<tr>
<td>Mean (range) sec/10m</td>
<td>23 (8.6- 80)</td>
<td>6- 40</td>
<td>23.1(12-55)</td>
<td>15- 32</td>
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<tr>
<td>Median</td>
<td>16</td>
<td></td>
<td>19</td>
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<td><strong>Timed walk at last visit (n= 36)</strong></td>
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<tr>
<td>Mean (range) sec/10m</td>
<td>21 (8.5- 40)</td>
<td>13.5- 28.5</td>
<td>30 (1-95)</td>
<td>13.8- 47</td>
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<tr>
<td>Median</td>
<td>21</td>
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<tr>
<td><strong>Timed walk difference</strong></td>
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<tr>
<td>Mean (range) sec/10m</td>
<td>3.7 (1- 11)</td>
<td>1.6- 7.8</td>
<td>16.6 (0.4-81)</td>
<td>11- 53</td>
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<tr>
<td>Median</td>
<td>2.7</td>
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<tr>
<td><strong>Rate of timed walk if worsened</strong></td>
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<tr>
<td>Mean (range) sec/10m</td>
<td>2.7 (0.5- 8)</td>
<td>0.4- 5.1</td>
<td>3.4 (0.05-16)</td>
<td>1.75- 8.6</td>
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<tr>
<td>Median</td>
<td>2</td>
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<td>0.63</td>
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Table 5 continued: Timed walk (tw), rate of progression, HTLV-1 viral load (VL) by age of onset in those patients whose need for aid did not change during follow up.

<table>
<thead>
<tr>
<th>Rate of timed walk if improved</th>
<th>&lt;50 years</th>
<th>95% CI</th>
<th>≥50</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (range) sec/10m</td>
<td>2.7 (0.01- 8.1)</td>
<td>1.3- 4.2</td>
<td>4 (0.05- 15.6)</td>
<td>0.4- 7.6</td>
<td>1</td>
</tr>
<tr>
<td>Median</td>
<td>2</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV-1 VL at first visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) copies/100PBMCs</td>
<td>21 (7.5- 41.5)</td>
<td>9.8- 32.4</td>
<td>27.5 (0.14- 140)</td>
<td>2.7- 52.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Median</td>
<td>18.2</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV-1 VL at last visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) copies/100PBMCs</td>
<td>20 (2.6- 55.2)</td>
<td>0.1- 40</td>
<td>19.2 (4- 65)</td>
<td>4.4- 34</td>
<td>0.8</td>
</tr>
<tr>
<td>Median</td>
<td>9.6</td>
<td>12.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6: Timed walk (tw), rate of progression, HTLV-1 viral load (VL) by age of onset in those patients whose need for aid did change during follow up.

<table>
<thead>
<tr>
<th>Age of onset</th>
<th>&lt;50</th>
<th>95% CI</th>
<th>≥50</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (range) years</td>
<td>n = 12</td>
<td>38 (10- 49)</td>
<td>31-45</td>
<td>57 (51- 66)</td>
<td>47-68</td>
</tr>
<tr>
<td>Median</td>
<td>42</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) years</td>
<td>6.7 (0.9- 12)</td>
<td>4-9.4</td>
<td>6.3 (0.1- 9.5)</td>
<td>- 0.4 -13</td>
<td>0.6</td>
</tr>
<tr>
<td>Median</td>
<td>6.7</td>
<td>7.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of onset to 1st visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) years</td>
<td>8.3 (0.2- 18)</td>
<td>4.5-12</td>
<td>9.9 (2-21)</td>
<td>0 3.2 -23</td>
<td>0.8</td>
</tr>
<tr>
<td>Median</td>
<td>8.5</td>
<td>7.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of onset to last visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) years</td>
<td>13.7 (1.2- 23.4)</td>
<td>9.3-18</td>
<td>16 (5.5- 30.9)</td>
<td>- 1.3 -34</td>
<td>0.03</td>
</tr>
<tr>
<td>Median</td>
<td>13</td>
<td>14.1</td>
<td></td>
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</tbody>
</table>
Table 6 continued: Timed walk (tw), rate of progression, HTLV-1 viral load (VL) by age of onset in those patients whose need for aid did change during follow up.

<table>
<thead>
<tr>
<th></th>
<th>&lt;50</th>
<th>95% CI</th>
<th>≥50</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timed walk at first visit (n= 14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>17.5 (8.3-33)</td>
<td>10.8-24</td>
<td>12.7 (8.2-16)</td>
<td>2.7-22.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Median</td>
<td>15.7</td>
<td></td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Timed walk at last visit (n= 10)</strong></td>
<td></td>
<td></td>
<td></td>
<td>-46 -120</td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>31 (9.5-71)</td>
<td>14-47</td>
<td>37 (17.4-76)</td>
<td>1.7 (0.003 -5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Median</td>
<td>25</td>
<td></td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Timed walk difference</strong></td>
<td></td>
<td></td>
<td></td>
<td>-51 -100</td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>13 (1.2-38)</td>
<td>2-24</td>
<td>24 (4-60)</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6</td>
<td></td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Timed walk rate/year</strong></td>
<td></td>
<td></td>
<td></td>
<td>-5.5 -8.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean (range) sec/10m/y</td>
<td>0.07 (0.004-0</td>
<td>-0.25</td>
<td>1.7 (0.003 -5)</td>
<td>- 5.5 -8.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Median</td>
<td>0.02</td>
<td></td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HTLV-1 VL at first visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean copies/100PBMCs (range)</td>
<td>21.7 (0.002-7</td>
<td>5.7 -38</td>
<td>1.8 (1.2-2.4)</td>
<td>0.8 -2.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Median</td>
<td>11.2</td>
<td></td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HTLV-1 VL at last visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean copies/100PBMCs (range)</td>
<td>13.8 (3.3-25.3</td>
<td>9.6 -18</td>
<td>6.1 (1.2-12.1)</td>
<td>- 0.2-13.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Median</td>
<td>14.9</td>
<td></td>
<td>5.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 7: Timed walk, rate of progression, and HTLV-1 VL by risk factors.

<table>
<thead>
<tr>
<th></th>
<th>Other Risk factors</th>
<th>95% CI</th>
<th>Blood transfusion</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 43</td>
<td>n= 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age of onset</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>46 (10- 72)</td>
<td>42-50</td>
<td>44 (29-60)</td>
<td>24 - 65</td>
<td>0.8</td>
</tr>
<tr>
<td>Median</td>
<td>48</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Follow up</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>3.5 (0.25- 5.3)</td>
<td>3.4- 5.8</td>
<td>3.5 (0.25- 5.3)</td>
<td>- 0.2 - 7</td>
<td>0.6</td>
</tr>
<tr>
<td>Median</td>
<td>4</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Timed walk at first visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>17.9 (8.2- 33)</td>
<td>15- 21</td>
<td>26.1 (8.6- 42)</td>
<td>-1.5 - 54</td>
<td>0.09</td>
</tr>
<tr>
<td>Median</td>
<td>17.6</td>
<td>26.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Timed walk at last visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>19.4 (16.8- 22)</td>
<td>8.5- 31</td>
<td>27 (9.6- 38.4)</td>
<td>-11 - 66</td>
<td>0.1</td>
</tr>
<tr>
<td>Median</td>
<td>18.8</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Timed walk difference</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>2.1 (-3- 9.2)</td>
<td>0.8- 3.5</td>
<td>5.9 (-8- 25)</td>
<td>-8 - 25</td>
<td>0.3</td>
</tr>
<tr>
<td>Median</td>
<td>1.8</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Rate of progression of tw</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>2 (0.01- 6.6)</td>
<td>1.1- 3</td>
<td>2.7 (0.5- 4.9)</td>
<td>-25 - 30</td>
<td>0.7</td>
</tr>
<tr>
<td>Median</td>
<td>1.2</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HTLV-VL at first visit</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>10.8 (0.002- 30)</td>
<td>7.9- 13.8</td>
<td>26.9 (0.3- 70)</td>
<td>- 23 - 77</td>
<td>0.4</td>
</tr>
<tr>
<td>Median</td>
<td>9.6</td>
<td>18.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HTLV-1 VL at last visit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range) sec/10m</td>
<td>12 (0.014- 34)</td>
<td>9.3- 14.6</td>
<td>14.7 (9.6- 17.9)</td>
<td>3.6 - 26</td>
<td>0.4</td>
</tr>
<tr>
<td>Median</td>
<td>11.4</td>
<td>16.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2.4.15 Multivariate analyses of risk of progression

The following values were analysed in the multivariate analyses of risk of progression: gender, age of onset of HAM, risk factor for HTLV-1 acquisition, duration of disease, presence or absence of pain at first visit, HTLV-1 viral load at first visit. Early age of onset (<50 years) alone predicted 75% of those patients who progressed in aid during follow up ($p=0.05$) however it did not predict the rate of change of tw ($p=0.8$). None of the other parameters were significantly predictive of progression of HAM as measured by need for additional aid or rate of tw/year. In Cox regression analysis none of the afore-mentioned parameters contributed significantly to time of progression to AID1 or to AID4 from first to last visit.

2.4.16 Treatment offered at the HTLV-1 clinic

2.4.16.1 Treatment for HAM

Data were available on 46 (95.5 %) patients (Table 8). Two (4.2%) and eight (18%) of patients were receiving disease-modifying medication at the first and last visit. More data are available on response to oral prednisolone and tenofovir in Chapter 2. Three patients were recruited to a ciclosporin trial for patients with early, progressive HAM. Data analysis from this trial is pending. However one patient (TCM) reported less spasticity and loss of spontaneous clonus and cramps of both legs at last follow up.

Of the 42 (87.5%) patients for whom data for musculoskeletal relaxants use were available 13 (27.1%) and 18 (37.5%) were taking baclofen at first and last follow up.

2.4.16.2 Treatment for symptomatic relief

All patients who were suffering from chronic pain had tried a combination of analgesics: paracetamol, NSAIDs, oral and intravenous corticosteroids,
opiates and anti-epileptics such as gabapentin and pregabalin. At last follow up three of those patients whose pain had improved were taking ciclosporin \((TCK, TCJ, TAN)\) as part of a clinical trial and one patient was taking oral methotrexate \((TAS)\).

Most patients were actively trying to prevent constipation: at first and last visit 14 [36%] v 15 [39%] patients were using fibres, 10 [26%] v 15 [38%] were using laxatives, and 1 [3%] v 5 [13%] were using enemas.

At first and last visit 11 [28%] v 13 [33%] patients were receiving oxybutinin for spastic bladder dysfunction and nine (23%) needed urinary catheterisation to empty their bladder fully. Therefore 22 (48%) patients were in need of medical intervention for urinary symptoms at last follow up.

Table 8: Summary of treatment received for symptomatic relief and HAM.

<table>
<thead>
<tr>
<th>Treatment for HAM, number (%)</th>
<th>First visit</th>
<th>Last visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral prednisolone</td>
<td>1 (2.1%)</td>
<td>3 (6.3%)</td>
</tr>
<tr>
<td>Anti-retrovirals</td>
<td>1 (2.1%)</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>0</td>
<td>1 (2.1%)</td>
</tr>
<tr>
<td>Ciclosporin</td>
<td>0</td>
<td>3 (6.3%)</td>
</tr>
<tr>
<td>Treatment for spasticity of legs, number (%)</td>
<td>42 (87.5%)</td>
<td>42 (87.5%)</td>
</tr>
<tr>
<td>Baclofen</td>
<td>13 (27.1%)</td>
<td>18 (37.5%)</td>
</tr>
<tr>
<td>Treatment for urinary frequency, number (%)</td>
<td>42 (87.5%)</td>
<td>42 (87.5%)</td>
</tr>
<tr>
<td>Oxybutinin</td>
<td>12 (25%)</td>
<td>14 (29.2%)</td>
</tr>
<tr>
<td>Treatment for constipation, number (%)</td>
<td>40 (83.3%)</td>
<td>42 (87.5%)</td>
</tr>
<tr>
<td>Fibre</td>
<td>14 (29.2%)</td>
<td>16 (33.3%)</td>
</tr>
<tr>
<td>Laxative</td>
<td>11 (22.9%)</td>
<td>15 (31.3%)</td>
</tr>
<tr>
<td>Enema</td>
<td>1 (2.1%)</td>
<td>6 (12.5%)</td>
</tr>
</tbody>
</table>

2.5 Summary

2.5.1 Deterioration during follow up

Prospectively collected data was analysed on 48 patients with HAM who were followed up from 1993 to 2007 at St Mary’s Hospital. Most patients were female and of Afro-Caribbean origin. The median duration of follow up at St Mary’s Hospital was 3.8 years and the median duration of HAM up to
last follow up 11.6 years. More than 80% of patients had been breast-fed and had a history of relevant unprotected sexual intercourse. Four (8%) patients recalled blood transfusions as their only risk factor for HTLV-1 acquisition. An association between blood transfusions and HTLV-1 viral load at first visit or progression of HAM could not be established most probably because of the small sample size. Most patients recalled unilateral leg weakness as their first HAM associated symptom. During follow up patients progressed to one walking stick within a median time of 11 years and to wheelchair dependence within 18 years. Three (6%) patients lost their ability to walk within two years of onset and were defined as rapid progressors. 33% of all patients progressed in their need for additional aid during follow up and timed walk worsened in all these patients. At last follow up timed walk had worsened in 77% of all patients with an overall deterioration in tw of the whole group of + 1.98 sec/10m/y.

The rate of progression in timed walk was 2.32 sec/10m/y for patients in group AID0, 1.95sec/10m/y in group AID1, 0.48 sec/10m/y in group AID2. Significantly more patients of younger age of onset required additional aid during follow up (<50 years: 12 patients v >50 years: 4 patients) and had higher median HTLV-1 viral loads at both visits (first visit: 11.2 v 1.7, p = 0.09; last visit: 14.9 v 5.5, p = 0.05).

At first and at last visit 60% and 53% of patients had pain, mostly located in the back. Of these patients 69% had permanent pain at first visit and 81% at last visit. Although the median pain score on a visual analogue scale was 5.9 at first and 6.4 at last visit (p=0.2) the pain score changed considerably for different subgroups over time. During follow up the pain worsened significantly in 23%, improved in 58% of the cohort, of which 27% reported themselves to be pain free at last follow up. There was no significant difference in the need of aid, timed walk and presence or absence of pain or pain score at either time points. However the clinical impression was that time walked improved with better pain management for individual patients during follow up (data not presented).
16% of patients reported bladder problems as their first symptom of HAM. At first visit 90% of all patients and at last visit 85% reported bladder dysfunction and were most commonly diagnosed with a spastic bladder (86%). Significantly more patients needed bladder catheterisation at last follow up and one patient had developed obstructive nephropathy. 63% of patients were constipated at both time points.

The mortality rate was 2.4/100 py follow up including one death due to ATLL and 1.9/100 py follow up when this was excluded. In the univariate analysis the rate of deterioration of timed walk, irrespective of additional need for walking aid, was non-significantly associated with higher HTLV-1 viral load at first visit ($p=0.2$).

In a multivariate analysis age of onset predicted 75% of those patients who progressed during follow up in their need for aid. None of the demographic or laboratory parameters showed a statistical measurable association with progression of HAM over time.

### 2.5.2 Improvement during follow up

At last follow up two (4%) patients needed less aid for mobilisation. One patient was using one instead of two walking sticks and one patient was able to transfer from bed to wheelchair. Mean timed walk quickened in six (23%) of patients, one in group AID0 by 2.4 sec/10m (0.92 sec/10m/y), two in AID1 by 4.7 sec/10m (9.66 sec/10m/y), two in AID2 by 35.4 sec/10m (130 sec/10m/y) and one in AID3 8 sec/10m (33.6 sec/10m/y). However all bar one patient were followed up for less than a year which makes the improvement seem more significant.

This initial improvement could also be due the fact that many patients were assessed and newly treated with pharmaco- and/or physio-therapy which they often had not previously received. However the underlying deterioration of HAM resulting in a steady deterioration may obscure transient or fluctuating improvements in patients followed up for longer as only the final outcome (last follow up compared with first assessment) is documented.
Therefore transient improvement or stalling of the disease process might have been missed.

At last follow up 16 (62%) patients reported improvement of pain, eight were pain free and eight patients had reduced pain scores. Constipation had improved in 25% and bladder symptoms had resolved in 17% of patients at last visit.

2.6 Discussion

Due to the ethnicity of our cohort our data on the natural history of HAM is most comparable with data from the Caribbean countries and from Brazil (93;96). Similarities are the female dominance in disease prevalence, heterosexual intercourse and being breast-fed as the route of acquisition of infection, age of onset before 50, gait impairment as the most common first symptom and slow but continuous progression of disability as well as high but stable median HTLV-1 viral load through out follow up (14 copies/100 PBMCs, 95% CI 13-28).

HTLV-1 viral load was not associated with increased use of walking aids in cohort as a whole but was higher in patients with early age of onset, who progressed in their need for aid and rate of timed walk.

The rate of deterioration of timed walk was associated with higher HTLV-1 viral load at first visit irrespectively of an increase of aid usage although only weakly in our cohort. This association might be more significant in a larger cohort. These data cannot be compared with other observational studies since to our knowledge timed walk measurements have not been included in longitudinal outcome studies so far.

On average patients are diagnosed earlier after the onset of HAM in the UK (mean 3.8 and median 2 years) than in Martinique (mean 5.3 and median 3 years). This might be due to patients’ ability and/or choice to access healthcare earlier. From the time of onset a patient with HAM is able to
remain ambulant and without the need for an aid for the first 10 years in the UK. Then he/she continues walking for another 10 years with a walking aid until eventually he/she becomes wheelchair bound. Although our patients could walk without an aid for a longer period compared with patients in Martinique (10 y v 6 y), patients in both cohorts stayed ambulant for about two decades (18 y v 21y). The difference in the time to first walking aid could be due to earlier diagnosis of HAM in the UK. It is also possible that the delay in need of aid was due to a more intense physiotherapy and symptomatic treatment of pain and spasticity in the UK. This hypothesis however needs to be verified. The similarity between time from onset to a single walking aid and to bilateral aid was unexpected suggesting that this may not be a outcome good measure in our setting. We report the progression of walking disability by timing the patient’s walk along a straight line of 10 meters. Comparing a mean of the first two with the last two timed walks can reflect change in speed of walking within an aid group despite fluctuations at different time points. This is most meaningful for those patients who were not using any aid and were followed up for more than one year. Our data gives an expected rate of deterioration in walking speed of about 2.32 sec/10m/year.

A large Japanese cohort study showed that 202 HAM patients had significantly higher HTLV-1 viral load than 234 HTLV-1 asymptomatic carriers (51). Our prospectively collected longitudinal data on HTLV-1 viral load of patients with HAM shows that the mean and median of HTLV-1 viral load did not change significantly over time, more importantly a patient who developed HAM during follow up had a high set point of HTLV-1 viral load prior to onset of symptoms which fluctuated only in-significantly over time. A previous publication reported that asymptomatic carriers followed up at the same HTLV-1 clinic from 1991 to 1998 had a median HTLV-1 viral load of 1.4 copies/100 PBMCs (0.003-70) compared to a median of 14 copies/100 PBMCs patients with HAM at their first clinic visit (111). This confirms that the HTLV-1 viral load of patients with HAM is 10 times higher compared to those who are asymptomatic but we do not have enough data to proof a significantly higher VL preceding the development of HAM.
A report from Health Protection Agency documented a higher mortality rate associated with HTLV-1 infection in the UK in patients of Afro-Caribbean ethnicity and in those living far from centres looking after HTLV-1 positive patients (225). HTLV-1 infection was associated with a 1.3 times increased risk of death from all causes excluding ATLL in Japan (221). The mortality rate was 1.7 for HTLV-1 infected compared to a HTLV-1 uninfected population in Guinea-Bissau and was associated with higher HTLV-1 viral loads. However this publication does not specify the cause of death and the prevalence of ATLL (222). 25% of 123 patients with HAM from Martinique died within 20 years of disease onset (96). In our population 10.6% of the patients with HAM died within a mean of 9.7 years from the onset of HAM. The mortality rate in our cohort, when excluding one death due to ATLL, was 1.9/100 py follow up compared to an age-adjusted mortality rate of 1/100 in general UK population. Therefore a higher rate of all cause non-neoplastic mortality is associated with HAM in the UK. Deaths in our cohort were more common in younger, more disabled patients.

This study has many of the limitations that a cohort study would have: small numbers, in some cases a follow up period < 1 year, no comparable arm, and not controlled for medication (including potentially disease modifying ones) and physiotherapy. Especially when reporting improvement of tw all bar one patient with documented improvement were followed up for less than a year, which may exaggerate the improvement. However it also implies that improvement, even if temporary, is possible, whether by disease modifying or symptomatic therapy, even in patients with chronic disease.

In order to be able to prevent HAM from developing we need to discover the factors that determine the HTLV-1 viral load and mediators that eventually, after a clinically silent period lead to tissue damage. Nature has provided us with a working model, the asymptomatic HTLV-1 carrier, in whom the virus and host appear to co-exist in harmony.
In patients with HAM the goal remains the early diagnosis post onset and the provision of a combination of anti-inflammatory/ immuno-modulatory drugs that can dampen the bystander tissue damage and/or a second agent that can effectively reduce the HTLV-1 viral load. These therapies can be identified through national and international clinical trials. Until then the quality of life of patients with HAM can be effectively improved through physiotherapy, muscular relaxants, analgesics, bladder care and laxatives as observed in our cohort.
Chapter 3

3 Incidence of new inflammatory events in patients with existing HTLV-1 associated inflammatory disease compared to asymptomatic carriers.

3.1 Background

Many of the inflammatory conditions associated with HTLV-1 infection have been described mainly in patients with HAM (see Chapter 1.2.2). HTLV-1 infection and HAM have both been associated with a higher rate of mortality compared to the non-infected population (see Chapter 1.3). The incidence of any conditions with an inflammatory nature as well as those conditions already strongly associated with HTLV-1 infection or HAM have not been reported for the infected population in the UK.

