CMV retinitis in HIV-positive patients in the pre-HAART era

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CHAPTER I

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

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

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HISTORICAL BACKGROUND

Cytomegalia was reported for the first time in 1921 by Goodpasture in a 6 week old child as large mononuclear inclusions disseminated in multiple organs. [1] In 1925 von Glahn suggested that cytomegalia was caused by a virus of the herpes group.[2] In 1954 successful growth and isolation of Cytomegalovirus (CMV) was finally achieved. [3] A mononucleosis-like syndrome occurring in adults was added to the disease spectrum in 1965 by Klemola.[4] CMV was increasingly reported in the following years. Particularly in immunocompromized patients CMV disease occurred with a more severe course, even life threatening. [5] For example patients using immunosuppressive therapy for kidney transplantation, or patients treated for systemic vasculitic disorders such as systemic lupus erythematosus.

Although the retina was recognised in 1959 as the primary site of ocular CMV disease in infants with disseminated CMV, the first case of CMV retinitis in an adult was described by Smith in 1964. [6; 7] In 1982 Moeller reviewed the literature and could only trace 50 reported cases of adult CMV retinitis.[8]

This was soon to change dramatically. 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. [9-14] If left untreated CMV retinitis invariably lead to blindness in a relatively short time, an unbearable burden for AIDS patients then and now. [15]

Since 1980, therapy for CMV was first introduced on a compassionate basis in unrandomized studies of ganciclovir, followed by FDA approval in 1989 after a randomised study. [16; 17] In 1991, a randomised controlled trial demonstrated efficacy of foscarnet, another anti-CMV drug. [13; 18] More recently cidofovir has been added to the list of effective anti-CMV drugs.[19; 20] All three medications had to be delivered intravenously to reach effective intraocular doses, but in urge to avoid systemic toxicity, strategies for direct intraocular treatment have been developed for all three. The success of this strategy lead to the elaboration of a sustained intravitreal delivery device, which came on the market in 1997.[21] Inhibition targeted to the CMV virus itself by antisense therapy is a recent development in an ongoing battle to prevent replication and progression of the virus. [22]

Anti retroviral treatment with a combination of two reverse transcriptase inhibitors and a protease inhibitor, so called Highly Active Anti Retroviral Therapy (HAART) or triple therapy, has been proven to be very effective. HAART seems to restore the immune system of AIDS patients, at least in part. This resulted in a dramatic decline in incidence of newly diagnosed and recurrent CMV retinitis. [23]

EPIDEMIOLOGY

Humans are believed to be the only reservoir for HCMV. Transmission of CMV appears to require close contact with an individual excreting virus through body fluids. Sexual contact plays an important role especially in the homo-bisexual population.[24-26] Other transmission pathways include blood products, transplantation with CMV infected organs, and transplacental infection of infants from mothers with a primary, or rarely a recurrent, CMV infection during pregnancy. Symptomatic disease in the new-born is believed to be more frequent when maternal infection occurs earlier in pregnancy.[27]

More than half of the adult population is seropositive for CMV.[25] Prevalence increases with age and differs geographically with higher numbers in the developed countries. Closeness of contacts within population groups appears to be the most important factor for the transmission rate. In the HIV-positive population the prevalence is much higher and largely due to an almost 100% seropositivity among the homo / bi-sexual HIV-positive individuals whereas in the heterosexual HIV-positive population, the frequency is equal to the general population frequency. [25; 28-30]

A clinical manifest primary infection from CMV occurs in only a fraction of immunocompetent adults. CMV is estimated to be the cause in 6 to 8% of all infectious mononucleosis-like syndromes. After primary infection CMV remains latent and infected individuals may periodically or chronically excrete CMV for the rest of their lives, in saliva, urine, semen, cervical excretions, and breast milk.[31; 32]

In immunosuppressed post transplantation patients the frequency of clinical manifest disease is much higher. After a kidney transplantation patients can either suffer from a primary CMV infection, in subjects without antibodies against CMV before transplantation, or from secondary infection, in a person already seropositive for CMV. The latter consists of reactivation or superinfection. [26] Following transplantation an average of 83% of patients, with primary CMV infection, developed clinical manifest disease and 44% of the patients, after a presumed reactivation of CMV infection.[33] The manifestation of CMV disease is associated with the

organ of transplant. In bone marrow and lung transplant patients pneumonia is a major problem, whereas hepatitis more often is seen after liver transplantation. The most common clinical presentation is a febrile mononucleosis.[26] CMV retinitis is reported in a minority of clinical manifest CMV disease after transplantations, the most frequent in renal allograft recipients (1 to 5%).[34]

In HIV-positive patients the incidence of clinical manifest CMV disease is 24% / year in patients with CD4+ lymphocyte counts less than 50 cells / mm^3. In over 90% of these cases the eye is involved, showing a necrotizing retinitis.[35] In 1996 Hoover et al. reported about the prevalence of CMV disease in a prospective study of a cohort of 367 HIV-positive patients. At 4 years after CD4+ cell count dropped below 100 cells / mm^3 the probability of these patients to (1) remain living without CMV retinitis was 11%, (2) dye without experiencing CMV retinitis was 66%, (3) experience CMV retinitis and be living 6%, and (4) experience CMV retinitis and died was 18%.[36] CMV disease is an opportunistic infection developing most often late in the course of HIV disease. The number of patients with CMV disease increased with better treatment of other opportunistic infections. For example the cumulative lifetime occurrence of CMV disease in patients before effective prophylaxis against PCP was 24%, compared to 45% after the introduction of *Pneumocystis Carinii* Pneumonia prophylaxis.[37] It can be an AIDS defining disease. In 2% of patients CMV disease is the single present first manifestation of AIDS.[38;39] The mean duration of AIDS before the development of CMV retinitis in patients with other AIDS defining diagnoses is 18 months (range between 0 and 45 months).[40;41]

RISK FACTORS

In immunocompetent patients CMV retinitis has been described in otherwise healthy individuals with a clinical picture of Acute Retinal Necrosis.[42;43] There are no known risk factors for the development of CMV retinitis. In immunosuppressed patients after transplantation a longer duration of CMV viremia was associated with a higher rate of CMV retinitis.[44] Other risk factors reported more recently in immunosuppressed post transplantation patients include CMV antigenemia, CMV DNA-emia, positive blood and or urine cultures.[45-47]

In HIV-positive patients a large number of risk factors for developing CMV retinitis have been reported. The search for risk factors became important with the availability of potentially useful prophylactic therapy for prevention of CMV retinitis. Selection of high risk patients became mandatory because results of studies on the effectiveness of primary prophylaxis were ambiguous in patients, selected on the basis of CD4+

lymphocyte counts. (see Prevention section, page 62).[35; 48]

Risk factors have been reported, which are related to the immune status of the patient, like CD4 positive lymphocyte count, CD8 positive lymphocyte count, and the presence of specific HLA types related to T-cell reactivity against CMV. 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 promising risk factor, not the presence of positive CMV cultures out of blood or urine, but especially CMV antigenemia, and CMV DNA-emia.

