

From DEPARTMENT OF MICROBIOLOGY, TUMOR AND
CELL BIOLOGY

Karolinska Institutet, Stockholm, Sweden

CMV-INFECTION IN GAY MEN WITH AIDS

Implications for diagnosis and treatment in
all co-infected patients

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CMV-infection in Gay men with AIDS

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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In memory of Alfredo

1952-02-26 – 1992-12-07

For my patients

There's are starman waiting in the sky

He'd like to come and meet us

But he thinks he'd blow our minds

There's a starman waiting in the sky

He told us not to blow it

Cause he knows it's all worthwhile

He told me:

Let the children lose it

Let the children use it

Let all the children boogie

David Bowie

ABSTRACT

Background: Before the era of combination therapy (c-ART) more than 90 % of the patients with HIV-infection died of one or more opportunistic infections (OI). We and others noted early on that CMV was an important pathogen in these patients. We investigated the OIs related to causes of death in all Venhälsan patients, with these specific questions 1) All OIs and opportunistic cancers (OC)s in patients who died with CD4+ counts below $100 \times 10^6/\text{mL}$; 2) The prevalence of CMV encephalitis (CMV-E) and Korsakoff syndrome (Paper 1); 3) CMV retinitis (CMV-R) in relation to CMV-E (Paper II); 4) CMV adrenalitis (CMV-A) and its relation to CMV-R and CMV-E, including comparison of CMV assessment by PCR in blood and the Synacthen test; 5) The correlation between CMV disease and other OIs and OCs (Papers I, II and III); 6) The interaction between CMV, Epstein Barr virus (EBV) and other human herpes viruses in a case of anaplastic large cell lymphoma (ALCL) (Paper IV)

Material and methods: We followed all patients that died at Venhälsan from 1989-1996, with intensive blood testing, biopsies, culturing, X-ray, computer tomography CT, magnetic resonance imaging MRI as well as autopsy when permission was provided. We focused especially on Synacthen tests to diagnose CMV-A, neurological examinations to diagnose CMV-E and Korsakoff, and ophthalmologic examinations to diagnose CMV-R. In a case of anaplastic large cell lymphoma, we did extensive analysis of all human herpes viruses in the course of the disease.

Results: Of all 219 patients, that died with $\text{CD4}^+ < 100 \times 10^6/\text{mL}$ 87% showed signs of reactivated CMV-infection. CMV-R was found in 84, CMV-E in 65, CMV-A in 41 and CMV in the gastrointestinal tract in 21. Mycobacterial infection was found in 87 and toxoplasmosis in 29. Kaposi's sarcoma was the most common tumour (68 cases) followed by 22 patients with malignant lymphoma and 20 with CNS-lymphoma. CMV-reactivation was seen in most diagnostic. A case of primary CMV-infection leading to a malignant lymphoma by interaction with two other herpes viruses was also seen.

Conclusion: CMV-infection was the main OI in AIDS-patients during the pre-c-ART era and also the main cause of death by itself or together with other OIs. Reactivation of CMV was found in 87%. The most important CMV manifestations were CMV-R, CMV-E and CMV-A that seemed to occur at the same time. All of them have characteristic symptoms. Thus, when one of these is diagnosed investigation for the other two should be done. This is still today important in patients with $\text{CD4}^+ < 100 \times 10^6/\text{mL}$ without access to modern HIV-treatment. These findings reveal the intimate interaction between HIV and CMV which should be considered in all co-infected patients also today.

LIST OF SCIENTIFIC PAPERS

- I. Impairment of short-term memory and Korsakoff syndrome are common in AIDS patients with cytomegalovirus encephalitis. Pirskanen-Matell R, Grützmeier S, Nennesmo I, Sandström E, Ehrnst A. *Eur J Neurol*. 2009 Jan;16(1):48-53. doi: 10.1111/j.1468-1331.2008.02337.x.

- II. CMV-retinitis in relation to CMV-encephalitis in HIV-infected patients with CD4 cell counts < 100 x 10⁶/mL. Sven Grützmeier^{1,3}, Bo Hedquist², Paniz Erfani^{1,3}, Börje Åkerlund⁴. Eric Sandström¹ and Ingemar Ernberg³ (submitted)

- III. Cytomegalovirus-adrenalitis (CMV-A) in HIV-patients with CD4 counts < 100 x 10⁶/mL. The value of ACTH-stimulating tests and blood CMV-PCR analysis. Sven Grützmeier^{1,2}, Johan Bergström^{1,2}, Börje Åkerlund³, Torbjörn Lindström⁴ Eric Sandström¹, Inger Nennesmo.⁵ and Ingemar Ernberg² (manuscript)

- IV. Fulminant anaplastic large cell lymphoma (ALCL) concomitant with primary cytomegalovirus (CMV) infection, and human herpes virus 8 (HHV-8) infection together with Epstein-Barr-virus (EBV) reactivation in a patient with asymptomatic HIV-infection. Grützmeier S, Porwit A, Schmitt C, Sandström E, Åkerlund B, Ernberg I. *Infect Agent Cancer*. 2016 Aug 22;11:46. doi: 10.1186/s13027-016-0094-5.

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LIST OF ABBREVIATIONS

HIV	Human Immunodeficiency Virus
AIDS	Acquired Immunodeficiency Syndrome
CMV	Cytomegalovirus
EBV	Epstein Barr-Virus
HSV	Herpes Simplex Virus
VZV	Varicella Zoster Virus
HHV-6	Human Herpes Virus 6
HHV-7	Human Herpes Virus 7
HHV-8	Human Herpes Virus 8
ALCL	Anaplastic Large Cell Lymphoma
Hb	Haemoglobin
WBC	White Blood Cell count
ANC	Absolut Neutrophil Count
PLT	Platelets
CD4/CD4+	CD4 lymphocytes/helper cells
CD8/CD8+	CD8 lymphocytes/suppressor cells
OC	Opportunistic Infection
OI	Opportunistic Cancer
MAC	Mycobacterium Avium Intracellulare Complex
KS	Kaposi's Sarcoma
PML	Progressive multifocal leukoencephalopathy
CNS	Central Nervous System
CNS Lymphoma	Central Nervous System Lymphoma
PNS	Peripheral Nervous System
PNP	Polyneuropathy
CSF	Cerebrospinal fluid
LP	Lumbar puncture
CT	Computer Tomography Scan
MRI	Magnetic Resonance Imaging
PCR	Polymerase Chain reaction

Ag	Antigen
Ab	Antibody
Pp65	Major Human Cytomegalovirus Structural Antigen
H and E	Hematoxylin and Eosin staining
DHEA	Dehydroepiandrosterone
AZT	Zidovudine
ddI	Didanosine
ddC	Zalcitabine
d4T	Stavudin
3TC	Lamivudine
SHBG	Sexual Hormone Binding Globulin
CXCR4	HIV-virus binding to co-receptor CXCR4
CCR5	HI-virus binding to co-receptor CCR5

1 INTRODUCTION

1.1.1 HIV infection

The first cases of what would later become known as Human Immunodeficiency Virus (HIV) infection were described in the Centers for Disease Control's (CDC) Morbidity and Mortality Weekly Report in June 1982 (1). The disease occurred in clusters of homosexual males in New York, Los Angeles and San Francisco. Many had Kaposi's sarcoma (a rare tumour in high-income countries at the time). Several had also had *Pneumocystis carinii* pneumonia (PCP), an opportunistic infection known to infect patients with lymphatic leukaemia and immunosuppressed bone marrow-transplant patients.(2-4) A few months later, Masur et al found that all these apparently previously healthy patients had signs of a compromised immune system. (5)The same infections were subsequently discovered in Haitians and haemophiliacs and an increasing number of papers on different opportunistic infections (OI) in these patients appeared during the following years (5-9) (*Pneumocystis carinii* has since been renamed *pneumocystis jirovecii* (10) but the term in use during the investigation period will be used in this thesis).

1.1.2 Effect on the immune system and decrease in CD4+ count

At the time, research concerning differentiation of human leukocytes was developing rapidly and labelling with antibodies led to the discovery of OKT3 cells, later named CD4+ cells. (9, 11) It was very early revealed, that the patients with the new immune deficiency had low and decreasing CD4+ counts. The term AIDS (Acquired Immune Deficiency Syndrome), used to denote the disease, was established in September 1982 by CDC. The virus that caused the disease was described by Barre Sanoussi et al already in 1983 (12). Both the term HIV and the HIV infection were confirmed at the 2nd World AIDS conference in Paris in 1986.