3.2 Objectives

We hypothesise that:

- Patients with an existing HTLV-1 associated inflammatory disease (HAID) will develop further HTLV-associated inflammatory diseases more commonly than HTLV-1 asymptomatic carriers (AC).
- Laboratory markers associated with HAM (i.e. elevated serum globulin (229), serum tumour necrosis factor-alpha (TNF-α) (120;230;231), HTLV-1 viral load (51;111;232) and levels of Tax expression ex vivo (112), will correlate with the development of other inflammatory disease.
- HTLV-1 infected patients who develop inflammatory events will have a higher rate of mortality.
We therefore aimed to determine the following outcome measures comparing patients with HAID to ACs:

- The incidence of all inflammatory events (IE)
- Markers of disease activity
- Mortality

3.3 Material and Methods

Data were collected prospectively on all patients, attending the HTLV clinic at St Mary’s Hospital in a clinical database and validated from the patients’ medical records. Patients who were either ACs or already had an HAID at the time of presentation were included in the study. The minimum follow-up was one month. Asymptomatic carriers were routinely followed up every 6 to 12 months while patients with inflammatory conditions every 4 weeks to 3 months. From this study patients with malignancy, immunosuppression and HIV co-infection were excluded. New IEs were only included if they could not be attributed to any other aetiology (e.g. metabolic, vascular, autoimmune or neoplastic). HTLV-1 associated IEs were defined as those already described in the literature as outlined in chapter 1. Changes in HTLV-1 viral load (VL), lymphocyte count, CD4+ T lymphocyte count, plasma globulin levels were analysed at two time points for both groups, first and last clinic visit. Ex vivo Tax expression by CD4+ T lymphocytes and plasma soluble TNF-α receptor I (sTNFα RI) were measured on one occasion during follow-up. The data collection was initiated in 1992 and censored in December 2006.

3.3.1 Routine laboratory markers

Full blood counts were measured on a Coulter LH750 Analyzer (Beckman Coulter Inc, Fullerton, CA) and T cell differentiation markers by flow cytometry (FACScalibur). The renal, liver and bone chemistry assays were
performed on an AU2700 Olympus Analyser (Olympus Diagnostica GmbH, Hamburg, Germany). These analyses were performed by St. Mary’s Hospital departments of haematology and biochemistry as part of routine clinical care.

3.3.2 Plasma globulin

The total plasma globulin level was calculated subtracting serum albumin from the total serum protein. IgG, IgM and IgA levels were determined directly on the Beckman Image, (Beckman Coulter Inc., USA) which utilises serum/plasma rate turbidimetry. These analyses were conducted by the department of biochemistry, St. Mary’s Hospital as part of routine clinical care. (http://www.beckman.com/products/testmenu/immage.asp).

3.3.3 HTLV-1 viral DNA

HTLV-1 viral DNA was quantified by ‘in house’ real-time PCR using the Roche LightCycler (Roche, Mannheim, Germany) (233). Genomic DNA extracted from 1 x 10^3 peripheral blood mononuclear cells (PBMCs) (52) was used as a template for amplification. In addition, the reaction mixture contained 2 µl of 10x LightCycler-DNA Master SYBR Green 1(Roche, Mannheim, Germany), 0.5 µM of each HTLV-1 primer [SK43 and SK44; (234)] and a final MgCl₂ concentration of 3.5 mM, made up in water to a volume of 20 µl. After denaturation at 95°C for 30 s, the DNA was amplified for 40 cycles: denaturation at 95°C for 10 s, annealing at 58°C for 5 s and extension at 72°C for 8 s. At the end of each cycle, the SYBR Green 1 incorporated into dsDNA was quantified by measurement of fluorescence at 525 nm. Fluorescence was detected at 85°C, below the melting temperature of the specific product but above the melting temperature of primer-dimers. The β-globin copy number of each sample was similarly quantified, using primers PC03 and PC04 (235) and a final MgCl₂ concentration of 4 mM.
Standard curves were generated for both PCRs using genomic DNA from C10-PBL cells, which contains 1 Tax copy per cell. Reactions containing $10^4$, $10^3$, $10^2$ and $10^1$ copies were used to generate the standard curves. The copy numbers in the samples were estimated by interpolation from the standard curves. The HTLV-1 viral load was then calculated as: 

\[
\text{Tax copies} / \left[ \beta_2\text{-globulin copies} \right] \times 100 = \text{Tax copies/100 PBMCs.}
\]

Later standard curves were generated using a MT-2 cell line containing 7 copies of the amplified Tax sequence. These assays were performed by Silva Youshya in the department of GU Medicine, Imperial College as part of routine clinical care.

3.3.4 Soluble tumour necrosis factor α- receptor I

Plasma soluble Tumour necrosis factor α-R1 (sTNF α-R1) levels were measured by Quantikine Human sTNF α-R1 Immunoassay (R&D System, Quantikine Minneapolis, USA), a 4.5 hour solid phase ELISA, according to the manufacturer’s instructions by the author on blood samples donated by the participants for HTLV research.

3.3.5 Tax expression

*Ex vivo* Tax expression was measured as previously described (236): CD8+ cells were positively selected from thawed cryo-preserved PBMC using magnetic micro-beads (Miltenyi Biotec, Germany). The CD8+ fraction was washed twice and re-suspended in standard culture medium (total volume 1 ml) in 5 ml round-bottomed, vented capped tubes. After 18 hours’ culture at 37°C, 5% CO2, the cells were washed in PBS, fixed for 20 minutes at room temperature in 2% para-formaldehyde (pH 7.4; Sigma), washed then surface-stained for CD4 and CD8 antigens by incubation at room temperature for 20 minutes in PBS/ 7% normal goat serum with relevant mAbs (15 μg/ml of PC5-conjugated anti-CD4 and ECD-conjugated anti-
CD8; Beckman Coulter). The cells were washed once and stained intra-cellularly for Tax protein (237) using the Tax monoclonal antibody Lt-4 (238), then analysed by flow cytometry on a Coulter EPICS XL. All assays were done in duplicate and the proportion of CD4+ lymphocytes that were Tax positive was calculated. The average purity of CD8+ cells was 96% with a minimum purity of 88%. These assays were performed by Angelina Mosley in the department of Immunology, Imperial College on samples donated by the participants for HTLV research.

3.3.6 Statistical methods

The data were originally recorded in an Excel spreadsheet. Data was analysed in SPSS-14 through parametric (t-test) and non-parametric tests (Mann-Whitney Test and Wilcoxon Signed Ranks Test) for continuous variables and chi-square (Pearson Chi-Square Test for large and Fisher’s Exact test small sample size) and odds ratio/relative risk for categorical variables. Kaplan Mayer survival analysis was used for survival data (Tarone –Ware Test was used if the survival curve for the two groups crossed over time). A multivariate logistic regression model was used to identify independent predictors of inflammatory events. Backward Wald was used for the best-fit model. Data of patients with and without IE were compared between the two groups and within each group.

3.4 Results

3.4.1 Description of the cohort

Twenty /160 patients were excluded from the study: 17 due to insufficient follow-up time or incomplete data, two due to HIV co-infection and one had a past history of malignancy (non-Hodgkin lymphoma).
140 patients were included in the study (Table 1): 95 were AC and 45 had HAID. The pre-existing HTLV-1 associated inflammatory conditions of the patients with a HAID were: HAM in 41 (91%), polymyositis in two (4%), encephalitis in one (2.5%) and uveitis in another patient (2.5%).

Table 1: The demographic details of the AC and the HAID groups.

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>95</td>
<td>45</td>
<td>140</td>
</tr>
<tr>
<td>Age median years (range)</td>
<td>40 (3-75)</td>
<td>57 (19-75)</td>
<td>48 (3-75)</td>
</tr>
<tr>
<td>Age at first clinic visit</td>
<td>45 (3-72)</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Age diagnosed with HTLV-1</td>
<td>X</td>
<td>48 (11-72)</td>
<td>X</td>
</tr>
<tr>
<td>Duration of follow up, median years (range)</td>
<td>1.1 (0.1-14)</td>
<td>4.4 (0.2-12)</td>
<td>1.5 (0.1-14)</td>
</tr>
<tr>
<td>Gender/Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>75 (79%)</td>
<td>35 (78%)</td>
<td>110 (79%)</td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>62 (65%)</td>
<td>35 (78%)</td>
<td>97 (69%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>18 (19%)</td>
<td>3 (7%)</td>
<td>21 (15%)</td>
</tr>
<tr>
<td>Other (African, mixed, Iranian)</td>
<td>15 (16%)</td>
<td>7 (15%)</td>
<td>22 (16%)</td>
</tr>
<tr>
<td>Risk Factors for HTLV-1 infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>3 (3.2%)</td>
<td>1 (2.2%)</td>
<td>4 (2.9%)</td>
</tr>
<tr>
<td>Sexual intercourse</td>
<td>19 (20.2%)</td>
<td>3 (6.7%)</td>
<td>22 (15.7%)</td>
</tr>
<tr>
<td>Breastfeeding + Sexual intercourse</td>
<td>58 (60.7%)</td>
<td>38 (84.4%)</td>
<td>96 (68.6%)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>14 (14.9%)</td>
<td>3 (6.7%)</td>
<td>17 (12.1%)</td>
</tr>
<tr>
<td>Unknown risk factors</td>
<td>1 (1%)</td>
<td>0</td>
<td>1 (0.01%)</td>
</tr>
</tbody>
</table>

Table 1: X= The mean time of diagnosis of HTLV-1 infection could not be ascertained for the whole HAID cohort since most patients were referred to our centre after the diagnosis had been made elsewhere and the date could not be determined. AC= asymptomatic carrier, HAID= HTLV-1 associated inflammatory conditions.

A larger proportion of ACs than patients with HAID were Caucasian (AC 18 [19%], HAID 3 [7%], p= 0.05) and blood donors (AC 14 [15%], HAID 3 [6.7%], p= 0.14) since they were referred to the clinic by the National Blood Service. The median age of the cohort was 48 years but the ACs were 17 years younger than the patients with HAID (40 [3-75] v 57 [19-75] years, p< 0.0001) at first clinic visit. The majority of patients, in both groups were female (AC 75 [79%], HAID 35 [78%], p= 0.5).
Most AC were either referred to the clinic by the National Blood Service or were diagnosed through contact tracing at the clinic and therefore had an exact date of first diagnosis of HTLV-1 infection. However the care of most patients with HAID was transferred from other medical centres and although HAID patients could remember (approximately) when they had developed first symptoms, finding documentation of the exact date of diagnosis was more difficult and this date is therefore not included in the final analysis. By definition the patients with HAID had developed at least one inflammatory condition prior to their first clinic visit and the self-reported median age of onset of the presenting symptom was 49 years (mean 47 [11-72]). This matched the median age of onset of an IE in the ACs (49 years (mean 49 [32-72]) which was directly observed during follow up.

One or more risk factors for HTLV-1 infection were identified in 139 patients. In the majority (127 [91%]) two risk factors were identified which could be neither confirmed nor refuted - having been breastfed by a mother from an endemic region and having had sexual intercourse with someone originating from an endemic region. In a minority, unprotected sexual intercourse, with a partner from an endemic region (18 [13%]), or blood transfusion (12 [9%]) were the sole identifiable risk factors. Five (3.6%) patients were blood product recipients as well as originating from an endemic region. 18/140 (12.9%) patients had been blood product recipients in the past and three had developed HAM before presentation. During follow up one of these patients with HAID and two ACs developed an IE. This gives a prevalence of 17% (3/18) IEs during follow up and 33% (6/18) of total IEs, including three cases of HAM prior to first clinic visit, among all HTLV-1 positive blood transfusion recipients. Therefore compared to the whole cohort the risk of an IE ($p < 0.0001$), and especially the development of HAM ($p < 0.0001$), was increased among blood product recipients.

The median duration of follow up of ACs was 1.1 (0.6-14) years as compared to 4.4 (0.2-12) years for the HAID group. ACs who had remained asymptomatic were followed up for the shortest period (median 1 year, [0.1-12.8]), mainly because blood donor screening for HTLV-1 was introduced in the UK in August 2002 which led to a surge of newly identified ACs and
referrals to the outpatient clinic. However nearly twice as many ACs as HAID patients were followed up and the cumulative follow up was 262 person-years (py) for the ACs and 239 for the patients with HAID.

### 3.4.2 Incidence of new inflammatory events

17/95 (18%) ACs developed 23 IEs during a median of 1.1 years (mean 2.3 years, [range 0.1-14]) and 262 py follow up. 15/45 (33%) patients with an existing HAID developed 22 new IEs during a median of 4.4 years (mean 4.4 years [range 0.2-12]) and 239 py follow up. 23% (32/140) of the whole HTLV-1 infected cohort developed at least one new IE during a median of 2.2 years (mean 3.4 years [0.1-14]) and a total of 501 py follow up.

13 (14%) AC developed one new event, three (3%) developed two, and one (1%) developed four different new IEs during follow up. In the HAID group eight (18%) patients developed one and seven (16%) patients developed two new IEs during follow up. 7.9% (11/140) of the whole cohort developed a second IE during follow up and this was observed significantly more often in patients with HAID (OR 4.2 95% CI: 1.2-15, \(p= 0.03\)). Note that for this analysis ACs who developed an IE are not reclassified as patients with HAID.

Taking into account, that HAID patients already had at least one IE before their presentation to the clinic, 46% (64/140) of HTLV-1 infected patients had two or more IEs by the end of the observation period.

The overall incidence of inflammatory disease was 9.0/100 py (45/501 py follow up) for the whole cohort: 8.8 /100 py (23 IEs/262 py follow up) for ACs compared to 9.2/100 py (22 IEs/239 py) for patients with HAID (Relative Risk: AC/HAID= 0.96). The incidence of an IE among the ACs fell to 7.7 /100 py (19 IEs/246.5 py) if the AC who developed four new IEs was excluded from the analysis.
The incidence rates, when only IEs historically associated with HTLV-1 infection are considered, was 4.6/100 py (23 IEs/ 501 py) for the entire HTLV-1 infected cohort, 3.4/100 py (9 IEs/ 262 py) for ACs and 5.9/100 py (14 IEs/239 py) for patients with HAID (Relative Risk: AC/HAID= 0.58).

3.4.3 Survival analysis: Estimated time to develop a new IE

The median time to diagnosis of a new IE from first clinic visit was 2.7 years (mean: 4.8 years [0.7-17]). Of all first IEs recorded 66% (21/32) were diagnosed in the first two years of follow up (71% [12/17] in ACs and 60% [9/15] in patients with HAID). Keeping in mind the median follow up for AC is 1.1 years these result are not surprising. It is possible that the diagnosis of new IEs would be evenly distributed throughout the years of follow up if ACs were followed up longer.

The mean estimated time for a patient with HAID to develop an additional IE during follow up is 8.0 years (95% CI: 6.3-9.8). For ACs the mean estimated IE free survival time is 8.2 years (95%CI: 6.4-10.1). This difference is not statistically significant (p=0.43, Figure 1).

Figure 1: Kaplan Maier curve for time to any first IE comparing each group.
Figure 1: AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease.

However there was a significant difference in IE free survival time between patients with HAID and AC when only IEs strongly associated with HTLV-1 infection were considered ($p = 0.044$, Figure 2). So that although the mean estimated event free time for a patient with HAID stayed roughly the same (8.2 years; 95% CI: 6.5-9.9), ACs remained disease free for longer (9.4 years, 95%CI: 7.4-11.5).

Figure 2: Kaplan Maier curve for time to an IE strongly associated with HTLV-1 when comparing each group.
Figure 2: AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease.
3.4.4 New inflammatory events

To avoid excluding IEs that had not been described in literature as being associated with HTLV-1 at the outset of the study, data on every IE that remained idiopathic after investigation were collected. A complete list of observed IEs is given in Table 2. The most common internationally acknowledged IEs associated with HTLV-1 infection that were diagnosed in the two groups were HAM (1AC), uveitis (3AC, 4HAID), alveolitis (1AC, 2HAID), arthritis (2AC, 2HAID), myositis (1 HAID), Sjögren’s syndrome (1HAID) and conjunctivitis (1AC). Patients with HAID develop IE’s that are more strongly associated with HAM and HTLV-1 infection than AC patients (9 AC, 14 HAID: OR: 4, 95%CI: 1.6-10, p= 0.002). In the HAID group 21/23 (91%) IEs were diagnosed in HAM patients and 12/21 (57%) of these were strongly associated with HTLV-1 infection. One patient with polymyositis was diagnosed with two IEs, Sjögren’s disease and bronchiectasis.

Table 2: All IEs documented during follow up of this cohort.

<table>
<thead>
<tr>
<th></th>
<th>AC (17)</th>
<th>HAID (15)</th>
<th>Total (32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with IE</td>
<td>17</td>
<td>15</td>
<td>32</td>
</tr>
<tr>
<td>Number of IEs developed</td>
<td>23 (100%)</td>
<td>22 (100%)</td>
<td>45 (100%)</td>
</tr>
</tbody>
</table>

Neurological disease

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of vibration sense in feet</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Anosmia</td>
<td>0</td>
<td>1 (4.5%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Bell’s Palsy</td>
<td>0</td>
<td>1 (4.5%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Radiculopathy</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Vertigo</td>
<td>1 (4%)</td>
<td>1 (4.5%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Paresis L arm and leg</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Cognitive dysfunction</td>
<td>2 (8.7%)</td>
<td>0</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

Eye disease

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uveitis</td>
<td>3 (13%)</td>
<td>4 (18%)</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>Keratoconjunctivitis</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Sjögren's Syndrome</td>
<td>0</td>
<td>1 (4.5%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Musculoskeletal disease

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arthritis</td>
<td>2 (8.7%)</td>
<td>2 (9%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>Myositis</td>
<td>0</td>
<td>1 (4.5%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Bursitis/Tendonitis</td>
<td>1 (4%)</td>
<td>2 (4.5%)</td>
<td>3 (7%)</td>
</tr>
</tbody>
</table>
### Respiratory disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolitis</td>
<td>1 (4%)</td>
<td>2 (9%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>0</td>
<td>4 (18%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>2 (8.7%)</td>
<td>1 (4.5%)</td>
<td>3 (7%)</td>
</tr>
</tbody>
</table>

### Hepatobiliary disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis</td>
<td>3 (12.7%)</td>
<td>2 (9%)</td>
<td>5 (11%)</td>
</tr>
</tbody>
</table>

### Mucocutaneous disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lichen planus</td>
<td>2 (8.7%)</td>
<td>0</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

Table 2: IE = Inflammatory event. AC = asymptomatic carrier. HAID = HTLV-1 associated inflammatory disease.

Table 3: List of inflammatory events known to be strongly associated with HAM that occurred in the cohort.

<table>
<thead>
<tr>
<th>Condition</th>
<th>AC</th>
<th>HAID</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with IE</td>
<td>7</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Number of IEs developed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 (100%)</td>
<td>14 (100%)</td>
<td>23 (100%)</td>
</tr>
<tr>
<td>HAM</td>
<td>1 (11%)</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Loss of vibration sense – feet</td>
<td>1 (11%)</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Uveitis</td>
<td>3 (33%)</td>
<td>4 (29%)</td>
<td>7 (30%)</td>
</tr>
<tr>
<td>Keratoconjunctivitis</td>
<td>1 (11%)</td>
<td>0</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>0</td>
<td>1 (7%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>0</td>
<td>4 (29%)</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>Alveolitis</td>
<td>1 (11%)</td>
<td>2 (14%)</td>
<td>3 (14%)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>2 (23%)</td>
<td>2 (14%)</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>Myositis</td>
<td>0</td>
<td>1 (7%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

Table 3: IE = inflammatory events. AC = ACs. HAID = HTLV-1 associated inflammatory disease, HAM = HTLV-1 associated myelopathy.

#### 3.4.4.1 HTLV-1 associated uveitis

Overall the most commonly recorded IE was uveitis/iritis (7/140 [5%]), at a mean age of 51 (34-74) years. Uveitis was diagnosed by split lamp examination by an ophthalmologist. Amongst ACs uveitis was found in 3/95 (3.2%) and amongst HAID patients 4/45 (8.9 %, p=0.15). All patients with HAID who developed uveitis had HAM. The incidence of HTLV-1 associated uveitis was 1.1 /100 py follow up for the AC group (3 IEs/ 262 py follow up), 1.7 /100 py follow up for the HAID group (4 IEs/ 239 py follow up) and 1.4/100 py follow up for the whole cohort (7 IEs/501 py follow up). Taking follow up into account the mean estimated uveitis free time for the AC group was
14.5 years (95% CI: 13.6-15.5), 12 years (95% CI: 10.8-13.2, p = 0.3) for the HAID group and 14 years (95% CI: 13.5-15.1) for the whole group.

### 3.4.4.2 Idiopathic transaminitis

So far hepatitis or transaminitis have not been reported to be caused by, or associated with, HTLV-1 infection. However, 3.6% (5/140) of our patients had persistently elevated liver enzymes. There was no statistical difference in the occurrence of transaminitis in the two study groups (p = 0.52). This was documented in 3/95 (3.2%) patients in the AC group (two male, one female, mean age 42 [36-58] years) of whom two had raised alanine transaminase (ALT) and gamma-glutamyl transpeptidase (γ-GT) and one had raised ALT only. Two out of 45 (4.4%) patients with HAID (one male, one female, mean age 47 [43-52] years) had both raised ALT and γ-GT (Table 4). The incidence rate was 0.9 / 100 py follow up for the whole group (5/501 py follow up), 1.1 / 100 py for ACs (3/262 py follow up) and 0.8 for patients with HAID (2/239 py follow up). Taking follow up into account the mean estimated hepatitis free time for the AC group was 14 years (95% CI: 13.3-14.5), 11.6 years (95% CI: 11-12, p = 0.3) for the HAID group and 14 years (95% CI: 13.4-14) for the whole group.

All patients were asymptomatic and no cause, such as viral, autoimmune, drug toxicities (including alcohol) or metallic (copper/iron) overload for the elevated ALT could be found in these patients. Of the four patients who had abdominal ultrasounds two were reported as normal, one demonstrated fatty infiltration of the liver and the fourth showed evidence of liver cirrhosis.

Either the patients did not agree to, or the hepatologist did not recommend, a liver biopsy.
Table 4: Liver function tests and findings of the ultra-sound and liver biopsies in patients with ideopathic hepatitis.