CD4+, CD8+ lymphocyte counts and CD4+/CD8+ ratio

In many studies the presence of a low CD4 positive lymphocyte count, generally less than 50 cells / mm^3, has been associated with the development of clinically manifest CMV disease. The prevalence of CMV retinitis in patients with a CD4+ cell count less than 50 cells / mm^3 is reported to vary between 30% to 40%. In patients with CD4+ cell counts less than 100 cells / mm^3 reported prevalence varied between 6.8% and 11.3%. [41; 49-53]

Hoover et al. in his prospective cohort study of 367 HIV-positive patients after a first drop of the CD4+ cell count below 100 cells / mm^3, reported CMV retinitis in 19% (n=73) of patients. In over 80% of patients CD4+ cell count was less than 50 cells / mm^3 at the time of diagnosis of CMV disease. [36]

Gerard et al. prospectively followed 192 patients for one year. CMV disease developed in 21 patients. The probability to develop CMV disease within 6 months was 13% for patients with baseline CD4+ cell count less than 50 c/mm^3, 3% for those with baseline CD4+ cell count between 50 and 100 cells / mm^3, and zero for those with cell counts above 100 cells / mm^3.[54]

Although association with low CD4+ cell counts is very strong, occasionally patients are reported with much higher cell counts: Baglivo reported a patient with a CD4+ cell count of 355 cells / mm^3, Fekrat reported two patients one with a cell count of 255 and the other with 235 cells / mm^3.[55 56]

Cut off levels for CD4+ cell counts below 50 cells / mm^3 and CD8+ cell counts below 520 cells / mm^3 were both equally predictive for the presence of CMV retinitis.[57] Both counts were highly correlated and addition of low CD8+ cell counts into regression analysis did not add substantially to the predictive value of low CD4+ counts alone. However patients with CMV retinitis had significantly lower CD8+ cell counts compared to the other patients, an observation confirmed by a second study.[58] Suggestions that specifically a sharp drop in CD8+ cell counts could precede a CMV retinitis have been made, but not substantiated.

Butler et al. presented some evidence that a low CD4 / CD8 ratio would predict CMV retinitis, but other authors were unable to confirm this observation. In the prospective study of Macgregor the CD4+ / CD8+ ratio was not significantly related to the development of CMV retinitis.[59-61]

HLA association

Schrier et al. reported that HIV-positive patients with a low T-cell responsiveness to CMV are at higher risk for developing a CMV retinitis than patients with normal T-cell proliferative responses.[62] In the same study an association was found between certain HLA alleles and the occurrence of CMV retinitis, especially the combined association of either B44, B51 or DR7 was highly significant (p=.008, relative risk of CMV retinitis = 15).

The presence of HIV related microangiopathy

HIV related microvasculopathy - microaneurysms, haemorrhages, vasculitis, and cotton wool spots - has been implicated in the pathogenesis of CMV retinitis (see pathogenesis section). It is generally agreed that HIV related microangiopathy precedes the occurrence of CMV retinitis.[63] Spaide et al. found the presence of cotton wool spots also highly related to CD4+ cell counts: 32.7% in patients with less than 50 cells / mm^3, 23% in patients with counts between 50 and 100 cells / mm^3, and 10 % in patients with cell counts over 100 cells / mm^3.[64] Unfortunately the time period between the presence of HIV related vasculopathy and the development of CMV retinitis can be highly variable. The vasculopathy can be present for a relatively short time, at a time the patient's CD4+ cell counts are rather high and the risk of CMV retinitis low, and becomes almost undetectable by normal ophthalmoscopic observation later on. Perhaps for these reasons the presence of HIV related microangiopathy has never been reported as an independent risk factor for the development of CMV retinitis.

Epidemiological risk factors

The incidence of CMV disease is higher in the homo-bi sexual group of patients.[14;64;65] Spaide et al. reported that CMV retinitis was present in 17% (63/348) homo-bisexual patients and in only 4.7% (4/68) of the intra-venous drug users, while patients in both groups were comparable with regard to their CD4+ counts.[64] This can be explained by the higher frequency of previous exposure to CMV infection in this group of patients, reflected in the high rate of CMV seropositive patients.[50] In addition homo-bisexual patients suffer more frequently from superinfection with different CMV strains, either successively in time or simultaneously present at different sites.[50]

Finkelstein et al. studied the pattern of development of opportunistic infections in a cohort of 1530 HIV-positive patients and found that the occurrence of *Pneumocystes Carinii* pneumonia, or *Mycobacterium avis* complex, and to a lower extend the occurrence of a systemic mycosis predisposes a patient to the subsequent development of CMV retinitis, even after adjusting for the CD4+ cell counts.[66]

Treatment with corticosteroids increases the risk for the occurrence of CMV disease. Nelson et al. compared 130 HIV-positive patients receiving corticosteroids with a case control cohort and found CMV disease in 11 of 130 steroid treated patients and in only 2 patients of the control cohort in a period of 28 days following steroid treatment. Mean dose of corticosteroid treatment was 4477 units in the patients with CMV disease, and 2017 in the patients without CMV disease. One unit dose was equivalent to 1 mg prednisolon, 0.8 mg methylprednisolon, and 0.13 mg dexamethason. The duration of the corticosteroid treatment was not specified. [67]

Verbraak et al. demonstrated an incidence of 85% of CMV retinitis in HIV-positive patients with an immuno-histologically confirmed diagnosis of extra-ocular CMV disease after a mean follow-up of 6.4 months. [68] Extra-ocular CMV disease is a major risk factor for developing CMV retinitis. CMV retinitis occurred despite the fact that the extra-ocular disease seemed to be completely healed after 3 to 5 weeks of anti viral treatment.

Presence of positive CMV cultures out of blood or urine

Sensitivity of positive blood culture as a predictor of future CMV disease varies between 66% and 76%, and specificity between 88% and 95%. Likewise the positive predictive value is relatively low (60%), but negative predictive value high (95%). So even though positive viral cultures, conventional or by shell vial method, seem to have a very low sensitivity in predicting CMV disease, patients with a negative viral culture have a very low risk for subsequent development of CMV disease. [54; 69] The same conclusion was made concerning urine cultures. [70]

CMV antigenemia and PCR based DNA-emia

The CMV pp65 antigenemia test is a quick method for identifying CMV in peripheral polymorphonuclear leukocytes. It is a more sensitive marker of viremia than virus isolation in standard cell cultures or on shell vial cultures.[71] The test is quantitative and performed with an indirect immunofluorescence technique, using a monoclonal fluorescein labelled anti-viral-pp65 antibody, counting fluorescent nuclei in aliquots of cytocentrifuged isolated peripheral blood leukocytes. The number of positive staining cells per 10^5 leukocytes is used.[72] Antigenemia assays

can differ in the technical details of the procedure: the number of polymorphonuclear granulocytes cytocentrifuged onto the microscopic slides, the type of fixation, the type of monoclonal antibodies, or the timing for sample collection and processing. This is one of the reasons different authors use different cut-off levels, and can explain the variability in some of the results.

Determination of CMV DNA in peripheral blood is considered the most promising test in use as a predictive marker for CMV disease. Detection of CMV DNA can be performed on whole blood, testing the peripheral blood cells for the presence of CMV, and on plasma or serum, testing the cell free fraction of the blood. The test can be qualitative merely showing the presence of CMV DNA in a sufficient amount to be above detection level of the technique used, or quantitative in which case the test uses an internal reference to calculate the number of CMV DNA copies present in the specimen tested. Most laboratories first perform a qualitative assay which is followed by a quantitative test if positive. In quantitative tests a threshold is defined, depending on the sensitivity of the test, above which a result is considered to be positive.

Results of both tests are influenced by the inclusion criteria of the patients, especially the CD4+ cell count as indicator of the immunesuppresion present, the length of follow-up, the scheduling of follow-up visits, the way patients are examined during follow-up (eye examination included or not), and the total number of CMV events diagnosed during follow-up. Another important factor is the definition of a positive test as predictive. Some authors included patients with only one positive test just before or even at the time of diagnosis of CMV disease, which can hardly be considered to be of any predictive value.[73] Results of recent studies, which all included regular eye examinations during follow-up, are summarised in Table 1 (page 15).