Today it is well established that the HIV-1 virus was transferred to humans from chimpanzees, while HIV-2, which is less pathogenic, derived from macaques (13). The HIV-1 epidemic originally followed the route from Africa, via Haiti, to the USA, as well as directly from Africa to Europe described by Pepin (13) and confirmed by Worobey et al (14)

1.1.3 HIV treatment

From 1982 to 1986, it was only the OIs in AIDS patients that could be treated. Zidovudin (AZT), a nucleoside analogue, was introduced in 1986; it was very effective in patients with HIV dementia as first described by (15) and then confirmed by Portegies (16). This drug also led to improved quality of life and weight gain, as well as decreased mortality and frequency

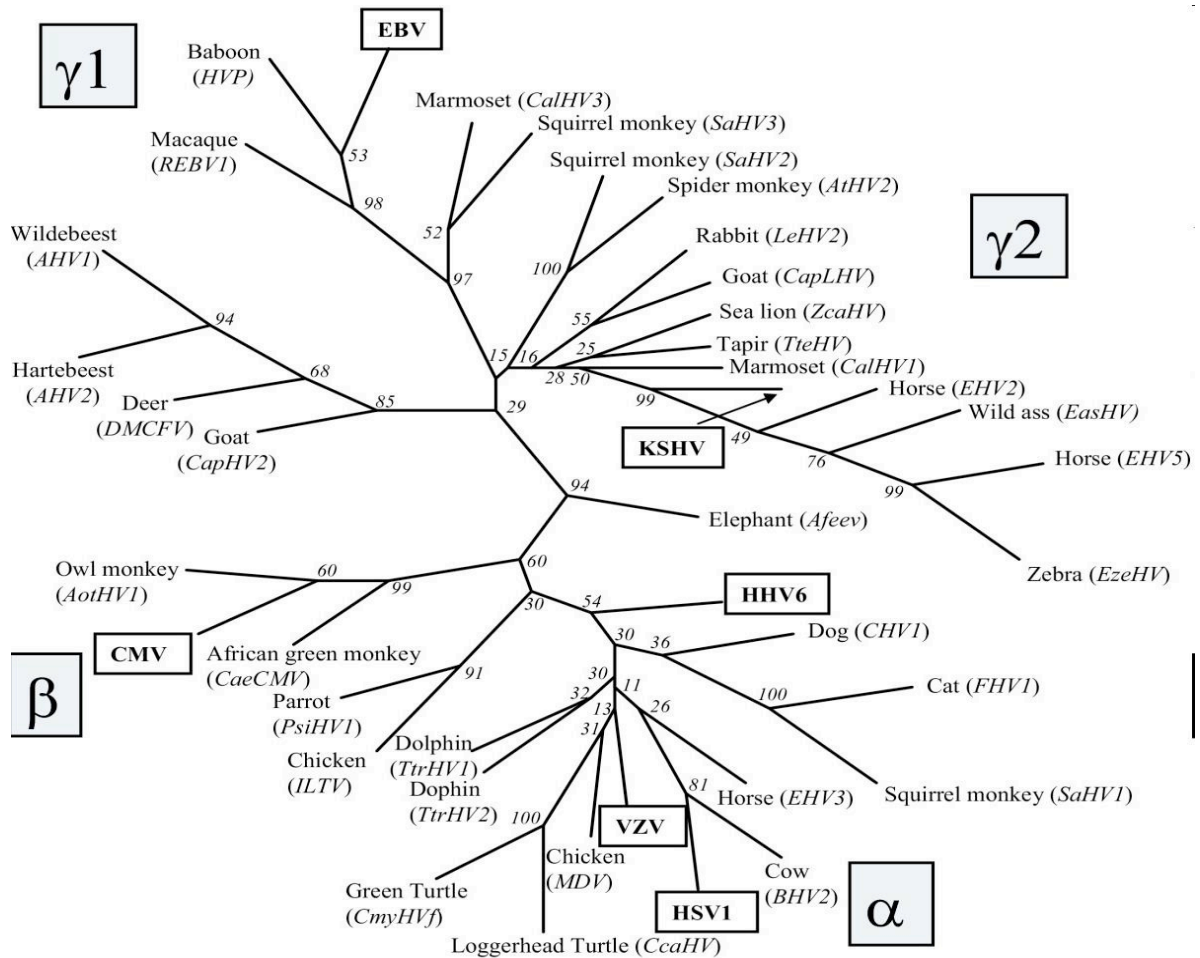
of OIs as summarized by Fischl et al (17). The HIV infection, however, usually progressed after a year with more OIs and related deaths. The effect of AZT on HIV dementia seemed to persist after more nucleoside analogues were introduced in 1991-1994, and it was discovered that combination therapy had a better effect on the disease. In 1996, a new combined antiretroviral treatment (ART) was introduced with dramatic effects on the quality and life expectancy of AIDS patients. This combination included a new drug class, the protease inhibitors (PI). HIV infects T-cells, especially CD4+ cells, dendritic cells, macrophages and microglia of the brain. It has several effects on the immune system; the killing of CD4+ T-cells (CD4+ cells) is a direct effect on the cellular immunity function and activation of B-cells producing antibodies that can interfere with body antigens, leading to autoimmunity.(18) Many of these B-cells do not produce effective antibodies against microbes, as seen in a deficient vaccine response study (Amu et al)(19). These defects in B-cells also influence the function of T-cells. Finally, even in patients undergoing successful ART treatment, there is a massive killing of CD4+ cells in the lymph glands (i.e. pyroptosis) described by Doitsh et al and Munoz-Aries et al (20, 21) leading to release of cytokines producing a chronic inflammatory reaction that can drive arteriosclerosis and cancer development.

1.1.4 CMV infection

Cytomegalovirus (CMV) is a beta-herpes virus that replicates in almost all cell types, such as blood cells, epithelial and endothelial cells and hepatocytes. The typical inclusion bodies seen in nuclei of CMV-infected cells were first described in 1881 by Ribbert. (22) A viral agent was suspected to cause these changes. The term Cytomegalia was used for the first time by Goodpasture and Gilbert in 1921. Mother-to-child transmission *in utero* was described in 1950(18). Three different research groups originally isolated CMV strains in 1956. In 1960, the name CMV was proposed by Weller, who also isolated the virus from the urine of children with general CMV disease. Since then, many different detection methods have been developed. It also became apparent that CMV is an important pathogen in mother-to-child transmission and in patients with immunodeficiency. Organs that can be infected with CMV s include: Multiple infection of ductal epithelial cells such as salivary gland, gastrointestinal tract and epithelial cells of the inner ear, Eyes (retina), brain and peripheral nerves, pancreas, adrenal glands kidney, lung and thyroid glands

CMV infection is ubiquitous and occurs in many animals, including primates. The oldest known CMV discovered is an oyster CMV believed to be 500 million years old. A phylogenetic tree of alfa, beta and gamma herpes viruses from (McGeoch et al)(23, 24) is shown in Figure 1

Figure 1.



The phylogenetic tree shows the relationship between alpha-, beta- and gamma- herpes viruses in different species. The names of human herpes viruses are in bold.

In humans, CMV is transmitted from 50% of infected mothers to their babies in connection with breastfeeding. After infancy, the virus is transmitted by kissing among teenagers (CMV accounts for 10% of clinical mononucleosis cases). The infection rate gradually increases during adulthood, leading to 70-80% eventually being infected with the virus. In certain groups, such as gay men with HIV infection, more than 95 % are infected. Children do not have serious symptoms of CMV infection if they are not infected during pregnancy; however, congenital infection can lead to serious encephalitis with severe sequelae. After infection, CMV remains latent, usually in blood cells. The virus usually does not cause any problems except in cases of immune deficiency, related for instance to chemotherapy, especially for lymphoma and leukaemia(25), or therapy associated with bone marrow or solid organ transplantation. (26-30). The lymphocyte antibody therapies used for malignancies and autoimmune diseases also cause severe immunosuppression (29, 31). Especially antibodies

against T-cells can generate an immunodeficiency similar to that in advanced HIV infection. It can also be a problem in patients ill with critical burns.(32-34).

1.1.5 HIV and CMV co-infection

In HIV-patients CMV emerged as a major pathogen, it was already described in the first reports as a possible cause of the new immune deficiency (2, 3) and during the following years numerous reports on CMV-disease was published (35-46). Interaction between HIV and CMV was discussed in some of the papers and CMV has also been reported to influence the natural history of HIV-infection (47). The main focus during this period was on CMV-Retinitis (CMV-R) that was the easiest to diagnose (36, 48-51) but many case reports and autopsy studies have also been published on CMV-encephalitis (CMV-E)(7, 35, 37, 40, 52) CMV-polyneuropathy (53) and CMV-colitis (54, 55). Case reports on CMV-adrenitis (CMV-A) has been published as well as autopsy studies (56-61). But no cohort studies or other studies trying to get a full overview of the development of CMV-infection in these patients had been published.

After the introduction of c-ART in 1996-7 the incidence of CMV-disease has decreased. One reason is the rapid restoration of CMV specific CD4+ cells (62). However, CMV-disease is still an important issue in low income countries (63-66). In recent years Freeman et al, has published a paper on CD8 cell expansion and inflammation linked to CMV- co-infection in patients with well controlled HIV-infection (67, 68) a sign that CMV-infection might still contribute to morbidity in HIV-infected patients. Ndumbi et al have found that delay in ART treatment results in persistent immune defects for many years especially if the patient initiate treatment with CD+ counts < 200x 10⁶/mL (69). Gianella et al has found that replication of human herpes viruses, especially CMV is associated with higher HIV DNA levels during ART treatment. (70-73). They also found that replication of CMV in semen is associated with higher HIV-DNA levels (72, 74). A study by Lichtner et al of more than 6000 patients with HIV-infection has also shown that CMV-co-infection is associated with an increased risk of severe Non-AIDS defining events (75).