<table>
<thead>
<tr>
<th>Hepatitis</th>
<th>ALT (0-40iu/L)</th>
<th>GGT (&lt;50 iu/L)</th>
<th>Bili (0-17μmol/L)</th>
<th>ALP (30-130u/L)</th>
<th>CK (150-0u/L)</th>
<th>LDH (150-450 u/L)</th>
<th>USS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAID</td>
<td>119</td>
<td>250</td>
<td>-</td>
<td>-</td>
<td>49</td>
<td>921</td>
<td>-</td>
</tr>
<tr>
<td>HAID</td>
<td>111</td>
<td>457</td>
<td>6</td>
<td>180</td>
<td>48</td>
<td>540</td>
<td>Fatty liver</td>
</tr>
<tr>
<td>AC</td>
<td>61</td>
<td>40</td>
<td>11</td>
<td>110</td>
<td>167</td>
<td>249</td>
<td>Normal</td>
</tr>
<tr>
<td>AC</td>
<td>103</td>
<td>110</td>
<td>39</td>
<td>78</td>
<td>345</td>
<td>422</td>
<td>Normal</td>
</tr>
<tr>
<td>AC</td>
<td>87</td>
<td>48</td>
<td>9</td>
<td>104</td>
<td>148</td>
<td>354</td>
<td>Cirrhosis</td>
</tr>
</tbody>
</table>

Table 4: ALT= alanine-transaminase, γ-GT= γ-glutamyl transferase, Bili= bilirubin, ALP= alkaline phosphatise, CK=creatinine kinase, LDH= lactate dehydrogenase, USS= liver ultrasound.
3.4.4.3 HTLV-1 associated bronchiectasis

Four (8.9%) patients with HAID, but none of the ACs, were investigated for chronic cough or abnormal lung function. All four were reported to have changes in lung parenchyma in keeping with bronchiectasis \((p=0.024)\) on high-resolution pulmonary computerised tomography. The mean age of diagnosis was 54 (41-63) years, three patients were male and one female, three had HAM and one had polymyositis at first clinic visit. Three patients were diagnosed with bilateral basal and the fourth with right middle lobe bronchiectasis. With an incidence of 1.9 /100 py follow up bronchiectasis was nearly as common as uveitis in this group. The mean estimated bronchiectasis-free time for patients with HAID was 11 years (95%CI: 9.8-12) during follow up.

Figure 3: Computer tomography of chest showing bronchiectasis in both lung fields but mainly the middle lobe.
### 3.4.4.4 Neurological conditions

Only one patient developed HAM at the age of 39 within 14 months of follow up in the AC group. The incidence of HAM in ACs was 0.4/100 py follow up. She was also subsequently diagnosed with alveolitis, sinusitis and encephalitis. The majority of patients in the HAID group had HAM as their presenting disease.

However a variety of other, less common, neurological conditions were documented: Loss of vibration sense (1AC), radiculopathy (1AC) and cognitive dysfunction (2AC) have been associated with HAM previously (239). Other, rather atypical, neurological conditions were rare events in either group: acute encephalitis (1AC), vertigo (1AC, 1HAID), anosmia (1HAID), Bell’s palsy (1HAID) and left hemiparesis (1AC). The patient with left hemiparesis was young and only mildly affected with no evidence of acute vascular event or CNS malignancy.

### 3.4.5 Mortality

Seven/ 140 (4.3 %) patients infected with HTLV-1 died during follow up. All were in the HAID group (7/45, 16%, \( p = 0.001 \), Figure 3). Five deaths were associated with HTLV-1 infection and all but two patients had HAM. This gives an incidence/ mortality rate for patients with HAID of 3/ 100 py follow up (7 cases/ 239 py). The causes of death were lymphomatous ATLL (n=1) in a patient with HAM, encephalitis in a patient who had no other infections but HTLV-1 (n=1), respiratory failure due to HTLV-1 associated polymyositis (n=2) and rapidly progressive HAM with disseminated inflammation (n=2).
The cause of death of a wheel-chair dependent patient with HAM older than 70 years was not reported. Death in these seven patients occurred after a mean of 3.3 years (median 2.9, range 0.9-6) of follow up. Median age at time of death was 48 years.

Although at last follow up the ACs were significantly younger than the HAID group (median 48 [3-73] v 63 [19-82], \( p<0.0001 \)), the patients who died were significantly younger than the HAID patients who remained alive (HAID died: median 48 [21-78] v HAID alive: 67 [19-82], \( p= 0.03 \)). Three out of 15 HAID patients who had developed a new IE during follow up died compared to four out of 30 who did not (\( p= 0.4 \)). None of the deaths could be attributed to the new IEs.

Figure 4: Kaplan Meier survival curve for time to death comparing each group in months.

Figure 3: Cum Survival= cumulative survival. IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease.
3.4.6 Laboratory results

3.4.6.1 Lymphocyte count

Data for the lymphocyte count (* 10^9 /L) was available on 79% ACs and all patients with HAID for both time points. There was no significant difference in mean lymphocyte count between the two groups at first (p=0.8) and last clinic visits (p = 0.2).

First visit:
AC: 1902 (900- 4500), 95% CI: 1767-2038, median 1800
HAID: 1958 (800-4500), 95% CI: 1729-2186, median 1800

Last visit:
AC: 1969 (110-4200), 95% CI: 1809-2128, median 1900
HAID: 2045 (800-4000), 95% CI: 1818-2272, median 1800

In a non-parametric two related samples test comparing the mean lymphocyte count of each group at first visit with last visit, there was no significant difference in the AC group (p= 0.5). However in patients with HAID the mean lymphocyte count was significantly higher at last visit (p= 0.01). This effect was also seen when considering those patients who did not develop an additional IE (p= 0.03) but did not reach statistical significance for those who did develop an IE (p=0.2).

As demonstrated in Table 5 and Figure 4 there was no significant difference when comparing the mean lymphocyte count of the patients within each group, those without IE with those with IEs, at first (AC: p= 0.3, HAID: p=0.4) or at last visit (AC: p= 0.1, HAID: p=0.3).
Table 5: Mean lymphocyte counts of each study group with or without IE at two time points.

<table>
<thead>
<tr>
<th>Lymphocyte count (×10⁹/L)</th>
<th>First clinic visit (range)</th>
<th>First clinic visit median</th>
<th>95%CI</th>
<th>p</th>
<th>Last clinic visit (range)</th>
<th>Last clinic visit median</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC without IE</td>
<td>1998 (900-4800)</td>
<td>1900</td>
<td>1550-2300</td>
<td>0.3</td>
<td>2122 (900-7300)</td>
<td>1900</td>
<td>1900-2400</td>
<td>0.1</td>
</tr>
<tr>
<td>AC with IE</td>
<td>1817 (900-2800)</td>
<td>1800</td>
<td>1450-2150</td>
<td></td>
<td>1817 (1200-3000)</td>
<td>1700</td>
<td>1700-2150</td>
<td></td>
</tr>
<tr>
<td>HAID without IE</td>
<td>1983 (800-4500)</td>
<td>1750</td>
<td>1664-2302</td>
<td>0.4</td>
<td>2224 (900-4400)</td>
<td>1850</td>
<td>1877-2571</td>
<td>0.3</td>
</tr>
<tr>
<td>HAID with IE</td>
<td>1900 (800-2900)</td>
<td>1800</td>
<td>1598-2202</td>
<td></td>
<td>2253 (1400-3300)</td>
<td>2500</td>
<td>1840-2668</td>
<td></td>
</tr>
</tbody>
</table>

Table 5: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. p≤ 0.05 = significant difference. CI= confidence interval.
3.4.6.2 CD4+ T Lymphocyte count

CD4+ T lymphocyte count (x 10^9/L) for both time points was available on 79% of ACs and all patients with HAID. There was no significant difference in mean peripheral venous CD4+ T lymphocyte count between the AC and the HAID patients at first (p = 0.7) or last clinic visit (p = 0.4). In a non-parametric two related sample test the mean CD4+ T lymphocyte count was compared between first visit and last visit within each group. It was lower in the ACs (p = 0.12) and significantly so for patient with HAID group (p = 0.02) at first compared to last follow up (Table 6). This was mainly attributable to higher CD4+ T lymphocyte counts in patients with HAID who developed a new IE (Figure 6) during follow up.
First visit:

**AC:** 948 (294-3690), 95% CI: 832-1064, median 855  
**HAID:** 930 (290-1940), 95% CI: 781-1079, median 935

Last visit:

**AC:** 1021 (407-6680), 95% CI: 840-1202, median 898  
**HAID:** 1159 (300-2450), 95% CI: 941-1378, median 1060

As demonstrated in Table 6 and Figure 5 there was no difference in median CD4+ count within each group, comparing patients without and with IEs at the first (AC: \( p = 0.5 \), HAID: \( p = 0.7 \)) or at last clinic visit (AC: \( p = 0.6 \), HAID: \( p = 0.9 \)).
Table 6: Median CD4+ T lymphocyte counts for both groups at two time points.

<table>
<thead>
<tr>
<th></th>
<th>First clinic visit mean (range)</th>
<th>First clinic visit median 95% CI</th>
<th>Last clinic visit mean (range)</th>
<th>Last clinic visit median 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC without IE</td>
<td>962 (300-2450)</td>
<td>1380 (130-170)</td>
<td>930 (668-1120)</td>
<td>860 (695-1115)</td>
</tr>
<tr>
<td>AC with IE</td>
<td>710 (460-1230)</td>
<td>690 (715-986)</td>
<td>674 (692-1088)</td>
<td>774 (695-1115)</td>
</tr>
<tr>
<td>HAID without IE</td>
<td>690 (610-1650)</td>
<td>620 (930-1088)</td>
<td>690 (692-1088)</td>
<td>690 (710-1120)</td>
</tr>
<tr>
<td>HAID with IE</td>
<td>962 (300-2450)</td>
<td>1380 (130-170)</td>
<td>930 (668-1120)</td>
<td>860 (695-1115)</td>
</tr>
</tbody>
</table>

Table 6: IE = inflammatory event. AC = asymptomatic carrier. HAID = HTLV-1 associated inflammatory disease. p<0.05 = significant difference within groups. CI = confidence interval.
Table 7: HTLV-1 viral load for patients with or without IE in each study group at two time points.

<table>
<thead>
<tr>
<th>HTLV-1 VL % PBMC</th>
<th>First clinic visit mean (range)</th>
<th>First clinic visit median</th>
<th>95%CI</th>
<th>p</th>
<th>Last clinic visit mean (range)</th>
<th>Last clinic visit median</th>
<th>95%CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC without IE</td>
<td>7.4 (0-77)</td>
<td>2.1</td>
<td>2.8-12</td>
<td>0.7</td>
<td>5.0 (0.0-56)</td>
<td>1.6</td>
<td>2.5-7.5</td>
<td>0.5</td>
</tr>
<tr>
<td>AC with IE</td>
<td>4.7 (0.01-21)</td>
<td>0.7</td>
<td>0.7-8.7</td>
<td>0.7</td>
<td>6.4 (0.0-30)</td>
<td>0.7</td>
<td>1.2-5.5</td>
<td>0.8</td>
</tr>
<tr>
<td>HAID without IE</td>
<td>24 (0.05-70)</td>
<td>17.4</td>
<td>15.4-33</td>
<td>0.5</td>
<td>18 (1.2-65)</td>
<td>12</td>
<td>11-25</td>
<td>0.8</td>
</tr>
<tr>
<td>HAID with IE</td>
<td>27.1 (0-140)</td>
<td>12.5</td>
<td>2.8-51</td>
<td></td>
<td>15.7 (1.4-44)</td>
<td>13.2</td>
<td>8.6-23</td>
<td></td>
</tr>
</tbody>
</table>

Table 7: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. p<0.05 = significant difference within each group. CI= confidence interval.
3.4.6.3 HTLV-1 viral load

Data on HTLV-1 viral load (% PBMCs) at both time points was available for 72% of the cohort. ACs had significantly lower mean HTLV-1 viral load than patients with HAID at first ($p < 0.0001$) and at last clinic visit ($p < 0.0001$).

There was no difference between HTLV-1 VL of ACs or patients with HAID at first visit and last visit (AC: $p = 0.7$, HAID: $p = 0.2$).

First visit:

**AC**: 6.5 (0-77), 95% CI: 3.1-10.5, median 1.5

**HAID**: 25.7 (0-140), 95% CI: 16-35, median 15.3

Last visit:

**AC**: 5.3 (0-56), 95% CI: 3.1-7.5, median 1.5

**HAID**: 16.8 (1.2-65), 95% CI: 11.5-22, median 13
There was no significant difference in the VL of patients in the AC group comparing those who did not, with those who did, develop an IE when analysed at first \((p= 0.7)\) or last clinic visit \((p = 0.5)\). This was also observed for patients in the HAID group at first \((p=0.5)\) and last presentation \((p=0.8,\) Table 7, Figure 6).

In a sub-analysis, within each study group, the mean VL of patients without any IE did not differ significantly with those who developed a “HTLV-1 strongly associated IE” at first \((AC: 6.9 \text{ v } 5.6, p= 0.9, HAID: 27 \text{ v } 18, p= 0.2)\) or last visit \((AC: 5.4 \text{ v } 4.7, p= 0.9, HAID: 19 \text{ v } 11, p= 0.5)\).

Figure 7: Box-plots of HTLV-1 viral load (%PBMCs) for both groups at two time points.

Figure 6: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. HTLV-VL= HTLV-viral load. PBMCs= peripheral blood mononuclear cells.
3.4.6.4 Serum globulin

Data on serum globulin (g/L) levels for both time points were available on 52% of the cohort. The mean serum globulin levels were significantly lower in ACs compared to the HAID group at first ($p = 0.005$) but not at last visit ($p = 0.3$). In the AC group the median serum globulin levels were lower at last follow up compared to first clinic visit ($p = 0.05$) and this was mainly seen in ACs without IE development. But for patients in the HAID group although the biological difference was greater it did not reach statistical significant ($p = 0.2$, Figure 7).

**First visit:**

**AC:** 35 (26-55), 95% CI: 33-37, median 35  
**HAID:** 38 (27-55), 95% CI: 35-41, median 36

**Last visit:**

**AC:** 34 (20-56), 95% CI: 32-36, median 34  
**HAID:** 35 (24-49), 95% CI: 33-38, median 36

As demonstrated in Table 8, **within** each study group, comparing patients with and without IEs, the mean serum globulin levels were not significantly different at the first visit (AC: $p = 0.9$, HAID: $p = 0.4$) or last visit (AC: $p = 0.2$, HAID: $p = 0.6$).
Table 8: Serum globulin levels for each study group at two time points.

<table>
<thead>
<tr>
<th>Serum Globulin (g/L)</th>
<th>First clinic visit mean (range)</th>
<th>First clinic visit median</th>
<th>95% CI</th>
<th>p</th>
<th>Last clinic visit mean (range)</th>
<th>Last clinic visit median</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC without IE</td>
<td>35 (26-55)</td>
<td>36</td>
<td>31-39</td>
<td>0.9</td>
<td>34 (20-56)</td>
<td>33</td>
<td>29-37</td>
<td>0.2</td>
</tr>
<tr>
<td>AC with IE</td>
<td>35 (30-45)</td>
<td>34</td>
<td>33-39</td>
<td>37</td>
<td>29-39</td>
<td>36</td>
<td>36-39</td>
<td>0.2</td>
</tr>
<tr>
<td>HAID without IE</td>
<td>39 (27-55)</td>
<td>40</td>
<td>32-45</td>
<td>0.4</td>
<td>37 (27-49)</td>
<td>36</td>
<td>36-41</td>
<td>0.6</td>
</tr>
<tr>
<td>HAID with IE</td>
<td>37 (31-50)</td>
<td>36</td>
<td>33-42</td>
<td>34</td>
<td>36-40</td>
<td>36</td>
<td>36-40</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 8: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. p<0.05 = significant difference within each group. CI= confidence interval.

Table 9: Percent of Tax expressing CD4+ / total CD4+ T lymphocytes cells for each study group.

<table>
<thead>
<tr>
<th>% Tax+CD4+/CD4+ lymphocytes</th>
<th>Mean (range)</th>
<th>Median</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC without IE</td>
<td>2 (0-10)</td>
<td>0.7</td>
<td>0.1-1.2</td>
<td>0.07</td>
</tr>
<tr>
<td>AC with IE</td>
<td>0.7 (0.4-4.6)</td>
<td>0.08</td>
<td>-0.6-2</td>
<td></td>
</tr>
<tr>
<td>HAID without IE</td>
<td>7.5 (0.12-25)</td>
<td>5.9</td>
<td>3-8.9</td>
<td>0.4</td>
</tr>
<tr>
<td>HAID with IE</td>
<td>8.9 (1.9-16)</td>
<td>6.7</td>
<td>3.8-11.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 9: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. p<0.05 = significant difference within each group. CI= confidence interval.
3.4.6.5 Tax expression

Results on *ex vivo* Tax expression (%Tax+ CD4+/CD4+ T lymphocytes) were available on 37 (39%) ACs and 37 (67%) patients with HAID. These experiments were conducted at non-standardised time points during follow up. The mean, single time point, percentage of CD4+ T lymphocytes expressing HTLV-1 Tax *ex vivo* was significantly lower in ACs than in patients with HAID *p* < 0.0001 (Figure 9).

**AC:** 1.7 (0-9.70), 95% CI: 0.9-2.5, median 0.4

**HAID:** 8 (0.12-25), 95% CI: 5.8-10, median 6.4,
As shown in Table 9 mean Tax expression was noticeably higher in ACs who remained IE free compared with those who developed an IE ($p=0.07$). This difference was not observed within the HAID group ($p=0.4$).

Figure 9: Box-plots of % of Tax expressing CD4+ T lymphocytes for both groups.

Figure 8: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. HTLV-VL= HTLV-viral load. PBMCs= peripheral blood mononuclear cells.

3.4.6.6 Soluble tumour necrosis factor-α receptor I

Single time point concentrations of soluble Tumour Necrosis Factor-α receptor I sTNF-αRI (pg/ml) were available on 36% ACs and 78% of patients with an HAID. sTNF-αRI levels did not differentiate between those who did and those who did not develop an IE when looking at all HTLV-1 infected patients (no IE: mean 1135 [130-5493], 95% CI 873-1398, median
921; with IE: mean 1991 [278-7766], 95% CI 934-3047, median 1041, p=0.15, Figure 9). However the ACs had significantly lower median plasma sTNF-αRI levels compared to the HAID group (AC: 833 pg/ml [230-1584], 95% CI: 704-963, median 846 vs HAID: 1865 pg/ml [130-7766], 95% CI: 1264-2466, median 1151, p=0.02). Taking into consideration that 91% of the patients with HAID had HAM plasma sTNF- αRI levels might be a biomarker for HAM. Our index case, who had developed HAM during follow up, had a plasma sTNF- αRI level of 975 pg/ml which is within the range but outside the 95% CI of patients with HAID. However the significant difference of sTNF- αRI levels between ACs and patients with HAID was lost once only those who developed IEs were included in the analysis (p=0.23):

**ACs with IE:** mean 936 (278-1541), 95% CI 509-1363, median 972

**HAID with IE:** mean 2566 (571-7766), 95% CI 970-4161, median 1637

This could either be because patients with IE do have higher sTNF- αRI levels or because the sample size was too small (AC with IE: n= 6, HAID with IE: 11).

As outlined in Table 10 there was a trend towards higher mean sTNF- αRI plasma concentrations in those patients who developed an IE **within** in each study group that did not reach statistical significance (IE vs no IE: AC: p=0.4, HAID: p=0.4).
Table 10: sTNF-α RI levels for each study group.

<table>
<thead>
<tr>
<th>STNF-α RI (pg/ml)</th>
<th>Mean (range)</th>
<th>Median</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC without IE</td>
<td>8011 (230-1584)</td>
<td>807</td>
<td>493-1049</td>
<td>0.4</td>
</tr>
<tr>
<td>AC with IE</td>
<td>936 (278-1541)</td>
<td>972</td>
<td>680-1166</td>
<td></td>
</tr>
<tr>
<td>HAID without IE</td>
<td>1530 (130-5493)</td>
<td>1104</td>
<td>247-1609</td>
<td>0.4</td>
</tr>
<tr>
<td>HAID with IE</td>
<td>2566 (571-7766)</td>
<td>1637</td>
<td>684-4533</td>
<td></td>
</tr>
</tbody>
</table>

Table 10: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. p<0.05 = significant difference within each group. CI= confidence interval.

Figure 10: Box-plots of sTNF-αRI for both groups.

Figure 9: IE= inflammatory event. AC= asymptomatic carrier. HAID= HTLV-1 associated inflammatory disease. HTLV-VL= HTLV-viral load. PBMCs= peripheral blood mononuclear cells.
3.4.7 Predicting an IE

The following co-variables were used in a multivariate logistic regression, testing their predictive value for IE development: study group, gender, ethnicity, risk factors, age at onset/diagnosis, age at first clinic visit, lymphocyte count, CD4+ lymphocyte count, % Tax expressing CD4+/CD4+ T cells, serum globulin, sTNF-αRI levels as well as age at first visit by gender and Tax expression by sTNF-αRI.

None of the categorical or continuous variables were significant predictors of a new IE. However Tax expression in conjunction with serum sTNF-αRI predicted 97% of patients without IE correctly but only 38% of patients with IE, so that 80% of the cases were categorised correctly (p=0.18, Table 11).

Table 11: Logistic regression and multivariate analysis predicting IE with Tax and sTNF-α RI together and comparing it to the observed cases.

<table>
<thead>
<tr>
<th>Observed cases as</th>
<th>Predicted cases as</th>
<th>% correct</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without IE</td>
<td>31</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>with IE</td>
<td>8</td>
<td>5</td>
<td>38</td>
</tr>
<tr>
<td>Overall %</td>
<td></td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

Table 11: IE= inflammatory event. Overall %= overall percentage of cases predicted with this model, % correct= percentage of correct predictions with this model, p <0.05 = significant contribution to prediction of correct cases with this model.

3.5 Discussion

3.5.1 Inflammatory events

A large Japanese cohort study conducted between 1984 and 1992 examined morbidity in HTLV-1 infected versus uninfected patients. This
study found that anaemia (OR= 1.3; 95% CI= 0.99-1.7), kidney disease (OR=1.6; 95% CI= 0.91-2.9), asthma (OR=3.4; 95% CI=1.2-9.8) and cardiac disease (OR=1.4; 95% CI=0.94-2.2) were more common in the infected population (240).

The American REDS Study Group (subsequently renamed the HOST study) found HTLV-1 infection to be significantly associated with a history of pneumonia (OR, 2.6; 95% CI, 1.2-5.3), minor fungal infection (OR, 2.9; 95% CI, 1.2-7.1) and bladder or kidney infection (OR, 1.6; 95% CI, 1.0-2.5) within the past 5 years as well as a lifetime history of tuberculosis (OR, 3.9; 99% CI, 1.3-11.6) and arthritis (OR, 1.8; 99% CI, 1.2-2.9). Only lymphadenopathy (≥ 1 cm) was associated with both HTLV-1 (OR, 6.6; 95% CI, 2.2-19.2) and HTLV-2 (OR, 2.8; 95% CI, 1.1-7.1) infection, although no case of adult T cell leukemia/lymphoma was diagnosed (241).