The antigenemia test is a very sensitive method to detect the presence of CMV in peripheral blood leukocytes. For this reason a cut-off level has to be specified to increase specificity of the test. Most authors define this cut-off level retrospectively and naturally will chose a level that will result in the best possible predictive values. By taking a high cut-off level the sensitivity decreases, and specificity increases. The reported sensitivity and specificity of the antigenemia test varied between 80 and 90%, and between 90 and 97% respectively. Positive predictive values, reflecting the number of CMV events in all patients with a positive test were always exceeded by negative predictive values. Patients with a negative test had a very low chance to develop a CMV event. [74-76]

The CMV DNA-emia assay in most studies is qualitative and uses plasma or serum samples of patients at risk for developing CMV disease. The sensitivity of the assays varies between 70% and 95%, and the specificity

between 60% and 88%. Like the antigenemia test the positive predictive value is much lower compared to the negative predictive value, meaning that a negative test makes disease highly unlikely, but a positive test is not invariably followed by disease.

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. In addition they can be performed in most laboratories. Multivariate analysis in a study comparing different tests in the same patient group have shown no additional gain in predictive accuracy by doing both tests. [69]

Unfortunately the time between a positive PCR assay or a positive pp65 -antigenemia test, and the occurrence of CMV disease can vary considerably. The interval can be in the order of 3 to 8 months or even longer. In spite of high expectations there is only a marginal gain in predictive value of a quantitative PCR assay, compared to a qualitative assay. In PBL's CMV DNA can be detected more often and in higher quantities. Using PBL's a quantitative assay is mandatory with definition of a cut-off level to differentiate between high and low risk patients. [77] Provided the assay is sensitive enough plasma or serum tests are slightly more accurate in predicting CMV retinitis than peripheral white blood cell tests. [78] Plasma and serum assays are equally sensitive. [69]

Quantitative assays have shown that the peak values of CMV viral load measured in many patients precede the occurrence of retinitis by 3 to 7 months, while in the same patients CMV viral load at the time of diagnosis is only slightly above normal, or even normal. The duration of a high CMV viral load seems to be an important additional risk factor. [78]

Most PCR protocols are time consuming and require a fair amount of expertise, especially when quantitative tests are performed, which is mandatory in tests using PBL's. Each laboratory performs its own assay to detect CMV DNA, using different ways in handling of the samples, different ways to extract DNA, different primers, different procedures of the PCR. For this reason results of tests can never be compared between different laboratories. There is a high need for standardisation of the PCR protocol used to detect CMV DNA. Recently a test has been developed, which allows simple batch testing of large numbers of samples and is commercially available. (Amplicor CMV test, Roche Diagnostic Systems, Inc,Branchburg, N.J.).[77; 79] Results of these tests compare favourably with previous reported test results, with a sensitivity and specificity of about 90%.

Why roughly 10% of patients with considerable CMV viral load measured

in peripheral blood do not proceed to develop CMV retinitis (or CMV disease elsewhere), and why CMV retinitis will develop in up to 20% of patients with negative PCR assays in peripheral blood, are questions that remain to be answered.[77; 78]

The occurrence of CMV retinitis is a multifactorial determined event, additional factors have to be considered: the presence of replicating infectious CMV in blood indicated by detection of mRNA's encoding structural or envelope proteins, specification of CMV strains more likely to cause retinitis, or immunologic host factors that make some individuals more susceptible for clinical manifest CMV infections. [62; 80; 81] Local factors in the eye like the presence of HIV related vasculopathy, and systemic factors like rheological abnormalities can add to the susceptibility of an individual to get an active ocular CMV infection.[82]

CLINICAL FEATURES

CMV is the most common opportunistic viral disease in HIV-positive patients and the eye the most commonly affected organ. Ocular CMV disease is primarily an infection of the retina. Positive cultures of CMV have been reported from conjunctival swabs of AIDS patients. [12; 83; 84] CMV has also been shown to be present by electron microscopy in conjunctiva, cornea, and tears, but the importance of these findings seem to be limited. A case of bilateral dentritic epithelial keratitis in an AIDS patient with a generalised rash has been described. Cytological examination and viral cultures of corneal scrapings showed CMV to be the involved pathogen. This patient went on to develop a stromal keratouveitis despite anti-viral treatment. [85]

Most of our knowledge about the clinical aspects of acquired CMV retinitis today comes from HIV-positive patients. 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.[51; 86; 87] Peripheral lesions can more likely remain silent. Complaints, if any, are blurring of vision (in 50% of cases), loss of visual field (20%), and photopsia or floaters (60%).[86] The severity of the symptoms is closely related to the location and the extent of retinal lesion(s) in the eye. Posterior lesions can be noticed by the patient as (para)central scotomas. Entoptic perimetry has been recommended to detect scotomas between the central 10 degree radius, evaluated by the Amsler grid, and the 30 degree radius.[88] Involvement of the macular area or the optic nerve head will lead to early complaints of blurred vision or even severe visual loss. Floaters reflect vitreous opacities as a result of the

Table 1 Predictive values of recently reported CMV antigenemia and CMV DNA-emia assays

Author (year)	Number of patients	Inclusion	Test / Sample	Sensitivity	Specificity	Positive Pred. value	Negative Pred. value	CMV events	Δ T * months	Follow -Up (months)	Comment
Podzamczer (1997)	-	-	Quantitative Antigenemia	-	-	65%	97%	13%	-	12	Cut-off level 10 positive cells / 10^5
Francisci (1997)	49	CD4 <150	Quantitative Antigenemia	77%	97%	91%	92%	26%	5.8 1 to 12	10 (mean) 1 to 25	Cut-off level 20 positive cells / 10^5
Dodt (1997)	200	CD4 <100	Quantitative Antigenemia	92%	88%	65%	98%	19%	1 (median) 0 to 4		Cut-off level 10 positive cells / 10^5 Range between first pos. test and event = 0 – 121 days. (concomitant included)
Shinkay (1997)	94	CD4 mean =53	Qualitative PCR Plasma (quantitative)	89%	75%	58%	94%	28%	6 (median)	12	Qualitative, followed by quantitative, if positive. Peak values some patients 4 months before CMV event.
Dodt (1997)	200	CD4 <100	Qualitative PCR Plasma Serum	95%	83%	57%	98%	19%	1.5 (median) 0 to 6	18	Serum and plasma samples gave identical results. Range between first positive test and event = 0 – 182 days. (concomitant included)
Laue	129	CD4 <100	Qualitative Plasma Serum	73%	61%	20%	94%	12%	2 (median)	4.5 (median) (0 to 16)	Relative short follow-up. Lower incidence. Range (199) between first pos. test and event =14 – 233 days. (concomitant excluded)
Rasmussen 75 (1997)	CD4 <100	Quantitative PCR		74% Plasma (PBL)	75%	67%	81%	40%	Range: 2 to 18	15 (mean) (+/- 4)	Plasma more accurate than PBL.
Spector	201	CD4 <100 mean=21	Qualitative PCR Plasma (quantitative)	71%	65%	43%	85%	27%	6 (median) 2 to 18	12	Qualitative, followed by quantitative, if positive. Author (1998) provides data as 12 months survival-curve. Clear correlation between CMV viral load and risk of development of CMV disease
Bowen (1997)	97	CD4 <50	Qualitative PCR PBL (quantitative)	80%	88%	60%	91%	21%	Not	12	Qualitative, followed by quantitative, if positive. Peak specified values in some patients several months before CMV event.

* D T = time between first positive test and occurrence of CMV retinitis

For additional explanation see text

inflammation, even in lesions in the far periphery of the retina. However inflammation is usually minimal in HIV-positive patients and more subtle opacities are easily ignored by the patient. CMV retinitis starts as a bilateral disease in the minority (20 to 40 %) of patients. [89-92] In unilateral cases the unaffected eye may "mask" the loss in the diseased eye. Pain is never a complaint.

