CMV retinitis in HIV-positive patients in the pre-HAART era
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SUMMARY AND GENERAL CONCLUSION
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SUMMARY CHAPTER 1

CYTOMEGALOVIRUS RETINITIS IN HIV-POSITIVE PATIENTS IN THE PRE-HAART ERA, A REVIEW.

Epidemiology

With the advent of the AIDS epidemic in 1981, it became clear that CMV was the most common opportunistic viral infection in HIV-positive patients. If left untreated, CMV retinitis invariably lead to blindness in a relatively short time, an unbearable burden for AIDS patients then and now. In HIV-positive patients, the incidence of clinical manifest CMV disease is 24%/year in patients with CD4+ lymphocyte counts less than 50 cells/mm³. In over 90% of these cases, the eye is involved, showing a necrotizing retinitis.

Risk factors

Risk factors have been reported, which are related to the immune status of the patient, like CD4-positive lymphocyte count (less than 50 cells/mm³), CD8-positive lymphocyte count (less than 520 cells/mm³), and the presence of specific HLA types related to T-cell reactivity against CMV (association with either B44, B51, or DR7). The presence of HIV-related microangiopathy has been considered a risk factor. Other factors are more epidemiological, like HIV acquisition through homo/bi-sexual contact, previous extra-ocular CMV infection, previous pattern of opportunistic infections, or treatment with corticosteroids.

Recovery of CMV from body fluids has become the most accurate predicting risk factor for the development of CMV disease, not the presence of a positive CMV culture, but especially CMV antigenemia, and CMV DNA-emia. Both tests, detection of CMV DNA by PCR, either in whole blood or in plasma/serum, and the quantitative pp65 antigenemia, are significant risk factors for developing CMV retinitis in HIV-positive patients, allowing a better discrimination between patients, with comparable CD4+ cell counts, who will and who will not develop CMV retinitis. Both tests have about the same sensitivity, specificity, and predictive values predicting CMV disease.

Clinical features

Initially symptoms caused by CMV retinitis are modest in most affected patients, and 20 to 40% of patients are totally unaware of the presence of an ocular disease, and are only diagnosed by ophthalmologic screening.
procedures for patients at high risk for CMV disease. Complaints, if any, are blurring of vision (in 50% of cases), loss of visual field (20%), and photopsia or floaters (60%).

The clinical appearance of untreated CMV retinitis has been described as a spectrum with two extremes: fulminant/oedematous type and indolent/granular type. At the lead edge of both lesions a dry-appearing granular border is present. Satellite lesions some at 500 μm or more of the main border can be seen. Spread of the retinal necrotic area is relatively slow in both types, marching on average 250 μm / week. In spite of large areas of necrotic retina the inflammatory reaction is minimal in both the vitreous cavity and the anterior chamber.

Healing of the retinitis after successful induction therapy with anti viral medications leads to a change in the aspect of the lesions in the eye. The fulminant/exudative type of inflammation subsides and changes into a granular/indolent type of lesion. Both the granular and the fulminant type lose their active border and the lesions do not expand any further. Affected retina is replaced by gliotic atrophic tissue without the prominent scarring and / or pigmentary changes.

Although anti viral treatment is reported to be successful in controlling CMV retinitis in most patients, patients do lose vision in a slow but continuous fashion even in the absence of complications or recurrence.

Complications

The most frequent complication of CMV retinitis is a rhegmatogenous retinal detachment. Retinal tears in the necrotic retina allows fluid to flow into the subretinal space leading to detachment of the retina. Prevalence of retinal detachment varies between 15 and 35%.

Treatment of retinal detachment in these patients is particularly challenging. For one, necrotic retina can easily tear, particularly if the vitreous base is not completely removed from its overlying surface. Secondly, progression of the retinitis is usually the norm. Thus any measure taken to reattach the retina must provide a permanent tamponade which not only takes in account presently involved retina, but also the unaffected retina.

Although final visual results in eyes after surgical intervention at first glance are rather disappointing, they are certainly better compared to eyes which are not treated. Mean visual acuity in treated eyes after a follow-up of 20 weeks is reported to be 20/200 (range 20/25 to no light perception), compared to hand motion level or worse in the untreated eyes.