1.1.6 Development of diagnostic methods for HIV and CMV

1.1.7 HIV

During the period 1985-1996, serology was used to diagnose and confirm the HIV infection. HIV antigen determination was also used and the syncytium-forming properties of the HIV virus were also studied. Rapid-high versus slow-low replication of HIV can determine affinity for CXCR4 or CCR5 receptors. However, these properties of the virus have not been

included in this thesis, as preliminary data had shown CXCR4-reactive virus in 90% of AIDS patients that had been previously treated (76). Detection of viral HIV-RNA in serum/blood became available in 1996. We started to perform HIV-PCR diagnosis during the second half of 1996.

1.1.8 CMV

Serology in blood, the pp65 antigen test, virus isolation, rapid antigen assays, CMV-PCR in whole blood with ethylene diamine tetra acetic acid (EDTA) as anticoagulant are the available analysis methods (25, 77-79). PCR in blood and cerebrospinal fluid (CSF) were introduced in the first months of 1992.(80) In 1996, quantitative CMV-PCR testing was gradually introduced in the clinical setting. Neither quantitative CMV-PCR assays nor quantification of HIV are covered in this thesis, since they were not available at the time.

1.1.9 Treatment of opportunistic infections

As HIV treatment had limited effect, we focused on prophylaxis against PCP and toxoplasmosis, as well as screening for and treating OIs and incident opportunistic cancers (OC) as soon as the patients presented with symptoms. When patients had been treated for OIs such as PCP, toxoplasmosis or Mycobacterium avium complex (MAC) infection, they were given lifelong secondary prophylaxis. CMV-infection was treated with foscarnet as first-line therapy from 1988 (81, 82) and ganciclovir in addition from 1989. Diagnostic tools consisted of blood tests, blood and cell cultures, X-rays, computed tomography (CT) scans, magnetic resonance imaging (MRI), as well as biopsies from skin and lymph glands and, occasionally, liver and bone marrow. We decided early on that we would not perform brain biopsies, as it would pose too big a risk and as the rapid development of PCR testing and other CSF analyses would provide more accurate diagnosis (83).

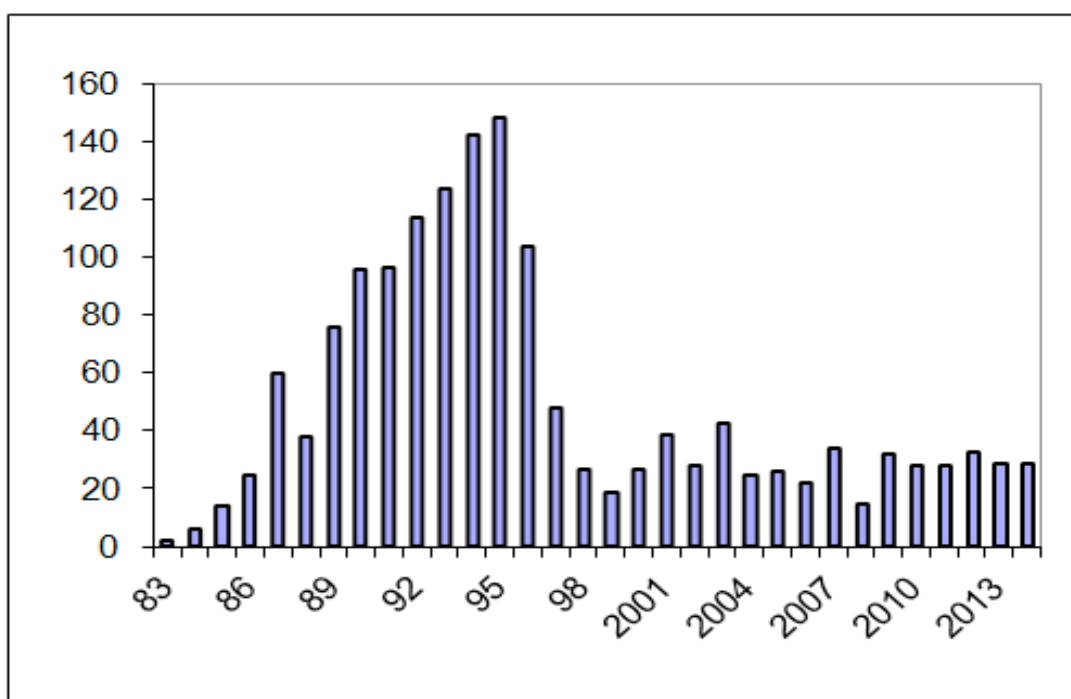
1.1.10. History of HIV and AIDS diagnosis at Venhälsan

One of the 41 first reported AIDS patients was a Danish citizen (1) and many of us thus expected that it was only a matter of time before this new disease would appear in the Scandinavian countries. In response to this, an initiative by several physicians, in collaboration with the Department of Venerology at Stockholm South General Hospital, The Swedish Institute for Infectious Disease Control and The Swedish Association for Sexual Equality led to the opening of an outpatient clinic called Venhälsan. Its purpose was to test gay men for sexually transmitted infections (STIs) and attempt to detect signs of the new immune deficiency syndrome. In the beginning, the clinic was only open in the evenings and all tests and examinations could be performed anonymously. All symptoms suspected to be

caused by STIs were treated at the clinic as described by Moberg et al (84), but if there were signs of AIDS the patients were referred to the Infectious Diseases Clinic at Roslagstull Hospital. Several of these first patients were described in Morfelt Månson’s thesis in 1989 (85). As mentioned above, HIV testing became available in 1985 and, beginning in 1986, Venhälsan was open five days a week in order to take care of the increasing number of HIV-positive patients. In November 1988, an inpatient ward for AIDS patients was opened; the care offered included treatment of all OIs, Kaposi sarcoma, and postoperative care after surgery as well as palliative care. The staff included a chef to make as good and nutritious food as possible for the patients. Initial treatment of patients with malignant lymphoma was in the haematological department. In January 1994, the Maria Regina Hospice began to care for some of our most ill patients. At the outset, over 90% of patients with diagnosed AIDS died within 2-3 years.

The number of patients with HIV infection that died each year in the Stockholm metropolitan area between 1983 and 2014 are shown in Figure 2. The decline in 1988 represents the introduction of AZT and the decline after 1996 represents the introduction of ART.

Figure 2



After 1997 most of the patients died of non-AIDS related diseases but still a few patients have died of AIDS during these years, mostly if they present with a long-lasting PCP or a malignant lymphoma. These patients are called “late testers” or “late presenters”.

.A graphic presentation of how AIDS developed in HIV-infected patients before the introduction of ART is shown in Figure 3 from Stewart (86).

Figure 3

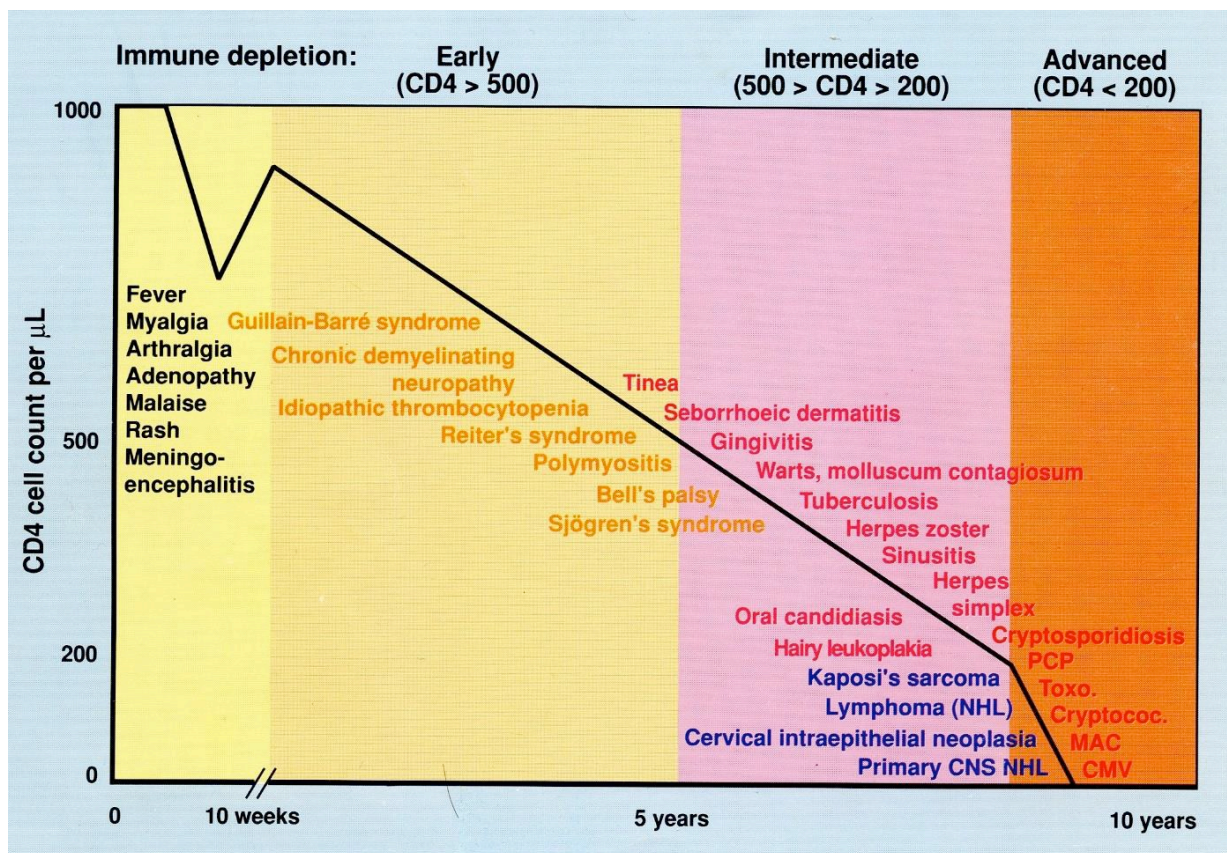


Figure 3 illustrates clinical symptoms and syndromes developing in cases of HIV infection. With decreasing CD4+ count, more serious opportunistic infections and cancers appear.