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 start of the AIDS epidemic CMV retinitis was mostly of the fulminant/oedematous type, which was more or less identical to the classical descriptions of CMV retinitis in immunocompromized patients in the pre-epidemic period. The retinal lesions are dense and whitish, obscuring all details of the underlying choroid. Haemorrhages are abundantly present, adding to the obscuration of deeper layers. This combination of signs has led to the descriptive term "pizza pie" lesion for obvious reasons. In the periphery lesions tend to be more of the granular/ indolent type, while the majority of the centrally located lesions are of the fulminant/ oedematous type, reflecting perhaps structural differences in the retinal anatomy at these sites. [89;92] Vasculitis and inflammatory sheathing can be present and the lesions follow the vascular tree, spreading along large vessels. Patients have been described with widespread involvement of the large vessels throughout the entire fundus, giving rise to a clinical picture comparable to so-called frosted branch angiitis.[93-95] Within 7 days this evolved into a typical necrotizing retinitis.

In later years the indolent/granular type of CMV retinitis became more frequent. This type of retinitis shows less dense retinal opacifications, allowing for some visibility of the underlying choroid. Haemorrhages, although present, are scarce, and the lesions do not show a tendency to follow the retinal vascular tree. In 1990/91, a multicenter study of 240 AIDS patients described the base line characteristics as indolent/granular in 54%, and fulminant/exudative in 46% of eyes.[86] At the leading edge of both lesions a dry-appearing granular border is present. Satellite lesions some at 500μm 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. [96]

Previous treatment of the HIV-positive patient, with medications like zidovudine or acyclovir, can also influence the appearance of the CMV lesions.[97] Some of these drugs have at least a partial anti CMV activity. Perhaps the availability of more effective drugs with time explains the shift from predominant fulminant type of CMV retinitis in the beginning of the HIV epidemic to predominant indolent type seen in later period. Lastly the extend of loss of immune-responsiveness of patients can dictate the way CMV disease presents itself in the eye. The severity of the disease

depending on the ability of patients to defend themselves against CMV infection.

CMV retinitis in the posterior pole of the eye can lead to foveal exudations, macular oedema and even frank serous detachments.[98-100] Lipid exudates and / or exudative detachments have been described in 35 of 618 eyes (6%) of patients with CMV retinitis. All these eyes had a centrally located retinitis.[98] An autopsy study revealed exudative retinal detachments in 10 out of 35 eyes of patients with CMV retinitis.[100] Visual loss due to central exudative lesions is reversible, and responds well to anti CMV therapy. Occasionally a parafoveal HIV related vasculopathy is present and causes cystoid macular oedema (CME) even in eyes with more peripheral lesions.[13] Cystoid macular oedema has also been described in a patient with CMV retinitis without foveal vasculopathy.[101] The CME disappeared after treatment with a non steroidal anti-inflammatory drug.

Optic disc swelling is present in 7% of patients with retinitis at presentation.[86] It can be seen in eyes with CMV retinitis in the peripapillary region. In these cases central vision remains good, although sometimes deep visual field defects may occur, even though histologic examinations do not show CMV to be present in the optic nerve. Nevertheless cases do exist where the optic nerve is secondarily affected by CMV infection through spread of adjacent retinal CMV disease. In a minority of patients the optic nerve and the peripapillary area is the primary site of inflammation by CMV. Central vision is deeply affected and sometimes permanently lost early in the course of the disease in the eyes of these patients.[102-106] However patients with papillitis treated aggressively with prolonged induction courses of ganciclovir and a prompt switch to foscavir after failure of ganciclovir, did respond much better, and useful vision (mean vision 20/68, range 20/400-20/25) was retained in 75% of eyes.[107]

At presentation most patients have one necrotic lesion in the eye(s), more than three foci is very uncommon. CMV retinitis most often starts adjacent to the vascular arcade bordering the macular area. Untreated the lesions expand relentlessly and affect the entire retina within a period of 6 months. The pattern in which the lesions expand and spread throughout the retina is depending upon the location where the lesions start.[91;96]

In spite of large areas of necrotic retina the inflammatory response is minimal in both the vitreous cavity and the anterior chamber. A variable amount of vitreous opacities develops with only a slight flare and almost no cells. In the anterior chamber cells are sporadic and flare again minimal. Flare measurements, with the laser flare photometer, have been advocated for screening of HIV-positive patients for the presence of CMV retinitis. The increase in flare was indeed measurable, but values (mean value 35.3 photon/msec), although above normal (mean value 6.8 photon/msec), were

barely detectable by slitlamp examination. The use of flare measurements for screening purposes is best kept for situations, where local circumstances prevent examination by an ophthalmologist. [108 109]

Small corneal endothelial deposits can be seen by careful slitlamp examination in 50 to 80% of patients with CMV retinitis, but can easily be overlooked, and best seen with retro-illumination. [86;110] These endothelial deposits are stellate in shape not unlike the deposits seen in Fuch's iridocyclitis. Post mortem examination in one patient disclosed a preponderance of large dendritic macrophages arranged in chains. There was no evidence of CMV infection in the endothelium. [111]

Comparison of the eyepressure between HIV-positive patients, with and without CMV retinitis, and normal controls, showed the intraocular pressure to be lower in HIV-positive patients, mean 12.6 mmHg, and lowest in patients with CMV retinitis, mean 9.8 mmHg (mean normal value 16.1 mmHg). [112] Eye pressures were significantly correlated with CD4+ lymphocyte counts, but no explanation could be provided for this phenomenon. It was argued that in evaluating HIV-positive patients for the presence of glaucoma one had to take this shift in the value of intraocular pressures into account. This observation was confirmed by a recent multicenter study in 240 patients at presentation of a CMV retinitis. The eye pressure in the unaffected eye in unilateral cases was significantly higher compared to the eye with retinitis, although the difference was small, 12.1 versus 13.2 mmHg, $p<.001$. [86]

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, seen in other inflammatory disorders of the retina. Sometimes a fine mottling of the retinal pigment epithelium can be appreciated. The fact that the entire retinal pigment epithelium is destroyed in the inflammatory process is probably the reason for this inconspicuous scarring. In most patients induction therapy is able to stop progression. However all available medications are virustatic rather than virucidal. Recurrence is inevitable when prophylaxis is stopped, unless there is restoration of the immunesystem. Until the advent of protease inhibitors and multidrug regimes, maintenance therapy was required life long to prevent recurrence. However in most instances maintenance was only able to delay recurrence. Successive reinductions lead to shorter relapse free periods and in many cases to frank resistance.

In some patients it can be difficult to make a distinction between smouldering slowly progressive retinitis and a quiet non progressive lesion.

[113] Only careful comparison of the location of the borders of the lesion between successive follow-up examinations is decisive. Many clinicians, including ourselves, advocate follow-up with base-line fundus photographs taken as soon as the lesion is fully quiescent. Atypical healing has been described in 12% of patients consisting of a white flat border opacification that does not advance for weeks to months. [114] Some of these patients received re-induction with all its side effects, without any effect on the lesion. Histology of one such case showed stable structures of dead cytomegalic cells within the retina.[114]

Visual function

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. It was estimated in the SOCA study that involved eyes lost vision at a rate of approximately one line of the eye chart (ETDRS chart) every 2 months. [89] The 6 months cumulative probability of a visual acuity worse than 20/40 in all involved eyes was 0.40 for foscarnet assigned patients and 0.34 for ganciclovir assigned patients. Visual fields also deteriorated and patients lost their visual field in the involved eye at a rate of 5% of the total visual field each month. Because the ability to function is determined by the visual acuity of the better eye, the SOCA study group also evaluated the visual acuity of the better eye and found a probability of 0.07 for ganciclovir, and of 0.12 for foscavir treated patients, at 6 months to have a vision less than 20 / 40. Initial visual acuity less than 20/40, decreased vision in the involved eye before treatment, fulminant / oedematous type lesions, and CD4+ cell counts less than 14 cells / mm^3 were all associated with a worse visual outcome. These factors were related to one another, because centrally located lesions were more often of the fulminant/ oedematous type, and more often associated with significant loss of vision from the start. Due to the central location these lesions had a greater chance of damaging the fovea or the optic nerve. It was found that patients with such lesions not only ended with the worse visual acuity, but also lost their vision at a faster rate. Slight progress of centrally located lesions can cause a dramatic loss of vision.