Pathogenesis and histopathology

After a first episode of CMV infection CMV will remain latent throughout life. In this latent state the CMV genome is present somewhere in the body, but does not replicate and gene expression is absent or minimal. The exact
Summary and general conclusion

The site of latency is still unknown. In the HIV-positive patients, retinal infection can occur during the primary infection, following reactivation of latent CMV, or following reinfection with a second CMV strain. It seems a realistic assumption that virus reaches the eyes through dissemination of virus via the blood during a period of viremia.

It has been hypothesised that HIV related microangiopathy allows entrance of CMV into the retina via damaged microvasculature. The pathogenesis of this microangiopathy has not been clearly defined and different hypotheses have been formulated. The most important one seems to be a change in bloodflow caused by haemorheological abnormalities.

Virology and immune response

Human CMV is a DNA virus with a capsid, a tegument and an envelope. CMV has the largest genome of the herpesviruses (230 kbp) encoding for over 200 genes. The genome can be divided into a long (L) and a short (S) segment. Each segment consists of unique sequences, $U_L$ and $U_S$, respectively, flanked by repeat sequences.

The envelope proteins are important antigens for both the humoral and the cellular immune response. The most important glycoproteins are gB complex (gpUL55) and gH (gpUL75). Both are targets for virus neutralising antibodies.

Compared to normal controls, AIDS patients had a significantly lower response following immunisation with pneumococcal polysaccharide and protein, suggesting a B-cell immune deficiency. Higher levels of neutralising antibodies against CMV did correlate with a more favourable clinical course.

Cellular immunity is important in HCMV infections, and has been shown to play a critical role in keeping latent infection in check. The target antigens to which the response is elicited are unknown. Structural proteins could be involved, like the tegument protein pp65.

There is evidence to suggest that herpesviruses including CMV could increase the pathogenicity of HIV by acting as a co-factor.

Differential diagnosis

Besides other infectious retinitis cases, like Toxoplasma, VZV and HSV retinitis, CMV retinitis has to be differentiated from retinal abnormalities caused by HIV associated vasculopathy and from intraocular neoplasms. Differential diagnosis has to be accurate and without delay, because therapy for each entity differs, delay can cause permanent visual loss, and most medications harbour serious toxic side effects.
Diagnosis of CMV retinitis relies on clinical findings, especially the fundus appearance, and the lack of inflammatory signs. However, in the early stages of retinitis and in atypical cases, it can be extremely difficult to differentiate between CMV and the other herpes viruses, or between CMV and non-viral pathogens such as Toxoplasma. Additional laboratory testing can be helpful in these cases. The laboratory diagnosis of CMV disease is hampered by the fact that CMV can be present without causing disease.

One of the most powerful tools in the differential diagnosis of necrotizing retinitis is the detection of DNA of the candidate pathogens in ocular fluid samples. Comparing the test results obtained from aqueous humour and vitreous samples, vitreous seems to be the most accurate sample in diagnosing the eventual involved pathogen. However, a paracentesis to obtain an aqueous humour sample is much easier to perform, and seems to be the safest procedure. Detection of DNA in ocular fluid samples is a highly sensitive and specific method to determine which pathogen is causing retinitis in a given patient, or exclude a pathogen in cases with non-infectious retinal pathology, like branch retinal vein occlusion, or HIV related vasculopathy resembling a beginning retinitis.

**Therapy**

Whatever systemic or local drug is chosen for the treatment of CMV retinitis, the treatment strategy is the same. First, control of retinitis is achieved with a higher dose of the drug (or more frequent administrations) for 2 to 3 weeks, induction therapy, followed by lifelong therapy with a lower dose (less frequent administrations) to prevent a relapse, secondary prophylaxis or maintenance therapy.

Control of CMV retinitis is characterised by the disappearance of oedematous necrotic borders changing the lesion into an inactive atrophic scar. After 2 weeks of induction therapy, the retinal lesions have to show a good response to therapy, but some activity can still be present and is acceptable. Present antiviral drugs suppress CMV replication but are unable to eliminate the virus from the eye. As a consequence, secondary prophylaxis has to be given for the rest of life.

