Cytomegalovirus infection in patients with HIV-1

Diagnosis, disease, and death in coinfected patients in Norway and Tanzania

Arne Broch Brantsæter



Faculty of Medicine

University of Oslo



Department of Infectious Diseases and Department of Acute Medicine

© Arne Broch Brantsæter, 2013

Series of dissertations submitted to the Faculty of Medicine, University of Oslo No. 1519

ISBN 978-82-8264-436-5

All rights reserved. No part of this publication may be reproduced or transmitted, in any form or by any means, without permission.

Cover: Inger Sandved Anfinsen. Printed in Norway: AIT Oslo AS.

Produced in co-operation with Akademika publishing. The thesis is produced by Akademika publishing merely in connection with the thesis defence. Kindly direct all inquiries regarding the thesis to the copyright holder or the unit which grants the doctorate.

Contents

A	CKNO	WLE	DGEMENTS	5
A	BBRE	VIAT	IONS	7
Ll	ST O	F PAP	ERS	9
1	IN	TRO	DUCTION	11
	1.1	HIV.		11
	1.2 CMV		,	12
	1.3	SIMIL	ARITIES BETWEEN HIV AND CMV INFECTION	13
1.4 EPIDEM			MIOLOGY	
	1.	4.1	Epidemiology of HIV infection	. 14
	1.4.2 1.4.3		Epidemiology of CMV infection	
			Epidemiology of HIV and CMV coinfection	. 15
	1.5	CLINI	CAL MANIFESTATIONS AND IMMUNE RESPONSE IN CMV INFECTION AND DISEASE	16
	1.	5.1	Definitions: CMV infection and disease	16
	1.	5.2	The immune response to CMV infection	. 16
	1.	5.3	Congenital CMV infection and disease	. 18
	1.	5.4	CMV disease in immunocompetent children and adults	. 18
	1.	5.5	CMV disease in immunocompromised individuals	. 18
	1.6	DIAG	NOSIS OF CMV DISEASE IN IMMUNOCOMPROMISED INDIVIDUALS	20
	1.7	PROP	HYLAXIS AND TREATMENT OF CMV DISEASE	22
	1.8 CMV		AS A COFACTOR FOR PROGRESSION OF HIV INFECTION	23
			Potential immunological mechanisms for the cofactor effect of CMV	. 24
	1.	8.2	Evidence of cofactor effect from epidemiological studies	. 24
	1.9	CMV	-RELATED IMMUNE ACTIVATION, INFLAMMATION AND IMMUNOSENESCENSE	25
2	A	IMS A	ND OBJECTIVES	27
	2.1	MAIN	AIM	27
	2.2	SPEC	IFIC OBJECTIVES	27
3	M	ATER	RIALS AND METHODS	29
	3.1	STUD	Y POPULATIONS, PERIODS, DESIGNS, AND OUTCOMES	29

3.2 DIA	GNOSTIC METHODS	30
3.2.1	Detection of CMV IgG antibody	30
3.2.2	Analysis of CMV viraemia by quantitative PCR	30
3.2.3	Diagnosis of CMV disease	31
3.3 STA	TISTICAL ANALYSIS	31
3.4 Етн	ICAL CONSIDERATIONS	32
4 SUMM	IARY OF RESULTS	33
4.1 PAP	ER I: CMV DISEASE IN PATIENTS WITH AIDS IN OSLO	33
	ER II: CMV QUANTITATIVE PCR FOR DIAGNOSIS OF CMV END-ORGAN DISEASE IN	
	ER III: CMV VIRAEMIA IN DBS IN RELATION TO SURVIVAL IN HIV-INFECTED PATIE	
5 DISCU	USSION	35
5.1 MET	THODOLOGICAL CONSIDERATIONS	36
5.1.1	CMV antibody	36
5.1.2	CMV quantitative PCR and assessment of test performance	36
5.1.3	Diagnosis of CMV retinitis and other end-organ disease	39
5.2 CM	V INFECTION AND DISEASE IN PATIENTS WITH AIDS	40
5.2.1	CMV seroepidemiology	40
5.2.2	Occurrence of CMV disease	40
5.2.3	Survival of patients with CMV disease	45
5.3 CM	V VIRAEMIA AS PREDICTOR OF CMV DISEASE AND DEATH	46
5.3.1	CMV viraemia as predictor of CMV disease	46
5.3.2	CMV viraemia as predictor of death	50
6 CONC	LUSIONS AND FUTURE PERSPECTIVES	53
6.1 Con	ICLUSIONS	53
6.2 Fut	URE PERSPECTIVES OF HIV-ASSOCIATED CMV MORBIDITY IN THE HAART ERA	54
7 REFEI	RENCES	57
Q DADEI	DC I III	75

Acknowledgements

This thesis would never have come to be without the encouragement, practical assistance, intellectual support, constructive criticism - and stamina - of many individuals. First, I would like to express gratitude to my PhD supervisors. The first step in the long march that eventually led to this thesis was taken in 1995 when Johan N. Bruun offered me a one-year research position. After that, Johan has followed my part-time CMV research through ups and downs – as high as Mount Kinabalu, and as low as the shores of the South China Sea. I am also deeply indebted to Dag Kvale for having included me into his scientific sphere in 2008. He convinced me that a PhD was within reach, showed me how it could be done, and walked me across the finishing line.

I would also like to thank my other co-authors in Norway and Tanzania. Oona Dunlop gave me much-needed support during the early phase of my CMV research. Her energy and enthusiasm was, and is, always inspiring. Anne Kristin Goplen was responsible for histopathological diagnosis of CMV disease, and her meticulous effort was essential for the success of this work. Mona Holberg-Petersen had main responsibility for performance of CMV quantitative PCR. I express deep gratitude for her patience, perseverance and soft-spoken, but always well-founded, suggestions. Stig Jeansson is due credit for intellectual support and constructive suggestions in matters of CMV virology. My deep-felt thanks also go to Halvor Rollag for enabling us to compare results of CMV viral load in plasma and in dried blood spots.

Asgeir Johannessen paved the way for our study of CMV viraemia in Tanzanian HIV-infected individuals. I thank him for making his collection of dried blood spots from Haydom Lutheran Hospital available for this purpose. This work was also totally dependent on the active involvement by Ezra Naman at the HIV Care and Treatment Centre. During my time at Haydom he gave me insight into the life of patients with HIV in Tanzania, and impressed me with his kind concern for their needs. It was always a pleasure to work with Sokoine L. Kivuya and meet him both in Tanzania and in Norway.

Trustworthy statistical analyses are key to any scientific paper. I would like to express gratitude to Knut Liestøl and Leiv Sandvik for statistical advice and quality control.

I am greatly indebted to Mette Sannes for providing numerous data files from the HIV database and for retrieving stored plasma samples from the biobank. I would also like to thank Marie Elisabeth Vad and Gunilla Lövgården for hands-on performance of PCR tests and for assistance with locating seemingly lost dried blood spots from the freezers.

Several employers have provided my daily bread during the many years that have passed by since I took an interest in CMV. Although it has often been hard to prioritise between research and more urgent daily tasks, their understanding have allowed me to press on. I would like to express gratitude to Harald Torsvik at Bærum Hospital for his understanding for my research needs when I was offered a position as infectious disease physician in 1997. There, I also received encouragement from unexpected quarters when, secretary Eva Finseth told me of a revelation she had in a dream. She had seen me wearing a dress suit defending my thesis. I also wish to thank Preben Aavitsland who employed me at the Norwegian Institute of Public Health in 2003, as SARS was about to break out. I express my deepest gratitude to Vidar Ormaasen and Helge Opdahl for offering me a position at Oslo University Hospital in 2008, thereby completing the circle, and allowing me to finish this PhD at the institution where it all began.

I would like to express deep gratitude to my mother and late father for supporting me and giving me confidence to meet the many challenges in life that are also necessary in research. A special word of thanks go to my late great grandmother, "Olde", who had a special place in my heart during early childhood years. Unwittingly, she may have stimulated my research interest by nick-naming me "the professor".

Above all, I thank my dear Anne Lise who is so much more than a wife and friend. As a researcher, she has offered me invaluable scientific advice and support over the years. She taught me much that was never covered in the PhD courses at the University. Apologies to our wonderful children Margrethe, Henrik and Thomas who have had to endure countless scientific discussion over dinner and during travels.

Oslo, December 2012

Abbreviations

AIDS Acquired immunodeficiency syndrome

AU Arbitrary unit

CMIA Chemiluminescent microparticle immunoassay

CMV Cytomegalovirus
DBS Dried blood spots

DNA Deoxyribonucleic acid

ELISA Enzyme-linked immunosorbent assay
HAART Highly active antiretroviral therapy
HIV Human immunodeficiency virus

HLA Human leukocyte antigen

IDU Injecting drug user

IL Interleukin

MHC Major histocompatibility complex
MSM Men who have sex with men

NASBA Nucleic acid sequence based amplification

NAT Nucleic acid test
NK cells Natural killer cells

PCP Pneumocystis carinii pneumonia (Pneumocystis jiroveci pneumonia)

PCR Polymerase chain reaction

RNA Ribonucleic acid

List of papers

Paper I

Brantsæter AB, Liestøl K, Goplen AK, Dunlop O, Bruun JN. CMV disease in AIDS patients: Incidence of CMV disease and relation to survival in a population-based study from Oslo. Scandinavian Journal of Infectious Diseases 2002;34:50-55.

Paper II

Brantsæter AB, Holberg-Petersen M, Jeansson S, Goplen AK, Bruun JN. CMV quantitative PCR in the diagnosis of CMV disease in patients with HIV-infection - a retrospective autopsy based study. BMC Infect.Dis. 2007; 7: 127.

Paper III

Brantsæter AB, Johannessen A, Holberg-Petersen M, Sandvik L, Naman E, Kivuyo SL, Rollag H, Bruun JN, Kvale D. Cytomegalovirus viremia in dried blood spots is associated with an increased risk of death in HIV-infected patients: a cohort study from rural Tanzania. Int.J.Infect.Dis. 2012; 16: e879-e885.

"The doubter is a true man of science; he doubts only himself and his interpretations, but he believes in science." Claude Bernard

1 Introduction

In June 1981 the Morbidity and Mortality Weekly Report (MMWR), Centers for Disease Control and Prevention (CDC), carried a report of five cases of Pneumocystis carinii pneumonia (PCP) in homosexual men (1). The cause of this outbreak was unknown, but all five patients had evidence of cytomegalovirus (CMV) disease or virus shedding within five months of the diagnosis of PCP. In the accompanying editorial note, it was stated that the role of CMV in the pathogenesis of PCP was unknown. PCP is associated with immunodeficiency, and these were the first reported cases of acquired immunodeficiency syndrome (AIDS). The cause of AIDS was subsequently demonstrated to be a previously unknown virus – today known as human immunodeficiency virus (HIV). However, the interest in CMV in relation to HIV infection was not over, as it soon turned out that CMV was a cause of serious disease in coinfected individuals. CMV disease has remained an AIDS-defining disease since 1981 (2). In addition, CMV was also early suspected of acting as a cofactor, inducing more rapid progression of HIV infection (3).

During the late 1980s and mid-1990s, patients with AIDS occupied many beds in the wards at the Department of Infectious Diseases, Ullevål University Hospital. Death from complications of AIDS was frequent, and CMV disease was among the most dreaded complications. CMV retinitis lead to impaired vision and blindness, and CMV colitis caused severe diarrhoea. As a junior doctor, I first met AIDS patients with CMV disease in 1988. These troubled patients kindled my research interest in CMV and HIV coinfection. At the time, the autopsy rate in the hospital was high, over 70% in HIV-related deaths, and this was an important asset in the study of CMV disease, which too often was diagnosed at the autopsy table.

1.1 HIV

It is generally acknowledged that Luc Montagnier and Françoise Barré-Sinoussi in 1983 were the first to isolate the retrovirus known today as HIV (4;5). The following year

Robert Gallo provided more convincing evidence that HIV was indeed the cause of AIDS. Today, we know that there are two types of HIV, named HIV-1 and HIV-2. Both viruses probably originated in non-human primates in Africa, where they jumped the species barrier in the late 19th or early 20th century, having evolved from the closely related simian immunodeficiency virus (SIV) (6;7). HIV-2 is less virulent and most prevalent in West Africa. In this thesis, I will for the sake of simplicity refer to HIV-1 as HIV.

HIV is a single-stranded ribonucleic acid (RNA) virus. As a retrovirus, HIV has the capacity to integrate into the host cell genome. It is dependent on several enzymes to convert RNA into deoxyribonucleic acid (DNA), and for subsequent integration into the host cell DNA. In the infected cells HIV may establish chronic infection with very high turnover, but may also lie dormant.

HIV can infect CD4+ T cells, macrophages, dendritic cells and microglial cells. HIV specific CD4+ T cells carrying the CCR5 and CXCR4 chemokine coreceptors are preferentially targeted (8). Infection with HIV is usually followed by a quantitative decline in both CD4+ T cell count and function (9).

In most HIV-infected individuals, HIV-specific CD4+ T cell mediated T helper function is relatively weak or absent (10). This abnormality in immune function is present at an early stage, before the loss of circulating CD4+ T cells that eventually is observed in most individuals. The preferential infection of HIV specific CD4+ T-cells provides a potential mechanism to explain the loss of HIV-specific CD4+ T-cell responses, which also contributes to the gradual loss of immunological control of HIV replication (8).

1.2 CMV

Human CMV is a double-stranded DNA virus that has several close relatives in animals. In this thesis, human CMV will for the sake of simplicity be referred to as CMV. CMV was first isolated in tissue culture in 1956 (11-13). Because the virus produced large, swollen, refractory cells, it was named cytomegalovirus, and it is also the largest known

Introduction

human virus. CMV is a member of the herpesvirus group. The human herpesvirus group members comprise:

- Alphaherpesviruses: Herpes simplex virus (HSV) 1 and 2, and varicella zoster virus (VZV)
- Betaherpesviruses: CMV and Human herpes virus (HHV) 6 and 7
- Gammaherpesviruses: Epstein-Barr virus (EBV) and Herpes virus 8

These viruses all have in common the capacity to establish latent infection. Viral latency can be defined as the persistence of the viral genome in the absence of production of infectious virions, but with the ability of the viral genome to reactivate under specific stimuli (14). Clinical manifestations of CMV reactivation are uncommon in immunocompetent individuals, but can result in severe disease in patients with immunodeficiency.

The CMV genome is highly conserved, but there is sufficient diversity to differentiate between several serotypes, mainly based on differences in the glycoprotein B constituent of the virion envelope. HIV-infected individuals are more frequently coinfected with different CMV strains than the general population (15).

CMV infects many cell types, including polymorphonuclear leukocytes, monocytes, macrophages, T lymphocytes, endothelial vascular cells, renal epithelial cells, smooth muscle cells, neuronal cells, fibroblasts, and salivary glands (16). Epithelial cells, endothelial cells, fibroblasts and smooth muscle cells are the predominant targets for virus replication. Infection of epithelial cells likely contributes to transmission between individuals. Infection of endothelial cells and hematopoietic cells facilitates systemic spread within the individual (17).

1.3 Similarities between HIV and CMV infection

Although HIV and CMV are fundamentally different viruses, they share some clinical features. Both viruses can be transmitted from mother to child, by sexual transmission,

and by blood or blood products. Both HIV and CMV infect T lymphocytes. Primary infection with both viruses can be asymptomatic or cause a clinical picture similar to mononucleosis, with prolonged fever, hepatitis, sore throat, enlarged lymph nodes and lymphopenia. Both viruses establish life-long infection in the host, although infection with HIV generally has far more severe long-term consequences.

1.4 Epidemiology

As a background to understanding HIV and CMV coinfection, the epidemiology of HIV and CMV infection will be briefly discussed.

1.4.1 Epidemiology of HIV infection

UNAIDS estimated that there were 33.3 million people living with HIV at the end of 2009, higher than at any time previously in the history of the HIV pandemic (18). In the same year it was estimated that there were 2.6 million new cases of HIV infection in the world, a considerable drop from 3.1 million in the peak year 1999. Countries with a decrease in incidence of >25 % were mainly located in Africa, India and South East Asia. In Western, Central, and Eastern Europe, Central Asia, and North America, the rates of annual new HIV infections were stable over the previous five years. However, in certain risk populations, such as men who have sex with men (MSM), the rate of HIV infection has increased in many high income countries, including Norway. In Eastern Europe and Central Asia, there are still high rates of HIV transmission among people who inject drugs and their sexual partners.

1.4.2 Epidemiology of CMV infection

CMV can be transmitted via saliva, urine, placental transfer, blood transfusion, breast milk, sexual contact, solid organ transplantation, or bone marrow transplantation. CMV seroprevalence varies with sex, age, socio-economic status and geographic location (19). Seroprevalence is lowest in Western Europe and in the United States, generally below 80%. Studies from both Europe and the US have identified non-white ethnicity and low socioeconomic status as risk factors for CMV infection. CMV seroprevalence is highest

in South America, Africa and Asia, where generally more than 90% of the populations are infected. Studies from non-HIV-infected individuals in Africa have found CMV seroprevalences between 78% and 100% (20-27).

Differences in age-related prevalence are probably due to varying child rearing practices, sexual behaviours, and living conditions. Breastfeeding, group care of children, crowded living conditions, and sexual activity have all been associated with high rates of CMV infection (28). A Norwegian study of CMV infection in married couples found that 64% were seropositive, and demonstrated an annual seroconversion rate of 1.7% (29). Significantly more women than men were seropositive, indicating that men are more likely to infect women than vice versa. Another study of Norwegian pregnant mothers found that 70% of mothers were seropositive, and estimated a mean annual seroconversion rate of 2.4% (30). Seroprevalence generally increases with age, and most studies have found that more than 60% are infected by age 50. A Norwegian study of kidney transplant recipients and donors found that prevalence was 50% at the age of 20 years, 80% at 30, and 90 -100% after 60 (31).

1.4.3 Epidemiology of HIV and CMV coinfection

In patients with HIV, the seroprevalence of CMV infection varies with the mode of acquisition of HIV, and with geographic location. In developed countries, studies of HIV-infected MSM have found CMV infection in all or nearly all individuals (32-34). This is likely due to the high risk of sexual transmission of CMV in this group. For other modes of acquisition of HIV, CMV seroprevalence more closely reflects the situation in the general population. In haemophiliacs infected with HIV by factor concentrates in the U.S., the prevalence of CMV antibody was 57% (33), and in injecting drug users (IDUs), CMV seroprevalence was 67% (34).

As CMV infection is highly prevalent in developing countries, and HIV and CMV share several modes of transmission, it is not surprising that CMV seroprevalence is extremely high in HIV-infected populations in this setting. A study from Ghana found that >90% of presumably mainly heterosexually acquired HIV positive patients, were also CMV IgG

positive (25). Compared to HIV negative individuals, CMV seroprevalence was significantly higher for symptomatic HIV-infected individuals, but not for asymptomatic individuals. In a study of HIV infection from rural Lesotho, all individuals were CMV seropositive (35).

1.5 Clinical manifestations and immune response in CMV infection and disease

1.5.1 Definitions: CMV infection and disease

In this thesis I will define CMV infection and CMV disease as follows:

CMV infection is the state of asymptomatic carriage of the virus in an individual, both in the context of latent infection and active replication. CMV infection is usually diagnosed by detection of CMV specific antibody.

CMV disease is the clinical manifestation of CMV caused by active replication or immune activation resulting in inflammation or tissue damage. CMV disease can be diagnosed by typical ophthalmological findings in the case or CMV retinitis, or by typical histopathological findings in tissue specimens (see also section 1.6). CMV disease is most common in immunocompromised individuals, but may occasionally occur in patients with seemingly well-preserved immunity, usually as a result of primary infection.