Bloom performed a study to the visual prognosis of 228 eyes with CMV retinitis of 147 AIDS patients, who were all treated according to standard protocol with intravenous ganciclovir. [90] At presentation visual acuity was above 6/12 in 80% and above 6/60 in 94% of eyes, just prior to death visual acuity was above 6/12 in 49% and above 6/60 in 75% of eyes. The vision in the better eye of the patients at last follow-up before death, was above 6/12 in 77%, above 6/24 in 92%, and below 6/60 in only 5% of patients.

Reduced vision was caused by macular involvement of retinitis in 36% of eyes, by optic nerve involvement in 23%, due to retinal detachment in 21%, and due to cataract in 4%.

Controversially an improvement of vision in patients with centrally located lesius 1 lesions can occur in case of central macular oedema secondary to retinitis lesions, adjacent but not involving the fovea, resolving after a favourable response to therapy. Improvement of vision has even been reported in 96% of such lesions. [98; 99] Some cases with lesions near the optic disc have also been reported to show visual improvement,. [106]

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%. [91; 99; 105; 115; 116] The median and mean time following the first diagnosis of CMV retinitis and the occurrence of a detachment is approximately 4 and 7 months, respectively. [117-119] The prevalence increases over time with a cumulative risk of around 20% after 6 months and around 38% after one year. [89; 119-121]

Several characteristics of CMV retinitis have been reported to be associated with the development of retinal detachments. Eyes infected in the peripheral retina, extending to the ora serata, are at higher risk for developing a detachment. In this location, the vitreous is more firmly adherent tot the retina. It also experiences more centipetal tractional forces. As the posterior hyaloid detaches, tears will easily form in this location.

The area and the location of CMV retinitis seem to be important determinants of the risk for developing retinal detachment. Patients with more than 50% of the retina involved at base line are at higher risk to develop a retinal detachment. [119] The cumulative probability developing a retinal detachment increased to 50% in 6 months in those patients with more than 50% of retina involved at initial examination. In a second study a relation between lesion size, even in lesions less than 50% of the retina, and the presence of a retinal detachment was found. [120] CMV retinitis extending to the ora serrata has a higher risk of developing a detachment[122] No firm association has been found between CMV retinitis related retinal detachment and the state of activity of the retinitis. CMV retinitis can be active or quiescent when the detachment is noted [119-121; 123]

The introduction of intraocular therapy modalities, especially the intraocular drug deliver devices do seem to increase the number of detachments or at least hasten their development. The surgical procedure to install such

a device disrupts the vitreous base, leading to local traction and an increased risk for the development of retinal tears. [21]

The presence of retinal detachment in one eye is a risk factor for the development of a detachment in the fellow eye. In 20% of patients there is bilateral involvement (reported bilaterally: in 17 to 67 % of cases). This could merely be a reflection of the symmetry of CMV retinitis in both eyes of the same patient, but other host factors could also play a role. [122] One such host factor could be myopia, but results of several studies were contradictory. [118; 122]

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. As with all detachments, localisation of the lesion and the presence of proliferative vitreoretinopathy (PVR) will influence the choice of therapy. Some degree of PVR can be seen in up to 20% of cases. [124; 125] Luckily high-grade PVR is a rare event, though with the advent of protease inhibitors, it can become more frequent. [121] Treatment of retinal detachment in AIDS patients can be broadly categorised in 2 groups. For retinal detachment not involving the retina affected with CMV, standard detachment procedures prevail. For detachments involving the affected retina or originating there, the preferred procedure has been a pars plana vitrectomy, and silicone oil tamponade, with or without a scleral buckle. Most studies have reported a high anatomic success rate, and a high macular reattachment rate. [118; 119; 121; 122; 126] Interestingly, repairs of the detachment before or just after macular detachment leads to the same end result. Use of silicone oil is not without complication. Cataract formation and optic atrophy have both been described. Cataract formation tends to occur within 2 to 15 months (mean 6 months) of the detachment procedure. [127] These patients benefit from a cataract extraction and intraocular lens (IOL) implantation. The IOL power calculation can be accurate, but axial length should be calculated for aqueous, lens, and silicone oil separately and summed, a constant should be added to compensate for the refractive index of silicone oil, and only convexplano IOL's should be used. [127]

Of greater concern is the higher rate of optic atrophy in vitrectomised eyes, which may also be responsible for the observed decline in vision after successful repair. [118; 123; 128] Optic atrophy may be due to direct toxicity of silicon oil. Another explanation is the high intraocular pressure occurring during the procedure, which, on top of the already compromised bloodflow of the optic nerve head in HIV-positive patients, leads to progressive loss of nerve fibres. [128] Other authors believe that progression of the retinitis is

responsible for the continuous decline in vision and the optic atrophy only follows the progressive loss of functional retina. [123; 127; 129] One argument in favour of this hypothesis is the observation in one patient, that the amount of optic atrophy was equal in both eyes, successfully repaired in one eye. [129]

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. [129; 130] Because of the high rate of bilateral disease and bilateral detachments there is another argument in favour of surgical intervention, even in patients with good vision in the fellow eye. The preserved vision in the operated eye could become the best vision in case the opposite eye suffers from the same or more serious complications.

Prophylactic argon laser coagulation for rhegmatogenous retinal detachment in eyes with quiescent CMV lesions seems to reduce the rate of progressive retinal detachment with no need for vitrectomy and silicone oil tamponade. In four out of 22 treated eyes a tear developed in the CMV retinitis scar with local retinal detachment, which stopped at the laser scar. [131]

PATHOGENESIS AND HISTOPATHOLOGY

CMV disease can be either acquired, congenital, or can be due to reactivation of latent virus. In immunocompetent patients CMV disease will be self limiting, only in the immunocompromized patients, especially the HIV-positive patients CMV disease can have serious consequences like loss of vision or even death. Once a patient is infected with CMV, infection remains latent and CMV can be shed intermittently or chronically in saliva, urine, semen, cervical excretions. Such patients serve as a source of CMV infection. After acquisition of the virus hematogenous cell-associated spread give rise to a disseminated presence of virus throughout the body. Molecular studies have shown virus to be present in granulocytes and to a lesser extend monocytes. [44; 132-134] Even viral RNA has been detected in these cells indicating active viral replication, although the pathophysiologic role of this phenomenon is unclear. [135; 136] During viremia early viral antigens can be detected in the nucleus of neutrophylic granulocytes. Many organs become involved and CMV can be detected in a variety of organs: kidney, spleen, salivary glands, brain, inner ear, lungs, gastrointestinal tract, adrenal glands, and testis. [33; 137; 138]

Immunohistochemical techniques using labelled monoclonal antibodies

against viral antigens showed CMV to be present in endothelial, epithelial, smooth muscle, and parenchymal cells. [139-141]

After a first episode of CMV infection CMV can remain latent probably 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 site of latency is still unknown. An important candidate site of latent CMV could be the leukocytes, but definite proof is lacking and evidence only indirect: the probability of seroconversion in individuals after bloodtransfusions is proportional to the amount of blood transfused and decreases with the transfusion of leukocyte-poor blood. [142; 143] In humans CMV DNA has been shown to be present in monocytes, macrophages, endothelium, and it is most probable that there is more than one cell type in which latency may occur.