Intravenous ganciclovir, foscavir and cidofovir have all been proven to be effective in the treatment of CMV retinitis. All three drugs are able to control CMV retinitis and to prevent relapse for a considerable length of time. Cidofovir seems even more effective and because administrations of the drug are infrequent, once every 2 weeks, instead of the daily intravenous maintenance treatment with either ganciclovir or foscavir, would be the preferred choice, if the therapeutical index was not very small, with serious nephrotoxicity as side effect. Nephrotoxicity is also the dose limiting side effect of foscavir, while ganciclovir can cause bone marrow suppression.
An oral formulation of ganciclovir is only slightly less effective as maintenance therapy, and forms a reasonable alternative for those patients without direct sight threatening lesions.

The frequent dose-limiting toxicity of the systemic anti-viral drugs stimulated the search for alternative treatment modalities. Regimes for local intra-vitreal administration of ganciclovir, foscavir, and cidofovir have been successfully applied. The lack of protection of the second eye in unilateral cases, and the lack of treatment of extra-ocular CMV disease is a major disadvantage of strictly local therapy. A combination of local therapy with oral ganciclovir could be a more optimal treatment.

The problems with serial local injections stimulated interest in an intraocular device for sustained release of ganciclovir. In a study comparing intravenous ganciclovir with the device, median time to progression was 71 days in the intravenous treated group and 221 days in the device assigned group. The observed large difference in time to progression probably reflects a real difference in efficacy of both treatments in controlling CMV retinitis. Retinal detachment is a major complication as a consequence of the surgical procedure to implant the device.

Long term treatment preventing progression in patients with CMV retinitis is the most difficult part of the management of patients. Time between relapses shortens and subsequent relapses are more difficult to control. Several strategies have been advocated in treating recurrences: increasing the dose of the drug, switching to another drug, using a combination of drugs and supplementing systemic therapy with local therapy.

Resistance to ganciclovir, foscavir, or cidofovir is a clinical important issue in AIDS patients with CMV retinitis who need prolonged, life-long maintenance therapy to prevent relapse of active retinitis. Viral drug resistance does not play an important role in the early phase of CMV retinitis. However CMV strains resistant to ganciclovir and/or foscavir and/or cidofovir have been reported in patients treated for CMV retinitis. Persistent CMV viremia or viruria during prolonged therapy should raise the possibility of a drug resistant mutant. The mechanism of resistance differs between the three drugs.

The ideal drug to use in prophylactic or pre-emptive therapy should be orally administered, have a high specific anti-CMV activity, a minimal toxicity, a minimal interaction with other drugs, and be potent enough not to select resistant strains. No such drug exists at the moment. Valaciclovir, in maximal tolerated oral dose of 2 gram / 4 times a day, reduced CMV event rate by 33% compared to low dose aciclovir receiving patients. Oral ganciclovir, 3 gram / day, reduced CMV event rate by 49% compared to placebo, in a study which included regular ophthalmic examinations in the follow-up. Unwanted toxic side effects were seen with both regimes, and with the modest efficacy, limit the wide spread use of prophylaxis.
Influence of HAART

The combination of two reverse transcriptase inhibitors and one protease inhibitor, triple therapy, has a profound effect on HIV viral load in patients. This combination therapy has been called Highly Active Anti-Retroviral Therapy (HAART). As a result, the CD4+ cell counts rise dramatically in most patients. In those patients with a substantial increase in the number of CD4+ cells and a sufficient extension of their repertoire, there is a drop in incidence of CMV retinitis and a better control of pre-existing CMV retinitis, as a consequence of the restoration of their immune system.

How long this trend will continue is not known. Unfortunately the number of patients who fail antiretroviral therapy increases, either because of the development of resistance or as a result of the inability to tolerate the drug regime.

SUMMARY CHAPTER 2

SEROLOGIC AND POLYMERASE CHAIN REACTION BASED ANALYSIS OF AQUEOUS HUMOUR SAMPLES IN PATIENTS WITH THE ACQUIRED IMMUNODEFICIENCY SYNDROME AND NECROTIZING RETINITIS.

In a HIV-positive patient with a necrotizing retinitis, usually the clinical ophthalmologic findings are sufficient to make a correct diagnosis. However, sometimes a diagnosis can not be made with certainty leaving the clinician with the question which therapeutic strategy to follow. The measurement of intraocular antibody production and detection of DNA by the polymerase chain reaction (PCR) in ocular fluid samples have been proven to be safe procedures for diagnosis of the causative micro-organism in patients with a necrotizing retinitis, with and without immune suppression. A combined approach has not yet been reported. In our study both methods have been used and results of the assays were compared with the final clinical diagnosis.