In 2004 we perceived HTLV-1 infection as a risk for the development of inflammatory conditions and this has been suggested in several publications since (84;96;167;242;243). The aim of the study was to establish the incidence of inflammatory conditions in HTLV-1 infected patients who have shown a tendency to develop inflammatory conditions and compare it to patients who were initially asymptomatic. For this study we used a broad definition of 'Inflammatory event' to capture conditions where a direct association with HTLV-1 infection has not been described yet in the literature as well as those conditions with an established HTLV-1 association. We hypothesised that during follow up patients with existing inflammatory conditions would be more likely to develop further inflammatory complications than ACs. The overall incidence of IEs for the
whole HTLV-1 cohort was 9/100 py follow up, almost one IE in 10 patient/years. The incidence rate did not differ significantly between the two groups when all first IEs were included but was nearly twice as high in the HAID compared to the AC group when only the recognised HTLV-1 associated disease were considered. This might suggest that the other events were simply common background inflammatory conditions observed in the general population and unrelated to HTLV-1 infection.

The incidence of HAM was low in our study (0.4/100 py follow up) and occurred in a Caucasian woman who acquired HTLV-1 through sexual intercourse in adulthood an Afro-Caribbean partner. This incidence rate is similar to Japanese cohorts (0.3% life-time risk) despite the fact that 65% of the ACs in our cohort were of Afro-Caribbean origin with reported life time risk of 3% when living in the West-Indies. One explanation could be that our ACs are younger and have therefore not had the time to develop HAM, which has a median onset age of 48 years. Also the seropositive patients in the first degree relatives cohort mentioned above were older at the time of testing (mean 46-52) compared to the relatives born in UK (mean 20) and have been at risk of heterosexual acquisition in endemic area for much longer (36). How much the higher rate of blood donors (15%) and Caucasians (19%) composing the AC group of our study are contributory factors to this low rate of HAM development is unclear but this could create bias.
In our study HTLV-1 associated uveitis was the most commonly diagnosed IE and was twice as common in patients with HAM as in ACs. Nevertheless it is worth emphasising that ACs may present with uveitis as a first manifestation of HTLV-I infection and therefore this should be considered in all patients with idiopathic uveitis.

The second most common IE in the whole cohort was idiopathic hepatitis. The diagnosis was made by measurement of liver enzymes as part of routine care of HTLV-1 infected patients. None of the patients were symptomatic and liver biopsies where either considered inappropriate by the hepatologist or declined. Although an increased risk of liver cirrhosis and development of hepato-cellular carcinoma has been reported in HTLV co-infected individuals HBV or HCV (244), and the case of a 54 year old woman with HAM and asymptomatic primary biliary cirrhosis has been reported in year 1993 (245), our study is the first to report abnormal laboratory liver function tests in isolated HTLV-1 infection. Since HTLV-1 inflammatory conditions are assumed to be caused by the infiltration of the affected tissue by HTLV-1 infected CD4+, CD8+ and cytotoxic CD8+ lymphocytes, it can be hypothesised that the liver tissue of patients with HTLV-1 is infiltrated with the virus and becomes a target of the host immune response. A prospective three-arm study comparing immuno-histo-chemistry staining of liver tissue of HTLV-1 infected patients with and without transaminitis compared to uninfected controls without transaminitis could answer this question. However an invasive procedure such as a liver biopsy might not be justifiable for a condition that is essentially asymptomatic. Alternatively regular follow up, non-invasive ultra- and fibro-scans would
show if idiopathic hepatitis in HTLV-1 positive patients carries the same risk of developing hepato-steatosis, cirrhosis and hepatocellular carcinoma as does hepatitis due to alcohol, or chronic HBV and HCV infections.

Until recently bronchiectasis has not been shown to be associated with HTLV-1 infection. By collecting data on all idiopathic conditions, we can report that bronchiectasis was seen only in patients with HAID, mostly with the pre-existing diagnosis of HAM, at a rate of one new case per five years. Considering that only symptomatic patients were investigated, the rate of asymptomatic bronchiectasis most probably exceeds this. In 2006, lymphocytosis was reported in the bronchoalveolar lavage and transbronchial biopsies of patients with bronchiectasis (103). A prospective study of bronchoalveolar lavage and high resolution chest computer tomography (CT) of HTLV-1 positive vs negative patients could establish the incidence of HTLV-1 associated lymphocytosis and bronchiectasis and help design new therapeutic approaches.

Although rare, loss of vibration sense, radiculopathy and cognitive dysfunction have been associated with HTLV-1 infection previously (239). Other, atypical, neurological conditions were rare events in either group but have been described in HIV positive cohorts (246-248).

Most of our patients are Afro-Caribbean women who are slightly older in the HAID group compared to the AC. They do not differ in the route of HTLV-1 acquisition. Interestingly the median age of onset of first HTLV-1 related symptoms of patients with HAID prior to the first clinic visit, was the same as
the age of diagnosis of the first IE in the AC group during follow up (49y).
This not only confirms data published on genetically related patients groups
from Jamaica and Martinique with HAM but also is the first prospective
report of age at onset for a variety of IEs in HTLV-1 infected ACs.
Considering that only 34% of ACs were older than 48 years old, that the
median follow up for the ACs was 1.1 years and that 71% of all IEs were
diagnosed within the first 2 years of follow up, it can be assumed that more
IEs, and especially HAM, might have been observed in an older population
of ACs followed up for at least 2 years.

In this study we could confirm that the acquisition of HTLV-1 through blood
products, which allows the transfer of a large number of virus infected cells
to the recipient, was not only a risk factor for HAM but for any IE
development.

From our study we can conclude that patients with a history of HTLV-1
related inflammatory disease and certainly patients with HAM tend to
develop a second or third IE more often than ACs (OR: 2.3 for any IE and
OR 4.1 if only strongly HTLV-1 associated IEs are considered).

However, 43% (10/23) of all the IEs known to be strongly associated with
HTLV-1 infection occurred in patients without HAM. This study highlights the
fact that even if a HTLV-1 infected patient has not developed HAM he/she is
not going to be spared from these other inflammatory conditions recognised
to be HAM associated.
3.5.2 Mortality

With an incidence of 3/100 py follow up, HTLV-1 related death was common in patients with existing HTLV-1 associated disease. As yet there has been no cure for ATLL which, despite aggressive chemotherapy, shows low remission and high relapse rates. In addition to the ATLL, five of the remaining six cases of death can be attributed to HTLV-1 infection, two (29%) were due to polymyositis and three (43%) due to disseminated HTLV-1 infection in this cohort. The AC group were younger than patients with HAID and the mortality rate amongst ACs could be higher as the studied population grows older. But the patients with HAID who died were of similar age when compared to ACs at last follow up. This suggests that the death was due to the severity of HAID rather than age or HTLV-1 infection per se.

3.5.3 Laboratory

We confirm that higher HTLV-1 viral load, raised sTNF-α RI levels and increased ex vivo CD4+ Tax expression can be directly linked with HAM. HTLV-1 viral load was significantly higher in patients with HAID compared to AC patients at both time points and did not distinguish patients who developed a “strongly HTLV-1 associated IE” from those who developed any IE. On the contrary the mean and median HTLV-1 VLs were lower in patients who developed an IE compared to those who did not, in both groups.
CD4+ T lymphocyte count normally decline with age yet the total lymphocyte count and CD4+ T lymphocyte count were significantly higher at last follow up compared to first visit, especially in the HAID patients that developed new IEs. This phenomenon has not been described previously. This rise in cell count does not seem to contribute to a rise in HTLV-1 viral load since this essentially remained stable over time in each study group. However, since viral load is measured as the % of total PBMC infected with HTLV-1 an absolute increase in lymphocytes at a constant viral load represents an increase in total HTLV-1 DNA copy number. Increased lymphocyte counts may be an indication of an active inflammatory process.

As previously reported the serum globulin levels were above the normal range in only 39% of ACs compared to 67% of patients with HAM at first visit (229). Patients with HAID tend to have higher serum globulin levels than ACs but intriguingly this difference was only significant at their first clinic visit and was lost with time. It is possible that there is a reduction in immunoglobulin production over time as observed in HIV infected individuals.

Both %Tax expressing CD4+/ CD4+ T lymphocytes and sTNF-α RI levels were significantly higher in patients with HAID. None of these parameters alone were predictive of IE development but the sTNF-α RI levels tended to be higher in patients who developed IEs in both groups. However in the multivariate model Tax expression combined with sTNF-α RI levels (but not HTLV-1 VL or belonging to the HAID group) could differentiate those patients who did not develop an IE correctly in 97% of cases and those who did in 38%. Therefore low ex vivo Tax expression combined with low in vivo
sTNF-α RI levels seem to reliably exclude future IE development (at least within the limited follow up period of observation).

This needs to be investigated further especially since our sample size was very small. Especially since not all samples for Tax and sTNF-α RI measurements were collected before the IEs were developed a prospective study on HTLV-1 infected patients > 48 years of age, followed up for more than 2 years collecting pre- and post IE samples for Tax and sTNF-α RI could confirm if these two laboratory markers together are truly predictive.

### 3.6 Conclusion

HTLV-1 infection can cause multi-systemic inflammatory disease not necessarily confined to the nervous system. Increased mortality is strongly associated with patients who have aggressive, progressive HAM and/or polymyositis. We can conclude that IEs occur not only in patients with HAID but also in ACs. HAM was uncommon but uveitis was the most common HTLV-1 associated condition diagnosed in this cohort. It is possible that uveitis occurs in HTLV-1 infected patients at a younger age than HAM.

So far we could not isolate a single inflammatory biomarker predicting the development of an IE. Whilst HTLV-1 viral load is higher in patients with HAID than in ACs at presentation and last follow up this did not predict new IE but the combination of sTNF-α RI levels and ex vivo Tax expression in CD4+ T-lymphocytes seems to accurately differentiate patients who do not develop a new IEs from those who do. sTNF-α RI was higher in patients with HAID especially in the presence of HAM and a study of anti-TNF-α in the treatment of HAM is has commenced at the NCHR.
Currently we are collecting data on the levels of serum $\beta_2$ microglobulin as an inflammatory marker for IEs in patients infected with HTLV-1 infection. In 2006, at the biannual meeting of the HTLV European Research Network in Verona, data presented from experiments with SELDI-TOF (surface-enhanced laser desorption ionisation time-of-flight) mass spectrometry performed at the Department of Immunology at Imperial College, raised the possibility of three plasma proteins as biomarkers of inflammatory disease in HTLV-1 infection patients for application both in clinical and basic research: Three proteins were up-regulated in patients with HAM: $\beta_2$-microglobin (11.7 kDa), calgranulin B (13.3 kDa) and apolipoprotein-AII (17.4kDa). One protein was up-regulated in ACs (apolipoprotein AII) but its significance is as yet unknown (249).

This study has several shortfalls: Limited patient number, especially in the HAID group, younger ACs; no comparison with a matched HTLV-1 negative group to compare the incidence and prevalence of new IEs with the general population; lack of tissue diagnosis of most IEs especially in those patients with transaminitis or bronchiectasis and incomplete sampling for all laboratory tests especially those that were non-routine.

The incidence rates for IEs and mortality rates in a HTLV-1 infected cohort need to be compared with a matched HTLV-1 uninfected and asymptomatic cohort. Also future studies need to address the direct link of HTLV-1 infection as a causative agent for chronically abnormal liver function test as well as bronchiectasis.
Chapter 4

4 Clinical, virological and immunological outcomes of patients with HAM treated with tenofovir or methylprednisolone.

4.1 Background

Currently there is no cure for HTLV-1 infection or for HAM. Studies of the treatment of HAM, which have focused on improvement of disability scores and reduction of HTLV-1 viral load, have had limited success (see Chapter 1.2.1.5).

At the NCHR patients have been offered a variety of treatments for chronic HAM depending on the available scientific data and clinical experience. Since tenofovir disoproxil fumarate (TDF) is known to be safe and well tolerated as monotherapy for patients infected with hepatitis B virus (250) as well as part of triple antiretroviral therapy for patients with HIV infection (251), patients with HAM were offered treatment with TDF at a time when it was still uncertain whether more potent HTLV-1 RT inhibitors would achieve clinical, virological or immunological benefits in vivo.

The potential role of steroid therapy based on published data (123;147;252), anecdotal reports in HAM/TSP and experience in multiple sclerosis (253) and other inflammatory conditions led to the decision to revisit steroid therapy for HAM. In order to reduce steroid associated side effects the treatment regime chosen was intermittent, high-dose, pulsed intravenous methylprednisolone for patients with chronic, late stage HAM.
4.2 Objectives

The aim of this longitudinal observational study is to describe in detail the clinical, immunological and virological effects of TDF and of pulsed methylprednisolone in patients with HAM.

4.3 Methods

All data were collected prospectively and all patients with HAM had symptoms for more than two years. The following outcomes were measured as part of routine clinical assessment: Timed 10 meter walk (tw: sec/10m); visual analogue pain score (VAS: 0-10); total, CD4+ and CD8+ T lymphocyte counts (10^9/L) and HTLV-1 viral load (VL: HTLV DNA copies/100 peripheral blood mononuclear cells [PBMCs]). Data were documented in the clinical notes, transferred into an Excel data sheet and analysed with SPSS 14.

Tenofovir disoproxil fumarate (TDF): Six patients were treated with TDF 245mg once daily orally between April 2004 and May 2005. Outcome variables at baseline were compared with those collected at last on-treatment assessment. All patients were informed that TDF was not licensed for the treatment of HAM and the reasons for treatment. All gave informed consent except one patient with encephalitis in whom informed consent was given by her guardian.

Methyl prednisolone: Fourteen patients received 1g intravenous methyl prednisolone infused over 2 hours, daily, on 3 consecutive days. Since treatment was not conducted as part of a clinical trial there were no
standardised criteria for recruitment of these patients. Most of these patients were complaining of severe chronic analgesic resistant backache and were offered steroid-therapy for its anti-inflammatory function. Treatment was given as an outpatient between January 2005 and September 2007. Pre-treatment clinical and laboratory data were compared with data collected on the third infusion day (Day 3), at four weeks follow up (Week 4) and the subsequent attendance (Month 3).

4.4 Results

4.4.1 Tenofovir disoproxil fumarate

Five patients with HAM, and one additional patient with HTLV-1 associated encephalitis, took treatment with TDF for a mean of 8.7 (± 2.3) months. At the time of TDF initiation the mean age was 44.8 (± 15) and the mean duration of HAM 8.3 (± 6.8) years. One patient (TBX) was co-infected with hepatitis B virus. Four (67%) patients were female and half of the cohort was originally from West Africa. Heterosexual intercourse was the most common attributed route of acquisition of HTLV-1. Unilateral leg weakness was the first presenting symptom in 50% of these patients (Table 1).

All patients tolerated TDF well and none of the patients stopped TDF due to intolerance or side effects.

TDF treatment did not cause any significant change in the mean and median continuous variables compared with baseline however there were significant
intra-patient variations. Change in aid usage was the only categorical variable. An overview of all analyses can be found in Tables 2 and 3.

Table 1: Demographics, risk factors for HTLV-1 acquisition and presenting symptom.

<table>
<thead>
<tr>
<th>Number ( %)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2 (33)</td>
</tr>
<tr>
<td>West-African</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Iranian</td>
<td>1 (17)</td>
</tr>
<tr>
<td><strong>Risk factor</strong></td>
<td></td>
</tr>
<tr>
<td>Heterosexual intercourse</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Breastfed</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Breastfed + Heterosexual intercourse</td>
<td>2 (33)</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
</tr>
<tr>
<td>HAM</td>
<td>5 (83)</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>1 (17)</td>
</tr>
<tr>
<td><strong>First symptom</strong></td>
<td></td>
</tr>
<tr>
<td>Leg weakness</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Urinary frequency</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Fall</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Confusion</td>
<td>1(17)</td>
</tr>
</tbody>
</table>

Table 1: %= percent, Risk factor= route of HTLV-1 acquisition, Diagnosis = HTLV-1 associated condition, First symptom= first symptom associated with HAM

Table 2: Clinical, virological and immunological outcomes of patients receiving tenofovir.

<table>
<thead>
<tr>
<th></th>
<th>TDF started (±SD)</th>
<th>TDF stoped (±SD)</th>
<th>Mean difference (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timed walk</td>
<td>14.2 (± 3.5)</td>
<td>16.1(±2.6)</td>
<td>+1.9 (± 2.7)</td>
<td>0.4</td>
</tr>
<tr>
<td>Visual analogue pain score</td>
<td>4.1 (± 1.7)</td>
<td>4.7 (± 2)</td>
<td>+0.6 (± 1.3)</td>
<td>0.3</td>
</tr>
<tr>
<td>HTLV-1 proviral load (copies/100 PBMCs)</td>
<td>39.8 (±14)</td>
<td>38.9 (±13)</td>
<td>-0.9 (±12.7)</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>35.7</td>
<td>26.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Clinical, virological and immunological outcomes of patients receiving tenofovir.

<table>
<thead>
<tr>
<th></th>
<th>TDF started (±SD)</th>
<th>TDF stopped (±SD)</th>
<th>Mean difference (SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte count (10^9/L)</td>
<td>Mean</td>
<td>2320 (±1070)</td>
<td>2882(±1395)</td>
<td>+562 (± 575)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1800</td>
<td>2710</td>
<td></td>
</tr>
<tr>
<td>CD4+ T lymphocyte (10^9/L)</td>
<td>Mean</td>
<td>962 (± 567)</td>
<td>1218(± 640)</td>
<td>+256 (± 582)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>840</td>
<td>1390</td>
<td></td>
</tr>
<tr>
<td>CD8+ T lymphocyte (10^9/L)</td>
<td>Mean</td>
<td>620 (± 399)</td>
<td>774(± 529)</td>
<td>+154(± 546)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>530</td>
<td>560</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: TDF = tenofovir disoproxil fumarate, SD = standard deviation, p = probability of difference being due to chance, PBMCs = peripheral blood mononuclear cells.

4.4.1.1 Effects of TDF on need for walking aid

Prior to treatment five patients were using a walking aid and one was wheelchair bound. By the end of therapy with TDF five patients had no change in walking aid and one needed an additional walking stick. This was a female, Caucasian patient who had acquired HTLV-1 through sexual intercourse, needed one walking stick 6.5 years post onset and had HAM for 9.2 years at the time of treatment initiation. She was treated with tenofovir for 8.3 months.

4.4.1.2 Effects of TDF on timed walk

Of the five ambulant patients the mean 10m timed walk (tw) measured in seconds /10m (sec/10m) had increased by 2.6% and 11.1% in two patients (TAN, TBJ) and decreased by 4.7%, 13.3% and 23.1% in three patients (TBX, TBW, TBU). In this small cohort the change in tw could not be
predicted by duration of tenofovir treatment ($p = 0.5$), change in HTLV-1 viral load ($p = 0.7$) or change in lymphocyte count in a multiple regression model. However the two patients with the greatest improvement in timed walk were each medicated for more than 12 months (TBW: 12.3, TBU: 16.6) and the patient who had the strongest response had also a significant decrease in VL (TBW: change in tw: -23.1%, change in VL: -40.6%).

4.4.1.3 Effects of TDF on pain

Prior to therapy two (33.3%) patients had no pain and one patient could not communicate. In those with pain it was continuous in nature and located in the back and lower limbs (1 [17%] lower back pain, 2 [33.3%] in lower back and leg). The distribution and 24 hour duration of pain did not change during treatment with tenofovir.

In one patient the visual analogue score of pain was reduced by 1/10 (TBU). This equated to a 14.3% reduction from baseline. In two patients the VAS increased by 2/10. For patient TAN this represented a 36.4% and for TBJ a 25% increase from baseline. The mean duration of tenofovir treatment for these three patients was 11.7 months; the patient who reported an improvement in pain took tenofovir for twice as long (16.6 months) as the other two.

4.4.1.4 Effects of TDF on HTLV-1 viral load

Compared with baseline VL (copies/100 PBMCs) had reduced by 1.48%, 16.1% and 40.6% in three patients and had increased by 11.9%, 28.4% and 312% in the other three by the time the treatment was discontinued. The
time spent on tenofovir treatment did not predict the percent change in VL from baseline for the whole cohort ($p=0.3$) or the subgroups of increased ($p=0.4$) and decreased ($p=0.2$) VLs compared to baseline. However apart from $TBU$ there seems to be a trend between duration of treatment and decrease in VL ($N5$, $TAN$, $TBW$) that occurred despite the significant increase in total lymphocyte and CD4 T-lymphocyte counts in these patients (Table 3). Longitudinal analysis of VL did not vary significantly from baseline (Figure 1).

Table 3: Percent change in clinical and laboratory parameters when tenofovir treatment was stopped compared to baseline values.

<table>
<thead>
<tr>
<th>Code</th>
<th>Months</th>
<th>$tw(%)$</th>
<th>$VAS(%)$</th>
<th>$VL(%)$</th>
<th>VL log change</th>
<th>Lymphocyte ($%$)</th>
<th>CD4+ ($%$)</th>
<th>CD8+ ($%$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N5$</td>
<td>2.08</td>
<td>$wc$</td>
<td>nk</td>
<td>-1.5</td>
<td>-0.007</td>
<td>+15</td>
<td>nk</td>
<td>nk</td>
</tr>
<tr>
<td>$TBX$</td>
<td>2.55</td>
<td>-4.7</td>
<td>0</td>
<td>+312.3</td>
<td>-0.23</td>
<td>-16.7</td>
<td>-60.7</td>
<td>-52.8</td>
</tr>
<tr>
<td>$TAN$</td>
<td>8.33</td>
<td>+2.6</td>
<td>+36.4</td>
<td>-16.1</td>
<td>+0.12</td>
<td>+33.3</td>
<td>+36.4</td>
<td>+15.2</td>
</tr>
<tr>
<td>$TBJ$</td>
<td>9.49</td>
<td>+11.1</td>
<td>+25</td>
<td>+11.9</td>
<td>+0.62</td>
<td>+8.1</td>
<td>+18.6</td>
<td>-12.5</td>
</tr>
<tr>
<td>$TBW$</td>
<td>12.26</td>
<td>-23.1</td>
<td>0</td>
<td>-40.6</td>
<td>-0.08</td>
<td>0</td>
<td>+414.8</td>
<td>+325.8</td>
</tr>
<tr>
<td>$TBU$</td>
<td>16.56</td>
<td>-13.3</td>
<td>-14.3</td>
<td>+28.4</td>
<td>+0.05</td>
<td>+43.8</td>
<td>+8.3</td>
<td>+5.4</td>
</tr>
</tbody>
</table>

Table 3: Code = patients’ study code, Months = duration of treatment with TDF, $tw$ = timed walk, $VAS$ = visual analogue pain score, $VL$ = HTLV-1 viral load, $wc$ = wheelchair, nk = not known.