1.5.2 The immune response to CMV infection

CD4+ and CD8+ T cells, natural killer (NK) cells and antibodies that recognise surface antigens play a crucial role in the immune response to CMV, preventing the development of CMV disease in the immunocompetent host (36). Cellular immunity is particularly important, and in non-HIV-infected individuals a high proportion of circulating CD4+ T cells and CD8+ T cells are dedicated to the control of CMV in seropositive individuals (37). In CMV and HIV-coinfected patients on highly active antiretroviral therapy

Introduction

(HAART), CMV-specific CD8+T cells have been reported to constitute up to 20% of circulating T cells (37), twice that of healthy HIV-negative individuals (38;39).

After allogenic bone marrow transplant patients received transfusion of CMV specific CD8+ cytotoxic T cells, the activity of these cells declined in patients deficient in CD4+ T cells specific for CMV, suggesting that helper T cell function is needed for the persistence of CD8+ T cells (40). In HIV-infected individuals it has been demonstrated that after the initial loss of CMV specific CD4+ T cells, there is a subsequent loss in function of CMV specific CD8+ T cells. The remaining CD8+ T cells are either unable to kill CMV effectively, or unable to proliferate effectively in the absence of CD4+ T cells (36;41).

Results of a recent vaccine trial in patients with solid organ transplantation indicate that antibodies play a more important role in conferring protection against CMV disease than previously thought (42). In this study, Griffiths et al. used a vaccine containing a recombinant form of the envelope glycoprotein B. They found that this vaccine reduced both the duration of viraemia, peak viral load, and the total days of ganciclovir treatment. There was an inverse relationship between the duration of viraemia and antibody titres, further indicating that the protection was mediated by the antibody response to the vaccine.

CMV has developed several ways for evading the host's immune surveillance and defence systems, including both innate and adaptive functions (43). Inhibition of Major histocompatibility complex (MHC) class I-restricted antigen presentation is a major mechanism, but antigen presentation by the MHC class II pathway is also hindered. In addition, CMV produces an interleukin (IL)-10 homologue that binds to the IL-10 receptor and down-regulates Th1 immune responses (44;45). CMV also produces chemokine receptors that bind chemokines and inhibit the immune response and NK cell function (46;47).

1.5.3 Congenital CMV infection and disease.

Infection of the fetus can result from active replicating of CMV in the mother during pregnancy, both during primary infection, reactivation, and reinfection. Congenital infection can cause congenital CMV disease - sensorineural hearing loss and neurological impairments being the most common manifestations (48). The fetus is most likely to suffer permanent damage if infected as the result of primary maternal infection. CMV disease is the most common congenital viral disease and occurs in between 0.2 and 2.2% of live births (49).

1.5.4 CMV disease in immunocompetent children and adults

In both children and adults, primary CMV infection is normally asymptomatic, after which the virus establishes lifelong latency with periodic reactivation (43). In some adolescents and adults, primary infection may present as a mononucleosis-like disease with prolonged fever, lymphadenopathy, mild hepatitis and lymphocytosis. Occasionally, pneumonitis, Guillain-Barré polyradiculitis, transverse myelitis, encephalitis, myocarditis, haemolytic anaemia, thrombocytopenia and other complications have been observed in immunocompetent individuals (16).

1.5.5 CMV disease in immunocompromised individuals

In immunocompromised individuals, CMV primary infection, reactivation and reinfection may cause severe disease with a high case fatality unless diagnosed and treated appropriately at an early stage. This is a well known complication after both bone marrow transplantation and solid organ transplantation (50;51). In addition to end-organ disease, CMV infection may have indirect effects, and is associated with allograft pathology, including atherosclerosis, bronchiolitis obliterans, vanishing bile duct syndrome, vascular disease, and both acute and chronic graft rejection (52).

Before HAART was available, CMV disease was the most common serious opportunistic viral disease in patients with HIV infection (53-58). CMV disease is rare in patients with CD4+ T cell counts >100 cells/mm³. The risk of CMV disease increases as CD4+ T cell

Introduction

counts drop <100 cells/mm³, and increases dramatically <50 cells/mm³ (57;59;60). Prior to the HAART era, the most common manifestation of CMV disease in patient with AIDS was retinitis, usually progressing to blindness within months unless appropriate therapy was given (61). Other commonly affected organs were the gastrointestinal tract, the nervous system, and the adrenal glands. CMV seropositive individuals with sexual exposure as the risk factor for acquisition of HIV were reported to have higher risk of CMV disease than persons infected by other modes of transmission, such as injecting drug use and transfusion of blood products (34).

During the pre-HAART era, increasing incidence rates for CMV disease were observed in patients with HIV. This was probably due to longer survival of patients with severe immunodeficiency, caused by improved PCP prophylaxis and advances in the treatment of opportunistic infections (62;63). Later, after the introduction of HAART, a dramatic fall in the incidence of CMV disease was observed (64-71). However, CMV disease continues to occur both in antiretroviral naïve and experienced individuals (69;72-74). A typical example of the situation in a developed country before and after the introduction of HAART in 1996 is shown in Figure 1.

In resource-poor settings, there are relatively few studies that have examined the occurrence of CMV disease in HIV-infected individuals (75). As CMV disease primarily occurs in patients with advanced immunodeficiency, it has been suggested that patients in developing countries generally die from opportunistic infections such as tuberculosis and pneumocystosis that present at less advanced stages of HIV infection, (24;76;77). However, CMV disease may represent a significant cause of morbidity in CMV seropositive patients with advanced HIV infection even in resource- poor countries (78;79).

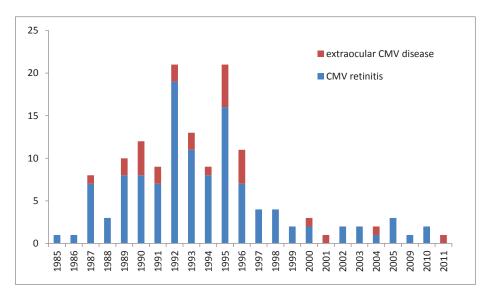
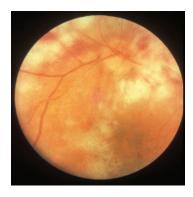


Figure 1. Number of cases of CMV disease in HIV-infected individuals at the Department of Infectious Diseases, Oslo University Hospital, during the period 1985 – 2011.

1.6 Diagnosis of CMV disease in immunocompromised individuals

Patients with CMV retinitis often experience blurring or loss of central vision, scotomata, floaters or photopsia ("flashing lights"). This manifestation can be reliably diagnosed by ophthalmoscopy by an experienced ophthalmologist. Typical features include white fluffy retinal infiltrates, and areas of haemorrhage (Fig. 2). CMV retinitis can also appear as a granular white area without haemorrhage (16).

The clinical features of CMV disease in other end-organs are generally non-specific, and symptoms such as fever, malaise, anorexia, diarrhoea, muscle and joint pain are common. In case of extraocular manifestations of CMV disease, histopathological findings typical of CMV disease are required for confirmation of the diagnosis. Typical findings include enlarged cells and nuclei with intranuclear inclusion bodies, often giving an "owl's eye appearance" (Fig. 2).



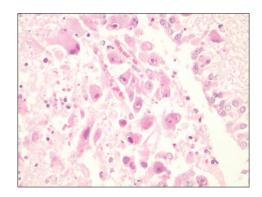


Figure 2. Diagnosis of CMV end-organ disease. The left photograph shows a typical example of CMV retinitis with white fluffy retinal infiltrates and areas of haemorrhage. The right photograph shows a typical example of CMV encephalitis with cytomegalic cells and inclusions.

Several studies have demonstrated that CMV disease is under-diagnosed in HIV-infected patients, and that the diagnosis is often first made at autopsy (53-56;58;80-88). With the aim of identifying patients at high risk, reducing the time to diagnosis and possibly eliminating the need for biopsies, a variety of microbiological tests for detection of CMV have been assessed.

Demonstration of CMV specific antibody is of limited utility for diagnosis of CMV disease, but the absence of anti-CMV IgG antibody makes the diagnosis unlikely (89).

CMV can be cultured from blood and other tissues, but is generally not very helpful in the diagnosis of CMV disease (90). Currently, culture is mostly performed for the purpose of susceptibility testing when antibiotic resistance is suspected. Isolation of CMV in culture demonstrates that the virus is present, but sensitivity and specificity for CMV disease is poor. Shedding of virus is common in the absence of disease, and the sensitivity is lower than newer nucleic acid amplification tests.

CMV antigenaemia assays using monoclonal antibodies to detect pp65 proteins in peripheral blood leukocytes have been widely used in patients at risk of CMV reactivation and disease, both in transplant and HIV-infected patients (91-98). Although these tests perform well, different nucleic acid tests (NATs), including polymerase chain reaction (PCR), hybrid capture, and nucleic acid sequence based amplification (NASBA), are now in more common use. They are fast and reliable, can provide a quantitative result, and have been used to detect CMV DNA in blood (including whole blood, peripheral blood leukocytes and plasma) (65;92;98-110), and cerebrospinal fluid (111-114). Several studies have found that detection of CMV DNA in blood by PCR is a risk factor for subsequent development of CMV disease (65;92;96;98;99;101-104;107;109;110;112;115-118). However, the reported sensitivities specificities, positive and negative predictive values have varied greatly (100;101;103;105;107;119).

1.7 Prophylaxis and treatment of CMV disease

In immunocompetent individuals there is generally no indication for treatment of CMV infection and disease. However, CMV infection may lead to life-threatening disease in immunocompromised individuals, in whom therapy is clearly warranted. Potential management modalities include prophylaxis to high risk individuals, pre-emptive therapy to patients with asymptomatic CMV viraemia, and treatment of manifest CMV end-organ disease. In solid organ and bone marrow transplant patients, all these modalities are used (51;120).

Drugs against CMV include the nucleosides ganciclovir and aciclovir, the nucleotide cidofovir, foscarnet and fomivirsen. With the exception of fomivirsen, these all act by inhibiting CMV DNA polymerase. Fomivirsen is an antisense compound for intravitreal use that acts by blocking the major immediate-early transactivator gene of CMV.

For treatment of CMV diseases in patients with HIV, intravenous foscarnet and ganciclovir have been shown to reduce the progression of retinitis and other end-organ manifestations (121-125). Intravenous cidofovir is also effective for treatment of retinitis, but is associated with serious nephrologic side effects (126;127). Oral valaciclovir, a

prodrug of aciclovir, has activity against CMV (118;128), but is no longer recommended for treatment, as oral valganciclovir is more effective. Valganciclovir is a prodrug of ganciclovir that has shown equal efficacy as intravenous ganciclovir for treatment of CMV retinitis (129). The ganciclovir intraocular device is also effective in the treatment of CMV retinitis (130;131), but this should preferably be combined with oral valganciclovir to prevent development of retinitis in the contralateral eye.

A recent trial concluded that pre-emptive therapy with oral valganciclovir was not warranted in HIV-infected individuals given the low incidence of CMV disease in patients with access to HAART (132).

Resistance to ganciclovir commonly results from mutations in the gene UL97 coding for protein kinase, and less commonly by mutations in the UL54 gene coding for DNA polymerase, which can lead to resistance to ganciclovir, cidofovir, and sometimes foscarnet (133).

1.8 CMV as a cofactor for progression of HIV infection

There are several potential ways in which CMV could act as a cofactor for HIV infection, defined as "an infectious agent which interacts at the molecular or cellular level to promote HIV pathogenicity" (134). By mechanisms outlined below, CMV replication may drive HIV replication to higher levels, which again may cause more rapid CMV replication, thus creating a vicious circle, as demonstrated in Figure 3.

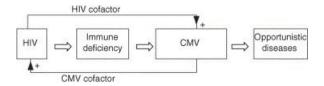


Figure 3. Pathways leading to opportunistic vs. cofactor relationships between CMV and HIV. (From: Griffiths PD. CMV as a cofactor enhancing progression of AIDS. J.Clin.Virol. 2006; 35: 489-92).

The result may be CMV disease, but also faster progression of HIV disease to new AIDS defining conditions or death. Studies solely focusing on CMV end-organ disease may underestimate the potential cofactor effect of asymptomatic CMV viraemia.

1.8.1 Potential immunological mechanisms for the cofactor effect of CMV

In vitro, several potential mechanisms for enhancing effects of CMV infection on HIV disease have been demonstrated (135-137):

- CMV activation of latent proviral HIV DNA by introduction of its transactivator proteins into the same cell (138;139).
- CMV activation of HIV latent provirus by release of cytokines from nearby cells by signal transduction (140).
- CMV antigen release from a nearby cell leading to activation of latent HIV
 provirus when HIV is latent in a T memory cell whose cognate antigen receptor is
 specific for a CMV-encoded protein (141).
- CMV-induced change in the tropism of HIV-infected cells. If CMV and HIV infect the same cell, the tropism of HIV could by altered by forming pseudotypes that would also be able to bind to other cells containing receptors for CMV (142). The US28 gene of CMV codes for a chemokine receptor that can permit entry of HIV into CD4+ T cells not depending on CCR5 (143). Alternatively, CMV could activate CD4 expression within cells that are CD4 negative (144). CMV could code for a molecule acting as an alternative receptor for HIV; for example HIV coated in non-neutralising antibodies can enter fibroblasts via an Fc receptor coded for by CMV (145).

1.8.2 Evidence of cofactor effect from epidemiological studies

There are some indications that CMV infection is linked to poor prognosis for patients with HIV infection, but results are conflicting. Some observational studies of HIV-infected adults in developed countries have reported that CMV seropositivity is

associated with more rapid rate of progression of HIV disease to a more advanced stage (3;135;146-148). Also, in children it has been demonstrated that HIV-1-infected infants who acquire CMV infection in the first 18 months of life have a significantly higher rate of disease progression and central nervous system disease than those infected with HIV-1 alone (149). In contrast, Rabkin et al. did not find that seropositivity was significantly linked to development of AIDS or risk of death in haemophiliacs (150). Shepp et al. also failed to find an association between CMV serological status and survival in a more heterogeneous HIV-infected population (34).

Many studies from developed countries have found that CMV viraemia is associated with poor prognosis (73;104;109;110;115;116;151;152). Detels et al. reported that patients with persistent excretion of CMV in semen had a significantly increased relative hazard of developing AIDS (153). However, few studies from developing countries have addressed the impact of CMV infection and viraemia on survival of HIV-infected individuals. In a study from Cambodia, serum CMV replication was highly prevalent among HIV-infected patients and was associated with increased mortality (154).

1.9 CMV-related immune activation, inflammation and immunosenescense

HAART has resulted in greatly improved life expectancy for individuals with HIV infection. Nevertheless, as a group these individuals are at greater risk of age-related disorders, such as certain malignancies, cardiovascular disease, type II diabetes, and cognitive impairment than persons without HIV infection. This may be related to the observation that HIV-infected individuals often have an immune risk phenotype that is more typical of older non-HIV-infected persons, and associated with poor prognosis (155). Many HIV-infected individuals continue to have abnormal T cell activation despite antiretroviral treatment (156-159), and this is associated with attenuated CD4+ T cell recovery (157).

Recent studies indicate that CMV infection increases all-cause mortality in the elderly non-HIV-infected population and raises the risk of many age-related diseases (155). This

Introduction

increased risk may at least in part be due to CMV mediated inflammation resulting in a disadvantageous immune risk phenotype (155). Chronic inflammation is also a probable explanation for the increased risk of age-related disorders observed in HIV-infected individuals (160). This may be aggravated by the deleterious immunological effects of CMV in coinfected individuals.

Fibrinogen and CRP are markers of inflammation that have been shown to be independent predictors of mortality in HIV-infected adults (161). The same has been reported for the inflammatory cytokine IL-6 (162). Inflammation is associated with depletion of CD4+ T cells and is also a likely cause of increased risk of cardiovascular disease in patients with HIV. Increased carotid intima thickness in HIV patients is associated with increased CMV specific T-cell response (163). Also, CMV IgG antibody titres have been shown to be positively associated with degree of coronary atherosclerosis, suggesting that immune activation and CMV-specific immune response may contribute to atherosclerosis in HIV-infected patients (164;165). In a recent clinical study by Hunt et al., valganciclovir reduced T cell activation in HIV-infected individuals with incomplete CD4+ T cell recovery on antiretroviral therapy (166). It was concluded that CMV and/or other herpesvirus replication is a significant cause of immune activation in this population.

2 Aims and objectives

2.1 Main aim

The overall aim of this thesis was to gain new knowledge of CMV infection in HIV-coinfected individuals, including aspects of epidemiology, diagnosis, clinical manifestations, and prognosis.

2.2 Specific objectives

- I. To study the incidence of CMV disease in patients with AIDS in Norway in the pre-HAART era (Paper I)
- II. To examine the impact of CMV infection and CMV end-organ disease on survival in patients with AIDS in Norway in the pre-HAART era (Paper I)
- III. To explore the utility of plasma CMV quantitative PCR in the diagnosis of CMV disease in patients with HIV-infection (Paper II)
- IV. To assess the utility of dried blood spots (DBS) for detection of CMV antibody and CMV viraemia in HIV-infected individuals in a resource-poor setting (Paper III)
- V. To study the prevalence of CMV infection and viraemia in relation to clinical outcome in patients with HIV infection and access to HAART in rural Tanzania (Paper III)

Aims and objectives

3 Materials and methods

Details of the study populations, study periods, study designs, diagnostic methods and outcome variables are provided in each paper, but an overview is shown in Table 1 and in the description below.

3.1 Study populations, periods, designs, and outcomes

In Papers I and II, potentially eligible patients were identified in the HIV database at the Department of Infectious Diseases, Oslo University Hospital. The studies presented in these papers comprised patients with advanced HIV infection in this hospital. In Paper I, 248 patients with a clinical diagnosis of AIDS during the years 1983-1995 were included. In Paper II, we included 125 patients with HIV-related death during the years 1991-2002 (53 with CMV disease and 72 without CMV disease) in whom autopsy was performed. Only 45 of 125 patients (36%) were tested for HIV viral load and 15 (12%) had HIV RNA <400 copies/ml, all in patients without CMV disease. Ten of 53 patients with CMV disease were tested for HIV viral load, all of whom had high-level viraemia.

In the study presented in Paper III, 168 HIV-infected and antiretroviral naïve patients presenting to Haydom Lutheran Hospital during 2008-2010 were included.

MSM comprised the majority of the Norwegian study populations (Paper I and II), whereas heterosexually infected women comprised the majority of the study population in Tanzania (Paper III).

In all studies, inclusion criteria and outcomes were verified by detailed review of patient files.

Table 1. Overview of study populations, periods, designs, diagnostic methods and main outcomes in Papers I, II, and III.

Paper no.	Study population	Study period	Study design	Diagnostic methods	Main outcomes
I	248 patients with AIDS in Oslo	1983- 1995	Retrospective cohort	Ophthalmoscopy, biopsy, autopsy	CMV disease Death
II	125 CMV and HIV seropositive deaths in Oslo with autopsy results	1991- 2002	Diagnostic study	CMV quantitative PCR in plasma, autopsy	CMV disease
III	168 antiretroviral naive patients in rural Tanzania	2008- 2010	Prospective cohort study	CMV antibody and quantitative PCR in dried blood spots	Death

3.2 Diagnostic methods

3.2.1 Detection of CMV IgG antibody

In all papers, CMV antibody was determined using commercially available and validated antibody tests in routine use at the time at the Department of Microbiology, Oslo University Hospital. For the years 1985-1996 the test used was Enzygnost (Enzymelinked Immunosorbent Assay, ELISA), from 2010 Abbott Axzym (ELISA), and from 2010 Abbott Architect (chemiluminescent microparticle immunoassay, CMIA).