For this study we obtained paired serum and aqueous humour samples from 28 patients with the acquired immunodeficiency syndrome and necrotizing retinitis, seen between January 1987 and March 1992. These samples were analysed for intraocular antibody production against cytomegalovirus, varicella zoster virus, herpes simplex virus, Epstein-Barr virus, and Toxoplasma gondii. Specific antibody titers in the inflamed eye and in the circulation were related to total IgG content in the aqueous humour and serum, by calculating the Goldmann-Witmer coefficient. The ratio of antiherpes viral antibody in serum and aqueous humour was compared to the ratio of total IgG in serum and aqueous humour. Additionally, PCR
Summary and general conclusion

Analysis was performed in 15 samples, using primers for CMV, VZV, HSV1, and Toxoplasma. Results were compared to the final diagnosis which was based on the subsequent clinical course. Results were also related to parameters describing the immune state of the patients: CD4 count, time between diagnosis of an AIDS defining illness and retinitis, and time of survival following the diagnosis of retinitis.

In 11 out of 28 patients (39%) local intraocular antibody production was found that correlated with the final diagnosis (1 out of 2 cases with acute retinal necrosis, 3 out of 5 cases with toxoplasma retinitis, and 8 out of 21 patients with cytomegalovirus retinitis). In all 13 patients with CMV retinitis PCR analysis detected CMV DNA. In one patient with the clinical diagnosis of toxoplasma retinitis, toxoplasma DNA could be determined, while in the same sample CMV DNA was also found. In yet another patient with toxoplasma retinitis only CMV DNA could be detected.

In 16 patients (57.1%) no positive Goldmann-Witmer coefficient could be measured. The failure to detect local antibody production could be due to the altered immune state of HIV-positive patients. However, a relationship between results of local antibody determination with either CD4 counts, or with time interval between AIDS defining illness and retinitis, or with survival time after diagnosis of retinitis, could not be established. CD4 counts were higher than 50 cells/mm\(^3\) in 8 out of 19 patients with CMV retinitis.

In two patients with toxoplasma retinitis PCR analysis yielded unexpected results: in both patients CMV DNA was present and in only one of them toxoplasma DNA could also be detected. A false positive result seemed unlikely. In one patient the clinical impression of a dual infection was indeed confirmed by the results of the PCR assay. In the second patient this was highly unlikely. The only explanation in this patient was an excessive breakdown of blood-aqueous humour barrier.

Detection of intraocular antibody production is a quick and safe procedure and may be decisive in patients with AIDS and a difficult to classify necrotizing retinitis. No complications of the paracentesis were seen in this study. In 11 out of 28 (39%) AIDS patients with necrotizing retinitis a positive Goldmann-Witmer coefficient was found indicating local antibody production against the involved pathogen. One additional case showed 2 equally borderline positive coefficients, one of which was in accordance with the final diagnosis.

Results of PCR analysis seems to be even more accurate: in all 13 patients with CMV retinitis tested CMV DNA could be detected, and no DNA of the other pathogens tested for. Especially in the early phase of a necrotizing retinitis detection of viral DNA by the polymerase chain reaction seems to be superior to determination of local antibody production.
SUMMARY CHAPTER 3

RISK OF DEVELOPING CMV RETINITIS FOLLOWING NON-OCULAR CMV END-ORGAN DISEASE IN AIDS PATIENTS.

Extra-ocular CMV disease has been considered to predispose for developing CMV retinitis, but exact data are not well known. Although maintenance therapy in case of CMV retinitis is mandatory, it is more questionable in case of other end organ disease. Even with the availability of oral ganciclovir as an alternative choice for maintenance therapy secondary prophylaxis is not routinely prescribed.

We tried to answer the question how often CMV retinitis occurs after an extra-ocular CMV disease, and whether there is a rationale for maintenance therapy after CMV end-organ disease outside the eye. A retrospective analysis was performed of all HIV-positive patients seen in the AIDS department, who had a biopsy proven non-ocular CMV disease between March 1989 and March 1995, without a previous diagnosis of retinitis. Patients did not receive protease inhibitors. The main outcome measures were incidence of CMV retinitis, time to development of CMV retinitis, relation to maintenance therapy, and survival.