Figure 1: Consecutive HTLV-1 viral loads of all patients receiving TDF.
4.4.1.5 Effects of TDF on lymphocyte count

The total lymphocyte count (10^9/L) rose by 8.1%, 15%, 33.3%, 43.8% in four patients, fell by 16.7% in one and remained unchanged in the sixth patient. CD4+ T-lymphocyte and CD8+ T-lymphocyte subset counts were not available in one patient. CD4+ T- lymphocyte count rose by 8.3%, 18.6%, 36.4% and 14.8% in four patients and dropped by 60.7% in the fifth patient. In three patients the CD8+ T-lymphocyte count rose by 5.4% and 15.2%, and in three these counts fell by 12.5%, 17.9% and 52.8%. In a univariate model no correlation between change in lymphocyte count and duration of treatment (p=0.2) or change in HTLV-1 VL (p=0.2) could be established.

4.4.2 Methylprednisolone pulsed therapy

14 patients with HAM who had completed at least one course of methyl prednisolone infusion (2g per day for 3 days) were included in the final analysis. Three patients received multiple infusions. Altogether 22 infusion regimes were started and 20 were completed. One patient (TBW) received only one 500mg infusion of methyl prednisolone but developed psychological symptoms and a second patient (TAW) refused the third day of infusion stating that the infusions made her feel unwell. These two patients were excluded from the final analysis. Three patients received multiple infusions (TAN =2, TBJ =2, TAS =5). In these cases data analysis was performed on the first infusions and compared with subsequent infusions (Table 4, 5).
Twelve patients were female (86%) and eleven (79%) were of Afro-Caribbean origin. At the time of first infusion the mean age was 55 (± 14.4) and the mean duration of HAM 12.1 (± 7.3) years. The most common first experienced symptom associated with HAM was unilateral leg weakness (6 patients [43%]). One patient (TBX) was co-infected with hepatitis B virus. Three patients were receiving additional treatment for HAM during the first infusion; two were taking tenofovir (TAN, TBJ) and one ciclosporin (TBR). One patient (TAS) was taking methotrexate during her fourth and fifth infusions. Data were collected at baseline, after the 3rd infusion on “Day 3”, at “Week 4” (mean: 1.2 [± 0.4], median 1.1 months) and at “Month 3” (mean: 3.2 [±1.8], median 2.8 months) after the first infusion.

Table 4: Demographics, risk factors for HAM acquisition and 1st experienced symptom associated with HAM.

<table>
<thead>
<tr>
<th>Number</th>
<th>Number of patients</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infusions</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>12 (86)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Afro-Caribbean</td>
<td>11 (79)</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>2 (14)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Risk factor</td>
<td>Breastfed + Heterosexual intercourse</td>
<td>11 (79)</td>
</tr>
<tr>
<td></td>
<td>Heterosexual intercourse</td>
<td>2 (14)</td>
</tr>
<tr>
<td></td>
<td>Breastfed</td>
<td>1 (7)</td>
</tr>
<tr>
<td>First symptom</td>
<td>Leg weakness</td>
<td>6 (43)</td>
</tr>
<tr>
<td></td>
<td>Back/leg pain</td>
<td>4 (29)</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>3 (21)</td>
</tr>
<tr>
<td></td>
<td>Bladder dysfunction</td>
<td>1 (7)</td>
</tr>
</tbody>
</table>

Table 4: %= percent, Risk factor= route of HTLV-1 acquisition, Diagnosis = HTLV-1 associated condition, First symptom= first symptom associated with HAM
Table 5: Clinical, immunological and virological outcome measures in patients who received methylprednisolone infusions.

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Day 3 (±SD)</th>
<th>Day 3 (±SD)</th>
<th>p</th>
<th>Week 4 (±SD)</th>
<th>Week 4 (±SD)</th>
<th>p</th>
<th>Month 3 (±SD)</th>
<th>Month 3 (±SD)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Timed walk</strong> (sec/10m)</td>
<td>Mean</td>
<td>20.7 (± 6.8)</td>
<td>18.8 (± 5.3)</td>
<td>-1.9 (± 4.9)</td>
<td>0.2</td>
<td>19.5 (± 5.3)</td>
<td>-1.2 (± 4.4)</td>
<td>0.9</td>
<td>19.7 (± 4.7)</td>
<td>-1 (± 5.4)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>20.5</td>
<td>19.5</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Visual analogue score</strong></td>
<td>Mean</td>
<td>7.2 (± 2.2)</td>
<td>2.7 (± 3)</td>
<td>-4.5 (± 3.8)</td>
<td>0.05</td>
<td>6 (± 2.4)</td>
<td>-1.2 (± 2.2)</td>
<td>0.2</td>
<td>5.8 (± 4)</td>
<td>-1.4 (± 4.2)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>7.5</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HTLV-1 VL</strong> (DNA copies/ 100 PBMCs)</td>
<td>Mean</td>
<td>24.4 (± 14)</td>
<td>15 (± 15.2)</td>
<td>-9.4 (± 13.4)</td>
<td>0.08</td>
<td>18.2 (± 23)</td>
<td>-6.2 (± 25)</td>
<td>0.7</td>
<td>24.7 (± 21.8)</td>
<td>+0.3 (± 6)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>22</td>
<td>9.3</td>
<td>11.3</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log HTLV-1 VL</strong></td>
<td>Mean</td>
<td>1.3 (± 0.3)</td>
<td>0.8 (± 0.7)</td>
<td>0.5 (± 0.6)</td>
<td>0.03</td>
<td>1 (± 0.5)</td>
<td>0.3 (± 0.4)</td>
<td>0.2</td>
<td>1.3 (± 0.3)</td>
<td>0 (± 0.3)</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5 continued: Clinical, immunological and virological outcome measures in patients who received methylprednisolone infusions.

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Day 3</th>
<th>Day 3 (±SD)</th>
<th>Day 3</th>
<th>Day 3 (±SD)</th>
<th>Day 3</th>
<th>Week 4</th>
<th>Week 4 (±SD)</th>
<th>Week 4</th>
<th>Week 4 (±SD)</th>
<th>Week 4</th>
<th>Month 3</th>
<th>Month 3 (±SD)</th>
<th>Month 3</th>
<th>Month 3 (±SD)</th>
<th>Month 3</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocyte count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^9/L)</td>
<td>Mean</td>
<td>2292</td>
<td>± 805</td>
<td>1290</td>
<td>± 661</td>
<td>-1002</td>
<td>± 599</td>
<td>2525</td>
<td>± 998</td>
<td>- 233</td>
<td>± 760</td>
<td>0.4</td>
<td>2371</td>
<td>± 405</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>2000</td>
<td></td>
<td>1100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2250</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ T lymphocyte</td>
<td>Mean</td>
<td>1205</td>
<td>± 398</td>
<td>437</td>
<td>± 127</td>
<td>- 768</td>
<td>± 107</td>
<td>1083</td>
<td>± 277</td>
<td>- 122</td>
<td>± 248</td>
<td>0.7</td>
<td>1304</td>
<td>± 278</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1135</td>
<td></td>
<td>415</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1205</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8+ T lymphocyte</td>
<td>Mean</td>
<td>629</td>
<td>± 409</td>
<td>218</td>
<td>± 239</td>
<td>+ 408</td>
<td>± 111</td>
<td>663</td>
<td>± 393</td>
<td>+ 34</td>
<td>± 74</td>
<td>0.8</td>
<td>673</td>
<td>± 117</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>485</td>
<td></td>
<td>180</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>555</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5: SD= standard deviation, p = probability of difference being due to chance, VL= viral load, PBMCs= peripheral blood mononuclear cell, Mean change*= change from pre-treatment measures**
4.4.2.1 Effect of methylprednisolone on walking aid requirements

At baseline eleven patients were ambulant and three were wheelchair bound. One patient (TBJ) who was using a tripod pre-treatment walked with only one walking stick the following day but reverted to her tripod by the end of the course (Day3). A second course of infusions given 2.5 months later did not have the same effect.

4.4.2.2 Effect of methylprednisolone on timed walk

The mean (Day3: 18.8 [± 5.3] sec/10m; Week 4: 19.5 [± 5.3] sec/10m) and median (Day3: 19.5 sec/10m; Week4: 19 sec/10m) timed walk did not differ significantly from the baseline (mean 20.7 [± 6.8] sec/10m, median 19 sec/10m). Table 6 and Figure 2 illustrate the individual fluctuations in timed walk post treatment. All but one patient’s (TBK) tw fluctuated within a range of 5 sec/10m from their baseline value at most time points. TBK’s tw improved by 10sec/10m and remained improved at all three follow up time points. One patient TCB was excluded from the analysis since the percent increase in tw made her an an outliner. Her tw gradually increased and the next follow up % change showed an increase of 96.9 sec/10m.
Figure 2: Timed walk of all ambulant patients on Day 1, Day 2, Day 3 of the infusions and four weeks later.

Table 6: Change in percent tw at 3 different time points post-treatment. Tw= timed walk.

<table>
<thead>
<tr>
<th>Code</th>
<th>%Change tw Day 1:Day 3</th>
<th>%Change tw Day 1:Week 4</th>
<th>%Change tw Day 1: Month 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS1</td>
<td>-4.41</td>
<td>5.82</td>
<td>4.06</td>
</tr>
<tr>
<td>TCB</td>
<td>14.68</td>
<td>30.05</td>
<td>96.89</td>
</tr>
<tr>
<td>TBI</td>
<td>-1.21</td>
<td>0.87</td>
<td>-12.58</td>
</tr>
<tr>
<td>TBU</td>
<td>12.45</td>
<td>0.42</td>
<td>8.83</td>
</tr>
<tr>
<td>TCF</td>
<td>-30.81</td>
<td>12.6</td>
<td>-6.38</td>
</tr>
<tr>
<td>TCL</td>
<td>-0.84</td>
<td>-5.43</td>
<td>5.73</td>
</tr>
<tr>
<td>TCO</td>
<td>-18.76</td>
<td>18.76</td>
<td>5.15</td>
</tr>
<tr>
<td>TBJ1</td>
<td>10.97</td>
<td>-21.91</td>
<td>-13.28</td>
</tr>
<tr>
<td>TAN1</td>
<td>-33.92</td>
<td>-18.42</td>
<td>-21.64</td>
</tr>
<tr>
<td>TBK</td>
<td>-34.38</td>
<td>-21.88</td>
<td>-31.25</td>
</tr>
</tbody>
</table>

Timed walk had improved in five patients' at day 3 and in six at month 3 but had deteriorated in four patients at day 3 and in five patients by month 3. Improvements were more striking than deteriorations at months 3 (Table 7).
Table 7: Summary of mean % change in tw at three different time points post infusion compared to baseline values. N = number of patients, tw = timed walk, SD = standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>% change when tw decreased</th>
<th>% change when tw increased</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3 (± SD)</td>
<td>Week 4 (± SD)</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Mean</td>
<td>-17.76 (±15.5)</td>
<td>-18.9 (±8.1)</td>
</tr>
<tr>
<td>Median</td>
<td>-18.76</td>
<td>-21.88</td>
</tr>
</tbody>
</table>

4.4.2.3 Effect of methylprednisolone on pain

Four (28.6%) patients were pain free throughout. One had mild pain (VAS = 3/10) and nine patients had severe (VAS ≥ 5/10) chronic pain. The pain was of a continuous nature and mainly located in the back and lower limbs (3 [21.4%] lower back pain, 3 [21.4%] leg pain, 4 [28.6%] in lower back and leg). In all patients therapy led to an immediate significant reduction in pain score by the third infusion day (pre-treatment: mean 7.2 [±2.2], median 7.5; Day 3: mean 2.7 [±3], median 2, \( p = 0.05 \)). Four patients (TCB, TCF, TCD, TBK) patients showed a 100% reduction, three (TBU, TCO, TAN) ≥ 50% reduction and in three patients > 17 % reduction (TAS1, TCL, TBJ1, Table 8). Although the mean and median VAS of the whole cohort did not differ significantly from baseline at 4 weeks (\( p = 0.2 \)) and at three months follow up \( (p = 0.4) \), a sustained reduction in pain was reported by six patients at 4 weeks and by five patients at three months. None of the patients reported increased pain by Day 3, two (TCF, TCD) reported an increase compared to baseline at four weeks, and a third (TAS1) at three months (Figure 3). In one
of these patients the pain score at three months was twice that at baseline.

Two patients requested repeated infusions (TAN, TBJ) and one patient (TAS) had four more treatments for severe pain (Figure 4). All three patients reported immediate relief but noticed a rapid rebound of pain.

Table 8: Percent change in pain score at three time points post –treatment in the 10 patients with pain at base-line. VAS= visual analogue pain score, nk= not known.

<table>
<thead>
<tr>
<th>Code</th>
<th>% Change VAS Pre-treatment : Day 3</th>
<th>% Change VAS Pre-treatment : Week 4</th>
<th>% Change VAS Pre-treatment : Month 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS1</td>
<td>-37.5</td>
<td>0</td>
<td>+ 25</td>
</tr>
<tr>
<td>TCB</td>
<td>-100</td>
<td>-71.43</td>
<td>-100</td>
</tr>
<tr>
<td>TBU</td>
<td>-50</td>
<td>-50</td>
<td>-66.67</td>
</tr>
<tr>
<td>TCF</td>
<td>-100</td>
<td>+ 33.33</td>
<td>nk</td>
</tr>
<tr>
<td>TCD</td>
<td>-100</td>
<td>+ 66.67</td>
<td>+ 200</td>
</tr>
<tr>
<td>TCL</td>
<td>-17.65</td>
<td>-5.88</td>
<td>nk</td>
</tr>
<tr>
<td>TCO</td>
<td>-81.25</td>
<td>0</td>
<td>nk</td>
</tr>
<tr>
<td>TBJ1</td>
<td>-20</td>
<td>-40</td>
<td>- 30</td>
</tr>
<tr>
<td>TAN1</td>
<td>-50</td>
<td>-20</td>
<td>- 30</td>
</tr>
<tr>
<td>TBK</td>
<td>-100</td>
<td>-20</td>
<td>- 10</td>
</tr>
</tbody>
</table>

Figure 3: Changes in visual analogue pain scores (0-10).

Figure 3: Day1/2/3 = One, two or three days after the infusion. Week 4= Four weeks after 1st infusion. Month 3= Three months after 1st infusion.
4.4.2.4 Effect of methylprednisolone on HTLV-1 viral load

A significant decrease in log VL was documented after the third day of methylprednisolone infusions (mean: 0.8 [± 0.7] HTLV DNA copies /100 PBMCs, median 1) compared with pre-treatment (mean: 1.3 [± 0.3], median 1.3, p=0.03). The mean and median VL remained below baseline (mean 1 [± 0.5], median 1), although not significantly, at week 4 (p=0.2) but had returned to pre-treatment levels at three months (mean 1.3 [± 0.3], median 1.1, p=0.6, Figure 5).

In those three patients that had multiple infusions over time the VL dropped repeatedly in two (TAN, TAS) and rose for one (TBJ) at each 3 month follow up (Figure 6).
4.4.2.5 Effect of methylprednisolone on lymphocyte counts

The total lymphocyte count (10^9/L) was significantly lower after the third infusion (Pre-treatment: mean 2292 [± 805], median 2000; Day 3 1290 [±661], median 1100, p= 0.01) but returned to pre-treatment levels at four
weeks (mean 2525 [± 998], median 2250, \( p = 0.4 \)) and at three months (mean 2371 [± 798], median 2250, \( p = 0.5 \)). The CD4+ T lymphocyte and CD8+ T lymphocyte subsets fell accordingly on Day 3 of the infusion by 768 (± 107) and 408 (± 111) cells/mm³. This reached statistical significance for both subsets (\( p=0.01 \)). Again these differences had reversed by Week 4 and at Month 3 (Mean difference of CD4 T lymphocytes: Week 4 -122 [± 248], \( p = 0.7 \); Month 3 +99 [± 278], \( p = 0.3 \); Mean difference of CD8 T lymphocytes: Week 4, +34 [± 74], \( p = 0.8 \); Month 3 +44 [±117], \( p = 0.3 \)). In fact lymphocyte counts were non-significantly higher at three months compared to baseline (Tables 5 and 9).
Table 9: Percent Change in lymphocyte count and T-cell subset at three different time points.

<table>
<thead>
<tr>
<th>% Change Lymphocytes from pre-treatment to:</th>
<th>% Change CD4+ Lymphocytes from pre-treatment to:</th>
<th>% Change CD8+ Lymphocytes from pre-treatment to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3          Week 4   Month3</td>
<td>Day 3          Week 4   Month3</td>
<td>Day 3          Week 4   Month3</td>
</tr>
<tr>
<td>TCD            nk       2.5   -10</td>
<td>-4.1          -63.4  -7.3</td>
<td>-63.13         nk       45</td>
</tr>
<tr>
<td>TBI            -47.06  -5.88  0</td>
<td>-2.6          nk      -15.4</td>
<td>nk                 nk     nk</td>
</tr>
<tr>
<td>TBU            -41.3   -41.3  -15.2</td>
<td>37.5          -38.5  51.3</td>
<td>-36.84         18.05  2.26</td>
</tr>
<tr>
<td>TBR            -60      0       35</td>
<td>nk               nk      nk</td>
<td>-38.46         25.64  51.28</td>
</tr>
<tr>
<td>TCP            -57.89  Nk     21.05</td>
<td>nk             Nk      -52.8</td>
<td>nk               nk     18.52</td>
</tr>
<tr>
<td>TCO            -57.14  28.57  0</td>
<td>2.1            nk      14.3</td>
<td>-63.41         -17.07 -7.32</td>
</tr>
<tr>
<td>TCL            -57.14  7.14   21.43</td>
<td>3.5            nk      23.3</td>
<td>-53.66         2.44   0</td>
</tr>
<tr>
<td>TCF            10.53   0      -10.5</td>
<td>54.4           -44.4  40.7</td>
<td>nk               -3.08  -15.38</td>
</tr>
<tr>
<td>TAS1           nk       Nk     4.76</td>
<td>X               nk      nk</td>
<td>nk               nk     23.26</td>
</tr>
<tr>
<td>TBK            -48.15  Nk     -7.41</td>
<td>15.4           -68.5  18.5</td>
<td>-65              3.33   2.25</td>
</tr>
<tr>
<td>TCB            nk       Nk     nk</td>
<td>nk               nk      17.8</td>
<td>-63.1           45     -44.44</td>
</tr>
<tr>
<td>TAC            nk       Nk     nk</td>
<td>nk               nk      nk</td>
<td>nk               nk     nk</td>
</tr>
<tr>
<td>TBJ1           -59.38  -21.88 12.5</td>
<td>0               -53.7  0</td>
<td>nk               -19.64 14.29</td>
</tr>
<tr>
<td>TAN1           nk       Nk     -26.7</td>
<td>-11              -65     -25</td>
<td>nk               nk     nk</td>
</tr>
</tbody>
</table>

Table 9: Pre-treatment = values before infusion, Day 3= values after the 3rd infusion, Week 4= values at four weeks follow up, Month 3= values at three months follow up, nk= unknown.
4.4.2.6 Correlations between methylprednisolone and outcome measures

An association between timed walk and pain both pre and post treatment was observed (Figure 7), but this did not reach statistical significance in this small cohort (pre-treatment: $p=0.28$, Day 3: $p=0.23$). Three patients stated that the treatment was beneficial and asked for it to be repeated (TAN, TBJ, TAS).

Figure 7: Timed walk plotted against visual analogue score comparing pre-treatment values with those collected after the third infusion.

A weak trend to higher HTLV-1 viral loads with higher lymphocyte count (Figure 8) was found pre-treatment ($p=0.7$). Both values were lower and the association inverted after the third infusion ($p=0.5$). A significant correlation between VL and T lymphocyte subsets was found in the regression model (Pre-treatment: $p=0.2$ and Day3: $p=0.05$).
Apart from the treatment none of the other measured values could predict a reduction in timed walk or pain score on Day3. In a linear regression model a reduction in timed walk could not be foreseen by a reduction in pain score \((p= 0.15)\), HTLV-1 viral load \((p=0.4)\) or lymphocyte count \((p=0.6)\) on Day3, although it was mostly associated to a reduction in pain. A reduction in HTLV-1 viral load \((p=0.8)\) and lymphocyte count \((p= 0.5)\) could not predict an improvement in pain on Day3.
4.5 Discussion

Based on published safety data on NRTIs in HIV-1 positive patients and the high potency of HTLV-1 RT inhibition of TDF in vitro, patients with HAM were treated with TDF. The aim of treating patients with HAM was to inhibit virus integration in vivo, assuming that this would reduce viral replication and block de novo infection and either reduce PBMC viral load or reduce the pool of newly infected cells. Even if the “measurable” PBMC viral load might not significantly change with RT inhibition, targeting the viral replication cycle and blocking viral expression within the cell could be of clinical relevance. The observed lack of significant overall disease modification with TDF treatment could be potentially attributed to the small sample size, a lack of clinically sufficient RT activity or irreversible disease in these patients with chronic-stable HAM.

It remains unclear if RT inhibitors could have a disease modifying effect in combination with immune-modulatory drugs when prescribed at an early stage of HAM. However in acute and chronic leukaemic ATLL the combination of zidovudine and INF-α normalises the lymphocyte count (82).

Interestingly in this cohort the lymphocyte count rose by 562 (± 575) cells x 10^9/L from baseline (p=0.07) and this was due to an increase in both CD4+ and CD8+ T lymphocytes. It is not clear why this occurred with TDF treatment in patients infected with HTLV-1 without any significant change in the viral load compared to HIV infection where a rise in CD+ T lymphocyte count is associated with HIV viral load decrease. To our knowledge there
are no data on change in lymphocyte counts in HIV or HTLV-1 uninfected patients treated with antiretroviral therapy.

In patients with HAM zidovudine did not cause a significant rise of lymphocyte counts (142). In patients with HAM receiving dual-therapy, with zidovudine and lamivudine, CD4+ T lymphocyte count rose, but the total lymphocyte count and the CD8+ T lymphocyte count fell (143). However in the same study the total lymphocyte as well as the subsets’ count rose in the placebo group at week 24 (Treatment group: total lymphocytes: -159.3 x 10^6/L, CD4+: +10.4 x 10^6/L, CD8+: -38.1 x 10^6/L; Placebo group: total lymphocytes: +148.2 x 10^6/L, CD4+: +32.6 x 10^6/L, CD8+: +65.8 x 10^6/L).