3.2.2 Analysis of CMV viraemia by quantitative PCR

In Papers II and III CMV quantitative PCR was performed in plasma and dried blood spot eluates, respectively. In paper III we performed an additional exploratory assessment of the performance of our CMV quantitative PCR methodology in DBS eluates, by comparing with results from 11 plasma samples prepared in parallel. All CMV PCR positive plasma samples were positive in DBS eluates, and negative samples were negative by both methods. There was good correlation between measured CMV viral

load in DBS and plasma (r = 0.87, p < 0.001) although detected viral load tended to be lower in DBS.

3.2.3 Diagnosis of CMV disease

In all papers we used a strict case-definition (see section 1.5.1). A diagnosis of CMV retinitis ante-mortem was based on typical ophthalmoscopic findings (see section 1.6). Routine ophthalmoscopy was not performed in individuals at high risk of CMV disease, but was performed in patients presenting with suggestive symptoms. The characteristic histopathological features of cytomegalocytes with inclusions were required for diagnosis of all other end-organ manifestations of CMV disease. Demonstration of CMV by culture or PCR of biopsies, blood or urine without histopathological verification was not accepted for diagnosis. In the autopsy group, a full necropsy including neuropathological examination was performed, except for the adrenal glands, the gastrointestinal tract, and the eyes in some cases. Paraffin-embedded sections were routinely stained with haematoxylin–eosin, but additional immunohistochemistry for CMV was performed in a few cases of doubt to confirm the presence of CMV. Histopathological examination was not available for diagnosis of CMV disease in study III.

3.3 Statistical analysis

For group comparison of continuous variables conforming to the normal distribution, two-sample t-tests were used. Non-parametric tests were used for group comparison of variables not conforming to the normal distribution, using Mann-Whitney U-tests for independent samples and Wilcoxon signed rank tests for related samples. χ^2 tests and logistic regression were used for comparison on categorical data. Correlation between variables was assessed by Spearman rank coefficients.

Paper I and III were cohort studies in which survival probabilities were presented as Kaplan-Meier plots. Cox regression analyses were used to calculate hazard ratios for mortality during the follow-up periods, entering baseline variables and time-dependent variables in the models, as appropriate. In paper III, we used a forward selection

technique, as outcomes were too few to allow us to enter all independent variables into the model simultaneously.

Paper II was a diagnostic study in which sensitivity and specificity, positive and negative predictive values for different cut-offs of CMV viral load were calculated. Results were also presented as a receiver operating characteristic (ROC) curve with the area under the curve representing the diagnostic accuracy of the test.

3.4 Ethical considerations

Approvals from the concerned authorities were obtained, as required. This included approval from the Regional Committee for Medical and Health Research Ethics in Norway, and the Medical Research Coordinating Committee in Tanzania. In paper II we used stored samples from the approved Biobank at Oslo University Hospital, Department of Infectious Diseases. In paper III, patients gave written consent to participate in the study.

4 Summary of results

4.1 Paper I: CMV disease in patients with AIDS in Oslo

- 92.9% of the tested sera were CMV seropositive. Seroprevalence in MSM was
 99.4%, in IDUs 71.1%, and was 83.3% in other risk groups combined. There was significantly higher CMV seroprevalence in MSM than in IDUs (p <0.0001).
- 95 of 248 patients (38.3%) developed CMV disease during the study period.
 Among autopsy cases, 73 of 152 patients (48%) had histopathological evidence of CMV disease, and 52 (71%) of these were first diagnosed at autopsy.
- Among patients who died (n =213), the most common manifestations of CMV disease were retinitis (23%), adrenalitis (20%), pneumonitis (16%), and gastrointestinal disease (9%).
- All patients diagnosed alive with CMV had evidence of CMV disease at autopsy, despite CMV therapy.
- The proportion of cases with CMV disease increased from 39% during the period 1983-89, to 51% during the period 1990-95.
- There was no significant difference in survival between CMV seropositive and seronegative individuals.
- CMV disease diagnosed alive was associated with increased risk of death.

4.2 Paper II: CMV quantitative PCR for diagnosis of CMV endorgan disease in HIV infection

• CMV viraemia was detected in at least one sample in 27 of 53 patients with CMV disease (51%) and in 10 of 72 patients (13%) without CMV disease (p <0.001).

Summary of results

- CMV viral load was significantly higher in patients with CMV disease than in patients without disease (p = 0.001). Among viraemic patients there was no significant difference in CMV viral load between the two groups, median 3420 copies/ml and 1705 copies/ml, respectively.
- The proportions of cases with viraemia were similar for patients diagnosed with CMV disease alive and at autopsy, 44% and 49%, respectively.
- In patients with multi-organ CMV disease and single-organ CMV disease the proportions with detectable CMV in plasma were 70% and 24% respectively, p = 0.005.
- Diagnostic sensitivity, specificity, positive predictive value and negative predictive values for patients with detectable CMV were 47%, 89%, 76%, and 70%, respectively.
- In patients with CMV PCR >10 000 copies/ml in a single sample, or any level of viraemia in two consecutive samples, diagnostic specificity and positive predictive values were 100%.
- CD4+ T cell count <100 cells/mm³ and high viral load were significantly associated with risk of CMV disease in multivariable analysis.

4.3 Paper III: CMV viraemia in DBS in relation to survival in HIV-infected patients in Tanzania

- All patients were CMV seropositive.
- 38 of 168 Tanzanian patients (22.6%) had detectable viraemia at baseline and 14 (8.3%) had CMV viraemia >200 copies/ml.
- CMV viraemia >200 copies/ml represented a significant risk factor for death in multivariable analysis.
- Among 51 patients with CD4+ T cell counts <100 cells/mm³, 11 (21.6%) had CMV viraemia >200 copies and were at high risk of CMV disease.

5 Discussion

The study presented in Paper I was initiated in the mid-1990ies, before the HAART era. At the time, the infectious disease wards at Oslo University Hospital provided care for a large number of HIV-infected patients with serious opportunistic diseases and other complications such as PCP, disseminated Mycobacterium avium intracellulare infections, candida oesophagitis, and Kaposi's sarcoma. CMV disease was also a common occurrence. During this period, Oslo University Hospital had a higher autopsy rate for HIV-related deaths than most similar clinics in other countries. This represented a clear advantage in the study of CMV disease – a complication of HIV infection that too often was diagnosed at the autopsy table. We found that almost one-half of the patients had evidence of CMV disease at autopsy, and that this diagnosis in most cases was not known before death. Furthermore, a diagnosis of CMV disease alive was identified as a significant risk factor for death.

Paper I clearly demonstrated that improved methods were needed for timely diagnosis of CMV disease. In the study presented in Paper II, we therefore assessed CMV quantitative PCR as a tool for early diagnosis of CMV disease. In this study, which covered a period both before and after introduction of HAART, we found that the diagnostic sensitivity of CMV viraemia was relatively poor, but that clinical specificity was good for patients with high CMV viral load or repeated viraemia.

After the introduction of HAART in 1996-97, a rapid drop in the incidence of CMV disease was observed in Norway and other developed countries. However, little is still known about the impact of CMV viraemia and disease on prognosis of HIV-infected individuals in developing countries. This is largely due to challenges with ensuring a correct diagnosis, be it with by fundoscopy by an experienced ophthalmologist, biopsy, or autopsy – procedures that are rarely available in these settings. Also, CMV viraemia assays are seldom available in resource-poor countries. This is partially due to unavailability of these advanced microbiological tests, but also related to logistical challenges with ensuring correct transport and storage of liquid specimens. DBS

specimens have potential logistical advantages, as they are more robust to ambient temperatures, and less prone to transport damage. DBS have previously proved useful for quantification of HIV viral load, and for HIV resistance testing (167;168). In Paper III, we report results of CMV antibody and viraemia assays performed in DBS eluates from HIV-infected individuals in Tanzania, and relate our findings to survival. We found that all patients were CMV IgG antibody positive and that CMV viral load >200 copies/ml in DBS eluates was a risk factor for death.

5.1 Methodological considerations

A detailed discussion of our methods can be found in each paper, but certain additional and general aspects are treated in more detail below.

5.1.1 CMV antibody

In Paper I, 4% of individuals were not tested for CMV antibody, and 7% were CMV IgG negative. As CMV antibody was not systematically retested in CMV negative individuals during the study period, it is possible that a few cases of seroconversion may have been missed. In this paper, we might therefore have slightly underestimated CMV seroprevalence. In Paper II, known CMV seropositivity was one of the inclusion criteria. In Paper III, all patients were CMV IgG positive in DBS eluates at baseline. Although this test has not been validated for use in DBS, all patients had high arbitrary unit (AU) values, and we find it unlikely that there should be a problem with false positive tests.

5.1.2 CMV quantitative PCR and assessment of test performance

Detection of CMV viraemia by PCR can be performed by both qualitative and quantitative tests. Today, quantitative tests are most commonly used, as knowledge of the amount of virus, not only presence or absence, may have clinical implications. In patients with HIV-infection and CMV viraemia, the risk of CMV disease or death has been shown to increase with rising viral load (104;115;116;152). Also, viral load can be used to monitor the effect of therapy and emergence of resistance (152).

In Paper II, we used a commercial CMV quantitative PCR test (Cobas Amplicor CMV Monitor Test). We assessed this test's clinical sensitivity and specificity, and calculated positive and negative predictive values for CMV disease.

It is important to distinguish between analytical and diagnostic sensitivity, and analytical and diagnostic specificity of a test. The analytical sensitivity represents the smallest amount of substance in a sample that can accurately be measured by an assay (169). Synonyms for "analytical sensitivity" are "limit of detection" and "minimal detectable concentration". Diagnostic sensitivity is the percentage of persons with a disorder who are identified by the assay as positive for the disorder. High analytical sensitivity does not guarantee acceptable diagnostic sensitivity.

In general, poor diagnostic sensitivity may have several explanations. First, it may be due to poor analytical sensitivity. In Paper II we used the Cobas Amplicor CMV Monitor Test. This is a well validated assay with relatively high analytic sensitivity. Second, diagnostic sensitivity can be reduced by failure to obtain the target substance in a processed sample from a person with the condition of interest. We tested frozen plasma samples taken at various intervals before CMV disease was confirmed or excluded. It is therefore possible that some patients did not have detectable CMV viraemia in a sample because it was taken too early or too late in relation to the final diagnosis, or that we failed to detect intermittent viraemia. Finally, diagnostic sensitivity may change with variation in the clinical spectrum of a disease. We found that diagnostic sensitivity was highest in patients with more severe disease, as determined by CMV disease in more that one organ (see section 5.3.1).

Analytical specificity refers to the ability of an assay to detect one particular organism or substance, rather than others. The diagnostic specificity represents the percentage of persons who do not have a given condition who are identified by the assay as negative for the condition. Poor diagnostic specificity may be due to poor analytic specificity, and failure to diagnose the disease in question when it is in fact present. Diagnosis of CMV end-organ disease may be challenging, as it often requires invasive procedures and examination of histopathological specimens. In addition, CMV viraemia may precede

manifest CMV disease by several months, and a sufficiently long observation period is therefore required in cohort studies. Failure to diagnose a condition due to short observation time or insufficient use of invasive procedures can potentially contribute to poor specificity. In Paper II we followed all patients to death, and autopsy results were available for all. We were therefore unlikely to miss cases with CMV disease and to underestimate the specificity of the test.

The positive predictive value is the probability that a person whose test result is positive truly has the disease or condition of interest. The negative predictive value is the probability that a person whose test result is negative does not have the disease. In addition to the diagnostic sensitivity and specificity, the predictive values are highly dependent on the prevalence of the condition of interest in the population being tested. High diagnostic sensitivity and low prevalence of the condition in question give a high negative predictive value. High diagnostic specificity and high prevalence of the disease result in high positive predictive value. In Paper II, we calculated positive and negative predictive values of CMV quantitative PCR for diagnosis of CMV disease. In this study, CMV disease was common, and this contributed strongly to the generally high positive predictive values and low negative predictive values. (See also section 5.3.1).

In Paper III, we used DBS as samples for detection of CMV viraemia. One aim was to study if CMV viraemia was associated with increased mortality in a rural Tanzanian population. It was not our ambition to directly compare results from DBS with results that might have been obtained if the analysis had been carried out using liquid plasma samples. We therefore chose not to perform an in-depth validation of CMV quantitative PCR in DBS eluates compared to plasma, but to limit our assessment to establishing satisfactory correlation between the two methods. In an exploratory study, performed prior to testing samples from Tanzanian HIV-infected individuals, the test performance of the artus CMV TM PCR Kit in DBS eluates was compared to the performance of the Cobas Amplicor CMV Monitor Test in plasma. We demonstrated good correlation between the tests (r = 0.87, p < 0.001), but found that CMV viral load in DBS eluates tended to underestimate viral load in plasma samples collected in parallel.

The most likely explanation for lower viral load in DBS than in plasma is that a smaller proportion of CMV DNA was successfully extracted from DBS than from plasma samples. Another possible explanation is degradation of CMV DNA in DBS due to storage, including freezing and thawing of specimens. However, CMV DNA is known to be robust to degradation (170;171), and this is unlikely to be a major factor. A last potential explanation for discrepancies between DBS and plasma results is that two different CMV quantitative PCR assays were used. However, the artus CMV TM PCR Kit and Cobas Amplicor CMV Monitor Test have generally shown good agreement (172).

In Paper III, our test of CMV viraemia in DBS may have failed to detect some patients with low viral load in plasma, and we may have underestimated the viral load of patients with detectable viraemia. This may reduce the diagnostic sensitivity of CMV quantitative PCR in DBS compared to plasma. However, the clinical significance of this is uncertain. In transplant patients, CMV viral load <100-500 copies/ml is of unknown significance and may be difficult to interpret (173). Similarly, low CMV viral load in HIV-infected patients may have low positive predictive value for CMV disease or death.

5.1.3 Diagnosis of CMV retinitis and other end-organ disease

In studies of CMV disease in HIV-infected individuals, clear case definitions are required and have been proposed (132). Definitions of CMV infection and disease in transplant recipients are not directly applicable to HIV-infected individuals (174), and for patients with HIV there is no universally accepted definition of CMV disease. Nevertheless, most studies of CMV disease in HIV coinfected individuals have applied stringent case definitions for the diagnosis of CMV disease. We required histopathological evidence of CMV disease by biopsy or autopsy, except for retinitis diagnosed by typical ophthalmological findings. Demonstration of CMV by culture of biopsies, blood or urine without histopathological verification was not accepted for diagnosis, as these are unspecific findings (16). Routine ophthalmoscopy was not performed in individuals at high risk of CMV disease, but was done when patients presented with suggestive

symptoms. It is therefore possible that we may have missed some cases of early CMV retinitis in all three studies.

5.2 CMV infection and disease in patients with AIDS

5.2.1 CMV seroepidemiology

In Paper I, 99.4% of HIV-infected MSM were CMV IgG positive, compared to 71.1% in IDUs (p <0.001). Our findings are in accordance with other studies that have found CMV infection to be almost universal in MSM (32-34). CMV infection in IDUs was slightly more common than in previous studies of Norwegian married couples and pregnant mothers (29;30), perhaps reflecting a higher seroprevalence associated with lower socioeconomic status (19). In addition, transmission of CMV by sharing of needles is possible.

In Paper III from Tanzania, all patients were CMV IgG seropositive, i.e. comparable to MSM in Norway. Other studies have also found CMV seroprevalence >90% in HIV-infected individuals in developing countries (25;35).

5.2.2 Occurrence of CMV disease

5.2.2.1 Occurrence of CMV disease in Norway

Paper I represents the only published Scandinavian study of the occurrence of CMV disease in HIV-infected individuals. This was a large population based study comprising the majority of AIDS patients in Oslo during the period 1983-95, before HAART was available. During this period, autopsy was performed in 71% of AIDS deaths. This proportion is higher than reported in all other autopsy-based studies of CMV disease (55;58;80;81), and reduces bias that may occur in more selected populations. Table 2 summarises results from previous autopsy-based studies in developed countries in which the prevalence of CMV disease has been assessed.

In Paper I, we found evidence of CMV disease at autopsy in 48% of the cases. In similar studies from other countries, the proportion with CMV disease at autopsy has varied

 Table 2. Major studies of cytomegalovirus disease at autopsy in HIV-coinfected individuals in developed countries

- · C · - · - · - ·				- I	/			I		
First author	No. of	Study	Country	Male	Main	Main risk	Autopsy	CMVD at	CMVD at	CMVD as
year	autopsies	period		%)	ethnicity	factors for	rate (%)	autopsy (%)	autopsy only	cause of death
(ref. no.)					(%)	HIV (%)			(%)	(%)
Wilkes MS, 1988	101	1981-87	USA	94	White 56	MSM 68	41	09	74.2	NS
(58)			(New York)		Black 22	IDU 16				
McKenzie R,	75	1982-88	USA	95	White 80	MSM 84	NS	81	46	11
1991 (86)			(Maryland)		Black 16	IDU 3				
Afessa B,	58	1985-89	USA	71	Black 97	MSM 41	NS	28	94.1	<10
1992(53)			(Wash. DC)		White 3	IDU 21				
d'Arminio	250	1984-91	Italy	82	NS	MSM 28	NS	49	2.09	NS
MA, 1992(54)						1DU 66				
Pillay D, 1993	47	1985-92	England	96	NS	MSM 53	NS	99	46	NS
(83)						BT 38				
Klatt EC, 1994	565	1982-93	USA	94	NS	MSM 54	NS	51	NS	17
(84)			(California)			IDU 23				
Lyon R, 1996	279	1984-93	USA (Texas)	95	White 55	MSM 30%	89	46	NS	11
(55)					Hisp 9	IDU 22%				
Markowitz GS,	252	1982-95	USA (New	74	NS	IDU 49	NS	30	NS	9.5
1996 (88)			York)			HS 19				
d'Arminio MA,	533	1984-96	Italy	78	NS	MSM 20	49	45	59	NS
1997(80)						IDU 64				
Dore GJ,	25	1992-93	Australia	100	NS	MSM 84	13	76	74	NS
1995(81)										
Hofman P, 1999	395	1983-96	France	89	NS	MSM 20	NS	38	29	NS
(82)						IDU 65				
Jellinger KA,	260	1993-99	Austria	88	NS	MSM 80	NS	40*	NS	NS
2000 (87)						IDU 14				
Masliah E, 2000	390	1982-98	USA	92	NS	MSM 70	NS	49	NS	NS
(56)			(California)			IDU 26				
Morgello S, 2002	394	1979-	USA (New	75	Black 40	MSM 27	NS	29	NS	NS
(85)		2000	York)		Hisp 33	IDU 36				
	Cre	٠.		2 503 5	-	17.			7	

^{*}extracerebral only. NS, not specified. His, Hispanic. MSM, men who have sex with men. IDU, injecting drug user. CMV, Cytomegalovirus

disease

from 28% to 81% (53-56;58;80-88). We found that retinitis was the most common manifestation of CMV disease, in agreement with other studies (61;69;80;175). Although most cases of retinitis were diagnosed before death, only a minority of the other CMV end-organ manifestations were diagnosed prior to autopsy. These results demonstrated a clear need for more timely diagnosis of CMV disease.

In Paper II, we included patients with AIDS who died during the period 1991-2002, i.e. encompassing years both before and after HAART was introduced. In this study, CMV disease was found in 42% of patients at autopsy, i.e. less commonly than in Paper I, which only included patients in the pre-HAART era. Furthermore, as CMV IgG positivity was one of the inclusion criteria in Paper II, our estimated prevalence of CMV disease at autopsy likely represents a slight overestimation of the prevalence in all HIV-related deaths. This overestimation is likely to affect IDUs most, as a not insignificant proportion of individuals in this group was CMV seronegative. Our results are consistent with other studies that have demonstrated a reduction in the incidence of CMV disease after the introduction of HAART (64-70).