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. Immunosuppressed patients already seropositive for CMV can still acquire new CMV strains. In the sexual active population reinfection is relatively common and in immunocompromized patients coinfection with multiple strains has been detected[25; 26]

It seems a realistic assumption that 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 granulocytes, reaches high levels especially in patients with gastrointestinal inflammation. This rise in viremia is probably relative to the extend of tissue injury and viral replication. The higher the level of viremia, the higher the chance of infection at other sites. This could explain the high incidence of CMV retinitis following gastrointestinal CMV disease in HIV-positive patients. [68]

It has been hypothesised that HIV related microangiopathy allows entrance of CMV into the retina via damaged microvasculature. [100] The pathogenesis of this microangiopathy has not been clearly defined and different hypotheses have been formulated, including deposition of immunecomplex, by chronically increased levels of products of immune activation (cytokines, interleukines), direct damage to endothelial cells caused by HIV, or by alterations in blood flow. [144]

The retina of AIDS patients has been found immunohistochemically positive for tumour necrosis factor (TNF-alpha), which suggests that cytokines may play a role in HIV-associated microangiopathy. [145] Endothelin-1 is a cytokine with a potent vasoconstrictive activity. [146] HIV-positive patients were found to have higher levels of endothelin-1 immunoreactivity in plasma compared to controls. Moreover, in HIV-positive patients with signs of microangiopathy in the retina, endothelin-1

immunoreactivity was higher compared to patients with a normal appearing retina. This findings suggests that of endothelin-1 may also play a role in the pathogenenis of HIV related angiopathy.

Post mortem examination of a patient with central retinal vein occlusion showed the absence of HIV in the retinal vessels.[147] The authors conclude that probably other hemorhologic abnormalities must be the cause of the vein occlusion.

Retinal blood flow indices as measured by scanning laser angiography showed a normal arteriovenous passage time, but a significantly reduced perifoveal capillary blood flow velocity in HIV-positive patients compared to normal controls.[148] This alteration may trigger events leading to ischaemia, and could be an initiating event in the pathogenesis of microvascular angiopathy. Another microvascular abnormality described in HIV-positive patients is conjunctival microvasculopathy visible as an increased sludge phenomenon. The presence of cotton wool spots and the conjunctival sludge phenomenon are both highly related to a reduced cerebral blood flow, both ascribed to HIV infection.[149; 150] Systemic hemorhologic abnormalities may lead to widespread microangiopathy. Increased sedimentation rate, increased fibrinogen, von Willebrand factor, and plasminogen activator inhibitor levels have been described in AIDS patients and may produce a sluggish blood flow.[82; 151] Altered blood flow could be caused by an increased rigidity of the leukocytes as a result of HIV infection, leading to slowing of blood flow through the capillaries.[152]

There is evidence for the presence of CMV in the eye long before a clinical manifest CMV retinitis presents itself. CMV DNA has been detected in vitreous of a patient with toxocara, without CMV retinitis, who went on to develop CMV retinitis 6 weeks later.[153] In a patient with CMV retinitis in the right eye, CMV could be cultured post mortem from the unaffected left eye.[154] In a post-mortem study it was shown by PCR detection that CMV DNA was present in retinal tissue of HIV-positive patients without clinical retinitis. There was a significant correlation between the presence of a cotton wool spot and CMV DNA. Ninety % of cotton wool spots were positive for CMV DNA versus 22% of normal appearing retina.[155] HIV related vascular abnormalities are separate from and preceding CMV retinitis in AIDS patients. The vascular abnormalities were more diffuse and more pronounced in eyes with CMV retinitis. While CMV retinitis and retinal vasculopathy are related topographically, it remains to be determined whether they are related pathogenically.[156]

In eyes with extensive CMV retinitis, CMV can be detected throughout all layers of the retina, including the retinal pigment epithelium.[100] Because of the presumed pathogenesis of CMV retinitis suggesting that the retinal vasculature is the first to become infected it seemed likely that retinal glia cells played an important role in the further spreading of disease. It has been

shown that retinal glial cells were indeed permissive for CMV replication and probably played an important role in the pathogenesis of CMV retinitis. Additionally, replicative intermediates of CMV were maintained in ganciclovir treated CMV infected retinal glial cells, capable of replication after removal of the drug. [157; 158]

Dual and even triple infections of the retina have been described with HIV-1, Herpes Virus type 6 (HHV-6), and CMV in AIDS patients, and some authors suggested that this was more than coincidence. Viral antigens and transcripts were analysed in 50 globes of AIDS patients with and without clinical signs of CMV retinitis. More than 50% of the retinas showed co-existence of HIV-1 or Human Herpes Virus type 6 (HHV-6) activity, without CMV activity suggesting that either virus alone or in combination could play a permissive role in the pathogenesis of CMV retinitis. [159] HHV-6 has been found to be an important pathogen in AIDS patients, which is disseminated throughout the body. [160] Active replication of HIV-1 has been demonstrated in the retinal vascular wall by in situ hybridisation of HIV-1 RNA. [161] Immunohistochemical staining coinfection of individual retinal cells have been demonstrated with HIV-1 and CMV. [162]

Animal model

Because of the extreme species -specificity of CMV it has been difficult to model in vivo. Laycock et al. succeeded in constructing a model in 1997. [163] Human retina was introduced into the anterior chamber of a-thymic rats and allowed to attach to the iris. Human CMV was then injected into the anterior chamber. After 4 weeks multiple foci of human CMV replication were found in the transplant. The CMV infected transplant sustained long enough to permit multiple cycles of viral replication and could be useful to evaluate antiviral therapies.

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 herpesvirusses (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 two segments are linked within the junctional area (J) through the internal repeat sequences, IR_L and IR_S and end in terminal repeat sequences (TR). Within the junctional area the a-region is located, a non-coding region which is thought to contain signals for cleavage and packaging essential for viral replication. [164]

Viral replication reflects the expression of three categories of genes termed

immediate early (IE) (alpha), early (beta), and late (gamma). These categories correspond to the timing of the appearance of messenger RNA or proteins in an infected cell. Many proteins of HCMV have been identified and classified. [165; 166] The capsid proteins are a relatively simple set of proteins. Antibodies to these proteins do not exhibit virus-neutralising activity and are unable to bind to either extracellular CMV or the surface of infected cells. [167] The region between the capsid and the surface of the virus is called the tegument and consists of at least 7 proteins, including pp65. The function of these proteins is unclear. Tegument proteins do not play a role in the induction of antibodies which are either neutralising or have the capacity to bind virus infected cells. The third category of proteins are the envelope glycoproteins. They play a pivotal role in the attachment to surface receptors, the penetration into a cell, the assembly of newly formed virions, and the egression of these virions out of the infected cells. The envelope proteins are important antigens for the humoral and perhaps also the cellular immune response. The most important glycoproteins are gB complex (gpUL55) and gH (gpUL75). Both are targets for virus neutralising antibodies. The gB homologue is immunodominant compared to gH and human neutralising monoclonal antibodies to gB have been generated. [26]

Compared to normal controls, AIDS patients had a significantly lower response following immunisation with pneumococcal polysaccharide and protein, suggesting a B-cell immunodeficiency. [168] A deficiency has been observed in antibody response to CMV gH glycoprotein in HIV-positive patients with CD4+ cell counts below 100 cells / mm3, in the presence of high titres of gB antibodies. Antibody titre of gB was comparable to titres found in HIV seronegative patients with CMV disease, and was equally high in HIV-positive patients with or without CMV retinitis. This would mean that precisely at a time these patients were at higher risk of developing active CMV disease their humoral response was less efficient. [169] However, in a study comparing patients with and without CMV retinitis, levels of anti CMV antibodies, including the two major envelope proteins gB and gH, were not specifically deficient. In this study higher levels of neutralising antibodies did correlate with a more favourable clinical course. [170]