These observations suggest a HTLV-1 independent increase in CD4+ T-lymphocyte counts in patients treated with nucleoside/nucleotide analogue RT inhibitors that is independent of obvious virological effect.

More persistent positive outcome effects are seen in the two patients who received TDF for more that 12 months, but again these patients were selected to receive treatment longer since in both cases the timed walk and in one the pain score were improving significantly from baseline. In fact in two patients the timed walk and pain score increased and both received TDF for more than 6 months and then stopped due to lack of benefit. In conclusion in this small group of patients with chronic, stable HAM no overall benefit in clinical or virological outcome of TDF treatment could be documented.
Corticosteroids have always been “major contenders” to modify the inflammatory component of HAM. Much of the disease activity has been attributed to high HTLV-1 viral load, Tax-protein-driven HTLV-1 specific lymphocyte expansion as well as HTLV-1 specific CTL activity, all of which are potential targets for corticosteroid therapy.

A recent study from Sao Paolo, Brazil, of 39 patients with HAM (67% female, mean age 47, mean age of onset 39, 79% lower limb weakness, 77% spasticity, 49% low lumbar pain) reported a significant improvement (24.5%) in the Incapacity Status Scale (which consists of 16 items: stair climbing, ambulation, toilet/chair/bed transfer, bowel and bladder function, bathing/dressing, grooming, feeding, vision, speech and hearing, medical problems, mood and thought disturbances, mentation, fatigability and sexual function) comparing baseline parameters with 1st visit (day 294) and 2nd visit (day 479) after receiving 1 g/ day methyl-prednisolone therapy for three days every 3 - 4 months. This difference was lost despite additional treatment at future visits. There was no difference in the Kurtzke Disability Status Scale (DSS) developed for Multiple Sclerosis and Osame’s Motor Disability Score (OMDS) specific for HAM at any follow up point. Those patients who received concomitant physiotherapy scored better. It is worth noting that none of the patients were evaluated immediately after the therapy and that data on change of experience of pain or any laboratory markers were not presented (148).

In our cohort of 14 patients with chronic and stable HAM, pain is one of the symptoms that causes most suffering and that seems to be perceived as the part of disease that is still “active”. Despite the chronicity of pain and disease duration of 12.1 (± 7.3) years there was a significant reduction in pain by
more than 50% by the third day of the infusion ($p=0.05$). This is the first report of a safe, effective, rapid and inexpensive palliative treatment for chronic HTLV-1 associated lower back and leg pain with pulsed methylprednisolone. This treatment has beneficial effects on patients’ quality of life since they can arrange to have an infusion therapy a few days before physically demanding activities such as travelling or attending festivities. Interestingly this small cohort of patients has an unusually low pre-treatment HTLV-1 VL (median 1%) which is atypical for a HAM population. This leads to two hypothesis: one that pain is not driven by high HTLV-1 VL, which was confirmed in this study and the second that methylprednisolone might cause a more impressive and longer lasting drop of HTLV-1 VL in a population with higher VL. Therefore the question remains if methylprednisolone could stop or slow down disease progression if offered to a selected group of patients with high HTLV-1 VL and early HAM disease.

The mechanism through which methylprednisolone led to an almost immediate decrease in CD4+ and CD8+ T lymphocytes from the peripheral venous blood is not fully understood. It may be due to induction of apoptosis of HTLV-1 infected lymphocytes, HTLV-1 specific lymphocytes or due to redistribution. The former might be suggested by the simultaneous reduction in HTLV-1 viral load but preferential compartmentalisation of infected cells remains possible. CSF studies of patients with HAM have shown pleocytosis, mainly lymphocytic, raised protein levels and the presence of oligoclonal bands (36;96). Histopathology of the CNS lesion of patients with HAM have revealed perivascular infiltration with CD4 + and CD8+ T lymphocytes as
well as of menigomyelitic changes of the spinal cord (35;36;254). Although there was no correlation between pain reduction and the venous lymphocyte count, it might be possible that methylprednisolone therapy also led to a fall in tissue lymphocyte counts and therefore reduced pain by decreasing inflammatory cytokine production in the CNS.

Although the immediate decrease of HTLV-1 VL together with the lymphocyte count on Day3 of the infusion therapy was significant, it most probably had no immediate effect on the clinical outcome of these patients with chronic HAM.

All these observations pave the way for a much needed randomised, multi-centre trial of oral high dose corticosteroid therapy in patients with early and progressing HAM.

A new treatment approach has been the modulation of chromatin condensation, an essential part of gene expression, which can be achieved with the histone deacetylase inhibitor, valproate. Essentially valproate reduces the affinity of histones for DNA and thereby increases cellular and viral gene transcription making infected T cells more accessible to CD8+ CTL and for reasons still unknown reinstates the apoptotic ability of the infected cells (255). An open-label non-randomised study of 20 patients with chronic HAM (7-19 years of duration) treated with valproate (20mg/kg/day) for three months, showed an almost 1.3 log reduction of HTLV-1 proviral load ($p= 0.001$). There was no change in CD4+ and CD8+ T cell lymphocyte count but a significant reduction of Tax expression of CD4+ T cell.
lymphocytes *ex vivo* was observed. Clinical data were only presented from four patients of whom one transiently showed some deterioration in timed walk at day 15 (256). According to the study coordinators about two thirds of patients reduced their dose to 500 mg daily due to side-effects (personal communication, A Lezin). We are therefore planning a dose-finding study of sodium valproate in patients with chronic stable HAM at the NCHR.

Other approaches are steroid sparing immuno-modulatory treatments similar to the modern treatment regimes for multiple sclerosis. An open study of ciclosporin for patients with early, progressing HAM at the NCHR has completed recruitment.

In summary there is little evidence from these observational data that TDF monotherapy is of clinical, virological or immunological benefit for patients with chronic, stable HAM. Conversely as a result of the dramatic reduction in pain with pulsed methylprednisolone, this treatment has become an established palliative treatment for chronic HAM-associated leg and back pain at the NCHR at St Mary’s Hospital. Due to its rapid onset of symptomatic relief, its prescription can be planned ahead of physically demanding life events improving patients’ life quality.
Chapter 5

5 Detection of episomal HTLV-1 circular DNA in vivo as a surrogate marker of viral reverse transcriptase (RT) activity and recent HTLV-1 infection at the cellular level.

5.1 Background

After cell entry retroviral RNA is transcribed into double stranded linear DNA in the cell cytoplasm using viral reverse transcriptase. This linear DNA is flanked by directly repeated sequences known as long terminal repeats (LTR). The linear DNA is packaged into the pre-integration complex and transported into the nucleus. There the linear DNA is either integrated into the genome through integrase or circularized prior to degradation.

The circular forms of retroviral DNA were discovered by Varmus and Guntaka in duck cells infected with Avian Sarcoma Virus (ASV) (257;258) and by Gianni et al in Moloney Leukemia Virus (MLV) infection (259) In the nucleus of ASV infected quail tumour cells, circular DNA was shown to constitute 50% of the nuclear viral DNA and 20–25% of the whole cell viral DNA (260).

Later two forms of circular viral DNA were discovered in the DNA of Rous Sarcoma Virus: The larger one is the 2-LTR DNA circle which is the same size as the linear DNA and a smaller 1-LTR-circle with a 300 bp deletion at one end. 1-LTR circles are present in great excess compared with 2-LTR
circles (261) Yoshimura and Weinberg confirmed these findings in Murine Leukemia Virus (262).

The bulk of research on circular viral DNA has been on HIV-1. The linear un-integrated form of the viral DNA is the direct precursor of both the integrated (263) and circularised forms (259;261). Once in the nucleus the concentration of un-integrated linear DNA remains low while integrated and circularised DNA increase (261;264;265) and although it is not clear how the circularised forms are built, it seems that there are two independent and competing pathways.

Later, other 2LTR circles were discovered. Different circular DNAs are now named depending on the number and position of LTRs inserted. In the nucleus 1LTR (261;266;267), tandem or simple 2LTR (268;269) and heterogenous 2LTR containing circles can be found (270;271).

The events leading to the formation and the functions of these circles remain uncertain. Circular DNA seems to be packaged into nucleoprotein complexes that are distinct from the pre-integration complexes containing linear DNA.

1LTR circles may arise through homologous recombination between the LTRs, leading to a single LTR flanked by pX and gag in HTLV-1 and env and gag in HIV.

A circle with two LTRs may be formed by ligation of the two ends giving rise to simple, tandem 2 LTR circle with a U5 - GTAC - U3 junction (272), or it may contain two separate, non-tandem LTRs after auto-integration. Auto-integration involves separation of linear cDNA and ligation of opposite DNA.
strands, leading to inversion of cDNA within the circle. Auto-integration seems to be integrase independent (273;274).

In HIV-1 infected cells the levels of total viral DNA are significantly higher than the amount of 2LTR DNA and the ratio of linear/1LTR/2LTR is suggested to be 20:9:1 respectively (275). However some authors claim that the levels of non-integrated HIV-1 DNA can reach 99% of total viral DNA (276).

It is still unclear if extra-chromosomal DNA itself has any function. It is unclear if they are dead end by-products or are functional, even cytotoxic once accumulated, as claimed by some authors (277;278). It has been suggested that 2LTRs have a short half life and can be used as a dynamic marker of new infection (275;279-281) whilst others suggest that they are long living especially in non-dividing cells such as macrophages (282-284). HIV-1 DNA circles have been used as an experimental marker of nuclear transport of linear cDNA (285;286) and as a marker of reverse transcriptase activity (272;280;283;287). Most published data are from HIV-1 since there are systems that allow the observation of infection through cell-free infectious supernatant.

Unlike HIV-1, cell-free virions are rarely detected in blood samples of HTLV-1 infected patients. For efficient infection body fluids containing whole blood cells such as cellular blood products or breast milk are required. Transmission of HTLV-1 from an infected cell to a previously uninfected cell is known to be a highly cell-associated process possibly through the ‘virological synapse’, a very tight “enclosure” between infected and
uninfected cell permitting env mediated cell to cell transmission of gag protein and viral RNA (18;288;289).

Although host cell mitosis leading to clonal expansion of HTLV-1 infected cells and conservation of HTLV-1 sequences is believed to be a significant, if not the dominant, determinant of HTLV-1 viral burden, some usage of the viral replicative cycle seems essential at least shortly after transmission of HTLV-1 to establish a new infection. For example a limited number of cycles of viral replication have been inferred from the development of sequence variation between generations following mother-to-child transmission.

The relative contribution of these two contrasting replication strategies remains unknown. Whether and to what degree ‘viral’, rather than cellular, replication of HTLV-1 occurs, early in the infection process or as a continuing process after the initial establishment of infection in a new host, is potentially of therapeutic importance, especially in the prevention of mother-to-child transmission and post exposure prophylaxis.

In vitro, un-integrated viral DNA is generally associated with super-infection and re-infection, after reverse transcription of newly infecting virions. In HTLV infection where there is hardly any cell-free infection there seems to be no correlation between the presence of un-integrated circular HTLV DNA and the presence of viral antigens in the culture supernatants so that the presence of extra-chromosomal DNA seems to be independent of virus exit and re-entry. It is possible that intracellular viral reverse transcriptase is used to transcribe viral RNA into linear extra-chromosomal DNA, without viral assembly, and re-infect the host cell (290).

Between 1986 and 1988 Hiramatsu et al published their studies on the mode of HTLV-1 transmission using HL60, a human promyelocytic
leukaemia cell line, by successive re-cloning and Southern blot analysis (291-293). They found that extra-chromosomal provirus molecules were present and maintained 24 hours after virus transmission and integration. Re-cloning of this HTLV-1 infected cell line showed fluctuation in the amount of extra-chromosomal provirus and a possible reintegration capacity of the virus. It was also noted that most of the clones with multiple integrated proviruses were those with persistent circular 1 and 2LTR DNA. The spontaneous initial increase in extra-chromosomal DNA was followed by its disappearance after two weeks of continuous culture. At the same time an 8.6% increase of integrated provirus was observed indicating the possibility of reintegration of extra-chromosomal DNA into the host genome (294). Two situations could have led to an increase in the integrated provirus levels: On the one hand this could have been due to re-infection of the cells by viral particles in the culture medium or through cell-to-cell contact. On the other hand this could have happened through reverse transcription of genomic viral RNA transcribed from the integrated pro-viral genome, followed by integration of the new provirus into the same host cell.

The first possibility was refuted after a three week culture of uninfected HL60 cells in the medium of HTLV-1 infected HL60 clones did not lead to the detection of extra-chromosomal DNA or integrated provirus in these cells (294). Hiramatsu et al had shown that transmission of HTLV-1 from HTLV-1 infected IMR90 cells to uninfected HL60 cells after three months of co-culture resulted only in a very low efficiency of transmission, <30% of clones were positive for integrated provirus. Therefore cell-to-cell transmission was also regarded as too inefficient to count for the significant rise in copy number of integrated provirus over time. The authors believe that a transient
increase in virus production and re-infection without cell exit was the most probable explanation for the increase in integrated virus copies. Following Hiramatsu’s work, Kitamura et al sought to determine the accumulation of un-integrated DNA in long-term T cell lines persistently infected with HLTV-I or HTLV-2 using a polymerase chain reaction-based assay. 2LTR DNA circles were chosen as a relative indicator of total extra-chromosomal viral DNA. 2LTR DNA circles were detected in all seven HTLV-1 infected and all eight HTLV-2 infected cell lines, with signal intensities of the un-integrated HTLV varying greatly among the cultures. Analysing the dynamics of 2LTR accumulation during acute infection of PBMCs from the HTLV-1 infected SP cell line, revealed that the steady state levels of extra-chromosomal DNA in a long-term infected cell line was substantially higher than those found in acutely infected cells. This suggests a lack of super-infection and or re-infection and makes viral RNA and DNA production within the infected cell without virion production more probable (290). This group could not detect 2LTR HTLV-1 DNAs in 2 asymptomatic carriers of HTLV-1 or five patients with HAM or any circularised LTR HTLV-2 DNA in asymptomatic carriers of HTLV-2. However, one out of seven ATLL patients was positive for HTLV-1 2LTR DNA. These results might indicate that 2LTR DNA exists only at very low concentration in patients’ PBMCs.

In 2004 the question, do currently licensed reverse transcriptase inhibitors inhibit the viral enzyme of HTLV-1 was still not clarified. Taylor et al had completed two clinical trials looking for the effects of lamivudine and subsequently lamivudine with zidovudine in patients with early, progressive and late, chronic HAM at St Mary’s Hospital, London (141;143). The first
observational trial showed a 10 fold reduction of viral DNA in five patients, which suggested that reverse transcriptase significantly contributes to the maintenance of HTLV-1 viral DNA load. The latter, a randomised double blind placebo controlled study of six months dual therapy in 16 patients, however, did not show a significant change in HTLV-1 viral DNA load in either arm.

Other publications reported sometimes a reduction and sometimes an increase in HTLV-1 viral load in vivo (142;295). In vitro studies demonstrated the susceptibility of HTLV-1 reverse transcriptase to an array of different nucleoside/nucleotide reverse transcriptase inhibitors (NRTI), of which tenofovir seemed to be the most potent (138;139;146).

5.2 Objectives

We therefore hypothesised that:

1. Extra-chromosomal circularised 1 or 2LTRs (EC 1/2LTR) should be present in peripheral blood mononuclear cells (PBMCs) of HTLV-1 infected patients.

2. EC 1/2LTR concentrations should correlate with HTLV-1 proviral loads in vivo and with different HTLV-1 clinical states.

3. EC 1/2LTR levels should be altered by therapy that alters viral replication.

4. If EC 1/2LTR circles represent recent infection then a viral entry inhibitor (peptide Pcr-400), or reverse transcription inhibitors will lead to a decrease in EC DNA in infected cell lines.
5.3 Material and Methods

5.3.1 Samples tested

For the in vitro studies MT-2 cells were used. These cell lines were cultured by Silva Youshya, Dr Graham P Taylor and Daniela Roemer. Cells were incubated at a concentration of 1 x 10^6 cells/ml in RPMI 1640 medium, supplemented with 100IU/ml penicillin, 100μg/ml streptomycin and 10% heat inactivated (56°C for 1 hour) foetal calf serum, at 37°C with 5% CO₂ (all regents from Gibco®). Cells were harvested every three to four days. MT-2 cells, which carry multiple incorporated HTLV-1 proviral copies were used to establish the polymerase chain reaction (PCR) assay for EC LTR detection and were subsequently used as positive controls.

For the in vivo studies patient samples were collected at the National Centre for Human Retrovirology and stored in the Jefferiss Trust Laboratories, Imperial College, London. PBMCs from HTLV-1 infected subjects were used for the ex vivo studies. All patients had tested positive for HTLV-1/2 antibody, had confirmed HTLV-1 infection by Western blotting (HTLV 2.4 Genelabs) and had negative HIV serology. All patients had, after giving written informed consent, donated blood for research purposes. Six patients were asymptomatic carriers, ten had HAM, four had ATLL, three had HTLV-1 infection with atypical neurology and one patient had polymyositis. EC LTR was quantified in sequential pre-, on and post-treatment PBMC samples of four patients with HAM who received corticosteroid and NRTI therapy.
5.3.2 Sample preparation

Classical DNA extraction: DNA was extracted from MT-2 cells as previously published (52). PBMCs were separated from EDTA blood by sucrose density centrifugation (Ficoll), washed twice in phosphate buffered saline and counted, after lysis of remaining erythrocytes with a lytic reagent (Zapoglobin™, Beckman coulter), using an automated counter (Coulter Electronics, Luton, UK). DNA was extracted from 2 x 10⁶ cells immediately after cell lysis in TST buffer (10mM Tris-HCL ph 7.5, 0.32 M sucrose, 5 mM MgCl₂, 1% Triton X-100) and immediate re-suspension in TE/TENT (10mM Tris-HCL ph 8.3, 1 mM EDTA/10 mM Tris-HCL ph 8.3, 1 mM EDTA, 10% NP40, 10% Tween 20) at 100µl per 2 x 10⁶ cells, and 2 hour incubation at 56°C with proteinase K (Sigma Chemicals, UK) at 1µl per 10⁶ cells. Proteinase K was then heat activated at 85°C for 10 minutes. The DNA was stored at -70°C. The cells that underwent this protocol are referred to as unpurified cells throughout this chapter.

DNA extraction and purification via QIAprep spin miniprep: DNA was extracted from MT-2 cells and PBMCs and low molecular weight DNA (circular DNA) separated from genomic, high molecular weight DNA through QIAprep spin miniprep kit (Qiagen) according to the manufacturer’s protocol. The DNA harvested from 6 x 10⁶ cells was eluted in 50µl EB buffer and stored at -70°C. The cells that underwent this protocol are referred to as purified cells throughout this chapter.

100bp DNA sequence: A 100bp DNA sequence was designed which contained the complementary sequences to the primers.
[U5forward/U3reverse] and [pXforward/gagreverse]. This was manufactured by Invitrogen /Illumina collaboration. DNA from MT-2 cells were spiked with this 100bp DNA. The DNA was then extracted and used or purified. Two copies of spiked DNA were used per PCR reaction. Spiked purified MT-2 cells were compared with un-spiked cells testing the primers and PCR conditions.

5.3.3 Primer design and synthesis (Table 1,2; Figures 1-3)

Primers for the classical and nested PCR were designed using the Primer3 website (waddlelab3.life.smu.edu/cgi-bin/primer3/primer3 www.cgi - 26k) by alignment of the AKT strain of the complete HTLV-1 genome (296). For the detection of EC 1LTR circle the primers were designed in the pX-forward and gag- reverse region in the inner and outer region (Figure 2). For the EC 2LTR circles primers were seated either side of the LTR-LTR, U5forward and U3reverse, junction (Figure 3). Reverse primers were in the anti-sense orientation (Table 1). An inner and outer primer was used for the detection of low copy number EC 1LTR in the nested PCR protocol (Table 2). The NCIB Blast database program was used to confirm that the designed sequences did not match other published DNA sequences. The primers were manufactured by Invitrogen /Illumina collaboration™.
Figure 1: Linear unintegrated HTLV-1.

<table>
<thead>
<tr>
<th>U3</th>
<th>gag</th>
<th>pol</th>
<th>env(pX)</th>
<th>U5</th>
</tr>
</thead>
</table>

Figure 1: LTR= linear unintegrated HTLV-1; gag, pol, env(pX)= gene regions coding for viral proteins.

Figure 2: Extra-chromosomal circularised 1LTR DNA (EC 1LTR).

Figure 2: 1LTR circle is formed by deletion of either LTRs. LTR= linear unintegrated HTLV-1; gag, pX= gene regions coding for viral proteins.

Figure 3: Extra-chromosomal circularised 2LTR DNA (EC 2LTR).

Figure 3: U3-U5 junction is the region where the LTRs from each end of the HTLV-1 DAN connect forming the 2LTR circle. LTR= linear unintegrated HTLV-1; gag, pX= gene regions coding for viral proteins.
Table 1: Designed EC 1 and 2LTR primers from AKT strain using classical PCR.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Product Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>pX forward (1LTR)</td>
<td>5'-CTC TCA CAC GGC CTC ATA CA-3'</td>
<td>940bp</td>
</tr>
<tr>
<td></td>
<td>8172 &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; 8192</td>
<td>812</td>
</tr>
<tr>
<td>U5 forward (2LTR)</td>
<td>5'-CCA AGT ACC GGC GAC TCC-3'</td>
<td>387bp</td>
</tr>
<tr>
<td></td>
<td>8976 &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; 8993</td>
<td>276</td>
</tr>
</tbody>
</table>

Table 2: Primers for detection of EC 1LTR from AKT strain using nested PCR.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Product Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>pX forward (1LTR)</td>
<td>5'-CTC TCA CAC GGC CTC ATA CA-3'</td>
<td>940bp</td>
</tr>
<tr>
<td></td>
<td>8172 &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; 8192</td>
<td>812</td>
</tr>
<tr>
<td>Inner pX forward</td>
<td>5'-CAC CAA CAT CCC CAT TTC TC-3'</td>
<td>840bp</td>
</tr>
<tr>
<td></td>
<td>8256 &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; 8275</td>
<td>276</td>
</tr>
<tr>
<td>Inner gag reverse</td>
<td>5'- AGA TTT GGC CCA TTG C -3'</td>
<td>821</td>
</tr>
<tr>
<td></td>
<td>836 &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; 821</td>
<td>821</td>
</tr>
</tbody>
</table>

5.3.4 PCR protocol

Classical PCR: 5μl of DNA solution containing DNA from 6x10^5 cells, was used for amplification in 50μl reaction volume containing 25 pmol of each of the primers pX/gag, 200μM dNTPs, 2 μM MgCL, 10x Buffer IV (Advanced Biotechnologies, Leatherhead, UK) and 0.2 μl Taq polymerase (Applied Biosystem-Six Paq, AmpliTaq Gold-N808-0243). The cycling conditions on a GeneAmp PCR System 2700 (Applied Biosystems Foster City, Ca.) were: denaturation step 5 minutes at 94 °C, followed by 35 cycles of amplification consisting of 1 minute at 95 °C, 30 seconds at 55 °C (TM), 2 minutes at 72
°C, and a final elongation step of a further 5 minutes. The PCR reaction and conditions were optimised at different MgCl concentrations and TM temperatures. One μl of the first round product was transferred to 49 μl of the second step of the nested PCR.