In Paper II, all cases with CMV disease with a known result of HIV viral load had high-level HIV viraemia. This is consistent with a cohort study by Erice et al. where all CMV end-organ events occurred in patients with high HIV viral load (109). Deayton et al. has demonstrated that effective HAART can suppress CMV viraemia (176). Currently, CMV disease is uncommon in HIV patients in Norway as in other developing countries. Nevertheless, CMV disease continues to be reported in low numbers in patients presenting late with advanced HIV infection, and may also occur in patients with poor treatment adherence, or resistant virus (69;72-74).

An epidemiological study from Europe for the period 1994-2001 found that CMV end organ disease was the cause of the death in 18% of cases (69), but the number of cases in which the cause of death was determined by autopsy was not reported. In the same study, the median survival was 10 months for patients with any CMV disease manifestation, 11 months for retinitis and seven month for other CMV organ disease. By multivariable analysis, the commencement of HAART was associated with a 37% decrease in mortality

(p <0.05). In a French study of HIV-infected individuals, median survival was seven months for CMV disease, and initiation of therapy with protease inhibitor was associated with increased survival (177).

Soon after the introduction of HAART, CMV retinitis was observed in patients with previously low CD4+ T cell counts even after counts rose >100 cells/mm³ (178). In addition, a new CMV-related manifestation of the eye was reported, known as immune recovery uveitis (179). The pathogenesis of both retinitis and uveitis in this setting is probably explained by immune reaction to low-level CMV replication or residual CMV antigens.

5.2.2.2 Occurrence of CMV disease in rural Tanzania

In Paper III, we identified no patients with a confirmed diagnosis of CMV disease, neither retinitis nor other end-organ disease. This may at least in part due to diagnostic constraints, as ophthalmoscopy was the only diagnostic modality available. Severe cases of symptomatic retinitis were unlikely to have been missed. However, mild cases of retinitis might have been overlooked, as routine ophthalmoscopy was not performed in patients with low CD4+ T cell counts. Due to non-availability of histopathological examination of tissue specimens, we were unable to diagnose patients with CMV disease in organs other than the eyes. However, we found that patients at high risk of CMV disease, by having both low CD4+ T cell counts and high CMV viral load, often had non-specific symptoms such as fever and diarrhoea that were compatible with CMV disease.

From other developing countries, we have some knowledge about the impact of CMV disease in HIV-infected individuals. In Thailand and India the prevalence of CMV retinitis in patients with HIV has been reported to be 33% and 17%, respectively (180;181). In Africa, CMV retinitis appears to be less common, with reported prevalence in cross-sectional surveys ranging from 0.0%–8.5% (76;182;183). It has previously been speculated that low prevalence of CMV disease in Africa may be due to high competing mortality from other opportunistic diseases (24;76;77). However, studies published in recent years indicate that other factors also may be at play. In two comparative cohorts of

patients starting HAART, the prevalence of CMV disease in Mumbai, India, was 11.4%, significantly higher than in Cape Town, South Africa, where the prevalence was 2.6% (184). A study from Tanzania also found a low prevalence of CMV retinitis (1.3%) in patients with CD4+ T cell counts <100 cells/mm³ (185).

The cause of the low prevalence or CMV retinitis in Africa is unknown, but genetic differences in the host or virus could be part of the explanation. A Canadian study found that some human leukocyte antigen (HLA) types were associated with increased risk of CMV retinitis, i.e. HLA-Bw4 and HLA-DRB115, whereas HLA-Cw7 was protective (186). Therefore, it is possible that Africans may have favourable genotypes. However, this explanation is challenged by a study from the US that found that the prevalence of CMV retinitis was higher in American-Africans (and Hispanics) than in the general HIVinfected population (187). In contrast, a study from London found that Africans born in sub-Saharan Africa had significantly lower incidence of CMV disease compared to non-African patients (188). Ben-Smith et al. found lower numbers of naïve T cells, higher numbers of antigen-experienced T cells and higher CMV seroprevalence in young Malawians compared to age-matched UK individuals, suggesting that immune responses in rural African settings may be induced and maintained in a different way than in developed countries (20). It is possible that early immune challenge in Africa, by unknown mechanisms, could reduce the risk of CMV retinitis in immunosuppressed individuals in later life.

As invasive procedures and histopathological examination of biopsies are unavailable to most HIV-infected individuals in developing countries, CMV disease is highly likely to be missed when present. Autopsy is also seldom performed, but there are some autopsy studies from sub-Saharan Africa (189). CMV disease was the presumed main cause of death in 4% of adults both in studies from Kenya and South Africa (190;191). Evidence of CMV disease was found in up to 18% in a study of adults and adolescents in Cote d'Ivoire, whereas 2% of deaths were ascribed to this condition (192). A necropsy study of adults in Botswana found evidence of CMV disease in 15% of HIV-positive patients, but CMV was a rare cause of death (193). In children, there is some indication that CMV disease may be more common. Autopsy studies of Botswanan children showed that

CMV disease was the cause of death in 20% of cases (CMV pneumonitis and disseminated disease combined), and was found in 66% of autopsies (194). In a study of respiratory infection in HIV-infected children in Zambia, CMV pneumonitis was found in 22% of cases, but most of these cases were mild (195).

To conclude, although we were unable to confirm CMV disease in any of our Tanzanian patients, other studies have shown that CMV disease does occur in sub-Saharan Africa. However, for unknown reasons CMV disease may be less common there than in HIV-infected individuals in other parts of the world, including Asian developing countries. Further studies are warranted to elucidate these findings.

5.2.3 Survival of patients with CMV disease

In Paper I, we found that CMV disease diagnosed alive was associated with increased risk of death. During the study period, ganciclovir and foscarnet were available to patients for treatment of CMV disease, except for two cases that died during the early part of the study period. Foscarnet and ganciclovir have been shown to reduce the progression of CMV retinitis and other end-organ disease (121-125). Nevertheless, in our paper evidence of CMV disease was found at autopsy in all cases, clearly indicating the inadequacy of this therapy alone. Our study did not allow us to study if CMV therapy was associated with increased survival, but results from other studies are conflicting. Some studies from the pre-HAART era found a survival benefit with treatment of CMV disease (177;196;197). A later study by Kempen et al. also found that despite HAART, CMV treatment reduced the risk of mortality by 65% in patients with CMV retinitis and poor immune-recovery (198). However, other studies have been unable to demonstrate a survival benefit with systemic CMV treatment (131;199).

In Paper I, we did not attempt to report the proportion of deaths in which CMV disease was the cause of death. Many patients had multiple opportunistic complications that may have contributed, and the role of CMV disease was difficult to discern. However, other autopsy-based studies in developing countries have reported CMV disease as cause of death in around 10 % (53;55;86;88).

5.3 CMV viraemia as predictor of CMV disease and death

Several studies have shown that detectable CMV viraemia by either qualitative or quantitative PCR is a risk factor for occurrence and progression of CMV disease, progression of HIV infection, and death. In Paper II, we assessed the performance of CMV quantitative PCR in the diagnosis of CMV disease in Norway. In Paper III, we studied if presence of CMV viraemia in DBS from antiretroviral naive patients in Tanzania was a risk factor for death.

5.3.1 CMV viraemia as predictor of CMV disease

Several studies have demonstrated that CMV viraemia is associated with statistically significant increased risk of CMV disease in HIV-infected individuals (65;92;96;98-104;107;109;110;112;115-118). However, in clinical practice this information is not very helpful. In the diagnostic work-up of a patient, the clinician needs to know the diagnostic sensitivity and specificity, and preferable also the positive and negative predictive values of the tests in use - and these are often not reported. In Paper II, we analysed the diagnostic sensitivity, specificity, positive and negative predictive values of CMV quantitative PCR under different assumptions in a population at high risk of CMV disease. Other studies that have addressed these parameters using CMV quantitative PCR in various blood compartments (serum, plasma, blood or polymorphonuclear leukocytes) are summarised in Table 3. Our study included a higher number of cases with CMV disease than any of these, and is unique in having assessed the performance of CMV quantitative PCR in a population with available autopsy results.

We found that a maximum overall diagnostic sensitivity of 51% was attained by defining a positive test as detectable viraemia in at least one of two plasma samples obtained before a diagnosis of CMV alive or at autopsy. Sensitivity was slightly lower (47%), when defining as positive only detectable viraemia in the final plasma sample. This is because some cases with previous CMV viraemia did not have detectable viraemia in subsequent samples, even though CMV therapy was not given. This has also been

demonstrated by others (100;108). Shinkai et al. found that peak plasma CMV viral load was detected median 4.1 months before the development of CMV disease (100).

In our study, the diagnostic sensitivity, based on the last samples before diagnosis, was lower than in most other studies, both for all cases combined (47%), cases diagnosed alive (44%), and at autopsy (49%). We used a validated test with good analytical sensitivity (linear range from 400 copies/ml), the time-interval between the last sample and diagnosis was approximately two months, similar to several previous studies, and the study population was at high risk of CMV disease. Therefore, differences in the analytical performance of the tests, differences in timing of samples in relation to diagnosis, and differences in study populations are unlikely to explain our relatively low diagnostic sensitivity. One possible explanation for low sensitivity is that close follow-up and availability of autopsy results enabled us to diagnose less severe cases with CMV disease at a very early stage while CMV replication was low and undetectable, cases that might have been missed in other studies. For cases first diagnosed at autopsy, the diagnostic sensitivity was higher for cases with multi-organ CMV disease (70%), than in cases with single organ disease (24%). This is in support of mild disease as an explanation for low diagnostic sensitivity.

In agreement with other studies, the specificity of our test was high under a wide range of definitions of a positive test. A false positive test in relation to presence of CMV disease occurred in $\leq 11\%$ of cases. Lower specificity reported in some studies might be explained by under-diagnosis of CMV disease, e.g. due to lack of autopsy results.

In our study population, 42% of the patients developed CMV disease, and this is higher than in other studies summarised in Table 3. We found an overall negative predictive value of 70%. The high prevalence of CMV disease in our population contributes to the relatively low negative predictive value.

The combination of high diagnostic specificity and high prevalence of CMV disease, explains the relatively high positive predictive value (76%) of detectable viraemia in our study. The positive predictive value could be further increased by raising the defined

viral load threshold for a positive result, or by defining as positive only cases with detectable viraemia in two out of two consecutive tests.

In a study conducted prior to the availability of HAART, Wiselka et al. found a positive predictive value of CMV quantitative PCR of only 44% (105). Poor predictive values (<50%) were also demonstrated in a study using CMV qualitative PCR (106). The low positive predictive values in these studies are due relatively low proportion of patients with CMV disease and low diagnostic specificity of the tests.

A study by Edwards et al. of acutely unwell HIV-1 positive individuals with access to HAART, found a positive predictive value of only 10% (119). HAART, may have prevented development of manifest CMV disease and contributed to the low diagnostic specificity (58%). In that study, only a small proportion (6%) of episodes of illness was followed by a diagnosis of CMV disease. Also, as autopsy was not performed, CMV disease might have been overlooked, resulting in potential underestimation of the true specificity and positive predictive value. However, it is likely that the positive predictive value for CMV quantitative PCR is low in HIV-infected individuals on effective HAART, or with access to treatment.

Using plasma, we found that CMV quantitative PCR had clear limitations in the diagnosis of CMV disease. Although studies of transplant recipients have found the analytical sensitivity to be higher in whole blood than in plasma, this does not necessarily mean that better positive predictive value is achieved (200;201). We did not test cerebrospinal fluid, but other studies have found high sensitivity and specificity using this specimen in patients with CMV neurological disease (111;113;114).

Studies have shown that foscarnet, ganciclovir, and valganciclovir reduce the risk of progression of CMV retinitis and other manifestations of CMV disease (121-125;129). In a U.S. study by Wohl et al. in patients with access to HAART, valganciclovir used as pre-emptive therapy did not significantly reduce the incidence of CMV disease (132). However, the power to detect a difference between groups given valganciclor and placebo was low due to few cases of CMV disease.

Table 3. Other studies reporting sensitivity, specificity, positive and negative predictive values for CMV quantitative PCR in serum, plasma,

blood or polymorphonu	clear leukoc	ytes in pati	ents with HIV i	infection at	blood or polymorphonuclear leukocytes in patients with HIV infection at high risk of CMV disease				
First Author, year	No. of cases	S	Study period	Country	Study period Country CMV viral load threshold	Sensitivity	Sensitivity Specificity PPV		NPV
(reference number)					(blood compartment tested)				
,	Total	CMVR			•				
	CMVD(%)	EOD(%)							
Shinkai M, 1997 (100)	94	19(73)	Before 1996	USA	10 ⁶ copies/ml	35	100	100	80
	26 (28)	7 (27)			10 ⁵ copies/ml (plasma)	73	06	73	06
Boivin G, 1998 (101)	106 27 (25)	21 (74) 7 (26)	Before 1998	Canada	1.6×10^4 copies/ml (PML)	93	92	92	86
Wiselka MJ, 1999 (105)	26 7 (27)	$\frac{7}{2}(100)^2$	1994-96	UK	1000 copies/ml (serum)	57	74	44	83
Pellegrin I, 1999 (103)	58	10 (77)	1995-97	France	400 copies/ml	85	100	100	96
	13 (77)	(52) 5			(piasilia)				
Yoshida A, 2001 (107)	70	11 (73)	1998-2000	Japan	3 x10 ³ copies/ml	98	85	63	95
	(12) C1	(77) +			(piasilia)				
Edwards SG, 2007 (119) 21	216^{3}	(20)	2001-05	UK	20 copies/ml	75	58	10	86
	12 (6)	6 (50)			(plasma)				

CMVD, Cytomegalovirus disease. CMVR, Cytomegalovirus retinitis. CMV EOD, other cytomegalovirus end-organ disease (other than retinitis). PPV, Positive Predictive Value. NPV, Negative Predictive Value. PML, polymorphonuclear leukocytes. One patient also had retinitis ²Two cases also had EOD ³No. of episodes of acute illness in 182 patients In contrast, studies using high-dose valaciclovir in the pre-HAART era when CMV disease was more common, showed reduced risk of CMV disease, but not reduced risk of death (118;128). In a small study from Italy, Foca et al. found that there was no difference in CD4+T cell count and HIV RNA response 52 weeks after starting HAART in patients with and without CMV viraemia at baseline (202). All patients had undetectable CMV viraemia after 52 weeks, even without specific CMV therapy. These results suggest that the risk of CMV disease occurring in patients with CMV viraemia and access to HAART in developed countries today is considerably lower than reported by us in Paper II.

In resource-poor countries, access to histopathological examination of tissue is often limited, as is availability of advanced microbiological assays such as PCR and other NATs. Therefore, the performance of CMV quantitative PCR in diagnosis of CMV disease in this setting is unknown.

5.3.2 CMV viraemia as predictor of death

In Paper III, we report that 22.6% of antiretroviral naïve patient in Tanzania had detectable viraemia at baseline, and 8.3% had CMV viral load >200 copies/ml. CMV viraemia >200 copies/ml was a significant risk factor for death in multivariable analysis, with a hazard ratio of 5.0. Only two previous studies from developing countries have assessed CMV viraemia as a risk factor for death, and both found that high CMV viral load was an independent predictor (154;203). To our knowledge, our paper is the first to demonstrate that a high CMV viral load is an independent risk factor for death in patients with HIV infection in Africa after wide-scale implementation of HAART.

Studies from developed countries in the pre-HAART era found that CMV viraemia was associated both with the development of CMV disease and death (104;116;152). Although the incidence of CMV disease in patients with HIV has been considerably reduced in patients with access to HAART, several studies have shown that CMV viraemia continues to represent a risk factor for death (73;109;110;115;151). Darwich et al. reported that HIV-infected patients with high specific interferon-gamma responses to CMV had better prognosis than patients with lower responses (204).

As CMV viraemia has been associated with poor prognosis in patients both before and after the introduction of HAART, a natural question to ask is if pre-emptive treatment of CMV can improve prognosis. No such studies have been conducted in developing countries, but Wohl et al. found no significant benefit on survival by the use of valganciclovir in a population with CMV viraemia, access to HAART and low incidence of CMV disease (132). Hunt et al. found that valganciclovir reduced immune activation in HIV-infected patients with low CD4+ T cell counts, but did not observe a significant increase in CD4+ T cell counts during a relatively short observation period (166). Present guidelines for the prevention and treatment of opportunistic infections in HIV-infected adolescent do not recommend CMV treatment for patients with asymptomatic CMV viraemia (89).

Previous studies from Australia and Europe have demonstrated a survival benefit with the administration of high-dose aciclovir to HIV-infected individuals, but have not found that aciclovir reduces the incidence of CMV disease (205;206). Studies from Africa have demonstrated that aciclovir treatment of HSV-2 in antiretroviral naïve HIV-infected patients is associated with a survival benefit (207;208). This effect may result from direct antiretroviral effect of aciclovir on HSV-2, but may also be mediated by a reduction in HSV-2-induced immune activation. In addition, a CMV-mediated effect on survival cannot be excluded. Valganciclovir is more active against CMV than aciclovir, and studies of the effect of this drug should therefore be considered in individuals with CMV viraemia in developing countries.

6 Conclusions and future perspectives

6.1 Conclusions

This thesis shows that CMV infection, CMV viraemia and CMV disease have serious consequences for HIV-infected individuals. Several challenges remain in the diagnosis and treatment of CMV disease, both in developed and developing countries.

I. Incidence of CMV disease in patients with AIDS in the pre-HAART era

Prior to the availability of HAART, CMV disease occurred frequently in patients with HIV infection in Oslo, and was diagnosed in almost one half of the patients at autopsy. More than two-thirds of these cases were undiagnosed prior to death. CMV infection was significantly more frequent in MSM than in IDUs. Retinitis was the most common manifestation.

II. Impact of CMV seropositivity and end-organ disease on survival in patients with AIDS in the pre-HAART era

There was no significant difference in survival between CMV seropositive and seronegative individuals. CMV disease diagnosed alive was associated with increased risk of death.

Despite CMV therapy, there was evidence of CMV disease at autopsy in all patients, clearly demonstrating the inadequacy of CMV treatment alone.

III. Utility of plasma CMV quantitative PCR in the diagnosis of CMV disease in patients with HIV-infection

CMV viraemia was more common in patients with CMV disease than in patients without CMV disease. The test only correctly identified one half of all patients with CMV disease, but more than two-thirds of patients with multi-organ disease. Few patients had CMV viraemia in the absence of CMV disease. Patients with high CMV viral load, or detectable viral load in more than one sample, were highly likely to have CMV disease, but by applying such stringent criteria many cases of CMV disease would be missed. CMV quantitative PCR is a valuable adjunct to diagnosis of CMV disease, but is far from perfect in discriminating between individuals with and without CMV disease.

IV. Utility of DBS for detection of CMV antibody and CMV viraemia in HIVinfected individuals in a resource-poor setting

We found that DBS are suitable for detection of CMV antibody and viraemia in a resourcepoor area. CMV viral load in DBS tended to underestimate plasma viral load, but this may not be of great clinical significance.

V. Prevalence of CMV infection and viraemia in relation to clinical outcome in patients with HIV infection and access to HAART in rural Tanzania

All antiretroviral naïve HIV-infected patients in the rural study area of Tanzania were CMV seropositive. Detectable CMV viraemia was common, and presence of viral load >200 copies/ml in DBS eluates was a significant risk factor for death, despite availability of HAART. Many patients had low CD4+ T cell counts and were at risk of CMV disease. A subgroup of these patients also had high CMV viral load and symptoms compatible with CMV disease. Although no cases were diagnosed with confirmed CMV disease, this may be due to diagnostic constraints.