A CMV retinitis was diagnosed in 17 of 20 (85%) patients with an immuno-histologically confirmed diagnosis of extra-ocular CMV disease after a mean follow-up of 6.4 months. Four patients received maintenance therapy. Three of them developed retinitis after a mean of 9.6 months (range 2 - 16 months). Sixteen did not receive maintenance and retinitis was diagnosed in 14 of them after a mean of 5.7 months (range 2 - 11 months). Mean survival was 9.9 months after the diagnosis of extra-ocular disease, and 4.5 months after the diagnosis of retinitis. In the four patients receiving maintenance therapy, mean survival was 11.5 months, and in the 16 other patients mean survival was 9.5 month.

Considering the 85% of patients with a diagnosis of CMV retinitis, following a first episode of extra-ocular CMV disease, after a mean follow-up of 6.4 months, found in this study, it seems obvious that extra-ocular CMV disease strongly predisposes for a subsequent development of CMV retinitis. CMV retinitis occurred despite the fact that extra-ocular CMV disease seemed to be completely healed after 3 to 5 weeks of anti-viral treatment. Although maintenance treatment did not prevent the occurrence of CMV retinitis the time interval between both events was considerably longer in patients receiving maintenance therapy. One patient receiving maintenance therapy with foscavir, 90 mg / kg / day, had to stop, due to drug toxicity. This patient developed CMV retinitis within one month after stopping the maintenance therapy. This patient is a good example of both
the desirability of an effective maintenance therapy and the unwanted toxic side effects of the drugs available today.

The difference in mean survival after a diagnosis of extra-ocular CMV disease, between maintenance receiving and non-receiving groups of patients, was not statistically significant.

Although our study does not provide conclusive evidence in favour of maintenance therapy after an initial extra-ocular CMV disease, frequent ophthalmic examinations are definitely warranted in such patients.

**SUMMARY CHAPTERS 4 AND 5**

**CYTOMEGALOVIRUS STRAIN DIFFERENCES BETWEEN THE EYE AND THE BLOOD IN AIDS PATIENTS WITH CMV RETINITIS.**

and

**CYTOMEGALOVIRUS GLYCOPROTEIN B GENOTYPING IN OCULAR FLUIDS AND BLOOD OF AIDS PATIENTS WITH CYTOMEGALOVIRUS RETINITIS.**

The polymerase chain reaction based assays enabled for the first time direct analysis of ocular fluid samples for the detection of CMV DNA in the eye with a necrotizing retinitis. Detection of CMV DNA is a valuable tool in the differential diagnosis of a necrotizing retinitis in case of ambiguous clinical findings. More detailed analysis of intraocular CMV is also possible with the help of PCR assays. For example analyse of genomic make-up of intraocular CMV and peripheral blood CMV and detection of mutations in the CMV genome conferring the virus resistant to anti-viral drugs.

In **Chapter 4** CMV DNA sequences from aqueous humour and peripheral blood leukocytes, obtained from 13 AIDS patients with CMV retinitis, were compared. The CMV IE-1 sequence, a part of the Immediate Early -1 gene, and the a-sequence, located in the a region were amplified. The a-region is a non-coding region and is considered the most variable part of the CMV genome. It contains signals for cleavage and package essential for viral replication. In epidemiological studies the variability of this region has been used to detect PCR amplified product length polymorphisms to characterise differences between CMV strains. The IE-1 sequence is part of a coding region of the CMV genome, lacks variability, and is used in our laboratory as diagnostic PCR assay.
CMV DNA could be detected in all aqueous humour samples and in 10 blood samples. In 7 out of the 10 cases with CMV DNA detectable in both compartments, the amplified products showed differences in the amplified product lengths. Sequence analysis in two patients revealed that aqueous humour and blood of the same patient can harbour both identical, similar and highly divergent CMV α-sequences. In 3 patients at least two different strains were present in one compartment.

The differences in the α-sequences do not necessarily reflect differences in the remainder of the CMV genome. Nevertheless the different strains as observed can have different biological properties like tropism, virulence, and drug resistance.

In Chapter 5 PCR assays have been used to determine the frequency of CMV glycoprotein B (gB) genotypes in ocular fluid samples of AIDS patients with CMV retinitis and compare these with gB genotype of CMV from paired blood samples. Glycoprotein B (gB) has been determined in 29 ocular fluid samples and 9 paired blood samples from 27 patients, by PCR followed by restriction fragment length polymorphism analysis.