Through optimisation the PCR protocol was changed by increasing the annealing temperature (TM) to 60 °C and reducing the cycles to 25. A 5 µl aliquot from each reaction was separated on a 2% agarose gel (NuSieve 3:1, FMC Bioproducts) and visualised by ethidium bromide staining under UV light: EC 1LTR on classical PCR: 940 bp, nested PCR: 840 bp, 100bpDNA: 100bp. The identity of the amplicons were confirmed through purification of the PCR product from the gel through PCR Purification Kit (250) (Qiagen) according to the manufacturer’s protocol and sequencing 400bp on each side with the forward and reverse primers. The sequencing was carried out by the MRC CSC Genomic Core Laboratory (http://www.csc.mrc.ac.uk) at Hammersmith Hospital, London. Standard sequencing generates 700-800 bases of readable sequence from a single sequence. The NCIB Blast program was used to find the matching DNA sequence published on the database.

5.3.5 Quantification of EC LTR and HTLV-1 viral load

EC LTR was determined by serial dilution of purified sample DNA in distilled water followed by amplification of quadruplicates at each dilution. The quantity of EC LTR was determined from Poisson’s distribution, where load
= -logₐ Fo x dilution, and Fo is the number of negative tests/the number of tests.

HTLV-1 viral load measurements from the same samples were obtained, as part of routine clinical care, by Silva Youshya, using real-time PCR with the Roche LightCycler (Roche, Mannheim, Germany) as described in chapter 3.

5.3.6 Controls

Initially positive controls for the MT-2 cell line experiments were with 100bpDNA spiked HTLV-1 negative DNA and thereafter MT-2 samples since they contained EC LTR. Negative controls were HTLV-1 negative cord and donor blood and water.

5.3.7 Reproduction of detection of 2LTR DNA circles as published by Kitamura et al

Primers and PCR protocol used by Kitamura et al to detect EC 2LTR circles were used in order to replicate their published data (290). PCR primers bound to U5 and U3 regions of the LTR-LTR junction and were designed to amplify a 250bp product for HTLV-1. The primer sequence (Table 3) published were manufactured by Invitrogen /Illumina collaboration ™.
Table 3: Primers as published by Kitamura et al for the detection of EC 2LTRin HTLV-1.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>U5CAR</td>
<td>TCTGTTCTGCGCCGTTACAG</td>
<td>250bp</td>
</tr>
<tr>
<td>HTLV-1(^5)primer</td>
<td>91&gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; 72</td>
<td></td>
</tr>
<tr>
<td>U3CAR</td>
<td>CTGACCTTCTCAGACTTCTG</td>
<td></td>
</tr>
<tr>
<td>HTLV-1(^3)primer</td>
<td>598 &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; &gt; 620</td>
<td></td>
</tr>
</tbody>
</table>

DNA from 3x10^5 (2 µg), 1x10^5 un-purified and 1.2 x10^6 and 3x10^5 (2 µg) purified MT-2 cells were used for amplification in 50µl reaction volume containing 200 ng of each of the primers [U5CAR/U3CAR], 200µM dNTPs, 2.5 µM MgCl, 5x Buffer IV (Advanced Biotechnologies, Leatherhead, UK) and 0.2 µl Taq polymerase. Cycling conditions on a GeneAmpl PCR System 2700 (Applied Biosystems, Foster City, Ca.) were: denaturation step 5 minutes at 95 °C, followed by 45 cycles of amplification consisting of 1 minute denaturation at 94 °C,1 minute annealing at 45 °C (TM) and 30 seconds elongation at 72 °C with the final elongation step increased to 7 minutes.

5.3.8 EC 1LTR localization study

MT-2 cells were treated with triton-sucrose buffer (0.32 M sucrose, 1% Triton X-100, 5mM MgCl2, 10mM Trio-HCL, ph 7.5) in order to separate
cytoplasm from nucleus. DNA was extracted using Qiagen DNA Mini Kit (Qiagen, Ltd. Crawley, UK) according to the protocol of the manufacturer.

5.3.9 Reverse transcriptase inhibitory study

MT-2 cells in established culture were split. One half was cultured with 0.01µmol/l tenofovir disoproxil fumarate at inhibitory concentration 50 (IC50), the other half were cultured untreated. Growth rates were compared. Tenofovir disoproxil fumarate was obtained through the AIDS Research and Reference Reagent Program, NIAID, NIH. EC LTR was quantified in DNA extracted and purified from both mediums via QIAprep spin miniprep (Qiagen) and compared on days 0, 17 and 28.

5.3.10 Cell entry inhibitor study

MT-2 cells 12 x 10⁶ cells were cultured in 10ml RPMI medium at 37 C 5% CO2 for 10 days with and without 10 µM Pocr-400, an HTLV-1 inhibitory peptide (297). Aliquots of 6x 10⁶ cells were obtained on days 3, 7 and 10, span and re-suspended in culture medium. DNA was extracted and purified from both treated and control cells by QIAprep spin miniprep (Qiagen) and EC LTR quantified as above.
5.3.11 Statistical analysis

Data were collected in an Excel spreadsheet and analysed in SPSS-14 through parametric (t-test) and linear regression model for comparing two or more continuous variable. Non-parametric tests (Mann-Whitney Test) were used for correlating categorical with continuous variables and chi-square (Pearson Chi-Square Test for large, >5, and Fisher’s Exact test small, <5, sample size) and odds ratio/relative risk for two or more categorical variables.

5.4 Results

5.4.1 EC 2LTR

Using the U5forward/U3reverse primers 100bp bands could be amplified from cells spiked with control sequence only, but EC 2LTR circles (378bp) were not detected in un-purified or purified MT-2 cells. The 100bp bands were more visible on the 2% Agarose gel when DNA from MT-2 cells was purified (Figure 4).
Following Kitamura’s PCR protocol the published primers, [U5CAR/U3CAR] which had been shown to amplify high concentrations of 2LTR circles in unpurified MT-2 cells of 250bp length, were used. However these results could not be reproduced and only a 100bp primer-dimer was amplified in DNA from purified and un-purified MT-2 cells as well as HTLV-1 negative cord blood (Figure 5).
Figure 5: Using primers [U5CAR/U3CAR] published by Kitamura et al. Different copy numbers of unpurified and purified MT-2 cells were used for the detection of EC 2LTRs. In all samples only primer dimers could be detected. pMT-2= purified MT-2, neg= negative, dH2O= distilled H2O, bp= base pair, numbers equal DNA copies used for purification.

5.4.2 EC 1LTR

A 940bp product was amplified using the primers [gag reverse/ pX forward] in un-spiked purified MT-2 cells only and a 100bp product was amplified in the spiked MT-2 cells whether the DNA was purified and un-purified (Figure 4).

Using limiting dilution classical PCR EC 1LTR were detected at a concentration of 1copy/ 600 MT-2 cells. Using the nested PCR protocol with outer [pX forward/ gag reverse] and inner [pXforward/gag reverse] primers that amplify an 840bp product 1copy EC 1LTR was detected per 100 MT-2 cells.
5.4.3 Localisation of EC 1LTR

Nuclei and cytoplasm of MT-2 cells were separated, DNA extracted and EC 1LTR concentration measured in these two compartments. The concentration of EC 1LTRs was 1copy / 6000 MT-2 cells in cytoplasm (Figure 6) and 1copy/87 MT-2 cells in the nuclei (Figure 7).

5.4.4 EC 1LTR circles *in vivo*

5.4.4.1 Demographics of the patient cohort

PBMC from patients infected with HTLV-1 were examined for EC 1LTR. 58% of the cohort were female, 75% of black ethnic background and 71% had acquired HTLV-1 infection either through

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*Figure 6: Dilution series of EC 1LTR harvested from cytoplasm. Samples are in quadruples. pMT-2= purified MT-2 cells, bp= base pair. The numbers equal the DNA copy number used for purified.*
Figure 7: Dilution series of EC 1LTR in the nucleus. Samples are in quadruples. pMT-2= purified MT-2 cells, bp= base pair, neg= negative, dH₂O. The numbers equal the DNA copy number used for purified.

mother-to-child transmission or heterosexual intercourse. The mean age of onset of symptoms was 42 years for patients with HAM and the mean age at the time of presentation was 46 years for the whole cohort.
Table 4: Demographics of patients infected with HTLV-1.

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Frequency (%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>14 (58)</td>
<td></td>
</tr>
<tr>
<td>Afro-Caribbean/ West African</td>
<td>18 (75)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risk factor for HTLV-1 acquisition</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterosexual intercourse</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Heterosexual intercourse and breastfeeding</td>
<td>17 (71)</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>2 (8.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of HAM</td>
<td>42</td>
</tr>
<tr>
<td>Presentation to clinic</td>
<td>46</td>
</tr>
</tbody>
</table>

5.4.4.2 EC 1LTRs in PBMCs

Preserved DNA samples from 24 HTLV-1 infected patients who were not on treatment were thawed and purified. Six patients were asymptomatic carriers, 10 patients had HAM of whom one developed ATLL during follow up, 4 patients had acute ATLL, 2 patients had atypical neurology (one case each of motor neuron disease, encephalitis), one patient had HTLV-1 associated polymyositis and another atypical myopathy (Table 5).

Table 5: Clinical status of patients infected with HTLV-1.

<table>
<thead>
<tr>
<th>Clinical Status</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic carrier</td>
<td>6 (25)</td>
</tr>
<tr>
<td>HAM</td>
<td>10 (42)</td>
</tr>
<tr>
<td>ATLL</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Atypical neurology and polymyositis</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (100%)</td>
</tr>
</tbody>
</table>
The median HTLV-1 viral load varied significantly between patient groups, with the highest seen in patients with ATLL (87 [77-125]) and the lowest in asymptomatic patients (3.2 [0.02-129]) (Table 6 and Figure 8).

Table 6: HTLV-1 viral load of patients infected with HTLV-1.

<table>
<thead>
<tr>
<th>HTLV-1 proviral load</th>
<th>%PBMCs</th>
<th>Range</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>Mean</td>
<td>26.5</td>
<td>0.02-129</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>HAM</td>
<td>Mean</td>
<td>27.6</td>
<td>10 -77</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>ATLL</td>
<td>Mean</td>
<td>94</td>
<td>77-125</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Mean</td>
<td>37.5</td>
<td>3.4-95</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

Figure 8: HTLV-1 viral load by clinical status of patients infected with HTLV-1.

EC 1LTRs were detected in 16/24 (67%) patients, 4/6 (67%) asymptomatic carriers, 7/10 (70%) patients with HAM, 3/4 (75%) patients with ATLL, and 2/4 (50%) of the remaining cohort tested positive for EC 1LTR (Figure 9).
In 10 patients the EC 1LTR copy numbers/100PBMCs (%) could be quantified at very low levels (Table 7). The median EC 1LTR copy number /100PBMCs was 0.0023% and the mean 0.01377% (range 0.00005 - 0.1155%, SD: 0.0358, 95%CI: -0.99 to 0.036).

The presence or absence of EC 1LTR did not differ between asymptomatic carriers and those patients who had HAM (Fisher’s Exact test, \( p = 0.15 \)) or ATLL (Fisher’s Exact test, \( p = 0.6 \)). The EC 1LTR concentration did not differ significantly between the HAM and ATLL group (Binary logistic regression, \( p=0.93 \)) and predicted only 50% of the clinical status correctly. HTLV-1 VL did not correlate with either the presence absence of EC 1LTRs (Mann-Whitney, 2 tailed \( p=0.95 \)) nor with the EC 1LTR copy number /100 PBMCs (Linear regression model, \( p = 0.2 \)) (Table 7, Figures 10-12).
Table 7: Detection of EC 1LTR in HTLV-1 infected patients: Patients are coded. VL% = HTLV-1 proviral load/100 PBMCs, 1LTR% = concentration of EC 1LTR 1 copy/ PBMCs as detected through serial dilution and calculated by the Poisson distribution. Code H= asymptomatic carrier, Code L= ATLL, Code T= HAM, Code P= Polymyositis, Code N= other Neurology.

<table>
<thead>
<tr>
<th>Patient</th>
<th>VL%</th>
<th>1LTR</th>
<th>1LTR%</th>
<th>Patient</th>
<th>VL%</th>
<th>1LTR</th>
<th>1LTR%</th>
<th>CODE</th>
<th>VL%</th>
<th>1LTR</th>
<th>1LTR%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBU</td>
<td>3.94</td>
<td>+</td>
<td>+</td>
<td>TAF</td>
<td>76.8</td>
<td>+</td>
<td>0.0048</td>
<td>P4</td>
<td>47.51</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>HBK</td>
<td>129</td>
<td>+</td>
<td>0.0023</td>
<td>TAS</td>
<td>17.4</td>
<td>+</td>
<td>0.00048</td>
<td>N2</td>
<td>3.4</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>HCT</td>
<td>0.08</td>
<td>+</td>
<td>0.0023</td>
<td>TAN</td>
<td>16</td>
<td>+</td>
<td>0.0048</td>
<td>N5/L</td>
<td>94.5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>HEF</td>
<td>2.39</td>
<td>+</td>
<td>+</td>
<td>TAQ</td>
<td>12.3</td>
<td>+</td>
<td>0.0023</td>
<td>N12</td>
<td>4.54</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>HT</td>
<td>23.4</td>
<td>-</td>
<td>-</td>
<td>TBJ</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HX</td>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>TBR</td>
<td>22.9</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEY</td>
<td>77.1</td>
<td>+</td>
<td>0.0048</td>
<td>TBU</td>
<td>10.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LER</td>
<td>76.75</td>
<td>+</td>
<td>0.00023</td>
<td>TBW</td>
<td>12.8</td>
<td>+</td>
<td>0.0023</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEV</td>
<td>124.7</td>
<td>+</td>
<td>0.11547</td>
<td>TBX</td>
<td>13.28</td>
<td>+</td>
<td>0.000048</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP4</td>
<td>96.3</td>
<td>-</td>
<td>-</td>
<td>TCD</td>
<td>43.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 10: Presence or absence of the EC 1LTR and disease status. Small diamonds equals absence and large diamonds presence of EC 1LTR.

![Figure 10: Presence or absence of the EC 1LTR and disease status. Small diamonds equals absence and large diamonds presence of EC 1LTR.](image)

Figure 11: Boxplot of EC 1LTR depending on disease status of patients with HTLV-1 infection.

![Figure 11: Boxplot of EC 1LTR depending on disease status of patients with HTLV-1 infection.](image)
Figure 12: Scatter plot correlating HTLV-1 viral load with EC 1LTR per 100PBMCs.

5.4.4.3 EC 1LTR concentration in patients treated for HAM

Pre-, during and post treatment frequencies of EC 1LTR circles as well as HTLV-1 VL were measured in four patients with HAM (Table 8, Figure 17). These patients had been treated with long term oral or short term intravenous corticosteroids in order to reduce the inflammation caused by the immune response to HTLV-1 infection in tissue. Patients were also treated with long term oral nucleoside analogue reverse transcriptase inhibitor (NRTI) lamivudine (3TC) and nucleotide reverse transcriptase inhibitor tenofovir in an attempt to inhibit HTLV-1 viral reverse transcription by viral RT enzyme or for HBV therapy in a co-infected patient.

Patient 1 (TBW, Figure 13 a -d): HTLV-1 VL was reduced (26.2%) compared to baseline (43.1%) during therapy with oral corticosteroids with no change in the EC LTR concentration (0.00048%). HTLV-1 VL was further reduced during treatment with tenofovir (17.5%) and increased when this
was discontinued (25.13%). The EC 1LTR copy /100 PBMCs increased independent of NRTI treatment (on tenofovir 0.001155%, off tenofovir 0.0023%).

Figure 13: Detection of EC 1LTR in four replicates of 10-fold serial dilutions of DNA from PBMCs of patient TBW starting with $6 \times 10^5$ PBMCs.

a: Pre-treatment.
b: During oral prednisolone.

c: During tenofovir.
d: After tenofovir.

Patient 2 (TBX, Figure 14 a -c): This patient was co-infected with HBV and on long-term antiretroviral/HBV treatment. During follow up he developed ATLL and was also treated with chemotherapy: CHOP-Z [Cyclophosphamide, Hydroxydoxorubicin, Oncovin, Prednisolone and the anti-CD25-monoclonal antibody daclizumab (zenapax)] and intra-thecal methotrexate. No baseline/ pre treatment blood samples were available. The EC LTR copy number became undetectable when the patient was switched from the NRTI lamivudine (0.000048%) to tenofovir (0%) and rose above the first frequency measurement when the patients received additional chemotherapy (0.00016%). In contrast the HTLV-1 VL dropped from 13.28% to 8.3% eventually to 5.45% during these three interventions.
Figure 14: Detection of EC 1LTR in four replicates of 10-fold serial dilutions of DNA from PBMCs of patient TBX starting with $6 \times 10^5$ PBMCs.

a: During lamivudine.
b: During tenofovir.

c: After tenofovir.
Patient 3 (TAN, Figure 15 a-f): In the third patient HTLV-1 VL initially rose during short course pulsed intravenous corticosteroids and fell immediately after treatment (16% to 22.5% to 10.5%) while the EC 1LTR concentration decreased continuously with intervention (0.0048 to 0.0023 to 0.0012%). During treatment with tenofovir HTLV-1 VL was not affected by NRTI’s (2.8, 18.2, 13.4%) whilst the EC 1LTR decreased 3 fold during treatment with tenofovir and by 10 fold after treatment was stopped (0.0048 to 0.00166 to 0.00048%).

Patient 4 (TAS, Figure 16 a-c): The fourth patient with HAM received only short course of intravenous corticosteroids for pain relief. There was no change in HTLV-1 VL, but a 5 fold reduction (0.0012% to 0.00023%) in EC 1LTR concentration followed by a 4 fold rebound (0.0048%).

To summarise 3/4 patients showed a noticeable, but not statistically significant decrease in EC 1LTR frequencies when on corticosteroids or tenofovir (p=0.27).
Figure 15: Detection of EC 1LTR in four replicates of 10-fold serial dilutions of DNA from PBMCs of patient TAN starting with $6 \times 10^5$ PBMCs.

a: Pre treatment.

b: On intravenous methyl-prednisolone.
c: After intravenous methyl-prednisolone.

d: On lamivudine.
e: On tenofovir.

f: After tenofovir.
Figure 16: Detection of EC 1LTR in four replicates of 10-fold serial dilutions of DNA from PBMCs of patient TAS starting with $6 \times 10^5$ PBMCs.

a: Pre treatment.

b: On intravenous methyl-prednisolone.
c: After intravenous methyl-prednisolone.
Table 8: The EC 1LTR, HTLV-1 proviral load and EC 1 LTR per infected PBMC of each of the four patients (TBW, TBX, TAN, TAS). CHOP-Z= Cyclophosphamide, Hydroxydoxorubicin, Oncovin (Vincristine), Prednisolone, Z= anti-CD25-monoclonal antibody, iv = intravenous.

<table>
<thead>
<tr>
<th></th>
<th>TAN</th>
<th></th>
<th></th>
<th>TBW</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LTR/100 PBMCs</td>
<td>VL/100 PBMCs</td>
<td>LTR/100 infected PBMCs</td>
<td>LTR/100 PBMCs</td>
<td>VL/100 PBMCs</td>
</tr>
<tr>
<td>Pre-Therapy</td>
<td>0.0048</td>
<td>16</td>
<td>0.03</td>
<td>0.00048</td>
<td>43.1</td>
</tr>
<tr>
<td>Oral prednisolone</td>
<td>0.0048</td>
<td></td>
<td></td>
<td>0.00048</td>
<td>26.2</td>
</tr>
<tr>
<td>Prednisolone iv</td>
<td>0.0048</td>
<td>22.5</td>
<td></td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Post iv prednisolone</td>
<td>0.00115</td>
<td>10.5</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine</td>
<td>0.0012</td>
<td>2.8</td>
<td>0.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenofovir</td>
<td>0.00048</td>
<td>18.2</td>
<td>0.003</td>
<td>0.001155</td>
<td>17.5</td>
</tr>
<tr>
<td>CHOP-Z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-Therapy</td>
<td>0.00167</td>
<td>13.4</td>
<td>0.012</td>
<td>0.00231</td>
<td>25.13</td>
</tr>
</tbody>
</table>
Table 8 continued: The EC 1LTR, HTLV-1 proviral load and EC 1 LTR per infected PBMC of each of the four patients (TBW, TBX, TAN, TAS). CHOP-Z= Cyclophosphamide, Hydroxydoxorubicin, Oncovin (Vincristine), Prednisolone, Z= anti-CD25-monoclonal antibody, iv = intravenous.

<table>
<thead>
<tr>
<th></th>
<th>TBX</th>
<th>TAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LTR/100 PBMCs</td>
<td>VL/100 PBMCs</td>
</tr>
<tr>
<td>Pre-Therapy</td>
<td>0.00116 10.2 0.011</td>
<td>Oral prednisolone</td>
</tr>
<tr>
<td>Prednisolone iv</td>
<td>0.0048 10.5 0.046</td>
<td>Post iv prednisolone</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>4.80E-05 13.28 0.0004</td>
<td></td>
</tr>
<tr>
<td>Tenofovir</td>
<td>0.00001 8.3 0.0001</td>
<td></td>
</tr>
<tr>
<td>CHOP-Z</td>
<td>0.00017 5.45 0.003</td>
<td></td>
</tr>
<tr>
<td>Post-Therapy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.4.4.4 EC 1LTR concentration in MT-2 cells treated with TDF (IC50)

MT-2 cell were cultured with and without tenofovir disoproxil fumarate (TDF) at the inhibitory concentration 50 (IC50). MT-2 cell lines showed equal growth over time with and without TDF (Figure 17).

Figure 17: Growth of MT-2 cell line during 28 days of culture with or without tenofovir.