6.2 Future perspectives of HIV-associated CMV morbidity in the HAART era

Currently, CMV disease represents a relatively small threat to HIV-infected patients with access to HAART. However, even in these populations, CMV disease will continue to occur in individuals first diagnosed after the development of severe immunodeficiency, and in patients with suboptimal treatment response due to poor treatment adherence or viral resistance. To prevent this serious opportunistic disease, priority should be given to early diagnosis of HIV-infection and timely administration of HAART. At the same time, clinicians need to be vigilant to symptoms of CMV disease and CMV immune recovery uveitis in HIV-infected patients at risk.

CMV disease remains a difficult diagnosis to make, and there is a clear need for future studies aiming to improve diagnosis of CMV disease. For this purpose a multifaceted approach is warranted, using information from patient history and clinical examination, in addition to results of immunological, microbiological, radiological, and histopathological tests. However,

Conclusions and future perspectives

as CMV disease has become a rare disease in populations with access to HAART, such studies will be hard to perform. CMV disease is therefore likely to be a challenging diagnosis to make in HIV-infected individuals for many years to come.

Analysis of CMV viraemia in DBS has several potential advantages compared to liquid samples, and DBS are particularly suitable for use in developing countries. However, there remain methodological challenges that have not yet been fully solved. Further validation of CMV quantitative PCR in DBS is needed before DBS can replace plasma samples in routine practice.

In developing countries, there is a need for further studies on the impact of CMV infection, viraemia and disease, including the role of CMV in accelerating the progression of HIV disease. Trials are needed to assess if diagnosis and treatment of CMV disease and viraemia can improve prognosis for patients in this part of the world.

Conclusions and future perspectives

7 References

- 1. CDC. Pneumocystis pneumonia Los Angeles. MMWR 1981; 30: 250-2.
- 2. Update on acquired immune deficiency syndrome (AIDS)--United States. MMWR Morb Mortal Wkly Rep 1982; 31: 507-4.
- 3. Webster A, Lee CA, Cook DG, et al. Cytomegalovirus infection and progression towards AIDS in haemophiliacs with human immunodeficiency virus infection. Lancet 1989; 2: 63-6.
- 4. Gallo RC. Historical essay. The early years of HIV/AIDS. Science 2002; 298: 1728-30.
- Montagnier L. Historical essay. A history of HIV discovery. Science 2002; 298: 1727 8.
- 6. Gao F, Bailes E, Robertson DL, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature 1999; 397: 436-41.
- 7. Hirsch VM, Olmsted RA, Murphey-Corb M, et al. An African primate lentivirus (SIVsm) closely related to HIV-2. Nature 1989; 339: 389-92.
- 8. Douek DC, Brenchley JM, Betts MR, et al. HIV preferentially infects HIV-specific CD4+ T cells. Nature 2002; 417: 95-8.
- 9. Epstein JS, Frederick WR, Rook AH, et al. Selective defects in cytomegalovirus- and mitogen-induced lymphocyte proliferation and interferon release in patients with acquired immunodeficiency syndrome. J Infect Dis 1985; 152: 727-33.
- Rosenberg ES, Billingsley JM, Caliendo AM, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. Science 1997; 278: 1447-50.
- 11. Smith MG. Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. Proc Soc Exp Biol Med 1956; 92: 424-30.
- 12. Rowe WP, Hartley JW, Waterman, et al. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc Soc Exp Biol Med 1956; 92: 418-24.
- 13. Craig JM, Macauley JC, Weller TH, et al. Isolation of intranuclear inclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc Soc Exp Biol Med 1957; 94: 4-12.

- 14. Sinclair J, Sissons P. Latency and reactivation of human cytomegalovirus. J Gen Virol 2006; 87: 1763-79.
- 15. Steininger C, Puchhammer-Stockl E, Popow-Kraupp T. Cytomegalovirus disease in the era of highly active antiretroviral therapy (HAART). J Clin Virol 2006; 37: 1-9.
- 16. Mandell GL, Bennett JE, Dolin R. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, 7th Edition Chapter 135 and 138 2009.
- 17. Sinzger C, Digel M, Jahn G. Cytomegalovirus cell tropism. Curr Top Microbiol Immunol 2008; 325: 63-83.
- 18. Global Report: UNAIDS report on the global AIDS epidemic. UNAIDS 2010.
- Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol 2010; 20: 202-13.
- 20. Ben-Smith A, Gorak-Stolinska P, Floyd S, et al. Differences between naive and memory T cell phenotype in Malawian and UK adolescents: a role for Cytomegalovirus? BMC Infect Dis 2008; 8: 139.
- 21. Njeru DG, Mwanda WO, Kitonyi GW, et al. Prevalence of cytomegalovirus antibodies in blood donors at the National Blood Transfusion Centre, Nairobi. East Afr Med J 2009; 86: S58-S61.
- Adjei A, Armah H, Gbagbo F, et al. Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. BMC Infectious Diseases 2008;
 111.
- 23. Ali F, Abdel-Aziz A, Helmy MF, et al. Prevalence of certain sexually transmitted viruses in Egypt. J Egypt Public Health Assoc 1998; 73: 181-92.
- 24. Cochereau I, Mlika-Cabanne N, Godinaud P, et al. AIDS related eye disease in Burundi, Africa. Br J Ophthalmol 1999; 83: 339-42.
- Compston LI, Li C, Sarkodie F, et al. Prevalence of persistent and latent viruses in untreated patients infected with HIV-1 from Ghana, West Africa. J Med Virol 2009; 81: 1860-8.
- Ghebrekidan H, Ruden U, Cox S, et al. Prevalence of herpes simplex virus types 1 and 2, cytomegalovirus, and varicella-zoster virus infections in Eritrea. J Clin Virol 1999; 12: 53-64.
- 27. Rodier MH, Berthonneau J, Bourgoin A, et al. Seroprevalences of Toxoplasma, malaria, rubella, cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou, Republic of Benin. Acta Trop 1995; 59: 271-7.

- 28. Boppana SB, Fowler KB. Persistence in the population: epidemiology and transmisson. Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis Chapter 44 Cambridge: Cambridge University Press; 2007.
- 29. Flesland O, Solheim BG. [Cytomegalovirus. Presence of antibodies in married couples]. Tidsskr Nor Laegeforen 1989; 109: 957-8.
- 30. Eskild A, Jenum PA, Bruu AL. Maternal antibodies against cytomegalovirus in pregnancy and the risk of fetal death and low birth weight. Acta Obstet Gynecol Scand 2005; 84: 1035-41.
- 31. Skar AG, Hoddevik G. Prevalence of cytomegalovirus antibodies in Norwegian kidney-transplant recipients and their living donors. A comparative study of two different methods. Acta Pathol Microbiol Immunol Scand B 1984; 92: 1-5.
- 32. Kryger P, Gerstoft J, Pedersen NS, et al. Increased prevalence of cytomegalovirus antibodies in homosexual men with syphilis: relation to sexual behaviour. Scand J Infect Dis 1984; 16: 381-4.
- 33. Jackson JB, Erice A, Englund JA, et al. Prevalence of cytomegalovirus antibody in hemophiliacs and homosexuals infected with human immunodeficiency virus type 1. Transfusion (Paris) 1988; 28: 187-9.
- 34. Shepp DH, Moses JE, Kaplan MH. Seroepidemiology of cytomegalovirus in patients with advanced HIV disease: influence on disease expression and survival. J Acquir Immune Defic Syndr Hum Retrovirol 1996; 11: 460-8.
- 35. Rabenau HF, Lennemann T, Kircher C, et al. Prevalence- and gender-specific immune response to opportunistic infections in HIV-infected patients in Lesotho. Sex Transm Dis 2010; 37: 454-9.
- 36. Emery VC. Cytomegalovirus: recent progress in understanding pathogenesis and control. QJM 2012; 105: 401-5.
- 37. Naeger DM, Martin JN, Sinclair E, et al. Cytomegalovirus-specific T cells persist at very high levels during long-term antiretroviral treatment of HIV disease. PLoS One 2010; 5: e8886.
- 38. Sylwester AW, Mitchell BL, Edgar JB, et al. Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. J Exp Med 2005; 202: 673-85.
- 39. Lenkei R, Andersson B. High correlations of anti-CMV titers with lymphocyte activation status and CD57 antibody-binding capacity as estimated with three-color, quantitative flow cytometry in blood donors. Clin Immunol Immunopathol 1995; 77: 131-8.

- 40. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med 1995; 333: 1038-44.
- 41. Bronke C, Jansen CA, Westerlaken GH, et al. Shift of CMV-specific CD4+ T-cells to the highly differentiated CD45RO-CD27- phenotype parallels loss of proliferative capacity and precedes progression to HIV-related CMV end-organ disease. Clin Immunol 2007; 124: 190-9.
- 42. Griffiths PD, Stanton A, McCarrell E, et al. Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial. Lancet 2011; 377: 1256-63.
- 43. Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev 2009; 22: 76-98, Table.
- 44. Kotenko SV, Saccani S, Izotova LS, et al. Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci U S A 2000; 97: 1695-700.
- 45. Spencer JV, Lockridge KM, Barry PA, et al. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol 2002; 76: 1285-92.
- 46. Seow HF. Pathogen interactions with cytokines and host defence: an overview. Vet Immunol Immunopathol 1998; 63: 139-48.
- 47. Vink C, Beisser PS, Bruggeman CA. Molecular mimicry by cytomegaloviruses. Function of cytomegalovirus-encoded homologues of G protein-coupled receptors, MHC class I heavy chains and chemokines. Intervirology 1999; 42: 342-9.
- 48. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 2007; 17: 355-63.
- 49. Demmler GJ. Infectious Diseases Society of America and Centers for Disease Control. Summary of a workshop on surveillance for congenital cytomegalovirus disease. Rev Infect Dis 1991; 13: 315-29.
- 50. Bowden RA, Sayers M, Flournoy N, et al. Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. N Engl J Med 1986; 314: 1006-10.
- 51. Hart GD, Paya CV. Prophylaxis for CMV should now replace pre-emptive therapy in solid organ transplantation. Rev Med Virol 2001; 11: 73-81.
- 52. Legendre C, Pascual M. Improving outcomes for solid-organ transplant recipients at risk from cytomegalovirus infection: late-onset disease and indirect consequences. Clin Infect Dis 2008; 46: 732-40.

- 53. Afessa B, Greaves W, Green W, et al. Autopsy findings in HIV-infected inner-city patients. J Acquir Immune Defic Syndr 1992; 5: 132-6.
- d'Arminio MA, Vago L, Lazzarin A, et al. AIDS-defining diseases in 250 HIVinfected patients; a comparative study of clinical and autopsy diagnoses. AIDS 1992; 6: 1159-64.
- 55. Lyon R, Haque AK, Asmuth DM, et al. Changing patterns of infections in patients with AIDS: a study of 279 autopsies of prison inmates and nonincarcerated patients at a university hospital in eastern Texas, 1984-1993 [see comments]. Clin Infect Dis 1996; 23: 241-7.
- 56. Masliah E, DeTeresa RM, Mallory ME, et al. Changes in pathological findings at autopsy in AIDS cases for the last 15 years. AIDS 2000; 14: 69-74.
- 57. Moore RD, Chaisson RE. Natural history of opportunistic disease in an HIV-infected urban clinical cohort. Ann Intern Med 1996; 124: 633-42.
- 58. Wilkes MS, Fortin AH, Felix JC, et al. Value of necropsy in acquired immunodeficiency syndrome. Lancet 1988; 2: 85-8.
- 59. Pertel P, Hirschtick R, Phair J, et al. Risk of developing cytomegalovirus retinitis in persons infected with the human immunodeficiency virus. J Acquir Immune Defic Syndr 1992; 5: 1069-74.
- 60. Saillour F, Bernard N, Dequae-Merchadou L, et al. Predictive factors of occurrence of cytomegalovirus disease and impact on survival in the Aquitaine Cohort in France, 1985 to 1994. Groupe d'Epidemiologie Clinique du SIDA en Aquitaine. J Acquir Immune Defic Syndr Hum Retrovirol 1998; 17: 171-8.
- 61. Saillour F, Bernard N, Ragnaud JM, et al. Incidence of cytomegalovirus disease in the Aquitaine cohort of HIV- infected patients: a retrospective survey, 1987-1993. Groupe d'Epidemilogie Clinique du SIDA en Aquitaine (GECSA). J Infect 1997; 35: 155-61.
- 62. Hoover DR, Saah AJ, Bacellar H, et al. Clinical manifestations of AIDS in the era of pneumocystis prophylaxis. Multicenter AIDS Cohort Study. N Engl J Med 1993; 329: 1922-6.
- 63. Selik RM, Chu SY, Ward JW. Trends in infectious diseases and cancers among persons dying of HIV infection in the United States from 1987 to 1992 [see comments]. Ann Intern Med 1995; 123: 933-6.
- 64. Brodt HR, Kamps BS, Gute P, et al. Changing incidence of AIDS-defining illnesses in the era of antiretroviral combination therapy. AIDS 1997; 11: 1731-8.

- 65. Casado JL, Arrizabalaga J, Montes M, et al. Incidence and risk factors for developing cytomegalovirus retinitis in HIV-infected patients receiving protease inhibitor therapy. Spanish CMV- AIDS Study Group. AIDS 1999; 13: 1497-502.
- 66. Mocroft A, Sabin CA, Youle M, et al. Changes in AIDS-defining illnesses in a London Clinic, 1987-1998. J Acquir Immune Defic Syndr 1999; 21: 401-7.
- 67. Moore RD, Chaisson RE. Natural history of HIV infection in the era of combination antiretroviral therapy. AIDS 1999; 13: 1933-42.
- 68. Goossens VJ, Wolffs PF, van Loo IH, et al. CMV DNA levels and CMV gB subtypes in ART-naive HAART-treated patients: a 2-year follow-up study in The Netherlands. AIDS 2009; 23: 1425-9.
- 69. Yust I, Fox Z, Burke M, et al. Retinal and extraocular cytomegalovirus end-organ disease in HIV-infected patients in Europe: a EuroSIDA study, 1994-2001. Eur J Clin Microbiol Infect Dis 2004; 23: 550-9.
- 70. Varani S, Spezzacatena P, Manfredi R, et al. The incidence of cytomegalovirus (CMV) antigenemia and CMV disease is reduced by highly active antiretroviral therapy. Eur J Epidemiol 2000; 16: 433-7.
- 71. Salzberger B, Hartmann P, Hanses F, et al. Incidence and prognosis of CMV disease in HIV-infected patients before and after introduction of combination antiretroviral therapy. Infection 2005; 33: 345-9.
- 72. Jacobson MA, Stanley H, Holtzer C, et al. Natural history and outcome of new AIDS-related cytomegalovirus retinitis diagnosed in the era of highly active antiretroviral therapy. Clin Infect Dis 2000; 30: 231-3.
- 73. Wohl DA, Zeng D, Stewart P, et al. Cytomegalovirus viremia, mortality, and endorgan disease among patients with AIDS receiving potent antiretroviral therapies. J Acquir Immune Defic Syndr 2005; 38: 538-44.
- 74. Jabs DA, Ahuja A, Van NM, et al. Course of cytomegalovirus retinitis in the era of highly active antiretroviral therapy: five-year outcomes. Ophthalmology 2010; 117: 2152-61.
- 75. Heiden D, Ford N, Wilson D, et al. Cytomegalovirus retinitis: the neglected disease of the AIDS pandemic. PLoS Med 2007; 4: e334.
- 76. Kestelyn P. The epidemiology of CMV retinitis in Africa. Ocul Immunol Inflamm 1999; 7: 173-7.
- 77. Maartens G. Opportunistic infections associated with HIV infection in Africa. Oral Dis 2002; 8 Suppl 2: 76-9.

- 78. Chakravarti A, Kashyap B, Matlani M. Cytomegalovirus infection: an Indian perspective. Indian J Med Microbiol 2009; 27: 3-11.
- 79. Nissapatorn V. Lessons learned about opportunistic infections in southeast Asia. Southeast Asian J Trop Med Public Health 2008; 39: 625-41.
- 80. d'Arminio MA, Mainini F, Testa L, et al. Predictors of cytomegalovirus disease, natural history and autopsy findings in a cohort of patients with AIDS. AIDS 1997; 11: 517-24.
- 81. Dore GJ, Marriott DJ, Duflou JA. Clinico-pathological study of cytomegalovirus (CMV) in AIDS autopsies: under-recognition of CMV pneumonitis and CMV adrenalitis. Aust N Z J Med 1995; 25: 503-6.
- 82. Hofman P, Saint-Paul MC, Battaglione V, et al. Autopsy findings in the acquired immunodeficiency syndrome (AIDS). A report of 395 cases from the south of France. Pathol Res Pract 1999; 195: 209-17.
- 83. Pillay D, Lipman MC, Lee CA, et al. A clinico-pathological audit of opportunistic viral infections in HIV- infected patients. AIDS 1993; 7: 969-74.
- 84. Klatt EC, Nichols L, Noguchi TT. Evolving trends revealed by autopsies of patients with the acquired immunodeficiency syndrome. 565 autopsies in adults with the acquired immunodeficiency syndrome, Los Angeles, Calif, 1982-1993 [corrected]. Arch Pathol Lab Med 1994; 118: 884-90.
- 85. Morgello S, Mahboob R, Yakoushina T, et al. Autopsy findings in a human immunodeficiency virus-infected population over 2 decades: influences of gender, ethnicity, risk factors, and time. Arch Pathol Lab Med 2002; 126: 182-90.
- 86. McKenzie R, Travis WD, Dolan SA, et al. The causes of death in patients with human immunodeficiency virus infection: a clinical and pathologic study with emphasis on the role of pulmonary diseases. Medicine (Baltimore) 1991; 70: 326-43.
- 87. Jellinger KA, Setinek U, Drlicek M, et al. Neuropathology and general autopsy findings in AIDS during the last 15 years. Acta Neuropathol 2000; 100: 213-20.
- 88. Markowitz GS, Concepcion L, Factor SM, et al. Autopsy patterns of disease among subgroups of an inner-city Bronx AIDS population. J Acquir Immune Defic Syndr Hum Retrovirol 1996; 13: 48-54.
- 89. Kaplan JE, Benson C, Holmes KH, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. MMWR Recomm Rep 2009; 58: 1-207.