2 AIMS

The studies in this thesis aimed at investigating:

1. The causes of death in all Venhälsan patients, from diagnosis of the HIV infection to death, between 1989 and 1996
2. All OIs and OCs in patients who died with CD4+ counts below $100 \times 10^6/\text{mL}$
3. The prevalence of CMV encephalitis (CMV-E) and Korsakoffs syndrome (Paper I)
4. CMV retinitis (CMV-R) in relation to CMV-E (Paper II)
5. CMV adrenalitis (CMV-A) and its relation to CMV-R and CMV-E, including comparison of CMV assessment by PCR in blood and the Synacthen test
6. The correlation between CMV disease and other OIs and OCs (Papers I, II and III)

7. The interaction between CMV, Epstein Barr virus (EBV) and other human herpes viruses in a case of anaplastic large cell lymphoma (ALCL)

2.1.1 Materials and Methods

2.1.2 Study cohort

This study cohort was followed prospectively and analysed retrospectively. It consists of 254 HIV-infected patients at Venhälsan who were inpatients and/or outpatients from diagnosis to death during 1989-1996. When patients had a fever and a CD4+ count of $<100 \times 10^6/\text{mL}$, isolation of CMV pp65 antigen (beginning in 1989) or CMV-PCR (beginning in 1992) in leukocytes were performed. Patients received toxoplasmosis prophylaxis if their CD4+ cell count was $<100 \times 10^6/\text{mL}$ and they were seropositive for toxoplasmosis antigen in blood. From 1988, patients were given PCP prophylaxis when the CD4+ count was $<100 \times 10^6/\text{mL}$.

As mentioned above, all patients that had been treated for PCP, toxoplasmosis, MAC infection, CMV-R or CMV-E received lifelong secondary prophylaxis. All patients were given HIV treatment, consisting of AZT from 1989, didanosine (ddI) from 1991 and then in combination with zalcitabine (ddC), stavudine (d4T) and/or lamuvidin (3TC) from 1992 and onwards. Autopsy, including the brain, was performed if permitted by the patient or his next of kin. Patients were diagnosed with AIDS according to the CDC criteria (87), including occurrence of OIs (bacterial infections, candidiasis, CMV, toxoplasmosis, PCP, tuberculosis (TB), or OCs (Kaposi's sarcoma, malignant lymphoma, etc) and fever or diarrhoea lasting one month or a 10% body weight loss within a month.

2.1.3 Database

The database was constructed as an Excel file. Coded identification numbers were routinely given to the Venhälsan patients at the time of the investigation. Data on OIs/OCs in the central nervous system (CNS) and results of lumbar puncture (LP), CT, MRI, and neurological consultations, as well as demographic data, were compiled in the database. CD4+ counts analysed within three months prior to death were also registered. The cohort was divided into two groups, the first comprising patients who died with a CD4+ count of $<100 \times 10^6/\text{mL}$ after a long disease course with increasing OIs/OCs and gradually diminishing CD4+ count. The second group consisted of patients who died with a CD4+ count of $>100 \times 10^6/\text{mL}$ after a short disease course and no apparent OIs/OCs. All data from CNS autopsy reports, body autopsy reports and patient records were retrieved.

2.1.4 Autopsies

Body autopsies were performed according to standard protocols (Rokitansky technique) at the Department of Pathology, Karolinska University Hospital in Huddinge. Visceral organs were fixed in formaldehyde and paraffin-embedded. CMV, bacterial infections, MAC, Cryptococcus, Candida, Toxoplasma, Aspergillus, Cryptosporidium and Pneumocystis carinii were assessed microscopically. Non-infectious diseases assessed at autopsy were CNS lymphoma, Kaposi's sarcoma, liver failure, heart failure, cardiovascular disease (CVD) and cachexia.

CNS autopsies were performed, according to standard protocols, by Inger Nennesmo, pathologist at the Department of Pathology, Karolinska University Hospital in Huddinge. Brain biopsies or brain slices were fixed in 4% formaldehyde and paraffin-embedded for 2 weeks to 2 months (to reduce infectivity). The brain was sectioned in the coronal plane. Tissue samples from frontal, temporal and occipital cortex; medulla oblongata; pons; midbrain; cerebellum; hippocampus; basal ganglia; thalamus and white matter were collected and analysed. Macroscopic focal lesions were sampled for histological analysis. Each tissue was stained with haematoxylin-eosin and the white matter was stained with Luxol fast blue. Basal ganglia, caudate nucleus, mesencephalon, pons, medulla oblongata and cerebellum were also examined macroscopically.

Microscopic examination, immunohistochemistry (IHC) and *in situ* hybridization (ISH) were performed on each tissue sample that was stained with haematoxylin-eosin for diagnosis of OIs, OCs and HIV-E. Special histochemical staining (Periodic acid-Schiff, Gomori-Grocott, Ziehl-Neelsen, Gram staining, Giemsa) was performed if bacterial, fungal or MAC infections were suspected.

CMV, lymphoma and HIV were routinely tested by IHC, performed with monoclonal antibodies against CMV (monoclonal antibody CCH2), HIV (anti-p24 antibody) and B-cells (monoclonal antibody L26 against CD20). Toxoplasma and syphilis infection were identified with polyclonal antiserum for IHC. CMV, JC virus (named after the first patient)-DNA and EBV-DNA were also confirmed by ISH if suspected in the brain. JC-virus is the cause progressive multifocal leukoencephalopathy (PML).

2.1.5 Lumbar puncture and cerebrospinal fluid, samples and analysis

CSF was sampled when patients complained of headache and fever and no other cause could be found. LP was also done if patients had neurological symptoms such as seizures, memory

loss and confusion, especially when the CD4+ count was $<50 \times 10^6/\text{mL}$. LP was contraindicated in patients showing symptoms of high intracranial pressure.

If LP was performed, the analyses included number of cells, glucose, proteins, cytology, bacterial culture, mycobacterial culture, Cryptococcus antigen, CMV-, EBV-, and JC-PCR, Toxoplasma gondii antigen and syphilis (Wassermann reaction).

2.1.6 Diagnosis of adrenal insufficiency with the Synacthen test

In patients with CD4 counts $<100 \times 10^6/\text{mL}$ and clinically suspected adrenal insufficiency a Synacthen test was performed. Several patients were tested at more than one occasion. The Synacthen test in our hands was the first pathological test obtained for each patient. The cortisone levels in blood were measured before injection of Synacthen, and 30 and 60 minutes thereafter. Interpretation of the test is basal and/or stimulated plasma concentration of cortisone above 500 nmol/L- standard level - is considered normal and an increase of 200 nmol/L at 30 or 60 minutes after stimulation is also considered to reflect normally functioning adrenal glands.(88, 89) A higher basal and/or stimulated level of 700 nmol/L - modified level - for normal response to ACTH has been used as recommended by Cooper and Henzen (90, 91). Cortone treatment was given to all patients with pathological Synacthen test at the standard level.

2.1.7 Computed tomography and magnetic resonance imaging

CT or MRI was performed if patients had neurological symptoms or if OIs/OCs in the brain were suspected. CT/MRI findings were retrieved from medical records.

2.1.8 Clinical examination

Neurological examination was performed on patients with symptoms from the central and/or peripheral nervous system by Ritva Pirskanen-Matell, neurologist at the Department of Neurology, Stockholm South General Hospital. Data was retrieved from medical records.

The neurological examinations assessed the cranial nerves, sensory and motor systems. The following cognitive tests were performed: orientation, talking, reading, writing, calculating, drawing a face, copying a three-dimensional drawing, ability to separate left from right, ability to understand abstract meanings, gnosis and praxis. Memory examination included repeating six numbers and the short-memory test included remembering five items after three minutes and re-reporting a text after reading it. The long-term memory test was to recall what had happened the preceding year. To differentiate HIV-E from other causes of encephalitis,

the finger tapping test, verbal fluency, connecting consecutive numbers, connecting consecutive numbers and letters and digit symbol tests were performed.

Ophthalmological examination was performed by an experienced ophthalmologist specialized in retinal pathology.

2.1.9 Treatment of opportunistic infections

The different OIs were treated according to national guidelines at the time of the study.