In the 29 ocular samples, 30 gB genotypes were found: gB1 in 8 samples (27%), gB2 in 9 samples (30%), gB3 in 6 samples (20%), and gB4 in 3 samples (10%). In one sample a mixed genotype was observed. In addition a new gB variant could be detected in 4 ocular samples. Partial sequence analysis revealed that this genotype was closely related to gB3, and was therefore named gB3'. In the paired blood samples only gB1, gB2, and gB3 genotypes could be detected, and 4 showed a difference in gB genotype between these compartments.

A previous study has suggested an increased risk for developing CMV retinitis in those patients from which CMV could be isolated with the gB2 genotype. Based on these findings one would expect the same preponderance of the gB2 genotype in intraocular samples. However, we were unable to confirm this. Remarkably, the gB3' genotype isolated from 4 out of 29 ocular samples was only found in the eye and was not present on the blood, this could point to an ophthalmotropism of this particular genotype.

The presence of multiple strains concurrently and serially, has also been observed in this study. In one patient the ocular gB genotype changed from gB4 to gB3' within 5 months, and in another patient from gB3 to a combination of gB3 and gB1, within a period of 10 months.

The difference in CMV strain distribution, as demonstrated in both studies, between the eye and the blood in samples drawn at the same time is an unexpected finding. These strain differences can be explained in several ways. Due to local differences in environment, continuing replication of CMV can lead to a divergent development of CMV in the eye compared
to extra-ocular CMV and ultimately to genomic different strains. Alternatively, after a first infection with CMV, infection with a second CMV strain occurs, which although present in the blood, does not reach the eye. The level of the ocular strain could also be below detection level in the blood, and the difference in distribution is established and maintained by tissue tropism or the blood-retinal barrier.

The conclusion must be that despite the haematogenous spread of CMV, the eye, being a relatively shielded organ, may contain CMV strains different from those found in the blood. These differences argue in favour of the use of ocular samples to determine drug resistance by PCR-based drug-sensitivity analysis.

**SUMMARY CHAPTER 6**

**INFLUENCE OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) ON THE DEVELOPMENT OF CMV RETINITIS IN HIV-POSITIVE PATIENTS AT HIGH RISK FOR CMV DISEASE.**

Recently it has been shown that Highly Active Anti-Retroviral therapy (HAART), usually a combination of two reverse transcriptase inhibitors and one protease inhibitor, is very effective in the treatment of the HIV infection. In the pre-HAART era, HIV-positive patients with CD4⁺ cell counts below 50 cells / mm³, and those with detectable CMV DNA in their peripheral blood, were considered to be at high risk for the development of CMV disease. Overall incidence of CMV disease during a follow-up of 12 months varied between 25 and 35% in these patients. In over 90% of cases CMV disease manifested itself as retinitis.

With the start of HAART, a restoration of immune function occurred in these patients, and as a consequence patients become less vulnerable to CMV disease. Since it is not exactly known how HAART influences CMV viral load in peripheral blood and the incidence of CMV disease in high risk HIV-positive patients we followed a group of patients before and after initiation of HAART.

We included 25 HIV-positive patients, seen in the first three months of 1996 at the AIDS clinic of the Academic Medical Centre, at high risk for development of CMV disease (positive CMV DNA assay in blood and / or CD4⁺ cell count below 50 cells / mm³), without a previous diagnosis of CMV disease, in a prospective cohort study. HAART was started in the second trimester of 1996. Patients were evaluated for the occurrence of
CMV retinitis, or CMV disease elsewhere, comparing the incidence of CMV events before and after the start of HAART. Following introduction of HAART, CD4+ cell counts, and quantitative PCR for CMV DNA in blood were monitored in all remaining CMV disease free patients (n=18). Follow-up was performed until August 1998, mean follow up after the start of HAART was 15.1 months (range 9 - 18 months).

In the pre-HAART period 3 patients developed CMV disease, and 4 patients died (without clinical manifest CMV disease). After the start of HAART not one patient developed CMV disease, or died. With HAART, mean CD4+ cell counts increased from 32 cells / mm³, to 190 cells / mm³ at the end of follow-up.