- 90. Zurlo JJ, O'Neill D, Polis MA, et al. Lack of clinical utility of cytomegalovirus blood and urine cultures in patients with HIV infection [see comments]. Ann Intern Med 1993; 118: 12-7.
- 91. Degre M, Bukholm G, Holter E, et al. Rapid detection of cytomegalovirus infection in immunocompromised patients. Eur J Clin Microbiol Infect Dis 1994; 13: 668-70.
- 92. Dodt KK, Jacobsen PH, Hofmann B, et al. Development of cytomegalovirus (CMV) disease may be predicted in HIV- infected patients by CMV polymerase chain reaction and the antigenemia test. AIDS 1997; 11: F21-F28.
- 93. Francisci D, Tosti A, Preziosi R, et al. Role of antigenemia assay in the early diagnosis and prediction of human cytomegalovirus organ involvement in AIDS patients. Eur J Clin Microbiol Infect Dis 1995; 14: 498-503.
- 94. Francisci D, Tosti A, Baldelli F, et al. The pp65 antigenaemia test as a predictor of cytomegalovirus-induced end-organ disease in patients with AIDS. AIDS 1997; 11: 1341-5.
- 95. Hoshino Y, Nagata Y, Gatanaga H, et al. Cytomegalovirus (CMV) retinitis and CMV antigenemia as a clue to impaired adrenocortical function in patients with AIDS. AIDS 1997; 11: 1719-24.
- Salmon-Ceron D, Mazeron MC, Chaput S, et al. Plasma cytomegalovirus DNA, pp65 antigenaemia and a low CD4 cell count remain risk factors for cytomegalovirus disease in patients receiving highly active antiretroviral therapy. AIDS 2000; 14: 1041-9.
- 97. Salzberger B, Franzen C, Fatkenheuer G, et al. CMV-antigenemia in peripheral blood for the diagnosis of CMV disease in HIV-infected patients [see comments]. J Acquir Immune Defic Syndr Hum Retrovirol 1996; 11: 365-9.
- 98. Wattanamano P, Clayton JL, Kopicko JJ, et al. Comparison of three assays for cytomegalovirus detection in AIDS patients at risk for retinitis. J Clin Microbiol 2000; 38: 727-32.
- 99. Bowen EF, Sabin CA, Wilson P, et al. Cytomegalovirus (CMV) viraemia detected by polymerase chain reaction identifies a group of HIV-positive patients at high risk of CMV disease. AIDS 1997; 11: 889-93.
- 100. Shinkai M, Bozzette SA, Powderly W, et al. Utility of urine and leukocyte cultures and plasma DNA polymerase chain reaction for identification of AIDS patients at risk for developing human cytomegalovirus disease. J Infect Dis 1997; 175: 302-8.
- 101. Boivin G, Handfield J, Toma E, et al. Comparative evaluation of the cytomegalovirus DNA load in polymorphonuclear leukocytes and plasma of human immunodeficiency virus- infected subjects [see comments]. J Infect Dis 1998; 177: 355-60.

- Spector SA, Wong R, Hsia K, et al. Plasma cytomegalovirus (CMV) DNA load predicts CMV disease and survival in AIDS patients. J Clin Invest 1998; 101: 497-502.
- 103. Pellegrin I, Garrigue I, Binquet C, et al. Evaluation of new quantitative assays for diagnosis and monitoring of cytomegalovirus disease in human immunodeficiency virus-positive patients. Journal of Clinical Microbiology 1999; 37: 3124-32.
- Spector SA, Hsia K, Crager M, et al. Cytomegalovirus (CMV) DNA load is an independent predictor of CMV disease and survival in advanced AIDS. J Virol 1999; 73: 7027-30.
- 105. Wiselka MJ, Nicholson KG, Rowley S, et al. Cytomegalovirus viraemia has poor predictive value for the development of cytomegalovirus disease in patients with advanced HIV-infection. J Infect 1999; 39: 187-92.
- 106. Blank BS, Meenhorst PL, Mulder JW, et al. Value of different assays for detection of human cytomegalovirus (HCMV) in predicting the development of HCMV disease in human immunodeficiency virus-infected patients. J Clin Microbiol 2000; 38: 563-9.
- 107. Yoshida A, Hitomi S, Fukui T, et al. Diagnosis and monitoring of human cytomegalovirus diseases in patients with human immunodeficiency virus infection by use of a real-time PCR assay. Clin Infect Dis 2001; 33: 1756-61.
- 108. Nokta MA, Holland F, De G, V, et al. Cytomegalovirus (CMV) polymerase chain reaction profiles in individuals with advanced human immunodeficiency virus infection: relationship to CMV disease. J Infect Dis 2002; 185: 1717-22.
- 109. Erice A, Tierney C, Hirsch M, et al. Cytomegalovirus (CMV) and human immunodeficiency virus (HIV) burden, CMV end-organ disease, and survival in subjects with advanced HIV infection (AIDS clinical trials group protocol 360). Clinical Infectious Diseases 2003; 37: 567-78.
- 110. Deayton JR, Sabin CA, Johnson MA, et al. Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet 2004; 363: 2116-21.
- 111. Bestetti A, Pierotti C, Terreni M, et al. Comparison of three nucleic acid amplification assays of cerebrospinal fluid for diagnosis of cytomegalovirus encephalitis. J Clin Microbiol 2001; 39: 1148-51.
- 112. Shinkai M, Spector SA. Quantitation of human cytomegalovirus (HCMV) DNA in cerebrospinal fluid by competitive PCR in AIDS patients with different HCMV central nervous system diseases. Scand J Infect Dis 1995; 27: 559-61.
- 113. Flood J, Drew WL, Miner R, et al. Diagnosis of cytomegalovirus (CMV) polyradiculopathy and documentation of in vivo anti-CMV activity in cerebrospinal

- fluid by using branched DNA signal amplification and antigen assays. J Infect Dis 1997; 176: 348-52.
- 114. Arribas JR, Storch GA, Clifford DB, et al. Cytomegalovirus encephalitis. Ann Intern Med 1996; 125: 577-87.
- 115. El Amari EB, Combescure C, Yerly S, et al. Clinical relevance of cytomegalovirus viraemia(*,dagger). HIV Med 2011; 12: 394-402.
- 116. Emery VC, Sabin C, Feinberg JE, et al. Quantitative effects of valacyclovir on the replication of cytomegalovirus (CMV) in persons with advanced human immunodeficiency virus disease: baseline CMV load dictates time to disease and survival. The AIDS Clinical Trials Group 204/Glaxo Wellcome 123-014 International CMV Prophylaxis Study Group. J Infect Dis 1999; 180: 695-701.
- 117. Gerard L, Leport C, Flandre P, et al. Cytomegalovirus (CMV) viremia and the CD4+ lymphocyte count as predictors of CMV disease in patients infected with human immunodeficiency virus. Clin Infect Dis 1997; 24: 836-40.
- 118. Griffiths PD, Feinberg JE, Fry J, et al. The effect of valaciclovir on cytomegalovirus viremia and viruria detected by polymerase chain reaction in patients with advanced human immunodeficiency virus disease. AIDS Clinical Trials Group Protocol 204/Glaxo Wellcome 123-014 International CMV Prophylaxis Study Group. J Infect Dis 1998; 177: 57-64.
- 119. Edwards SG, Grover D, Scott C, et al. Cytomegalovirus viral load testing of blood using quantitative polymerase chain reaction in acutely unwell HIV-1-positive patients lacks diagnostic utility. Int J STD AIDS 2007; 18: 321-3.
- 120. Castagnola E, Cappelli B, Erba D, et al. Cytomegalovirus infection after bone marrow transplantation in children. Hum Immunol 2004; 65: 416-22.
- 121. Combination foscarnet and ganciclovir therapy vs monotherapy for the treatment of relapsed cytomegalovirus retinitis in patients with AIDS. The Cytomegalovirus Retreatment Trial. The Studies of Ocular Complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group. Arch Ophthalmol 1996; 114: 23-33.
- 122. Jacobson MA. Treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome [published erratum appears in N Engl J Med 1997 Oct 2;337(14):1019]. N Engl J Med 1997; 337: 105-14.
- 123. Kim YS, Hollander H. Polyradiculopathy due to cytomegalovirus: report of two cases in which improvement occurred after prolonged therapy and review of the literature [see comments]. Clin Infect Dis 1993; 17: 32-7.

- 124. Masur H, Whitcup SM, Cartwright C, et al. Advances in the management of AIDS-related cytomegalovirus retinitis [see comments]. Ann Intern Med 1996; 125: 126-36.
- 125. Salmon-Ceron D. Cytomegalovirus infection: the point in 2001. HIV Med 2001; 2: 255-9.
- 126. Parenteral cidofovir for cytomegalovirus retinitis in patients with AIDS: the HPMPC peripheral cytomegalovirus retinitis trial. A randomized, controlled trial. Studies of Ocular complications of AIDS Research Group in Collaboration with the AIDS Clinical Trials Group. Ann Intern Med 1997; 126: 264-74.
- 127. Lalezari JP, Stagg RJ, Kuppermann BD, et al. Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS. A randomized, controlled trial. Ann Intern Med 1997; 126: 257-63.
- 128. Feinberg JE, Hurwitz S, Cooper D, et al. A randomized, double-blind trial of valaciclovir prophylaxis for cytomegalovirus disease in patients with advanced human immunodeficiency virus infection. AIDS Clinical Trials Group Protocol 204/Glaxo Wellcome 123-014 International CMV Prophylaxis Study Group. J Infect Dis 1998; 177: 48-56.
- 129. Martin DF, Sierra-Madero J, Walmsley S, et al. A controlled trial of valganciclovir as induction therapy for cytomegalovirus retinitis. N Engl J Med 2002; 346: 1119-26.
- 130. Martin DF, Parks DJ, Mellow SD, et al. Treatment of cytomegalovirus retinitis with an intraocular sustained-release ganciclovir implant. A randomized controlled clinical trial. Arch Ophthalmol 1994; 112: 1531-9.
- 131. Musch DC, Martin DF, Gordon JF, et al. Treatment of cytomegalovirus retinitis with a sustained-release ganciclovir implant. The Ganciclovir Implant Study Group. N Engl J Med 1997; 337: 83-90.
- 132. Wohl DA, Kendall MA, Andersen J, et al. Low rate of CMV end-organ disease in HIV-infected patients despite low CD4+ cell counts and CMV viremia: results of ACTG protocol A5030. HIV Clin Trials 2009; 10: 143-52.
- 133. Griffiths PD, Boeckh M. Antiviral therapy for human cytomegalovirus. Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis Chapter 66 Cambridge: Cambridge University Press 2007.
- 134. Griffiths PD. Studies to define viral cofactors for human immunodeficiency virus. Infect Agents Dis 1992; 1: 237-44.
- 135. Webster A. Cytomegalovirus as a possible cofactor in HIV disease progression. J Acquir Immune Defic Syndr 1991; 4 Suppl 1: S47-S52.

- Griffiths PD. CMV as a cofactor enhancing progression of AIDS. J Clin Virol 2006;
 35: 489-92.
- 137. Jabs DA, Martin BK, Forman MS, et al. Cytomegalovirus (CMV) blood DNA load, CMV retinitis progression, and occurrence of resistant CMV in patients with CMV retinitis. J Infect Dis 2005; 192: 640-9.
- 138. Davis MG, Kenney SC, Kamine J, et al. Immediate-early gene region of human cytomegalovirus trans-activates the promoter of human immunodeficiency virus. Proc Natl Acad Sci U S A 1987; 84: 8642-6.
- 139. Skolnik PR, Kosloff BR, Hirsch MS. Bidirectional interactions between human immunodeficiency virus type 1 and cytomegalovirus. J Infect Dis 1988; 157: 508-14.
- Clouse KA, Robbins PB, Fernie B, et al. Viral antigen stimulation of the production of human monokines capable of regulating HIV1 expression. J Immunol 1989; 143: 470 5.
- 141. Peterson PK, Gekker G, Chao CC, et al. Human cytomegalovirus-stimulated peripheral blood mononuclear cells induce HIV-1 replication via a tumor necrosis factor-alpha-mediated mechanism. J Clin Invest 1992; 89: 574-80.
- 142. Margalith M, D'Aquila RT, Manion DJ, et al. HIV-1 DNA in fibroblast cultures infected with urine from HIV-seropositive cytomegalovirus (CMV) excretors. Arch Virol 1995; 140: 927-35.
- 143. Pleskoff O, Treboute C, Brelot A, et al. Identification of a chemokine receptor encoded by human cytomegalovirus as a cofactor for HIV-1 entry. Science 1997; 276: 1874-8.
- 144. Lusso P, Garzino-Demo A, Crowley RW, et al. Infection of gamma/delta T lymphocytes by human herpesvirus 6: transcriptional induction of CD4 and susceptibility to HIV infection. J Exp Med 1995; 181: 1303-10.
- 145. McKeating JA, Griffiths PD, Weiss RA. HIV susceptibility conferred to human fibroblasts by cytomegalovirus-induced Fc receptor. Nature 1990; 343: 659-61.
- 146. Sabin CA, Phillips AN, Lee CA, et al. The effect of CMV infection on progression of human immunodeficiency virus disease in a cohort of haemophilic men followed for up to 13 years from seroconversion. Epidemiol Infect 1995; 114: 361-72.
- 147. Sinicco A, Raiteri R, Sciandra M, et al. The influence of cytomegalovirus on the natural history of HIV infection: evidence of rapid course of HIV infection in HIV-positive patients infected with cytomegalovirus. Scand J Infect Dis 1997; 29: 543-9.

- 148. Karlsson A, Bratt G, von Krogh G, et al. A prospective study of 115 initially asymptomatic HIV infected gay men in Stockholm, Sweden. Scand J Infect Dis 1991; 23: 431-41.
- 149. Kovacs A, Schluchter M, Easley K, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1-infected women. Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV Infection Study Group. N Engl J Med 1999; 341: 77-84.
- 150. Rabkin CS, Hatzakis A, Griffiths PD, et al. Cytomegalovirus infection and risk of AIDS in human immunodeficiency virus-infected hemophilia patients. National Cancer Institute Multicenter Hemophilia Cohort Study Group. J Infect Dis 1993; 168: 1260-3.
- 151. Jabs DA, Holbrook JT, Van Natta ML, et al. Risk factors for mortality in patients with AIDS in the era of highly active antiretroviral therapy. Ophthalmology 2005; 112: 771-9.
- 152. Bowen EF, Wilson P, Cope A, et al. Cytomegalovirus retinitis in AIDS patients: influence of cytomegaloviral load on response to ganciclovir, time to recurrence and survival. AIDS 1996; 10: 1515-20.
- 153. Detels R, Leach CT, Hennessey K, et al. Persistent cytomegalovirus infection of semen increases risk of AIDS. J Infect Dis 1994; 169: 766-8.
- 154. Micol R, Buchy P, Guerrier G, et al. Prevalence, risk factors, and impact on outcome of cytomegalovirus replication in serum of Cambodian HIV-infected patients (2004-2007). J Acquir Immune Defic Syndr 2009; 51: 486-91.
- 155. Barrett L, Fowke KR, Grant MD. Cytomegalovirus, aging, and HIV: a perfect storm. AIDS Rev 2012; 14: 159-67.
- 156. Hunt PW, Martin JN, Sinclair E, et al. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis 2003; 187: 1534-43.
- 157. Gandhi RT, Spritzler J, Chan E, et al. Effect of baseline- and treatment-related factors on immunologic recovery after initiation of antiretroviral therapy in HIV-1-positive subjects: results from ACTG 384. J Acquir Immune Defic Syndr 2006; 42: 426-34.
- 158. Goicoechea M, Smith DM, Liu L, et al. Determinants of CD4+ T cell recovery during suppressive antiretroviral therapy: association of immune activation, T cell maturation markers, and cellular HIV-1 DNA. J Infect Dis 2006; 194: 29-37.
- 159. Valdez H, Connick E, Smith KY, et al. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. AIDS 2002; 16: 1859-66.

- Samaras K, Gan SK, Peake PW, et al. Proinflammatory markers, insulin sensitivity, and cardiometabolic risk factors in treated HIV infection. Obesity (Silver Spring) 2009; 17: 53-9.
- 161. Tien PC, Choi AI, Zolopa AR, et al. Inflammation and mortality in HIV-infected adults: analysis of the FRAM study cohort. J Acquir Immune Defic Syndr 2010; 55: 316-22.
- 162. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 2008; 5: e203.
- 163. Hsue PY, Hunt PW, Sinclair E, et al. Increased carotid intima-media thickness in HIV patients is associated with increased cytomegalovirus-specific T-cell responses. AIDS 2006; 20: 2275-83.
- 164. Lo J, Abbara S, Shturman L, et al. Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men. AIDS 2010; 24: 243-53.
- 165. Parrinello CM, Sinclair E, Landay AL, et al. Cytomegalovirus immunoglobulin G antibody is associated with subclinical carotid artery disease among HIV-infected women. J Infect Dis 2012; 205: 1788-96.
- 166. Hunt PW, Martin JN, Sinclair E, et al. Valganciclovir Reduces T Cell Activation in HIV-infected Individuals With Incomplete CD4+ T Cell Recovery on Antiretroviral Therapy. J Infect Dis 2011; 203: 1474-83.
- 167. Johannessen A, Garrido C, Zahonero N, et al. Dried blood spots perform well in viral load monitoring of patients who receive antiretroviral treatment in rural Tanzania. Clin Infect Dis 2009; 49: 976-81.
- 168. Johannessen A, Garrido C, Zahonero N, et al. HIV-1 drug resistance testing from dried blood spots collected in rural Tanzania using the ViroSeq HIV-1 Genotyping System. J Antimicrob Chemother 2011; 66: 260-4.
- 169. Saah AJ, Hoover DR. "Sensitivity" and "specificity" reconsidered: the meaning of these terms in analytical and diagnostic settings. Ann Intern Med 1997; 126: 91-4.
- 170. Barbi M, Binda S, Caroppo S. Diagnosis of congenital CMV infection via dried blood spots. Rev Med Virol 2006; 16: 385-92.
- 171. Johansson PJ, Jonsson M, Ahlfors K, et al. Retrospective diagnostics of congenital cytomegalovirus infection performed by polymerase chain reaction in blood stored on filter paper. Scand J Infect Dis 1997; 29: 465-8.
- 172. Waggoner J, Ho DY, Libiran P, et al. Clinical Significance of Low CMV DNA Levels in Human Plasma. J Clin Microbiol 2012.

- 173. Kraft CS, Armstrong WS, Caliendo AM. Interpreting quantitative cytomegalovirus DNA testing: understanding the laboratory perspective. Clin Infect Dis 2012; 54: 1793-7.
- 174. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 2002; 34: 1094-7.
- 175. Surveillance for AIDS-defining opportunistic illnesses 1992-1997. MMWR Morb Mortal Wkly Rep 1999; 48: 1-23.
- 176. Deayton J, Mocroft A, Wilson P, et al. Loss of cytomegalovirus (CMV) viraemia following highly active antiretroviral therapy in the absence of specific anti-CMV therapy. AIDS 1999; 13: 1203-6.
- 177. Binquet C, Saillour F, Bernard N, et al. Prognostic factors of survival of HIV-infected patients with cytomegalovirus disease: Aquitaine Cohort, 1986-1997. Groupe d'Epidemiologie Clinique du SIDA en Aquitaine (GECSA). Eur J Epidemiol 2000; 16: 425-32.
- 178. Jacobson MA, Zegans M, Pavan PR, et al. Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet 1997; 349: 1443-5.
- 179. Goldberg DE, Smithen LM, Angelilli A, et al. HIV-associated retinopathy in the HAART era. Retina 2005; 25: 633-49.
- 180. Ausayakhun S, Watananikorn S, Ittipunkul N, et al. Epidemiology of the ocular complications of HIV infection in Chiang Mai. J Med Assoc Thai 2003; 86: 399-406.
- 181. Biswas J, Madhavan HN, George AE, et al. Ocular lesions associated with HIV infection in India: a series of 100 consecutive patients evaluated at a referral center. Am J Ophthalmol 2000; 129: 9-15.
- Lamzaf L, Ammouri W, Berbich O, et al. [Ocular complications of HIV infection: experience of the Northern Excellence Pole of Morocco]. J Fr Ophtalmol 2011; 34: 75-82.
- 183. Jaffar S, Ariyoshi K, Frith P, et al. Retinal manifestations of HIV-1 and HIV-2 infections among hospital patients in The Gambia, west Africa. Trop Med Int Health 1999; 4: 487-92.
- 184. Pathai S, Gilbert C, Weiss HA, et al. Differing spectrum of HIV-associated ophthalmic disease among patients starting antiretroviral therapy in India and South Africa. Trop Med Int Health 2011; 16: 356-9.
- 185. Nirwoth JP, Hall AB, Lewallen S. Prevalence of cytomegalovirus retinitis in Tanzanians with low CD4 levels. Br J Ophthalmol 2011; 95: 460-2.