Seroconversion is usually a good marker of primary CMV disease, but not useful in HIV patients because most patients are already CMV seropositive. CMV infection is ubiquitous in humans, 50 to 70 % of the adult population is seropositive for antibodies to CMV by the age of 40 years. [25] Primary infection is followed by persistent infection. The precise site of latency is unknown. [26] In HIV-positive patients other than the homo/bi-sexual population the prevalence of CMV seropositive patients equals the general population. In the homo/bi-sexual population CMV seropositivity is almost 100%. [25; 28-30; 50] Two cases have been reported of HIV-positive

patients, with histologically proven CMV disease, in which CMV serology was negative.[171]

The IgG titres may be very high in HIV patients with active CMV disease, but can not be used as a diagnostic tool. [172] IgM is presumed to be only present upon a primary infection, but titres have been shown to be almost continuously present in over 90% of HIV-positive individuals.[173] This high prevalence of positive IgM titres can be attributed to reactivation of previously latent infection, but is in most cases the result of repetitive exposure to different strains of virus. [174]

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. The tegument protein pp65 is identified as a target for CD8+ class I major histocompatibility complex (MHC) -restricted CMV specific cytotoxic T lymphocytes (CTL). [175] Recognition of pp65 on target cells occurs prior to viral gene expression. Apparently these proteins are available to MHC molecules for presentation at the cell surface directly after penetration of the cell, before the onset of viral gene expression, and the pp65 specific CTL could play an important role in the early limitation of CMV infection.

As already mentioned Schrier reported an association between certain HLA alleles and the occurrence of CMV retinitis, especially the combined association of either B44, B51 or DR7 was highly significant (p=.008, relative risk of CMV retinitis = 15). The patients with CMV disease also showed a lower T-cell responsiveness to CMV, compared to HIV-positive patients without CMV disease. [62]

Studies have been performed to relate a certain gB genotype of CMV with the development of CMV retinitis. In a first report retinitis was seen in 14 out of 18 patients with an isolate with gB2 genotype and in only 6 out of 26 isolates with one of the other genotypes (gB1, 3, or 4) This association suggested that this gene, or one linked to it, was an important virulence factor for CMV strains. [81] A second study could not confirm these findings, but did find an increased incidence of gB2 genotype in HIV-positive patients compared to allograft recipients. [176] Comparing gB genotypes of intraocular CMV strains and strains from paired blood samples of patients with CMV retinitis, in at least 50% of cases genomic differences could be detected between the eye and the blood compartment. The gB2 genotype was not more frequently seen in the eye compared to the other types, but a new variant gB type, gB3' was discovered in the intraocular samples, that was not detectable in the blood.[177] Geographical differences in prevalence of the gB genotypes do exist and can also account for the observed variability.[178]

There is evidence to suggest that herpesvirusses including CMV could

increase the pathogenicity of HIV by acting as a co-factor.[179;180] In vitro experiments have shown CMV to activate HIV gene expression or alter the cellular tropism of HIV through a variety of mechanisms (antigen presentation, cytokine release, pseudotype formation, CD4 cell surface upregulation, Fc receptor formation, transactivation). The beneficial effect of acyclovir on the survival of HIV-positive patients could well be related to the inhibition of the herpes viruses acting as co-factor in vivo. Some additional observations point to a role of CMV in the evolution of HIV infection. There is an increased rate of HIV infection progressing to AIDS in patients shedding multiple CMV strains in semen as compared to patients shedding a single strain or not shedding CMV.[181] The age adjusted relative risk of developing AIDS is 2.4 times higher for HIV-positive haemophilia patients, who are CMV seropositive, than for those who are seronegative for CMV.[182]

DIFFERENTIAL DIAGNOSIS

CMV retinitis is the most common cause of necrotizing retinitis in patients with the acquired immunodeficiency syndrome. Less frequent causative agents are Toxoplasma *gondii*, Varicella Zoster, and Herpes simplex virus.[183-191] Even more rarely other species are involved like *Pneumocystis Carinii*, Mycobacteria, *Treponema pallidum* or fungal species like *Cryptococcus neoformans*, *Histoplasmosis*, or *Candida albicans*.[192-197] Besides these other infectious retinitis cases 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.

Clinical differential diagnosis

Other infectious diseases of the retina in HIV-positive patients

The most frequent cause, besides CMV, of retinitis in HIV-positive patients is Toxoplasma *gondii*. Ocular toxoplasmosis accounts for 3 % of retinal infections in the HIV-positive population.[184;185;198;199] Toxoplasma retinitis is characterised by solitary, round yellow-white lesions, which have volume and seem to be prominent, with hazy or fluffy edges. Lesion size varies between less than one disc diameter to over 5 disc diameter.

Haemorrhages are absent or scarce and no vasculitis is normally seen. No pre-existing toxoplasma chorioretinitis scars can be observed. Retinitis is unilateral in 82% and most of the time unifocal in 83%, with half of the lesions starting in the posterior pole and the other half in the periphery.[190; 198] Very rarely a miliary pattern has been described. [183] The presence of inflammatory signs is the most helpful aid in the differential diagnosis. Anterior segment involvement, keratic precipitates and anterior flare or cells and even posterior synechiae, is present in 60% of eyes, and vitreous reaction, although to a much lesser extent than in the immunocompetent patient, exists in 72% of cases.[198 185] Fluorescein angiography can be of help in the differential diagnosis. In toxoplasma retinitis the fluorescence starts at the edge of the lesion and progresses to the centre, with the final area of hyperfluorescence larger than the lesion seen by fundoscopy. In CMV retinitis lesions the fluorescence starts in the centre of the lesion and spreads to the border, and final hyperfluorescence is less than the observed lesion. [198] Another important fact to consider is the associated cerebral toxoplasmosis, which can be found in 30% of patients. [198] HIV-positive patients with toxoplasma retinitis and / or cerebral toxoplasmosis have overall higher CD4+ lymphocyte counts, between 50 to 350 cells / mm^3, than patients with CMV retinitis, normally less than 50 cells / mm^3, but values do overlap. CT scan is mandatory in patients with (suspected) toxoplasma retinitis and can help in the differential diagnosis. Because of the relative frequent occurrence of toxoplasma retinitis and CMV retinitis both can develop in the same patient. In one third of patients with toxoplasma retinitis CMV retinitis has been described to develop, which, according to Cochereau ,et al., appears not to be more common compared to the whole group HIV-positive patients. [198]

Necrotizing retinitis can also be caused by Varicella Zoster Virus or Herpes Simplex Virus, both giving rise to two different clinical pictures. Either a retinitis which has the characteristics of Acute Retinal Necrosis (ARN) or a retinitis which has been described as Progressive Outer Retinal Necrosis (PORN). [200] Although most of the cases have been ascribed to VZV, there is indirect proof of Herpes Simplex Virus type 1 and type 2 in some cases of PORN and ARN. [201; 202] HSV and VZV are the third-most-frequent cause of retinitis in HIV-positive patients, diagnosed in 1.1% of AIDS patients. [184; 188] It is speculated that the severity of immune-deficiency determines the form in which the retinitis presents itself: ARN occurring in patients with a less affected immune system than PORN. [200] ARN has been described in patients with relatively high CD4+ lymphocyte counts, while PORN is exclusively reported in patients with CD4+ counts below 50 cells/mm^3, with a mean of 25 cells / mm^3. Both types occur in 50 to 80% of cases after or sometimes concomitantly with

an episode of Zoster or Herpes keratitis, or dermatitis, which may be remote, but generally falls in the 18 months before the development of retinitis.[200; 203]

ARN is characterised by yellow-white peripheral lesions, which compromise the entire circumference in a short time, accompanied by an occlusive vasculopathy, 80% of cases showed arteriitis.[203] ARN is much more rapidly progressive than CMV retinitis. ARN responds poorly to therapy, and is frequently complicated by retinal detachment, leading to blindness in over 80% of cases.[203] The eye is painful especially when the ARN is associated with scleritis. Visual loss may be acute and early in the course of the disease due to optic neuritis. Partial loss of visual acuity, restriction of visual fields, and dyschromatopsia can occur within a period of 8 days prior of the diagnosis of ARN, suggestive of a preceding optic neuritis.[203] In contrast with CMV retinitis there is a marked inflammation of the vitreous and sometimes also of the anterior segment.[184; 188; 204] ARN is bilateral in 54% of cases.