Comparing the incidence of CMV disease in the patients before and after the start of HAART, using the Kaplan-Meier method with the log rank test, we could not find a statistical significant difference between both observation periods (p = .10). A placebo controlled trial was considered to be unethical, withholding HAART to these patients. Nevertheless compared to historical controls the difference is highly significant.

During the years 1995 and 1996 the estimated annual incidence of CMV disease in HIV-positive patients with CD4+ cell counts less than 50 cells / mm³ was 17% in our centre. The difference between this pre-HAART event rate of CMV disease and the observed absence of events in the patients in this study following HAART is significant ($X^2$-test, p<.02).

In 7 patients a positive CMV PCR test was obtained during follow-up after the start of HAART. Quantification of the PCR test showed a decrease of the amount of CMV DNA detectable in the peripheral blood of all these patients. At the 7th examination, longest follow-up 10 months after the start of HAART, none of the tested patients had detectable CMV DNA in their blood. However, in three patients CMV DNA could incidentally be detected afterwards. In one patient, 12 months following start of HAART, 280 CMV copies / ml could be measured, and in another patient, 14 months following the start of HAART, 98 CMV copies / ml could be detected. At the last examination, after a mean follow up of 15.1 months, only one patient still tested positive, with a CMV viral load of 727 copies / ml. This was also the only patient with a CD4+ cell count less than 100 cells / mm³.

We concluded that in HIV-positive patients at high risk of CMV retinitis, either with a positive CMV PCR assay in blood and/or with CD4+ cell counts below 50 cell / mm³, HAART causes a dramatic decrease in occurrence of CMV disease. This decrease is paralleled by an increase in CD4+ cell count, and a decrease in the amount of CMV DNA in the blood, which decreases below detection levels in all patients with CD4+ cell counts above 100 cells / mm³.
GENERAL CONCLUSION

With the advent of the AIDS epidemic CMV retinitis has become the most frequent infectious cause of retinitis in the western world. In the pre-HAART era, the annual incidence of CMV retinitis in HIV-positive patients, with CD4+ cell counts less than 50 cells/mm$^3$, was around 30%. One in every three patients developed a serious sight-threatening infection of the eyes. Most HIV-positive patients, visiting the AIDS clinic of the Academic Medical Centre in Amsterdam, are well informed about the possible opportunistic infections, and the consequences of such a diagnosis. Ophthalmologic screening strategy, with an increased frequency of examinations with each substantial decrease in CD4+ cell count, also increased the fear of the patients to hear the verdict: CMV retinitis. Minor and innocent complaints became ominous signs of a much dreaded complication. Most patients were of an age that necessitated an addition for near sight, and it was no exception that we had to reassure frightened patients that their “loss of vision” was due to a normal ageing phenomenon.

The fear of patients to develop CMV retinitis was not only caused by the possible loss of their vision, but also by the knowledge, that life-long therapy had to be installed, that they needed an indwelling catheter, that frequent controls by the internal physician and the ophthalmologist would become the new routine of their lives, and above all, by the collapse of their life expectancy, survival could suddenly be measured in months instead of years. For many patients this seemed to be more than they could bear and some, especially those who lost their vision, even chose for euthanasia. Because of the enormous impact of CMV retinitis, one must be quite certain about its diagnosis.

Diagnosis of CMV retinitis relies on clinical findings, especially the fundus appearance, and the lack of inflammatory signs. However in the early stages of retinitis and in atypical cases it can be extremely difficult to differentiate between CMV and the other herpes viruses, or between CMV and non-viral pathogens such as toxoplasma. To improve the accuracy of our diagnosis we have analysed the diagnostic value of the measurement of intraocular antibody production and PCR based detection of DNA of different pathogens in aqueous humour samples. We concluded that the analysis of aqueous humour samples is a quick and safe procedure. PCR assays, detecting DNA of the candidate pathogens in ocular fluid samples, has become one of the most powerful tools in the differential diagnosis of necrotizing retinitis. Detection of DNA in ocular fluid samples is a highly sensitive and specific method to determine which pathogen is involved in the retinitis of a given patient. Likewise, it can also exclude a pathogen in cases with non-infectious retinal pathology, such as branch retinal vein occlusion, or HIV related vasculopathy, resembling a beginning retinitis.
One of the risk factors for developing CMV retinitis often mentioned in literature is a diagnosis of extra-ocular CMV disease. However, literature does not provide exact data on this subject. In a retrospective study to the risk of developing CMV retinitis in HIV-positive patients with a previous biopsy proven extra-ocular CMV disease, we could confirm our clinical impression of a very high frequency of retinitis in these patients. One conclusion could be that this group of patients would benefit of some form of treatment to prevent CMV retinitis. However, maintenance therapy is not routinely prescribed to patients after healing of the extra-ocular CMV disease. Although not statistically significant, we were able to demonstrate a beneficial effect of maintenance treatment following extra-ocular CMV disease, resulting in at least a considerable delay in the occurrence of retinitis.