2.1.10 Statistical analysis

The IBM Statistical Package of Software Science (SPSS) 23 was used for analysis of the means and standard deviations. Calculations were undertaken with Fisher's two-tailed exact test. The Mc-Nemar's test was used to analyse differences between two CMV methods in Paper I.

2.1.11 Ethical considerations

The study was approved by the Ethics Committee at the Karolinska Institute, Stockholm (Dnr 168/01)

3 RESULTS AND DISCUSSION

Table one shows all the patients in the cohort. All were males and from the Stockholm area. Median age of diagnosis of HIV was median 35 years (range 23-65), Death with CD+ counts of $> 100 \times 10^6/\text{mL}$ was 45 years (31-65) and death with CD4+ counts $< 100 \times 10^6/\text{mL}$ was 42 years (28-73)

Table 1 Total cohort

Patients that died with CD4 + counts $< 100 \times 10^6/\text{mL}$	219
Patients that died with CD4+ counts $> 100 \times 10^6/\text{mL}$	23
Patients not eligible for the cohort or lost to follow-up	12
Total	254

Table 2 shows the causes of death in the smaller group of patients with a better preserved immune system.

Table 2. Causes of death in patients with CD4+ counts > 100 x 10⁶/mL

Diagnosis	No. of patients	No. of patients with alcohol as contributing factor to death
Cardiomyopathy	5	5
Suicide	5	5
Cancer with metastases	3	0
Malignant lymphoma	2	0
Cerebral infarction	2	0
Sudden cardiac death	2	0
Dissecting aortic aneurysm	1	1
Encephalitis of unknown cause	1	0
Hypoglycaemic coma	1	1
Accident	1	0
Total	23	12

And Table 3 shows causes of death in the patients with severe immune deficiency.

Table 3. Causes of death in patients that died with CD4+ counts < 100 x 10⁶/mL n=219

Cause of death	No. of patients	No. of CMV-PCR+ patients
CMV disease	106	104
MAC infection	58	54
Kaposi's sarcoma	49	46
Toxoplasmosis	29	20
Malignant lymphoma	22	22
CNS lymphoma	20	19
Cardiovascular disease	12	11
PCP	11	10
Progressive multifocal leukoencephalopathy (PML)	11	5
Cryptosporidium infection	9	8
Suicide	5	4
Possible adverse reaction to drugs or unknown factor	5	5
HIV encephalitis	4	2
Cryptococcal meningitis	3	3
Other cancers	3	3
HIV wasting	2	1
Cysticercosis	1	0
Herpes simplex encephalitis?	1	1
Aspergillus pneumonia	1	1
Pulmonary tuberculosis	1	1

In the table, all patients with suspected CMV-disease are included, many died of CMV-E. If not diagnosed by a neurologist, they were not included in the Paper I, II and III.

Table 4. CMV infections in HIV-infected patients who died with CD4+ counts < 100 x 10⁶/mL

CMV manifestation	n
CMV-PCR positive	187
CMV retinitis	84
CMV encephalitis	65
CMV adrenalitis	41
CMV in the gastrointestinal tract	21
CMV colitis	13
CMV esophagitis	5
CMV gastritis and/or duodenitis	3

CMV-E, CMV-R and CMV-A will be described in Paper I, II and III.

3.1.1 CMV-infection in the gastrointestinal tract

Eight of the patients with CMV colitis have been reported by Söderlund et al.(92) These patients underwent surgery, four hemicolectomy or four ileocecal resection with good results and no sequelae in spite of their severe immune deficiency. Of the 21 patients with CMV in the gastrointestinal tract three died of PCP, one of toxoplasmosis, one of cryptosporidiosis, and one of K S and pneumonia not long after diagnosis of CMV-disease.

Of the last 15 patients nine got CMV-R and nine CMV-E. Thus, the course of the CMV-reactivation in these patients was first diagnoses by CMV-PCR in the blood, then gastrointestinal CMV within one to six months and finally CMV-R and CMV-E within 18 months after the gastrointestinal CMV. Most of these patients got treatment with foscarnet in courses of seven days every 42 days (six weeks) but that did not prevent development of

CMV-A, E and R, however it might have delayed the development of the late manifestations of CMV.

3.1.2 CMV in the blood

Table 5. Blood parameters at the first positive CMV-antigen or CMV-PCR test, n = 187

Test	Median (range)
Haemoglobin, g/L	113 (70-157)
White blood count $10^9/\text{mL}$	3,2 (1,0-23,80)
Absolute neutrophil count, $10^9/\text{mL}$	1,9 (0-9.3)
Platelets, $10^{12}/\text{mL}$	170 (13-606)
CD4+ count, $10^6/\text{mL}$	20 (1-220)
CD8+ count, $10^6/\text{mL}$	430 (20-1750)
CD4+/CD8 ratio	0,07 (0,0-0,7)

The blood parameters show (as expected) low CD4+ and CD8+ counts and very low CD4+/CD8+ ratios when the first sign of CMV infection appeared, Haemoglobin, white blood count (WBC) and absolute neutrophile count (ANC) were also low.

At the time of CMV testing, most patients were not taking medications that suppressed bone marrow function. These low values are thus probably due to the reactivated CMV infection in combination with advanced HIV-infection. AZT can suppress the bone marrow in a few per cent of patients, these patients, with the typical AZT related anaemia, were instead given another nucleoside analogue.

3.1.3 Causes of death in the whole cohort

In the patients that died with CD4+ counts of $> 100 \times 10^6/\text{mL}$, the primary causes of death were cardiovascular disease (CVD) in ten, cancer with metastases in five and suicide in five cases. Half of the patients had alcohol problems stated in their medical records. From the

beginning of the epidemic, we knew that gay men with alcohol or drug problems were at a high risk of becoming HIV-infected.

In the cohort of patients that died with CD4+ counts $< 100 \times 10^6/\text{mL}$, the dominant causes of death were CMV disease, MAC infection, malignant lymphoma and Kaposi's sarcoma.

All but 2% were CMV-seropositive at the study start, which explains the high rates of CMV activation and disease in our cohort. Our findings are similar to autopsy studies like Monforte (56, 93, 94) MAC is very common in water in the Nordic countries which can explain the many MAC infections.(95) Only one patient died from pulmonary TB. Five patients presented with TB, four with pulmonary TB and one with colitis. Four were cured from their TB infection and survived several years before they died of progressive HIV disease. Four of the five contracted the infection in another European country or in the US. Death from PCP only occurred in 5% of patients, probably due to effective PCP prophylaxis. Toxoplasmosis is not so common in the Swedish population (23% are seropositive) (96) which might explain the relatively low incidence in our material. We also gave prophylactic treatment to all Toxoplasma-seropositive patients.

It is interesting that there were 5% cardiovascular deaths, of which 92% were CMV-PCR positive in blood, indicating CMV reactivation. All patients in this group died outside hospital and underwent forensic autopsy, i.e. the presence of CMV in the heart or brain was not investigated. CMV is increasingly suspected of contributing to CVD in HIV-negative populations.(97, 98)

Five patients had committed suicide and they were all severely ill with several OIs at the time.

For a more detailed investigation of CMV role in the course of the HIV infection in these patients, we studied CMV-E and the Korsakoff syndrome (Paper I), CMV-E and CMV-R (Paper II), CMV-A and the correlation to CMV-PCR in blood, CMV-E and CMV-R (Paper III), as well as the consequences of an acute CMV infection in a CMV-seronegative HIV-infected patient with a well-preserved immune system (Paper IV).

4 CMV ENCEPHALITIS AND THE KORSAKOFF SYNDROME (PAPER I)

In the first study, we investigated why several of our patients contracted Korsakoff syndrome. The Wernicke-Korsakoff syndrome was first described in 1881 by Wernicke (99) as an acute encephalopathy with global confusion, memory disturbances, ophthalmoplegia, nystagmus

and unsteadiness of gait. If the patient is not treated when presenting with these symptoms, this condition will develop into a chronic encephalopathy, the so called Korsakoff syndrome, characterized by progressive dementia, apathy, confabulation, non-violent psychosis and peripheral polyneuropathy. Vitamin B1 (thiamine) deficiency is believed to be the cause of Wernicke encephalopathy and it can be reversed by thiamine substitution in large doses.

Victor (99) demonstrated neuropathological lesions around the third and fourth ventricle of the brain, especially in the surrounding thalamus, hypothalamus, mammillary bodies and olive nuclei. He found necrosis and haemorrhagic lesions in these areas. The affected areas also include the Papez circuit or limbic system. The limbic system was first believed to be the circuit that controls emotions, but is today considered to be the main circuit for establishing memory.(100)

The clinical diagnosis was made by a neurological examination combined with CSF analysis, because CT and MRI has not the sensitivity to diagnose Korsakoff syndrome. Typical macroscopic and microscopic signs of CMV infection in Korsakoff syndrome are shown in Figures 4 and 5.

To be as certain of the diagnosis as possible, we decided to study only the CMV-E patients who had been examined by a neurologist less than six months before death. CSF was taken from all patients and CT and MR scans were done in most patients.