- Hodge WG, Boivin JF, Shapiro SH, et al. Laboratory-based risk factors for cytomegalovirus retinitis. Can J Ophthalmol 2004; 39: 733-45.
- 187. Holland GN, Vaudaux JD, Shiramizu KM, et al. Characteristics of untreated AIDS-related cytomegalovirus retinitis. II. Findings in the era of highly active antiretroviral therapy (1997 to 2000). Am J Ophthalmol 2008; 145: 12-22.
- 188. Del AJ, Petruckevitch A, Phillips AN, et al. Spectrum of disease in Africans with AIDS in London. AIDS 1996; 10: 1563-9.
- 189. Cox JA, Lukande RL, Lucas S, et al. Autopsy causes of death in HIV-positive individuals in sub-Saharan Africa and correlation with clinical diagnoses. AIDS Rev 2010; 12: 183-94.
- Rana FS, Hawken MP, Mwachari C, et al. Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. J Acquir Immune Defic Syndr 2000; 24: 23-9.
- Martinson NA, Karstaedt A, Venter WD, et al. Causes of death in hospitalized adults with a premortem diagnosis of tuberculosis: an autopsy study. AIDS 2007; 21: 2043-50.
- 192. Lucas SB, Hounnou A, Peacock C, et al. The mortality and pathology of HIV infection in a west African city. AIDS 1993; 7: 1569-79.
- 193. Ansari NA, Kombe AH, Kenyon TA, et al. Pathology and causes of death in a group of 128 predominantly HIV-positive patients in Botswana, 1997-1998. Int J Tuberc Lung Dis 2002; 6: 55-63.
- 194. Ansari NA, Kombe AH, Kenyon TA, et al. Pathology and causes of death in a series of human immunodeficiency virus-positive and -negative pediatric referral hospital admissions in Botswana. Pediatr Infect Dis J 2003; 22: 43-7.
- Chintu C, Mudenda V, Lucas S, et al. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. Lancet 2002; 360: 985-90.
- 196. Holland GN, Sison RF, Jatulis DE, et al. Survival of patients with the acquired immune deficiency syndrome after development of cytomegalovirus retinopathy. UCLA CMV Retinopathy Study Group. Ophthalmology 1990; 97: 204-11.
- 197. Polis MA, deSmet MD, Baird BF, et al. Increased survival of a cohort of patients with acquired immunodeficiency syndrome and cytomegalovirus retinitis who received sodium phosphonoformate (foscarnet). Am J Med 1993; 94: 175-80.

- 198. Kempen JH, Jabs DA, Wilson LA, et al. Mortality risk for patients with cytomegalovirus retinitis and acquired immune deficiency syndrome. Clin Infect Dis 2003; 37: 1365-73.
- 199. Martin DF, Kuppermann BD, Wolitz RA, et al. Oral ganciclovir for patients with cytomegalovirus retinitis treated with a ganciclovir implant. Roche Ganciclovir Study Group. N Engl J Med 1999; 340: 1063-70.
- 200. Lisboa LF, Asberg A, Kumar D, et al. The clinical utility of whole blood versus plasma cytomegalovirus viral load assays for monitoring therapeutic response. Transplantation 2011; 91: 231-6.
- 201. Garrigue I, Doussau A, Asselineau J, et al. Prediction of cytomegalovirus (CMV) plasma load from evaluation of CMV whole-blood load in samples from renal transplant recipients. J Clin Microbiol 2008; 46: 493-8.
- 202. Foca E, Motta D, Pollara C, et al. Impact of detectable human cytomegalovirus DNAemia on viro-immunological effectiveness of HAART in HIV-infected patients naive to antiretroviral therapy. New Microbiol 2012; 35: 227-31.
- Fielding K, Koba A, Grant AD, et al. Cytomegalovirus Viremia as a Risk Factor for Mortality Prior to Antiretroviral Therapy among HIV-Infected Gold Miners in South Africa. PLoS One 2011; 6: e25571.
- 204. Darwich L, Cabrera C, Romeu J, et al. The magnitude of interferon-gamma responses to human cytomegalovirus is predictive for HIV-1 disease progression. J Acquir Immune Defic Syndr 2008; 49: 507-12.
- 205. Cooper DA, Pehrson PO, Pedersen C, et al. The efficacy and safety of zidovudine alone or as cotherapy with acyclovir for the treatment of patients with AIDS and AIDS-related complex: a double-blind randomized trial. European-Australian Collaborative Group. AIDS 1993; 7: 197-207.
- 206. Youle MS, Gazzard BG, Johnson MA, et al. Effects of high-dose oral acyclovir on herpesvirus disease and survival in patients with advanced HIV disease: a doubleblind, placebo-controlled study. European-Australian Acyclovir Study Group. AIDS 1994; 8: 641-9.
- 207. Lingappa JR, Baeten JM, Wald A, et al. Daily acyclovir for HIV-1 disease progression in people dually infected with HIV-1 and herpes simplex virus type 2: a randomised placebo-controlled trial. Lancet 2010; 375: 824-33.
- 208. Reynolds SJ, Makumbi F, Newell K, et al. Effect of daily aciclovir on HIV disease progression in individuals in Rakai, Uganda, co-infected with HIV-1 and herpes simplex virus type 2: a randomised, double-blind placebo-controlled trial. Lancet Infect Dis 2012; 12: 441-8.

8 Papers I-III

Paper I

Brantsaeter AB, Liestol K, Goplen AK, Dunlop O, Bruun JN. CMV disease in AIDS patients: Incidence of CMV disease and relation to survival in a population-based study from Oslo. Scandinavian Journal of Infectious Diseases 2002; 34: 50-55.

Brantsæter AB, Holberg-Petersen M, Jeansson S, Goplen AK, Bruun JN. CMV quantitative PCR in the diagnosis of CMV disease in patients with HIV-infection - a retrospective autopsy based study. BMC Infect Dis 2007; 7: 127.

BMC Infectious Diseases



Research article Open Access

CMV quantitative PCR in the diagnosis of CMV disease in patients with HIV-infection – a retrospective autopsy based study

Arne B Brantsaeter*1,2, Mona Holberg-Petersen³, Stig Jeansson³, Anne K Goplen⁴ and Johan N Bruun¹

Address: ¹Department of Infectious Diseases, Ullevaal University Hospital and Faculty of Medicine, University of Oslo, Oslo, Norway, ²Department of Internal Medicine, Asker and Baerum Hospital, Rud, Norway, ³Department of Microbiology, Ullevaal University Hospital and Faculty of Medicine, University of Oslo, Oslo, Norway and ⁴Department of Pathology, Ullevaal University Hospital and Faculty of Medicine, University of Oslo, Norway

Email: Arne B Brantsaeter* - abrant@online.no; Mona Holberg-Petersen - Mona.Holberg-Petersen@ulleval.no; Stig Jeansson - s.l.jeansson@medisin.uio.no; Anne K Goplen - AnneKristin.Goplen@ulleval.no; Johan N Bruun - j.n.bruun@medisin.uio.no * Corresponding author

Published: 6 November 2007

Received: 4 May 2007 Accepted: 6 November 2007

BMC Infectious Diseases 2007, 7:127 doi:10.1186/1471-2334-7-127

This article is available from: http://www.biomedcentral.com/1471-2334/7/127

© 2007 Brantsaeter et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: Patients with advanced HIV infection at the time of diagnosis and patients not responding to antiretroviral therapy are at risk of cytomegalovirus (CMV) disease. Earlier studies of patients with HIV infection have demonstrated that the diagnosis is often first made postmortem. In recent years new molecular biological tests have become available for diagnosis of CMV disease. Although clinical evaluation of tests for diagnosis of CMV disease in HIV-infected individuals is suboptimal without autopsy, no results from such studies have been published. The aim of this study was to explore the diagnostic utility of CMV quantitative polymerase chain reaction (PCR) in plasma from HIV and CMV seropositive patients who died during the period 1991–2002 and in whom autopsy was performed.

Methods: Autopsy was performed in all cases, as part of routine evaluation of HIV-infected cases followed at Ullevaal University Hospital. Of 125 patients included, 53 had CMV disease, 37 of whom were first diagnosed at autopsy. CMV disease was diagnosed either by ophthalmoscopic findings typical of CMV retinitis, biopsy or autopsy. One or two plasma samples taken prior to the first diagnosis of CMV disease (alive or at autopsy) or death without CMV disease were analysed by CMV quantitative PCR. Sensitivity, specificity, positive and negative predictive values were calculated for different CMV viral load cut-offs and according to detection of viraemia in one versus two samples.

Results: Twenty-seven of 53 patients with CMV disease (51%) and 10 of 72 patients without CMV disease (14%) had detectable viraemia in at least one sample. Sensitivity and negative predictive value (NPV) of the test, maximised with a cut-off at the test's limit of detection of CMV viraemia (400 copies/mL), were 47% and 70%, respectively. With cut-off at 10 000 copies/mL, specificity and positive predictive value (PPV) were 100%. With a requirement for CMV viraemia in two samples, specificity and PPV were 100% in patients with CMV viraemia above the limit of detection.

Conclusion: Our results indicate that quantitative CMV PCR is best used to rule in, rather than to rule out CMV disease in HIV-infected individuals at high risk.

Background

Although the incidence of cytomegalovirus (CMV) disease in HIV-infected patients has declined sharply after the introduction of highly active antiretroviral therapy (HAART) [1], many patients are still at risk of CMV disease [2,3]. Clinical features associated with CMV disease are, with the exception of CMV retinitis, unspecific. In cases with extra-ocular disease, histopathological findings typical of CMV are required for verification of the diagnosis.

Earlier studies, not using modern molecular biological methods for CMV disease, demonstrated that CMV disease was under-diagnosed in HIV-infected patients, and that the diagnosis was often first made at autopsy [4-10]. Newer diagnostic tests may improve the detection rate of CMV disease in this patient population. French guidelines recommend both ophthalmocsopy and testing for CMV viraemia by PCR every three months in patients with CD 4 cell counts below 50–100/mm³, whereas U.S. guidelines focus on the early clinical recognition of CMV disease [11,12].

A variety of microbiological tests for detection of CMV DNA in blood [3,13-27] and cerebrospinal fluid [28] have been used in the diagnosis of CMV disease. Previous studies have demonstrated that detection of CMV DNA in blood by PCR is a risk factor for subsequent development of CMV disease. Sensitivity, specificity, positive and negative predictive value of these tests have shown great variation [13,18-20,23,26,27]. In one recent study, plasma CMV viraemia was not found to be associated with later development of CMV disease, but was predictive of death [29].

Because diagnosis of CMV disease is often first made at autopsy, sensitivity and negative predictive value of diagnostic tests may be overestimated, and specificity and positive predictive value may be underestimated if autopsy results are not available. Furthermore, by using a quantitative method it may be possible to improve specificity and positive predictive value of CMV detection. However, to our knowledge, no previous studies have related the results of CMV quantitative PCR to autopsy findings. The objective of this study was to explore the diagnostic utility of CMV quantitative PCR in plasma samples from a group of HIV infected patients who died during the study period and in whom autopsy was performed.

Methods

Patients included in this retrospective study were HIV and CMV seropositive adolescents and adults who died during the period 1991–2002 while they were followed up at Ullevaal University Hospital. A total of 125 patients were included. Patients were generally not screened for CMV disease, even with low CD 4 cell counts, but fundoscopy

or biopsy of relevant organs was performed on the basis of suggestive clinical signs and symptoms. Autopsy was performed in all cases as part of routine practice in the evaluation of HIV-infected cases who died at Ullevaal University Hospital, unless this was objected to by relatives. Patient characteristics are shown in Table 1.

A full autopsy including neuropathological examination was performed in every case. Paraffin-embedded sections were routinely stained with haematoxylin-eosin (HE), and confirmatory immunohistochemistry for CMV was performed in cases where microscopy of HE stained biopsies were inconclusive. CMV disease was verified by demonstration of characteristic cytomegalocytes with inclusions in histopathological samples by light microscopy. However, ante-mortem diagnosis of CMV retinitis was defined as typical ophthalmoscopic findings in patients examined by an experienced ophthalmologist.

Among 53 patients diagnosed with CMV disease, sixteen patients were first diagnosed before death. Of patients diagnosed alive, twelve had retinitis and four had biopsy verified gastrointestinal infection (colitis 2, oesophagitis 1, stomatitis 1) as their first end-organ manifestation of CMV disease. Except for two patients diagnosed with retinitis alive, all had CMV disease confirmed in one or several organs at autopsy. Thirty-seven cases were first diagnosed at autopsy. In these patients the number of CMV end-organ manifestations at autopsy were: adrenalitis 26, pneumonitis 22, encephalitis 16, retinitis 14, gastrointestinal infection 14 (oesphagitis 11, gastritis 1, small intestine infection 1, colitis 1), and other end-organ disease 11 (prostate gland 5, ovaries 2, uterus 1, epididymis 1, lymph node 2, pancreas 5, spleen 3, liver 3, kidney 4, myocardium 1, thyroid gland 4, vascular endothelium 1). Twenty-patients first diagnosed at autopsy had more than one organ manifestations of CMV disease.

Only twenty-eight patients received HAART at the time of the last plasma sample tested for CMV DNA, either before the first diagnosis of CMV disease (alive or at autopsy), or before death in patients never developing CMV disease. This is because the majority of plasma samples were taken prior to the introduction of HAART in 1996. Seven cases with CMV disease (diagnosed 1997–2002) were on HAART when the last plasma sample was taken and later analysed with CMV PCR. However, HIV viral load was high in all, ranging from 17000 – >750 000 copies/mL. Two of these seven cases had CMV retinitis diagnosed alive, whereas the remaining five were first diagnosed with CMV at autopsy.

Plasma aliquots were routinely collected and stored at -20°C or colder during the study period, as part of clinical follow-up of patients. From cases with CMV disease, the

Table 1: Characteristics of 125 patients included in the study

Characteristic	Value		
	CMV disease	Without CMV disease	
	N = 53 (%)	N = 72 (%)	
Risk category			
Men who have sex with men	32/53 (60)	30/72 (42)	
Intravenous drug user	9/53 (17)	30/72 (42)	
Other	10/53 (19)	11/72 (15)	
Unknown	2/53 (4)	1/72 (1)	
Age (median) at time of death, years	39	41	
Sex			
Male	46/53 (87)	58/72 (81)	
Female	7/53 (13)	14/72 (19)	
CD4 cell count*			
Median count, cells/mm ³	17	69	
<100 cells/mm ³	47/53 (89)	41/72 (57)	
<50 cells/mm ³	43/53 (81)	32/72 (44)	
Highly active antiretroviral therapy*	7/53 (13)	21/72 (29)	
Plasma HIV RNA level*			
Tested	10/53 (19)	35/72 (49)	
Median among tested, copies/mL blood	305 000	35 000	
HIV RNA < 400 copies/mL blood	0/10 (0)	10/35 (29)	
HIV RNA < 50 copies/mL blood	0/10 (0)	5/35 (14)	

Data shown are number of subjects per no. of subjects with available data

last one or two (according to availability) plasma samples prior to diagnosis were retrospectively analysed by quantitative CMV PCR. In patients with more than one CMV diagnosis at different time points during life or at autopsy, plasma samples taken prior to the first CMV diagnosis were tested. Similarly, for patients without CMV disease, the last one or two plasma samples before death were tested. The samples were analysed by COBAS AMPLICOR CMV Monitor test (Roche Molecular System, Branchburg, NJ) according to the manufacturer's recommendations. One low-positive control, one high-positive control, and one CMV-negative control were processed with each batch of samples. The linear range of this assay is between 400 and 100 000 CMV DNA copies/mL plasma.

A total of 82 plasma samples were tested before diagnosis in patients with CMV disease, and 127 samples were analysed from patients without CMV disease. Median time interval between the last plasma sample and the date of the first (or only) CMV diagnosis (alive or post-mortem) was 69 days. Median time interval between the last plasma sample and date of death in patients not developing CMV disease at any time was 79 days. This difference was not statistically significant. For patients first diag-

nosed alive with CMV disease, the median time interval between the last sample and diagnosis was 64 days, as compared to 70 days for patients first diagnosed at autopsy (statistically not significantly different). Among 29 patients with CMV disease and 55 patients without CMV disease from whom two samples were tested, median time intervals between the two tests were 77 days and 90 days, respectively, again not statistically different.

The CMV quantitative PCR results were used to study sensitivity, specificity, PPV and NPV of the test with different cut-off values for CMV viral load, and with viraemia in one versus two samples.

CD 4 cell counts were available from electronic records and had been measured in blood samples taken on the same day that plasma had been taken for later quantitative CMV PCR.

SPSS statistical software version 13.0 (SPSS Inc. New York, 2004) was used to calculate Spearman rank correlation between CD4 cell counts and CMV viral load. Statistical differences between groups were calculated using non-parametric tests (Mann-Whitney and chi-square test as

^{*} at time of last sample prior to diagnosis of CMV disease or death without CMV disease

appropriate). Logistic regression was performed using presence or absence of CMV disease as dependent variable and last CD4 cell count (above or below 100/mm³, last CMV viral load (log transformed), HAART (yes or no), time in days between last plasma sample or outcome defined as CMV disease or death without CMV disease, sex, and age at time of death as independent variables. Confidence Interval Analysis statistical software (Wilson's method) was used to calculate sensitivity, specificity, PPV and NPV with confidence intervals. All confidence intervals (CI) are 95% CIs. The significance level was set at 5%.

Results

Twenty-seven of 53 patients with CMV disease (51%) and 10 of 72 patients without CMV disease (13%) had detectable viraemia in at least one sample (p < 0.001). Results of CMV quantitative PCR from the last plasma sample are shown in Table 2. There was significantly higher CMV viral load in patients with CMV disease compared to patients without CMV disease (p < 0.001). In 25 patients with CMV disease and 8 patients without CMV disease who had viraemia in the last sample, median CMV viral loads were 3420 (range 392–261 000) and 1705 (range 277–9620) DNA copies/mL, respectively (statistically not significantly different). Table 2 also shows the number of cases with detectable CMV viraemia according to the number of samples tested.

Among 16 patients diagnosed alive with CMV disease, 7 (44%) had detectable CMV viraemia in the last sample before diagnosis (median 1810 DNA copies/mL among viraemic cases). Among 37 patients first diagnosed with CMV disease at autopsy, 18 (49%) had CMV viraemia in the last sample (median 4430 DNA copies/mL among viraemic cases). There was no significant difference in the proportion of cases with viraemia (sensitivity) or level of viraemia between these two groups of patients.

In 37 patients first diagnosed with CMV disease at autopsy, 14 of 20 patients with CMV disease in more than one organ had detectable CMV viremia in the last sample (median 7035 copies/mL among viraemic cases), compared to 4 of 17 patients with CMV disease in a single organ, (median 2680 DNA copies/mL among viraemic cases). In other words, sensitivity of the test was 70% for cases with multi-organ disease and 24% for cases with single organ disease (p = 0.005). However, among viraemic cases there was no statistically significant difference in CMV viral load.

Results from Table 2 were used to calculate the corresponding sensitivity, specificity, PPV and NPV with increasing CMV viral load cut-off, and with one or two samples with detectable viraemia in individual patients (Table 3). The viral load cut-off is defined as the lowest

level of viraemia which is considered a positive result for diagnostic purposes. Sensitivity and NPV were generally low under a wide range of assumptions. Specificity and PPV were generally higher, and were improved further by raising the viral load cut-off. By setting the cut-off at 10 000 CMV DNA copies/mL plasma, a specificity of 100% was attained, but at the expense of sensitivity which fell to 17%. Also, a specificity and PPV of 100% was achieved by defining a positive test as CMV viraemia in two samples.