DNA was harvested and purified from both TDF exposed and unexposed cells at 3 time points, EC 1LTR detected by PCR amplification of serial dilutions and concentration calculated from Poisson’s distribution. The EC 1LTR concentration was the same in treated and untreated cells at all times. The levels were 3.5 times higher at baseline (day 0) at 1 copy/0.6 MT-2 cells compared to the other two time points. At day 11 and day 28 the measured concentration was 1 copy EC 1LTR/ 2.1 MT-2 in untreated as well as with TDF treated MT-2 cells (Figure 18).
Figure 18: Detection of EC 1LTR in four replicates of 10-fold serial dilutions of DNA from MT2 cells starting with $6 \times 10^5$ cells.

a: Pre treatment.

b: After 28 days culture with tenofovir.
5.4.4.5 Effect of HTLV-1 entry inhibitor $P^{cr}$-400 on extra-chromosomal 1LTR DNA

MT-2 cells incubated with or without $P^{cr}$-400 were harvested and DNA was extracted and purified at day 0 and after 10 days of culture. The frequency of EC 1LTR circles was the same (0.048 copies/100 PBMCs) with and without treatment at day 10. However, the baseline values were significantly lower EC 1LTR levels at 1 copy/2083 cells compared with previous measures from the same MT-2 cell line (Figures 19).

Figure 19: Detection of EC 1LTR in four replicates of 10-fold serial dilutions of DNA from purified MT2 cells when treated with Pcr-400 for 10 days.
5.5 Discussion

5.5.1 EC 2LTR

We were unable to detect EC 2LTRs in MT-2 cells despite using Kitamura et al’s as well as our own protocol. Although Kitamura reported to be able to readily detect EC 2LTRs in cell lines they could not detect EC 2LTRs in any patient PBMCs bar from one patient with ATLL. They postulated that EC 2LTRs might exist at very low concentration. Published data on circular DNA in HIV-1 infected cell lines show a ratio of 1:2:3 for 2LTR, 1LTR and linear DNA respectively (298). In general most publications are on HIV 2LTR circles, there are no publications on EC 1LTRs HTLV-1 positive cell lines or patients and only a few on EC 2LTRs.

5.5.2 EC 1LTR

Unlike 2 LTRs, EC 1LTR HTLV-1 DNAs were repeatedly detected in our MT-2 cells as well as some patient PBMCs. A whole circle was not amplified because of product size (~10kb) but the orientation of the primers allowed the amplification of an LTR that is flanked by HTLV-1 viral genome only. This makes an extra-chromosomal circle likely. Pauza et al hypothesised that DNA circles integrate into each other containing more than one LTRs in HIV-1 (298). This possibility has not been excluded but it is certain that the amplified product (840bp) contains one LTR as opposed to two tandem LTRs as
described by Kitamura from the smaller size of the harvested product (1LTR 840bp, 2LTR 940bp) and from the sequence of the amplified product.

Yoder *et al* reported that optimisation of PCR conditions may improve the amplification of HIV-1 1LRT products, especially if the concentration of target sample is below 500ng. An input of low concentration of DNA molecules reduces the risk of linear amplification, hybridization of these linear compounds and false exponential amplification as EC 1LTR circles (299). Using the current protocol HTLV-1 EC 1LTR circles were detected by classical PCR with a DNA input of 400ng.

During early HTLV-1 infection HTLV-1 reverse transcriptase is required to produce the complementary linear viral DNA that is transported into the nucleus and then integrated into the host chromosome. A very low proportion of linear DNA becomes a dead end product and is circularised. The formation of HTLV-1 1LTR circles seems to be much less common than in HIV-1 infected cells, which adds to the pool of evidence that HTLV-1 infected cells are not effective virion producing factories. Therefore the chances of intra- and extra-cellular re-infections are low. We assume that the detection of 2LTR circles in HTLV-1 infected cells is even less likely since they form less readily than 1LTRs in HIV-1 positive cells.

This might provide the virus with a survival benefit since it has been shown that an accumulation of EC DNA circles is highly cytopathic and a poor prognostic marker in HIV infected cells (298;300). The low concentration of EC HTLV-1 DNA circles might be one of the reasons why HTLV-1 infection has
little intrinsic cytopathic effect, is well tolerated and remains asymptomatic in most cases.

5.5.3 EC 1LTRs in HTLV-1 infected patients

EC 1LTR circles were found in asymptomatic and symptomatic HTLV-1 infected patients. Only two patients, both asymptomatic HTLV-1 carriers, had a HTLV-1 viral load of < 1%, and only one of them tested negative for HTLV-1 circles. HTLV-1 viral load in the remaining patients was high and this might be one of the reasons why 67% patients tested positive for EC 1LTRs. A correlation between HTLV-1 VL and 1LTR concentration could not be established as most patients (22/24) had high (> 2%) HTLV-1 VL and there was little viral load gradient. It is also possible that the low concentration of 1LTR circles contributed to a relatively high inter-assay variability.

EC 1LTR did not predict the clinical status of HTLV-1 infected patients. The chance of differentiating a patient with HAM from a patient with ATLL by the presence or absence of EC 1LTRs was 50%. Since 4/6 ACs were also EC 1LTR positive we conclude that EC 1LTR HTLV-1 DNA is a cellular by-product of HTLV-1 infection without clinical implications.
5.5.4 Treatment in vitro

Nucleoside and nucleotide analogue reverse transcriptase inhibitors have been shown to inhibit HTLV-1 reverse transcriptase, with tenofovir being the most potent and lamivudine the least potent in vitro using RT rather than whole cell assays. However with in vitro treatment of long term HTLV-1 infected MT-2 cells we did not find a significant reduction in EC 1LTR levels comparing tenofovir treated with untreated cells. Nevertheless there was a 3.5 fold decrease in EC 1LTRs concentration in both MT-2 cell lines during culture without any reduction in the rate of cell growth over time. MT-2 cells are known to have established HTLV-1 infection and we used freshly grown cells. These results suggest the possibility of early HTLV-1 reverse transcriptase activity when cell lines are first thawed and cultured which becomes quiescent as cells continue to grow and thereafter remains at a lower level.

Pcr-400 treatment of MT-2 cells did not reduce the EC 1LTR levels but these levels were significantly lower at baseline than in previous MT-2 studies. It is possible that there were inhibitory factors in the DNA medium harvested from these MT-2 cells so that EC 1LTRs could only be detected at higher DNA concentration. Also the cells might have a different lineage having been cultured over many months in a different laboratory.
5.5.5 Treatment \textit{in vivo}

HTLV-1 infected patients that require treatment mostly present once the infection has been clinically latent for several years prior to presentation. So far we have not been able to treat acutely infected patients. Patients who require treatment have mostly been diagnosed with HAM and/or ATLL. We therefore examined changes in EC 1LTR concentration over time in 4 patients with HAM one of whom developed ATLL during follow up. Two/three patients who received corticosteroids and 2/3 who received tenofovir showed a decrease in EC 1LTR concentration during these therapies. This decrease in EC 1LTR correlated with a decrease in HTLV-1 VL in only two cases. It is not known if corticosteroids have an effect on the HTLV-1 VL \textit{in vitro} or \textit{in vivo}. The presented data suggest that there is a transient decrease in EC 1LTRs during pulsed corticosteroid therapy. It is possible that corticosteroids have a direct inhibitory effect on HTLV-1 reverse transcriptase activity which has yet to be proven. Equally corticosteroid therapy could be more likely to induce apoptosis in HTLV-1 containing cells, especially those recently infected.

Although we could not demonstrate a reduction in EC 1LTR levels with tenofovir in the chronically HTLV-1 infected MT-2 cell line some reduction in EC 1LTR was seen in patients with HAM. However the significance of this finding remains uncertain and further studies of a larger cohort are needed to confirm or refute this observation.
No clinical response was documented in these patients and thus far it is not known if a reduction in EC LTRs has a dampening effect on Tax protein expression that could lead to clinical improvement.

5.6 Conclusion

Although we could not detected EC 2LTRs, we have confirmed that EC 1LTRs are present at low concentrations in chronically HTLV-1 infected cell lines and in PBMCs from chronically infected patients. They can be detected in patients with different clinical states and in the majority of patients with high HTLV-1 viral load. Their presence or absence did not predict a specific clinical state and could not be correlated to the HTLV-1 VL.

Tenofovir was associated with a reduction in EC LTR concentration in vivo but not in vitro which is consistent with a low level HTLV-1 ‘viral replication’ in vivo in addition to the dominant mitotic replication.

Corticosteroids might have an inhibitory effect on HTLV-1 reverse transcriptase in vivo or influence the survival of HTLV-1 infected cells especially if recently infected.

We were unable to demonstrate any effect of NRTI (tenofovir) or HTLV-1 entry inhibitor (Pcr-400) in vitro, on one HTLV-1 transformed cell line (MT-2), which may be due to the longevity of LTR circles in this cell line or Tax induced up-regulation of PgP expression leading to positive exclusion of tenofovir.
5.7 Future studies

EC 2LTRs seem to be present at even lower concentrations than EC 1LTRs. Multiple sets of primers for the detection of LTR-LTR junction through nested PCR could increase the chance of detection and quantification of EC 2LTR. PCR inhibitors could be excluded by spiking the DNA or quantifying the $\beta$ globin gene.

Detection and quantification of EC LTRs over time in freshly HTLV-1 infected PBMCs could give information on the dynamics of EC LTR circles over time. It is still unknown if HTLV-1 EC LTRs are functional in any way. HIV EC-DNAs have been shown to be transcriptionally active and producing functionally active proteins such as Tat, Enf, Nef (301-306).

HIV Nef protein production was evaluated in the absence of HIV viral integration in cells infected with recombinant integrase defective virus as well as cell treated with an integrase inhibitor. The viral DNA is shunted towards the formation of extra-chromosomal DNA when integration is not possible. In this setting Nef expressed from EC DNA down regulates CD4 surface expression on CD4+ T lymphocytes.

Protein production by HTLV-1 EC LTRs, if any, will be at very low levels and possibly insignificant. However if there is a correlation between higher levels of HTLV-1 EC LTRs and Tax protein expression, which has been associated with HAM, in asymptomatic carriers, an effective reverse transcriptase inhibitor might be of value in prevention of HAM.
Chapter 6

6 General Discussion and Conclusions

6.1 Discussion and Implications of the main findings

Since the 2nd World War many people from the West-Indies have migrated to the UK (http://yourarchives.nationalarchives.gov.uk/index.php?title=West_Indian_Immigration_and_Labour). Most 1st and 2nd generation Afro-Caribbean’s live in London and the surrounding counties, with significant populations in other major cities e.g. Bristol and Birmingham. Consequently UK physicians are occasionally confronted with conditions such as ATLL and HAM that are mostly seen in countries where HTLV-1 is endemic. Since 1992 patients with HTLV infections and associated diseases have been seen in a dedicated HTLV clinic within the Genitourinary and HIV medicine services at St Mary’s Hospital, Paddington. A decade later the National Blood Service introduced universal HTLV-1 screening of blood donors. This contributed to the recognition of the need for a dedicated centre providing specialised care for HTLV positive patients. Thus, in 2003 the National Centre for Human Retrovirology (NCHR) was established by the Department of Health with the remit to accept referrals from across the UK. In 2005 and 2007 the first outreach clinics of the NCHR were opened in Birmingham and Manchester respectively. Due to this centralisation of care, clinical expertise could be
developed and clinical, immunological and virological data on patients with HTLV-1 infection in the UK collated and analysed.

Of the 234 PhDs submitted to date in North America and Europe on aspects of HTLV infection none have addressed HTLV-1 associated myelopathy. http://proquest.umi.com/login. This thesis, the first in the UK on clinical aspects of HTLV-1 infection, focuses on HTLV-1 associated inflammatory conditions. The long-term outcome of patients with HAM living in UK was not known and therefore could not be compared to data from other regions.

We have observed that the UK patient cohort infected with HTLV-1 resembles cohorts in the Caribbean as opposed to Japan. Our patients are mainly female, of Afro-Caribbean origin and have acquired HTLV-1 infection either through being breast-fed or through unprotected sexual intercourse, or have both risk factors. In the UK the average time from onset to diagnosis of HAM is three years, one year less than in the Caribbean. UK patients remain ambulant and independent for four years longer than patients in the Caribbean. This might be due to better health care access, free of charge at the point of care, specialised care and patient awareness and education. Similar to endemic countries patients report unilateral leg weakness as the most common presenting symptom however 16% of patients reported urinary symptoms as their first HAM associated symptom. The gait of patients with HAM continues to deteriorate over decades as demonstrated by changes in timed walk and increasing use of walking aids. More than one third of the cohort became
wheelchair dependent within two decades. HAM long-term non-progressors are rare. A laboratory marker predicting progression of HAM could not be identified but in contrast to previous reports younger age at onset (<50 years) was associated with progression in our cohort. However, this is the first study to document timed walk, which seems to be more sensitive than traditional disability scores for detecting change, which showed a deterioration in 10m timed walk by 1.98 sec/year. Even though the risk of HAM development increases exponentially with viral loads above 1% (51) and patients with HAM had a significantly higher HTLV-1 viral load than ACs, this surprisingly did not predict deterioration of HAM. It seems likely therefore that there is a viral load threshold above which myelopathy is more likely to develop but once the inflammatory process has been initiated other factors contribute to the rate of progression.

There have been few publications on HTLV-1 associated mortality. Survival following ATLL is poor (82) but increased mortality has also been demonstrated in patients with HTLV-1 infection without malignancy (222;307). Compared to ACs patients with HAID were more likely to die during follow up. Although the HAID cohort was older than the ACs the median age of those who died was only 48 years and death was independent from IE development.

The incidence of inflammatory conditions in ACs and patients already diagnosed with an HTLV-1-associated disease, mostly HAM, is reported here for the first time. Although the overall incidence was similar, conditions known to be strongly associated with HTLV-1 were more commonly seen in patients
with an existing HTLV-1 associated inflammatory condition. However, idiopathic uveitits was as common in ACs as in patients with HAID and greater awareness of this as a presenting HTLV-1 associated symptom, especially in patients without myelopathy, is needed. The possible association of hepatitis with HTLV-1 infection whilst novel is not surprising given the multi-systemic nature of HTLV-1 associated inflammation.

HTLV-1 viral load and lymphocyte count did not correlate with IE development but low *ex vivo* Tax expression in conjunction with low serum sTNF-αRI predicted 97% of patients without IE correctly. This lack of correlation might be due to a too small sample size.

Thus far there is no cure for HAM and outside of clinical trials patients are mainly treated symptomatically. Prevention, or reduction, of HAM progression in patients treated with TDF or methylprednisolone was not demonstrated however the latter provides at least temporary improvement in the majority of patients and is now routinely offered to patients with HAM-associated severe, chronic lower back and leg pain, at the NCHR.

In the search for a marker of recent HTLV-1 infection at the cellular level EC 1LTR HTLV-1 DNA circles were detected and quantified. However their presence could not be correlated with HTLV-1 viral load or disease status. Equally the concentration of EC 1LTR DNA circles was not reduced by treatment with the HTLV-1 RT inhibitor TDF *in vivo* or *in vitro* or by blocking infection with the entry inhibitor, P<sub>etr</sub>-400. This is another indicator of HTLV-1’s
lack of ability or need to produce new virions for replication and adds to the pool of evidence that HTLV-1 viral load is predominantly maintained through virus induced cell division leading to polyclonal cell expansion rather than new cell infection and viral integration.

The CD4+ T-lymphocyte counts were higher after patients with HAM had been treatment with TDF for two months or more. This has not been reported in HIV negative/ HBV positive patients treated with lamivudine, adefovir or tenofovir. On the contrary patients with chronic ATLL are treated with zidovudine and interferon-α to normalise the high lymphocyte count. This incidental finding needs further examination in a larger cohort, however due to the lack of clinical efficacy of HTLV-1 RT inhibitors, monotherapy with these drugs might not be considered worth pursuing.

Only one patient with HAM was found to be a long-term non-progressor consistent with a mild or resolved inflammatory event. Conversely the progressive deterioration in gait (timed walk and walking aid usage), the occurrence of additional IEs, the HAID associated mortality and the transient, but repeated, response to pulsed methylprednisolone imply a persistent HTLV-1 driven inflammation.

Whilst high viral load is found in patients with HAM, HTLV-1 viral load did not significantly fluctuate during follow up nor correlate with disease progression in patients with HAM or with the development of IEs. This suggests that high HTLV-1 viral load may be associated with, but not causative of HTLV-1 associated inflammation. Furthermore, the low concentration of EC 1 LTR
DNA circles in PBMCs and the lack of effect of TDF therapy on their frequency in vivo suggest that active viral replication is of low importance in causing and or modifying these diseases. Thus, long-term anti-inflammatory, rather than antiretroviral, therapy may be the appropriate approach for HAID.

6.2 Future Directions

Currently there are no surrogate markers for HAM progression and we are unable to predict the development of HAM in ACs. The measurement of HTLV-1 VL in venous blood, CSF, aqueous humour and enlarged lymph nodes can be helpful in the diagnosis HAM, uveitits and lymphomatous ATLL but is not yet validated as a prognostic tool (156;157;308). A study of serial sampling at the sites of disease will improve our understanding of the role of HTLV-1 viral burden in the pathogenesis and natural course of these diseases.

Better markers of disease development for ATLL or HAID, comparable to the CD4+ T lymphocyte count and HIV-1 viral load in HIV infection are needed. Serial measurement of plasma β2 microglobin and T-cell activation markers (CD4+/CD25+ and CD4+ HLA DR+) is now part of the routine care of patients attending NCHR but there as yet are insufficient longitudinal data for prognostic analysis.
Idiopathic hepatitis in HTLV-1 infected patients needs to be investigated further, ideally with immuno-histochemistry for lymphocytic infiltration and exclusion of confounding toxicity with medication such as non-steroidal anti-inflammatory drugs. HTLV-1 infected patients need regular liver function tests and prolonged follow up to determine the possible risk of hepatic steatosis, cirrhosis and hepatocellular carcinoma as observed in hepatitis due to alcohol or chronic HBV and HCV infections.

The detection and quantification of EC 2LTR DNA circles would help to identify another piece of the puzzle of EC HTLV-1 DNA forms. In order to detect evidence of recent HTLV-1 infection at the cellular level, integration sites in serial patient samples could be analysed through pyro-sequencing (113).

Clinically there is evidence of mild neurocognitive impairment in patients with HAM in this cohort (data not presented), and depression and mild cognitive dysfunction associated with HAM has also been reported from Brazil (89;309). We intend to verify these observations in a collaborative study with clinical psychologists undertaking formal cognitive function tests, through a new, tailored batch of cognitive tests able to detect subtle changes in cognitive function over time. Patients will also be examined with advanced non-conventional magnetic resonance neuro-imaging (MRI: proton MR spectroscopy, magnetising transfer MRI, diffusion MRI and functional MRI) as used in multiple sclerosis and other white matters diseases (310).
At NCHR a 3rd clinical trial for HAM patients has concluded the treatment phase. Ciclosporin has been used specifically for patients with early, progressing myelopathy. The next efficacy trial will offer infliximab therapy for the same disease stage.

Patients with late or slow progressing disease will be recruited to a study of sodium valproate. Sodium valproate has been reported to reduce HTLV-1 VL in vivo and Tax expression in vitro in patients with HAM through histone deacetylase mediated transcriptional activation and increased accessibility of infected cells to CTL (256). The clinical efficacy of this treatment needs to be established.

### 6.3 Concluding remarks

HTLV-1 associated myelopathy remains a chronic, progressive, disabling, painful and incurable disease associated with psychosocial deprivation of patients and their carers. The pathogenesis of, and treatment options for, HTLV-1 associated diseases remain a scientific challenge.

Initial reports of HAM described a progressive phase of deterioration of walking disability up to two years followed by a plateau. However, we have demonstrated that patients with HAM have a slowly progressing and disabling disease and have established a baseline rate of deterioration in timed walk against which new therapies can be compared.
HTLV-1 viral load, lymphocyte count and CD4+ T lymphocytes were not predictive of the development of HAID or progression of HAM, but we found that patients with low Tax expression and serum sTNF-αRII production were at lower risk of developing an IE during follow-up.

Inflammatory events strongly associated with HTLV-1 infection other than HAM do occur in patients considered to be HTLV-1 asymptomatic carriers and need to be recognised as a possible presenting symptom of HTLV-1 infection.

Stronger histopatho-immunological evidence linking HTLV-1 infection with several observed IEs needs to be collected to distinguish these conditions directly from the background prevalence of IEs in the British population.

We have shown that with intermittent, anti-inflammatory treatment a transient improvement of chronic pain was achieved, which suggests continuing inflammation.

Although TDF failed to modify disease or viral load a novel observation of persistent significant increase in T-lymphocytes, both CD4 and CD8, raises new mechanistic questions that may be relevant to HIV medicine.

We could not demonstrate cell re-entry of HTLV-1 or an active usage of HTLV-1 RT through a dynamic change of 1LTR circles by treating infected cells with the nucleotide RT inhibitor TDF or viral entry inhibitor peptide Pcr-400. RT inhibitors alone did not lead to a reduction of cell infection once HTLV-1 DNA was integrated but it is still unclear if they are of value at the time of pre-
transcription/ integration and therefore prevention of mother-to-child-
transmission or post-exposure prophylaxis.

Due to a low global prevalence of HTLV-1 associated diseases compared to
HIV/AIDS, Tuberculosis and Malaria, there is a strong need to raise
international awareness of the severity of conditions such as HAM and ATLL.
International collaborative, suitably powered, randomised and controlled trials
to investigate promising therapies efficiently will provide the first evidence
based stepping stones towards future cures.
Declaration:

I collected all data for the original research chapters 2, 3 and 4 with the assistance of Dr Taylor and the clinic nurse Miss Alexandra Fedina. I performed the sTNF-αRI EIAs and the Tax expression assay was performed by Dr Angelina Mosley.

For Chapter 5, the cell cultures were performed by Miss Silva Youshya, Dr Graham P Taylor and Dr Daniela Roemer. Treatment of MT-2 cells with tenofovir and the peptide entry inhibitor Pdr-400 were performed by Dr Graham P Taylor and Dr Daniela Roemer. I designed the primers and performed all polymerase chain reactions.

I performed all statistical analysis after completing a training course in SPSS at Imperial College London. In addition specific statistical advice was sought from Mrs Elena Kulinskaya at the Statistical Advisory Service, Imperial College London.
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My name is on the cover of this thesis but I do not feel sole ownership. Without the support of the patients, co-workers, friends, family and supervisors I would have never found the strength to make these seeds grow.

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