4.1.1 Results

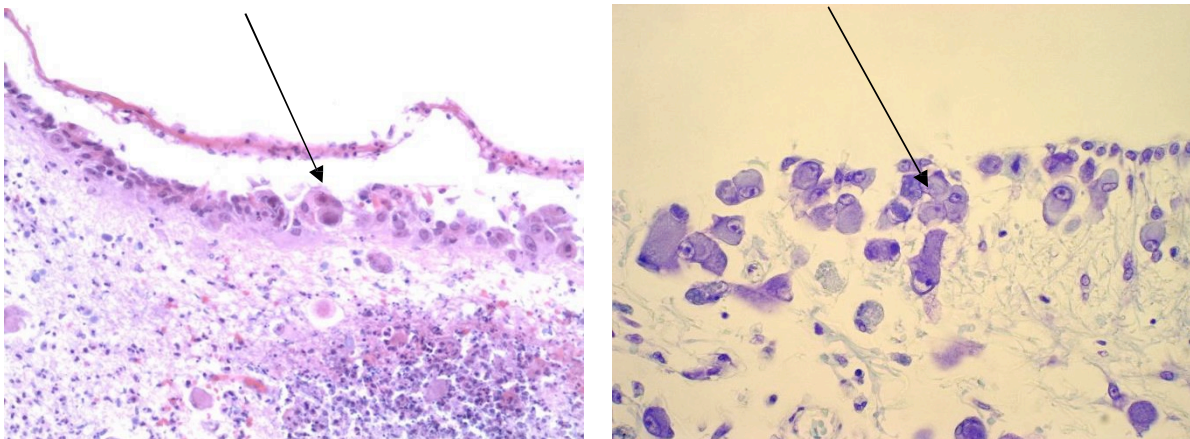
Half of the 34 patients had Korsakoff syndrome. Autopsy was performed in 24 of the 34 patients and in 13 of the 17 patients with Korsakoff syndrome. We found CMV infection with varying degrees of necrosis and micro nodules around the third and fourth ventricles in all 34 patients. Furthermore, we also found CMV infection in the hypothalamus and hippocampus in all patients and in the mammillary bodies and the olive nuclei in several patients, especially the Korsakoff patients. Figure 4 shows a section of the brain and Figure 5 shows typical microscopical findings in our patients with CMV-E

Figure 4



Cross-section of a human brain. The red lines indicate the lining of the third and fourth ventricles. These are typical locations for CMV-infected cells. In most patients, there was also CMV infection in other parts of the brain.

Figure 5



Microscopic image of CMV infection in the same area; haematoxylin-eosin staining on the left and Lyxol fast blue staining on the right. Typical CMV-infected cells and inflammation are seen in both slides.

We did not observe the haemorrhages and necrosis described by Victor; nor did we see other OIs in the same areas, with the exception of toxoplasmosis in some individuals. It seems as if the Korsakoff syndrome in these patients is caused by changes in the same areas as described by Victor and is part of a more generalised CMV infection in the CNS and the whole body.

Our patients often had fever, a slight headache and, in some cases, bizarre psychotic symptoms as well.

Table 6. Cerebrospinal fluid findings in 34 patients, at 0-6 months before death. Mean (range) is shown.

Mononuclear cells	Polynuclear cells	Protein	Glucose	CMV-PCR positive
2 (0-82)	0 (0-32)	0,34 (0,2-1,7) mg/mL	2,4 (0,14-2,9) mg/mL	25/31

The findings in CSF, including in patients with very advanced HIV and CMV infections, the CSF was often devoid of mononuclear and polynuclear cells and mostly exhibited a normal protein pattern. Overall, our results are in accordance with other studies by Sundström et al and Hagberg et al (101-104). We found that CMV-PCR was positive in 81% of the samples taken.

In Table 7, patients with CMV-E with and without Korsakoff syndrome are listed with their OIs, OCs and other intercurrent conditions. Some were heavy alcohol users, as noted in their medical records. There were no statistically significant differences between the respective occurrence of other OIs or OCs. There were more heavy alcohol users in the Korsakoff group but the difference was not statistically significant. CMV-A seems to be more prevalent in the CMV-E group without Korsakoff. A diagnosis of CMV-A infers that the patients had been given cortisone treatment, which might have had a protective effect on the brain. On the other hand, the Korsakoff patients might have had undiagnosed adrenal insufficiency, adding to the symptoms of CMV in the brain. An interesting finding is that there was more polyneuropathy in the Korsakoff group. Polyneuropathy is a common feature of CMV in HIV patients(105). However, the number of patients in this study is small, so it is difficult to draw any firm conclusions.

Table 7 Intercurrent conditions in patients with CMV encephalitis with and without Korsakoff syndrome

	CMV-E N=17	CMV-EK N= 17	Statistical significance P
Heavy alcohol drinker	1	6	0,078
Polyneuropathy	12	2	<0.001
CNS OI/OC			
PML	0	3	NS
Toxoplasmosis	0	1	NS
CNS lymphoma	1	2	NS
Systemic OI/OC			
Kaposi´s sarcoma	5	3	NS
MAC infection	9	10	NS
Other CMV disease	16	17	NS
CMV-R	16	16	NS
CMV-A	15	6	<0.01
CMV colitis	0	1	NS

p- value derived by Fisher´s exact test (two tailed)

Abbreviations: CMV-E: CMV encephalitis; CMV-EK: CMV-E with Korsakoff syndrome; OI: opportunistic infection; CNS: central nervous system; OC: opportunistic cancer; PML: progressive multifocal leucoencephalopathy; CMV-R: CMV retinitis; CMV-A: CMV adrenalitis

5 THE CORRELATION BETWEEN CMV RETINITIS AND CMV ENCEPHALITIS (PAPER II)

In this study, we investigated all patients with a diagnosis of CMV-R, focusing on the extent of the eye infection, the outcome of specific CMV treatment and possible correlations with CMV-E diagnosed ante mortem or at autopsy. We also correlated the CMV-R diagnosis to other OIs in these patients as well as to patients that did not have CMV-R. CMV-R was divided into unilateral and bilateral disease, in order to investigate any difference in therapy outcome or late complications. We also investigated whether central or peripheral CMV-R influenced the outcome.

We found that CMV-R was the most common CMV manifestation as well as the most common OI in our cohort. There was a highly significant correlation between CMV-R, CMV-E and CMV-A. There was a lower, but significant, correlation with MAC infection, but not with other OIs. There was a statistically significant correlation between patients that did not have CMV-R and toxoplasmosis. This might be explained by the fact that many patients contract toxoplasmosis at a higher CD4+ count, together with the bad prognosis of toxoplasmosis. Thus these patients did not live long enough to get the low CD4+ counts where CMV-R develops. There was no statistical difference with regard to response to treatment or late complications, such as retinal detachment, central retina destruction or CMV-E. Only 50 % had a regression at the first ophthalmological check-up at two- three weeks after initiation of treatment. Most patients progressed slowly despite combination treatment. A more positive outcome was that vision was maintained in 75%, while only 25% totally lost vision (visual acuity < 0.1) in the affected eye. Survival was poor in both groups and no statistically significant difference in survival duration was noted between them. In the whole group, median survival was 207 days (range 3-817). In the autopsied patients that had CMV-E, the CMV-infected cells and CMV antigen were clearly visible despite intensive antiviral treatment for CMV for more than half a year. The overall impression was that the specific CMV treatment had had a limited effect, in contrast to the CMV treatment effect in patients with immunodeficiency from other causes than HIV infection.

6 CMV-ADRENALITIS: THE VALUE OF SYNACTHEN TESTS AND CMV-PCR IN BLOOD (PAPER III)

In this study, we investigated all patients with a positive CMV-PCR for adrenalitis if they had fatigue, hypotension (blood pressure < 100/70 mm Hg), nausea and/or diarrhoea. The adrenal glands are shown in Figure 6. They consist of medulla, developed from the ectoderm embryological plate (same as the brain), and cortex, derived from the mesodermal embryological plate. The cortical cells secrete cortisone, aldosterone and dehydroepiandrosterone (DHEA); the medulla cells secrete epinephrine and nor epinephrine. All of these hormones are important for survival, but cortisone is the most important because insufficient cortisone secretion can be an acute life-threatening condition. Other organs, such as the liver, can take over the production of epinephrine and nor-epinephrine, for example, so insufficiency of the adrenal medulla will not produce symptoms as fast as insufficiency of the cortex. Addison's disease, first described by Thomas Addison in 1855, is characterized by fatigue, hypotension, nausea, diarrhoea and extreme pigmentation of the skin and the lines in the palm. The pigmentation is caused by compensatory release of adrenocorticotrophic hormone (ACTH) and melanocyte-stimulating hormone (MSH) from the pituitary. Classic Addison's disease is most often caused by an autoimmune disorder or by TB. The disease develops slowly over several years. Elevated serum potassium and decreased serum sodium are typical findings. The author Jane Austen and the American president J.F. Kennedy are two famous Addison's patients.