It seems a realistic assumption that CMV virus reaches the eyes through dissemination of virus via the blood during a period of viremia. Once clinical manifest CMV disease is present in a patient, the amount of viral DNA detectable in the blood, reaches high levels especially in patients with gastrointestinal inflammation. This rise in viremia is probably relative to the extent of tissue injury and viral replication. With increased levels of viremia, the chance of infection at other sites becomes more probable. This could explain the high incidence of CMV retinitis following gastrointestinal CMV disease in HIV-positive patients.

It is very difficult, if not impossible, to isolate CMV from ocular fluids of patients with CMV retinitis. This prevented studies to analyse ocular CMV. PCR enabled the direct analysis of CMV from ocular fluid samples of patients with CMV retinitis. In a first study we compared CMV from the eye with CMV from paired blood samples, using the α-sequence located in the a-region of the CMV genome. In the majority of cases genomic differences could be demonstrated between ocular and blood derived CMV strains. Despite haematogenous spread of CMV the eye may contain CMV strains different from those found in the blood. This could explain the clinical observations of development of gastrointestinal CMV disease in a patient with a totally quiet retinitis, or progression of retinitis in the absence of substantial drug resistance in blood derived CMV (despite adequate treatment). The observed differences argue in favour of the use of ocular samples to determine drug resistance by PCR based drug-sensitivity analysis.

In the literature an association between the occurrence of CMV retinitis with a certain glycoprotein B (gB) genotype, gb2, was reported, suggesting that this gene, or one linked to it, was an important virulence factor for CMV strains. In a second study, we determined the frequency of CMV glycoprotein gB genotypes in ocular fluid samples. The gb2 genotype was not more frequently seen in the eye compared to the other types, but a new variant...
**Summary and general conclusion**

gB type, gB3' was discovered in the intraocular samples, that was not detectable in the blood. We have also compared gB genotypes of intraocular CMV strains and strains from paired blood samples of patients with CMV retinitis, and confirmed our previous observation of genomic differences between ocular and blood derived CMV strains.

The combination of two reverse transcriptase inhibitors and one protease inhibitor, triple therapy, has a profound effect on HIV viral load in patients. This combination therapy has been called Highly Active Anti-Retroviral Therapy (HAART). As a result the CD4+ cell counts rise dramatically in most patients. We have conducted a prospective study to the effect of HAART in HIV-positive patients at high risk for developing CMV retinitis, and concluded that HAART caused a dramatic decrease in occurrence of CMV disease. This decrease was paralleled by an increase in CD4+ cell count, and a decrease in the amount of CMV DNA in the blood, which decreased below detection level in all patients with CD4+ cell counts above 100 cells / mm³.

At the moment we are in a period of transition. The positive change in immune response caused by HAART, brings up the questions which patients still have to be screened, and how often they must be seen. Another issue is the need for continuation of maintenance therapy in a patient with a quiet CMV retinitis, and a good response to HAART, with a rise in CD4+ cell count of over 100 cells / mm³. With the advent of HAART a dramatic improvement has been accomplished in the course of the HIV infection, with it a sharp decline in the incidence of CMV retinitis. How long this trend will continue is not known. Unfortunately the number of patients who fail antiretroviral therapy increases, either because of the development of resistance or as a result of the inability to tolerate the drug regime.

It is to be expected that patients who fail multiple drug anti retroviral therapy again will become victim of opportunistic infections like CMV retinitis. There is a need to monitor the efficacy of the immune system of the individual patient to control CMV. The CD4+ cell count and the HIV viral load are indirect and surrogate markers in this respect. Perhaps CMV viral load measurements, if standardised and commercially available, and immunologic tests to evaluate the response to CMV antigens, can more accurately determine the immune functionality against CMV of the patient.