The proportion of cases with CMV disease that have a positive tests (sensitivity) plotted against the proportion of cases without CMV disease that have a positive test (1-specificity) for all measured values of CMV DNA viraemia in the last plasma sample are shown as a receiver operating characteristic (ROC) curve in Figure 1. Using the lowest detectable level of viraemia as cut-off, a maximum sensitivity of 47.2% was attained. However, specificity of 1 (100%) (1-specifity = 0) was attained with a cut-off of 10 000 CMV DNA copies/mL as mentioned above. The diagnostic accuracy of the test, expressed by the area under the curve, was 0.69. This area corresponds to the probability that a random person with the disease has a higher CMV viral load than a random person without CMV disease.

CMV viraemia was negatively correlated with CD4 cell counts ($r_S = -0.24$ (p < 0.01). In the multivariable logistic regression model low CD4 cell counts and high CMV viral load were the only statistically significant positive predictors of CMV disease.

Discussion

With the exception of retinitis, end-organ manifestations of CMV disease in HIV-infected individuals have unspecific clinical characteristics, and histopathological confirmation is required for definite diagnosis. Because biopsy of affected organs may be difficult to obtain, there is a need for microbiological diagnostic methods that can be used on readily available specimens. A variety of tests to detect CMV nucleic acids have been evaluated in different patient populations. To our knowledge, no previous tests have been evaluated in relation to autopsy findings, an important shortcoming, as histological verification of CMV disease is often first obtained at autopsy. The main strength of this study is that it explores the utility of quantitative PCR in the diagnosis of CMV disease in HIVinfected individuals with available autopsy results. Also, it includes one of the highest reported number of cases of CMV disease in HIV-infected patients evaluated with CMV

Several qualitative and quantitative PCR methods for CMV in plasma in various patient populations have demonstrated sensitivity in the range 35–93% [13,18-20,23,26,27]. In our study, maximum sensitivity of 47%

for the total number of cases, using a cut-off at the limit of detection, was in the lower end of this range. In a small previous study on HIV-infected individuals using COBAS AMPLICOR CMV Monitor, sensitivity was 92% [27]. The relatively low sensitivity in our study could be due to samples in many cases having been taken as part of routine monitoring and therefore too long, a median of 69 days, before development of CMV end-organ disease. However, CMV viraemia is known to often precede development of CMV disease by several months [2,13,17]. Also, in contrast to previous studies, most of our cases of CMV disease were first diagnosed at autopsy, and it is possible that these post-mortem manifestations are associated with a shorter time with viraemia and/or lower level of viraemia. Interestingly, our results show that sensitivity was significantly higher for patients with CMV disease in more than one organ compared to patients with single organ disease.

Previous studies of CMV disease diagnosed before death have yielded specificities in the range 47–100% [13,18-20,23,26,27]. Our study shows specificity in the upper range (89%), with only 8 of 72 (11%) cases having detectable CMV viraemia but no diagnosis of CMV disease (false positive tests). Studies that do not include autopsy results may underestimate the specificity, as CMV end-organ disease may be a difficult clinical diagnose to make.

Our data were also analysed with regard to detection of CMV viraemia in one versus two plasma samples taken at different times. We found that cases with detectable CMV viraemia in both samples all developed CMV disease.

Thus, by requiring confirmation in a second test if the first was positive, a specificity of 100% was attained, but at the cost of lower sensitivity.

From a clinician's point of view, the predictive value of a positive and negative test result has greater practical implications than do sensitivity and specificity. The predictive value of any test is generally dependent on the prevalence of the disease in question in the study population, NPV decreasing and PPV increasing with increasing prevalence. Previous studies have reported NPV in the range 80-95% [20,23,26,27], which is somewhat higher than in our study. The prevalence of CMV disease in our study was comparatively high - 42% (53 of 125 patients) - thus contributing to a low NPV. For the same reason, the PPV demonstrated in our study was among the highest reported. In our patient population at high risk of CMV disease we attained a PPV of 100% with a cut off-value of 10 000 CMV DNA copies/mL or a requirement for viraemia in two consecutive tests. Studies that do not include autopsy results may underestimate the proportion of cases with CMV disease, resulting in an overestimation of the NPV and an underestimation of the PPV.

The study covers a period both before and after HAART became standard of care. Even in patients on HAART, antiretroviral treatment was suboptimal for many, probably due to HIV resistance having developed after previous ineffective therapy with single drugs, or combination of two antiretrovirals. This may affect the external validity of our results in the diagnosis of CMV disease in HIV-

Table 2: Results of CMV quantitative PCR in patients with and without CMV disease

Results of CMV PCR assay	Number of patients		
	with CMV disease N = 53	without CMV disease N = 72	
Viral load (CMV DNA copies/mL) (last samples)			
Below limit of detection	28	64	
Viraemia ^a	25	8	
≤I 999	9	5	
2 000–9 999	7	3	
≥10 000	9	0	
CMV DNA detection ^a by no. of tested samples before diagnosis			
One sample	24	17	
Not detected in any sample	13	15	
Detected in one sample	H	2	
Two samples	29	55	
Not detected in any sample	13	47	
Detected in one sample	9	8	
Detected in both samples	7	0	

^aAny viraemia above limit of detection.

Results of plasma CMV quantitative PCR in HIV patients with and without CMV disease by different viral load cut-offs and number of tested samples.

infected patients who have access to more effective HAART today and, as a result, have lower risk of CMV disease. However, results from our study are likely to be relevant for patients who interrupt treatment, who fail to respond to antiretroviral therapy, or who are diagnosed too late for effective HIV therapy to be initiated.

This was a retrospective study in which the time intervals varied considerably between the last plasma sample and CMV diagnosis (alive or at autopsy), or between the last plasma sample and death in cases without CMV disease, as samples were taken as part of routine monitoring of the patients rather than on clinical suspicion. It is likely that the sensitivity and PPV of CMV PCR will be higher in a setting where plasma is analysed at a time of clinical suspicion of CMV end-organ disease, rather than as part of routine monitoring of patients.

The availability of more than one plasma sample from individual patients at relevant time points was limited, but a high positive predictive value for CMV disease was found for patients with CMV viraemia in two consecutive tests with a median time interval of between two and three months. However, our data do not allow us to conclude when plasma CMV quantitative PCR should be performed, or how soon a patient should be retested after a positive test result.

In solid organ transplant patients and allogenic bone marrow transplantation, routine monitoring of virological markers for cytomegalovirus and administration of preemptive therapy has been shown to significantly reduce the risk of CMV disease [30,31], and post-transplant monitoring of CMV viraemia by PCR or other methods is used in many centres. Among HIV-infected individuals the value of pre-emptive therapy is less well documented, but

monitoring of high risk patients for this purpose is recommended in French guidelines [11].

Many studies have shown that the risk of CMV disease sharply increases when CD4 cell counts fall below 100/mm³ [2,3,32]. We suggest that HIV infected patients with low CD4 cell counts who have clinical features suggestive of CMV disease, should have plasma tested for CMV by quantitative PCR as part of the diagnostic procedure. In patients with repeated viraemia or viraemia above 10 000 CMV DNA copies/mL, pre-emptive therapy should be considered after a careful clinical examination for signs and symptoms of CMV end-organ disease. However, our findings need to be validated in prospective studies on patients living with HIV today.

Conclusion

Using CMV quantitative PCR, the sensitivity and NPV for CMV disease were low in this study. Our results indicate that in a population at high risk of CMV disease quantitative CMV PCR is best used to rule in, rather than to rule out CMV disease. Although CMV disease presently is relatively uncommon in HIV-infected patients who have access to antiretroviral therapy, these results may have implications for diagnosis of CMV disease in patients with low CD4 cell counts due to late HIV diagnosis or failure of antiretroviral therapy.

Competing interests

None of the authors declare any conflict of beyond those declared in the acknowledgements regarding the manufacturer of the diagnostic test.

Authors' contributions

All authors have made substantial contributions to conception and design of the study, have been involved in

Table 3: Sensitivity, specificity, positive and negative predictive values of plasma CMV quantitative PCR

Results of CMV PCR assay	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)
Viral load (CMV DNA copies/mL (last samples)				
Viraemia ^a	47 (34-60)	89 (80-94)	76 (59-87)	70 (60-78)
≥2 000	30 (20–44)	96 (89–99)	84 (62–95	65 (56–74)
≥10 000	17 (9–29)	100 (95–100)	100 (70–100)	62 (53–70)
CMV DNA detection ^a by no. of tested samples before diagnosis One sample				
CMV DNA detected	46 (30–65)	90 (69–97)	85 (58–96)	57 (39–73)
Two samples				
CMV DNA detected in at least one sample	55 (38–72)	86 (74–92)	67 (47–82)	78 (66–87)
CMV DNA detected in both samples	24 (12–42)	100 (94–100)	100 (65–100)	70 (61–80)

^aAny viraemia above limit of detection.

Sensitivity, specificity, positive and negative predictive value of plasma CMV quantitative PCR in the diagnosis of CMV disease in HIV patients by different viral load cut-offs and number of tested samples.

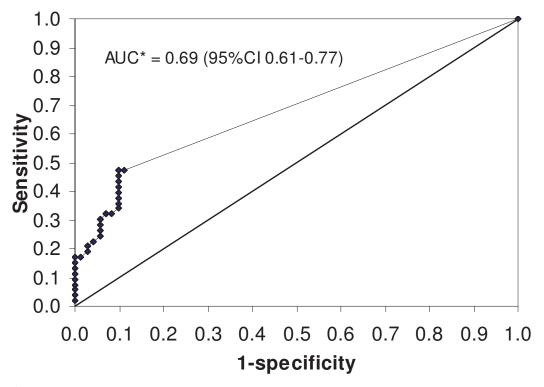


Figure I

CMV quantitative PCR results expressed as ROC curve. Receiver operating characteristic (ROC) curve based on quantitative CMV PCR results in the last available plasma sample prior to the first diagnosis of CMV disease (alive or at autopsy) or death without CMV disease. *AUC = area under the curve.

writing and revising the manuscript critically, have read and given approval of the final version of the manuscript submitted for publishing. ABB has had main responsibility for analysing the data and collecting clinical information about the cases. AKG has had main responsibility for the pathological diagnosis of CMV disease in tissue samples. MHP has been responsible for analysis of CMV viraemia by quantitative CMV PCR in plasma samples. All authors read and approved the final manuscript.

Acknowledgements

This work was presented in part at the 15th European Congress of Clinical Microbiology and Infectious Diseases (Copenhagen/Denmark, 2005; Abstract 1134_01_280). The cost of CMV PCR diagnostics was partially covered by Roche Norway.

References

Mocroft A, Sabin CA, Youle M, Madge S, Tyrer M, Devereux H, Deayton J, Dykhoff A, Lipman MC, Phillips AN, Johnson MA: Changes in

AIDS-defining illnesses in a London Clinic, 1987-1998. J Acquir Immune Defic Syndr 1999, 21:401-407.

- Salmon-Ceron D, Mazeron MC, Chaput S, Boukli N, Senechal B, Houhou N, Katlama C, Matheron S, Fillet AM, Gozlan J, Leport C, Jeantils V, Freymuth F, Costagliola D: Plasma cytomegalovirus DNA, pp65 antigenaemia and a low CD4 cell count remain risk factors for cytomegalovirus disease in patients receiving highly active antiretroviral therapy. AIDS 2000, 14:1041-1049.
- Deayton JR, Sabin CA, Johnson MA, Emery VC, Wilson P, Griffiths PD: Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. Lancet 2004, 363:2116-2121.
- Dore GJ, Marriott DJ, Duflou JA: Clinico-pathological study of cytomegalovirus (CMV) in AIDS autopsies: under-recognition of CMV pneumonitis and CMV adrenalitis. Aust N Z J Med 1995, 25:503-506.
- Hofman P, Saint-Paul MC, Battaglione V, Michiels JF, Loubiere R: Autopsy findings in the acquired immunodeficiency syndrome (AIDS). A report of 395 cases from the south of France. Pathol Res Pract 1999, 195:209-217.
- Lyon R, Haque AK, Asmuth DM, Woods GL: Changing patterns of infections in patients with AIDS: a study of 279 autopsies of prison inmates and nonincarcerated patients at a university hospital in eastern Texas, 1984-1993 [see comments]. Clin Infect Dis 1996, 23:241-247.

- Pillay D, Lipman MC, Lee CA, Johnson MA, Griffiths PD, McLaughlin JE: A clinico-pathological audit of opportunistic viral infections in HIV- infected patients. AIDS 1993, 7:969-974.
- Wilkes MS, Fortin AH, Felix JC, Godwin TA, Thompson WG: Value of necropsy in acquired immunodeficiency syndrome. Lancet 1988. 2:85-88.
- Brantsaeter AB, Liestol K, Goplen AK, Dunlop O, Bruun JN: CMV disease in AIDS patients: Incidence of CMV disease and relation to survival in a population-based study from Oslo. Scandinavian Journal of Infectious Diseases 2002, 34:50-55.
- d'Arminio MA, Mainini F, Testa L, Vago L, Balotta L, Nebuloni M, Antinori S, Bini T, Moroni M: Predictors of cytomegalovirus disease, natural history and autopsy findings in a cohort of patients with AIDS. AIDS 1997, 11:517-524.
- Yeni P: Prise en charge médicale des personnes infectées par le VIH.Rapport 2006. Recommandations du groupe d'experts. Paris, Flammarion; 2006:250-250.
- Masur H, Kaplan JE, Holmes KK: Guidelines for Preventing Opportunistic Infections among HIV-Infected Persons-2002: Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. Ann Intern Med 2002, 137:435-478.
- Blank BS, Meenhorst PL, Mulder JW, Weverling GJ, Putter H, Pauw W, van Dijk WC, Smits P, Lie AL, Reiss P, Lange JM: Value of different assays for detection of human cytomegalovirus (HCMV) in predicting the development of HCMV disease in human immunodeficiency virus-infected patients. J Clin Microbiol 2000, 38:563-569
- Bowen EF, Sabin CA, Wilson P, Griffiths PD, Davey CC, Johnson MA, Emery VC: Cytomegalovirus (CMV) viraemia detected by polymerase chain reaction identifies a group of HIV-positive patients at high risk of CMV disease. AIDS 1997, 11:889-893.
- Bowen EF: Cytomegalovirus reactivation in patients infected with HIV: the use of polymerase chain reaction in prediction and management. Drugs 1999, 57:735-741.
- Dodt KK, Jacobsen PH, Hofmann B, Meyer C, Kolmos HJ, Skinhoj P, Norrild B, Mathiesen L: Development of cytomegalovirus (CMV) disease may be predicted in HIV- infected patients by CMV polymerase chain reaction and the antigenemia test. AIDS 1997, 11:F21-F28.
- Spector SA, Wong R, Hsia K, Pilcher M, Stempien MJ: Plasma cytomegalovirus (CMV) DNA load predicts CMV disease and survival in AIDS patients. J Clin Invest 1998, 101:497-502.
- Wattanamano P, Clayton JL, Kopicko JJ, Kissinger P, Elliot S, Jarrott C, Rangan S, Beilke MA: Comparison of three assays for cytomegalovirus detection in AIDS patients at risk for retinitis. J Clin Microbiol 2000, 38:727-732.
- Yoshida A, Hitomi S, Fukui T, Endo H, Morisawa Y, Kazuyama Y, Osumi K, Oka S, Kimura S: Diagnosis and monitoring of human cytomegalovirus diseases in patients with human immunodeficiency virus infection by use of a real-time PCR assay. Clin Infect Dis 2001, 33:1756-1761.
- Boivin G, Handfield J, Toma E, Murray G, Lalonde R, Bergeron MG: Comparative evaluation of the cytomegalovirus DNA load in polymorphonuclear leukocytes and plasma of human immunodeficiency virus- infected subjects [see comments]. J Infect Dis 1998, 177:355-360.
- Casado JL, Arrizabalaga J, Montes M, Marti-Belda P, Tural C, Pinilla J, Gutierrez C, Portu J, Schuurman R, Aguirrebengoa K: Incidence and risk factors for developing cytomegalovirus retinitis in HIVinfected patients receiving protease inhibitor therapy. Spanish CMV- AIDS Study Group. AIDS 1999, 13:1497-1502.
- Nokta MA, Holland F, De G V, Emery VC, Jacobson MA, Griffiths P, Pollard RB, Feinberg JE: Cytomegalovirus (CMV) polymerase chain reaction profiles in individuals with advanced human immunodeficiency virus infection: relationship to CMV disease. J Infect Dis 2002, 185:1717-1722.
- Shinkai M, Bozzette SA, Powderly W, Frame P, Spector SA: Utility
 of urine and leukocyte cultures and plasma DNA polymerase
 chain reaction for identification of AIDS patients at risk for
 developing human cytomegalovirus disease. J Infect Dis 1997,
 175:302-308.
- Spector SA, Hsia K, Crager M, Pilcher M, Cabral S, Stempien MJ: Cytomegalovirus (CMV) DNA load is an independent predictor of CMV disease and survival in advanced AIDS. J Virol 1999 73:7027-7030

- Erice A, Tierney C, Hirsch M, Caliendo AM, Weinberg A, Kendall MA, Polsky B: Cytomegalovirus (CMV) and human immunodeficiency virus (HIV) burden, CMV end-organ disease, and survival in subjects with advanced HIV infection (AIDS clinical trials group protocol 360). Clinical Infectious Diseases 2003, 37:567-578
- Wiselka MJ, Nicholson KG, Rowley S, Bibby K: Cytomegalovirus viraemia has poor predictive value for the development of cytomegalovirus disease in patients with advanced HIVinfection. J Infect 1993, 39:187-192.
- Pellegrin I, Garrigue I, Binquet C, Chene G, Neau D, Bonot P, Bonnet F, Fleury W, Pellegrin JL: Evaluation of new quantitative assays for diagnosis and monitoring of cytomegalovirus disease in human immunodeficiency virus-positive patients. Journal of Clinical Microbiology 1999, 37:3124-3132.
- Bestetti A, Pierotti C, Terreni M, Zappa A, Vago L, Lazzarin A, Cinque P: Comparison of three nucleic acid amplification assays of cerebrospinal fluid for diagnosis of cytomegalovirus encephalitis. J Clin Microbiol 2001, 39:1148-1151
- Wohl DA, Zeng D, Stewart P, Glomb N, Alcorn T, Jones S, Handy J, Fiscus S, Weinberg A, Gowda D, van der HC: Cytomegalovirus viremia, mortality, and end-organ disease among patients with AIDS receiving potent antiretroviral therapies. J Acquir Immune Defic Syndr 2005, 38:538-544.
- Strippoli GF, Hodson EM, Jones C, Craig JC: Preemptive treatment for cytomegalovirus viremia to prevent cytomegalovirus disease in solid organ transplant recipients. Transplantation 2006, 81:139-145.
- Qamruddin AO, Oppenheim BA, Guiver M, Mutton KJ, Chopra R: Screening for cytomegalovirus (CMV) infection in allogeneic bone marrow transplantation using a quantitative whole blood polymerase chain reaction (PCR) method: analysis of potential risk factors for CMV infection. Bone Marrow Transplant 2001,
- Gallant JE, Moore RD, Richman DD, Keruly J, Chaisson RE: Incidence and natural history of cytomegalovirus disease in patients with advanced human immunodeficiency virus disease treated with zidovudine. The Zidovudine Epidemiology Study Group [see comments]. J Infect Dis 1992, 166:1223-1227.

Pre-publication history

The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2334/7/127/prepub

Publish with **Bio Med Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- · yours you keep the copyright

Submit your manuscript here: http://www.biomedcentral.com/info/publishing_adv.asp



Paper III

Brantsæter AB, Johannessen A, Holberg-Petersen M, Sandvik L, Naman E, Kivuyo SL, Rollag H, Bruun JN, Kvale D. Cytomegalovirus viremia in dried blood spots is associated with an increased risk of death in HIV-infected patients: a cohort study from rural Tanzania. Int.J.Infect.Dis. 2012; 16: e879-e885.