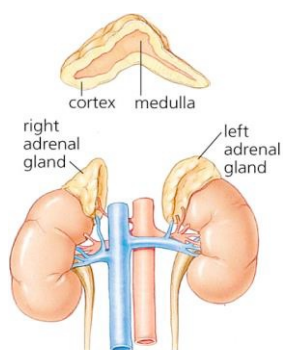


Figure 6. Adrenal glands, palmar hyper pigmentation

The position of the adrenal glands and the adrenal cortex and medulla are shown to the left. The typical hyper pigmentation of lines in the palm seen in Addison's disease is shown to the right.

To diagnose adrenalitis a Synacthen test (Figure 7) was performed in these cases.

Figure 7 Synacthen test

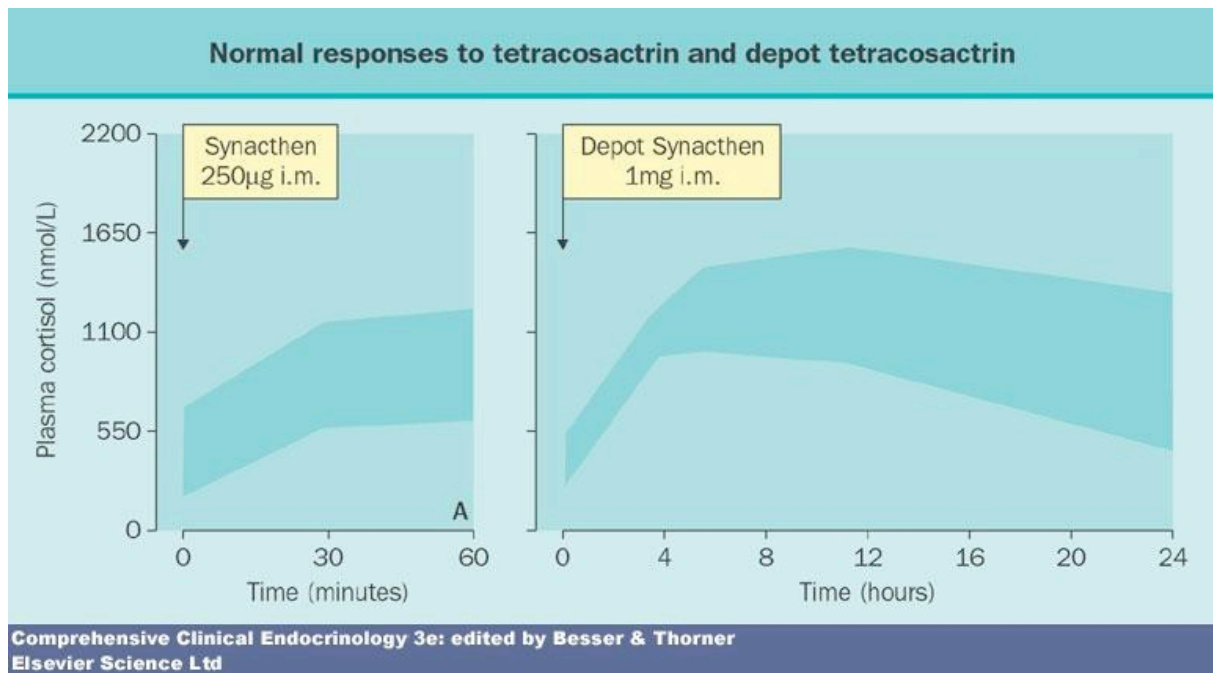


Figure 7 shows the increase in cortisone levels after injection of Synacthen. The left-hand curve shows the standard level values. The modified level would be a line 200 nmol/L above the dark blue line in the figure. The right-hand test was not used.

6.1.1 Clinical symptoms and findings

The majority of the patients had symptoms and therefore thus underwent a Synacthen test. At that time, we only treated patients if the Synacthen test was pathological based on the standard level. Even if the test was pathological according to only one of the two criteria for the standard level definition, treatment was also administered. All patients improved dramatically. Seven patients went into a typical Addison's crisis when medication was forgotten or another infection developed rapidly. These patients improved again on increased cortisone therapy.

6.1.2 Autopsy findings

Of the 46 investigated patients with CMV-A, 44 had macroscopically atrophic adrenal glands, according to the autopsy report. In 41 of the analysed adrenal glands, we discovered

typical signs of inflammation, necrosis and atrophy, and typical CMV cells were found in most of them. These findings were most prominent in the adrenal medulla but the structure of the cortex was also severely damaged (Figure 2 in Paper IV) and replaced by inflammation, CMV cells and atrophy.

6.1.3 Clinical symptoms correlated to other CMV-manifestations

Symptoms of adrenalitis were strongly correlated with positive CMV-PCR in blood $p < 0.001$, CMV-A $p < 0.001$, CMV-E ($p < 0.001$) and CMV-R ($p < 0.01$), but not with CMV in the gastrointestinal tract ($p = 0.08$). There was a minor significant correlation between CMV-A and malignant lymphoma ($p = 0.03$), (we found malignant lymphomas in two of the patients with CMV-A) but not with any other OI or OC. Thus, a positive CMV-PCR in blood is a good marker of CMV infection in the adrenal glands.

6.1.4 Synacthen tests

We considered two interpretations of the Synacthen test results in CMV-A. As the patients obviously had other OIs, a higher threshold could be applied when interpreting the test results (90, 91) have shown that a higher threshold can be applied in patients under stress. The higher threshold would also apply to the Synacthen tests that did not quite fulfil the criteria with the standard level definition. There was a better correlation between test results and CMV-A if the modified level was applied, but we still failed to detect all CMV-A with the Synacthen test. Indeed, seven (14%) cases of typical CMV-A had normal Synacthen tests. In all the Synacthen tested patients we did not see the typical increase in potassium, decrease in sodium or the increased skin pigmentation associated with longstanding adrenalitis. This may be because CMV-A seems to develop rapidly within months and is accompanied by other CMV manifestations, such as CMV-R and CMV-E. These other CMV-induced diseases may obscure the symptoms of CMV-A. Another explanation could be that CMV-A develops so rapidly that the patients only contracted minor symptoms of CMV-A before they died of CMV-E.

We found a high incidence (91%) of CMV-A, consistent with previous autopsy reports (106). In addition only two other OIs (PCP and Kaposi's sarcoma) were found. They caused minor pathology in the adrenal glands and were probably not the cause of the adrenalitis symptoms. We also found a very strong correlation between adrenalitis symptoms and reactivation of CMV infection, CMV-A, CMV-E and CMV-R.

The correlation between autopsy findings and the Synacthen test results was not very strong, but the rapidly developing CMV-A and the fact that we did not examine both adrenal glands

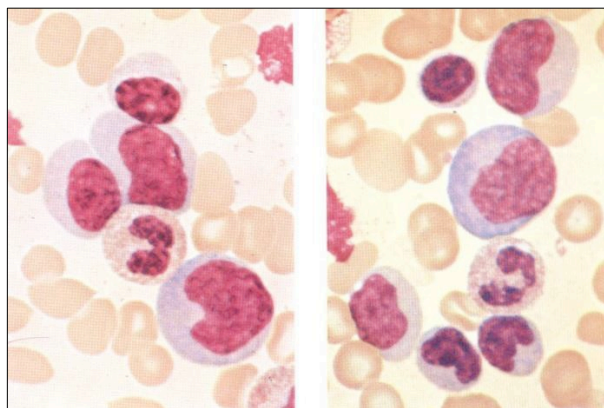
microscopically might be two reasons for this. In fact, there have not, to our knowledge, been any studies published during the last 50 years, comparing results of Synacthen tests with adrenal gland findings at autopsy (Pub med search 2017).

7 ANAPLASTIC LARGE CELL LYMPHOMA, PRIMARY CMV-INFECTION, HHV-8 INFECTION AND EBV REACTIVATION (PAPER IV)

In our first three studies, we investigated the role of reactivated CMV infection in patients with advanced HIV infection. In this paper, we describe another disease caused by a primary CMV infection and other human herpes viruses in a patient with an HIV infection and well-preserved immune status.

A young man with an HIV infection detected two years earlier, and who was healthy without treatment, fell ill with fatigue, fever and enlarged lymph glands and spleen. He was admitted to our inpatient unit. Blood tests, including WBC and differential count were performed. They revealed lymphocytosis and cells typical for mononucleosis, which might have been due to CMV or EBV infection. Blood tests for CMV and EBV were taken. However, the patient's health did not improve. The lymph glands in the neck, axillae and spleen continued to increase in size, as did the liver.

Figure 8 Blood differential count image (haematoxylin-eosin staining).



Red blood cells and enlarged Mackinlay cells are seen in both panels. Such abnormal cells are seen in acute or primary CMV and EBV infections and are of T-cell origin (activated CD8 cells).

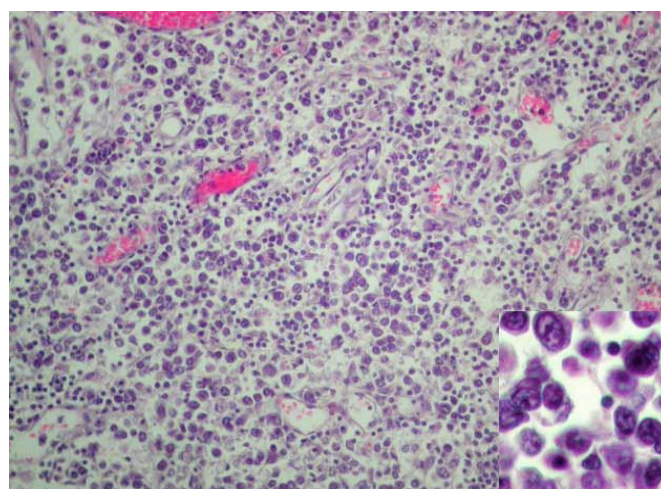
A malignancy such as lymphoma was suspected. A bone marrow aspirate, a liver biopsy and a biopsy from a neck lymph gland were performed. The lymph gland only showed reactive inflammation at initial assessment and no lymphoma was found in the liver or bone marrow aspirates at that time. Later test results from the blood samples taken at admittance showed positive CMV-PCR and the patient was treated with foscarnet, with good effect initially. His

fever disappeared and his health improved. Then he suddenly deteriorated and died. Autopsy revealed a generalized malignant lymphoma in the lymph glands, spleen, liver and bone marrow (Figure 9). The immediate cause of death was adult respiratory distress syndrome

The lymphoma was classified as an adult large cell lymphoma (ALCL). A re-evaluation of the lymph node from the neck showed a small focus of the same lymphoma. All blood samples taken before and after the lymphoma onset were analysed for CMV, EBV and HIV, since we suspected that these viruses might have influenced the lymphoma development. Later, herpes simplex virus (HSV) 1 and 2, varicella zoster virus (VZV) and human herpes virus (HHV)-6, -7 and -8 assays were added. It emerged that the patient had a latent EBV infection that was reactivated by a primary CMV infection. Moreover, he had a HHV-8 infection with rapidly increasing HHV-8 and EBV-PCR levels at the same time as the tumour developed. The development of IgM antibodies to HHV-8 suggested a primary HHV-8 infection. The strong inflammation induced by the primary CMV infection might have activated the carcinogenic properties of both EBV and HHV-8, causing the fatal lymphoma. EBV is believed to cause most of the lymphomas in HIV patients,(107) and HHV-8 also contributes (108). None of them have previously been described in connection with ALCL in AIDS patients. To our knowledge this is also the first time that an interaction between three herpes viruses causing a malignant lymphoma is described. The other five herpes viruses were not activated or believed to be involved in the development of the lymphoma.

Figure 9

Adult large cell lymphoma cells (magnification x 10, insert x 40)



8 CONCLUSIONS

Reactivated CMV infection was the most important OI in this cohort of gay men with HIV infection as 87% were CMV-antigen or CMV-PCR positive in blood. Ninety-eight percent of them had acquired the CMV infection previously, as determined by serology tests. The most important clinical manifestations were CMV-E, CMV-R and CMV-A. This is in contrast to most reports on AIDS-patients, which find CMV-retinitis and CMV-colitis to be the most common CMV-manifestations. In these reports CMV-A and CMV-E are not systematically investigate.

CMV also, in this study, seems to be the most important factor in causing death, often in combination with other OIs.

The typical cause of CMV-reactivation is The results of these studies are of importance for all HIV patients, irrespective of gender, transmission route of the HIV-infection, as the majority of HIV infected patients are co-infected with both viruses. It is also important to acknowledge that reactivated CMV infection is mainly manifested in the brain, retina and adrenal glands, in contrast to the CMV manifestations in induced immune deficiency during chemotherapy for tumours or immunosuppressive therapy after transplantation. One explanation for this could be that these two viruses, which both are neurotropic, interact differently compared to immune deficiency from different causes. The combination of CMV and HIV is considered a significant risk even in HIV patients taking ART both (109). As we have shown antiviral treatment for CMV had limited effects in these co-infected patients before ART for HIV infection became available. Thus, when CMV is detected in HIV-infected patients, ART treatment should begin as soon as possible. The natural history of HIV and CMV infection in co-infected patients has thus been described in this cohort of male HIV patients studied before ART became available. The findings have significance for:

1. All HIV-infected patients with CD4+ counts $< 100 \times 10^6/\text{mL}$
2. HIV infections discovered late, in so-called late testers
3. Patients with limited access to HIV treatment
4. Other patients treated with immunosuppressive therapy, especially those treated with anti-T-lymphocyte antibodies
5. Bone marrow transplant or solid organ transplant recipients

The studies in this thesis also add knowledge concerning the nature CMV - infection:

1. Knowledge about the natural course when treatment is not available
2. Knowledge about CMV-adrenitis and its relation to CMV-retinitis and CMV-encephalitis
3. Knowledge about the neurotropic nature of CMV in patients co-infected with HIV
4. Knowledge about the influence of primary CMV infection on the gamma herpes viruses
EBV and HHV-8 in rapidly progressing malignant lymphomas
5. Possible contribution to our understanding of the influence of CMV in the aging process

9 FUTURE PERSPECTIVES, RESEARCH ON CO-INFECTED PATIENTS

In recent years, the focus of CMV research has been on its possible role in arteriosclerosis in patients without HIV-infection. Most studies have been based on serology studies, showing that people with positive CMV serology carry a higher risk of arteriosclerosis. Furthermore, the interaction between HIV and CMV has been recently been highlighted by Gianella et al (71, 110).

In these studies patients with reactivation of CMV for shorter periods of time in HIV-patients has been investigated.

Many of the patients at Venhälsan that survived after being given ART during 1996-1998 were CMV-PCR positive in blood and some have also had CMV-R. A few have had CMV-E.

These patients have had a longer lasting CMV-reactivation (for months to a year) as measured by CMV-PCR. Thus we are planning a follow-up study of all these patients with focus on the late effects of CMV reactivation, including residual symptoms after subclinical CMV-E, CMV-A and also arteriosclerosis. We have noted an increased incidence of chronic pancreatitis, pancreatic insufficiency as well as gout in many of our patients and will investigate if that could be related to longstanding HIV and CMV co-infection. An investigation of patients that had had a significant CMV reactivation measured with CMV-PCR in blood might provide new information on the interaction between HIV and CMV.

We and others have noticed that 90% of the patients have the CXCR4 virus in the blood when they have AIDS-defining diseases (76). It would be interesting to know if it is the CXCR4 form that contributes to the development of CMV-infection in these co infected patients.

10 PRACTICAL GUIDELINES FOR DIAGNOSING CMV-ADRENALITIS, - ENCEPHALITIS AND -RETINITIS IN HIV-INFECTED PATIENTS AND RAPIDLY PROGRESSING LYMPHOMAS.

1. When a HIV-infected patient has a CD4+ count $< 100 \times 10^6/\text{mL}$, CMV-PCR should be analysed in blood. If positive, an ophthalmological examination is indicated, as well as a neurological examination, to detect signs of CNS involvement.
2. If a CMV-retinitis is diagnosed, the possibility of CMV-encephalitis should be investigated both by clinical neurological examination and lumbar puncture. A Synacthen test should also be performed if the patient has signs of adrenal insufficiency. ACTH and aldosterone should be analysed together with the 0 sample for cortisone. In case of pathological results, continue as in point 4.
3. If CMV encephalitis is diagnosed, both CMV and HIV encephalitis should be investigated by neurological examination and lumbar puncture, with analysis of HIV, CMV, EBV, toxoplasmosis and syphilis antigens. A Synacthen test should also be performed.
4. If CMV adrenalitis is suspected, a Synacthen test should be performed together with ACTH, aldosterone, testosterone and sexual hormone binding protein (SHBG) analyses.
5. The Synacthen test should be interpreted at the modified level presented in Paper III.
6. Do not forget that a patient with HIV and reactivated CMV-infection can have more opportunistic infections at the same time.
7. If a very rapidly progressing malignant lymphoma in an HIV patient is found, blood PCR for CMV, EBV and HHV-8 should be tested and the patient should be given initial treatment for CMV with foscarnet or ganciclovir. The patient should subsequently be administered chemotherapy appropriate to the type of lymphoma and be continuously monitored with PCR tests for virus infection.

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First all my patients and their relatives, struggling with disease and always allowing me to take blood tests, biopsies and allowing autopsy. They all wanted to contribute to research in this new and frightening disease. “You can take any blood test and biopsy. We must all learn from this and not suffer in vain” as several patients said.

All staff at Department 53 and G3 Stockholm South General Hospital during 1989-96 who worked so hard caring for the patients, easing physical and psychologic pain and finding solutions to all kinds of problems during these years.

Professor Ingemar Ernberg my main supervisor for endless support, patience, stimulating discussions about research, science and life and friendship. Your weekly research meetings have been an oasis and inspiration for 14 years. I’m very grateful that you could take over as main supervisor when Anneka died.

Professor Anneka Ehrnst My late supervisor for introducing me to lab research, stringent analysis of lab methods and how they should be used in the clinic. Also for teaching me statistics, especially Fishers exact test so I could do it in the middle of the night. Her generosity towards her students when we were visiting art museums, opera or bowling halls together is an example to follow.

Co supervisor Börje Åkerlund for big support, encouraging me to continue with the work after the half time seminar, supplying a lot of interesting articles on CMV in HIV-patients, the Synacthen test at the modified level and thorough revision of the manuscripts.

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