PORN is characterised by deep retinal multifocal lesions, with early perifoveal lesions, and a very rapid progression. Macular lesions were reported in 32% of cases at diagnosis.[205] PORN is not or only minimally accompanied by an inflammatory response of vitreous or anterior segment in contrast to ARN, and resembles CMV retinitis in this respect. Despite treatment, visual loss, to no light perception, has been reported in almost 70% of cases within 4 weeks after diagnosis.[205] [206] Second eye involvement is frequent and occurs early, within weeks of diagnosis of PORN in the first affected eye. Lesions in PORN lack a granular border, lack extensive haemorrhages and do spread very rapidly, these features help to distinguish PORN from CMV retinitis. The involvement of outer retina, the absence of occlusive vasculitis, and the absence of inflammatory signs differentiates PORN from ARN. PORN is also complicated by retinal detachment, detachments have been reported in 70% of patients.[205; 206] PORN is associated with an increased risk for VZV encephalitis.[200; 207]

Syphilis is more common in the HIV+ individuals compared to the general population. HIV modifies the course of the disease increasing the rate of cases complicated by neurosyphilis and accelerating the development of late manifestations. Ocular syphilis most commonly presents itself with anterior and intermediate uveitis, but occasionally posterior uveitis has been described with papillitis, and vasculitis. Retinitis also has been reported and can take the form of placoid chorioretinitis, which is located in the posterior pole, or necrotizing retinitis, which can start in the periphery.[195; 208-210] This last presentation of syphilis can resemble CMV retinitis. In syphilitic retinitis the inflammatory response in the eye is much more pronounced with a sometimes dense vitreous reaction and anterior

chamber cells and flare. CD4+ lymphocyte counts are generally higher, and one has to ask for a history of primary syphilis and look for the cutaneous signs of secondary syphilis. Almost all HIV-positive patients with ocular syphilis suffered from neurosyphilis as well, frequently with meningeal signs and symptoms, for this reason cerebrospinal fluid examination is mandatory.

Vascular abnormalities in HIV-positive patients

Although CMV is by far the most frequent cause of retinitis in HIV-positive patients, CMV retinitis is not the most frequent observed retinal abnormality in these patients. Non-infectious HIV related retinal vasculopathy is much more frequent, seen in about 70% of patients. This vasculopathy causes microinfarctions of the retinal vessels leading to small haemorrhages and cotton wool spots. This combination of haemorrhages and whitish lesions can make the differentiation between both lesions difficult. The prevalence of HIV related vasculopathy increases with a decrease in CD4+ lymphocyte count, and is much more frequent in patients with CD4+ lymphocyte counts below 100 cells / mm^3. [53] Nevertheless this non infectious vasculopathy tends to be seen in patients with higher CD4+ counts compared to patients at risk for CMV retinitis. Some authors believe that HIV related vasculopathy acts as a porte d'entree for CMV and plays an important role in the pathogenesis of CMV retinitis. [97] Other distinguishing features are the complete lack of inflammatory signs in HIV related vasculopathy, the involvement of only the superficial layer of the retina, the multifocal location of the cotton wool spots with a predilection of the posterior pole, and the lack of symptoms. In case of severe doubt the most prominent differing factor is the resolution of cotton wool spots in 3 to 4 weeks, whereas lesions of CMV retinitis will double their size in one month time. If central vision is not jeopardised one can simply wait and follow the patient closely, progression of the lesion excludes HIV related vasculopathy. [200]

Another vascular abnormality which has been described in HIV-positive patients is central or branch retinal vein occlusion. [147; 211; 212] Most authors believe this to be a vascular abnormality which is either primary, or which is related to HIV vasculopathy. Friedman et al.reported about the histopathologic findings in one patient with bilateral central retinal vein occlusion. He found no evidence of any structural or infectious cause for central retinal vein occlusion, and concluded that other hemorheologic factors are probably responsible for the predisposition to vascular thrombosis. [147] In a few patients branch retinal vein occlusion may present with a picture which resembles CMV retinitis. The presence of extensive intraretinal haemorrhage with mild retinal whitening and the absence of a

granular border distinguishes the two. Fluorescein angiography may also be of help, showing stasis or occlusion in case of venous occlusion. [212]

Intraocular malignancy, B-cell lymphoma

B-cell non-Hodgkin lymphoma is a common AIDS related cancer. Immune suppressed individuals have a 100-fold greater risk for systemic and primary CNS lymphomas, nevertheless reports of intraocular lymphoma in AIDS patients are rare. [200] The ocular manifestations are similar to those in immunocompetent patients: small retinochoroidal infiltrates, optic nerve head swelling , and some vascular sheathing. Vitreous involvement is common, but usually mild. The retinal lesions are creamy white, may have associated haemorrhages, and can be quite large in size, with sharply defined borders. These lesions may be confused with CMV retinitis. Most lesions are deeply situated in the retina, and show pigment epithelial alterations. Sometimes a serous detachment is present overlying the retinal lesions. There is a very high rate of central nerve system involvement and computed tomography scans or magnetic resonance imaging can help in the differential diagnosis. Diagnostic vitreous biopsy should be reserved for those patients with negative results in these tests.[184]

Other forms of retinitis

There are other forms of retinitis, or chorioretinitis associated with a variety of systemic infections. The clinical setting and the ocular findings of these patients normally do not cause much difficulty in differentiating them from CMV retinitis.

Ocular mycobacterial infections are thought to be a manifestation of systemic disease. Lesions of *Mycobacterium avium intracellulare* have only been described in patients at autopsy series and have consisted of isolated choroidal granolomas, discrete and slightly elevated. They do not cause complaints in the absence of vitritis and are not clinically important. [200; 213; 214] Mycobacterium tuberculosis can cause a prominent anterior chamber granulomatous reaction, a moderate vitritis, and yellowish-white choroidal lesions that can be slightly elevated and typically spare the overlying retina. [200; 215]

Two patients have been described with a clinical picture of fever, weight loss, and diarrhoea and retinal lesions. These lesions, multifocal yellow-white infiltrates with subretinal exudation regressed after instillation of therapy with doxycycline. The exact nature of this presumed endogenous bacterial retinitis was not determined. Gram positive organisms were implicated. [216]

Pneumocystis Carinii infection is common in AIDS patients, but ocular

involvement is rare. [217-219] Patients most often present with a multifocal choroiditis with numerous, deep, round, creamy yellow, slightly elevated ½ to 1 disc diameter in size lesions located in the posterior pole. [194] No inflammatory signs accompany the ocular infection, neither in the anterior segment nor in the vitreous. Even more seldom patients have been reported with unilateral, unifocal, yellow, 1 to 3 disc diameter in size, choroiditis. [192] A relation seemed to exist between the occurrence of pneumocystic choroiditis and the start of using aerosolised pentamidine prophylaxis against pneumocystic pneumonitis, which would be insufficient to protect the eye. In later years prophylaxis proved to be complete and very efficient. [200]

Intraocular *Cryptococcus neoformans* is far less common than the neuro-opthalmologic complications secondary to cryptococcal meningitis. Papiledema, abducens paresis, and optic atrophy are the most frequently occurring ophthalmic complications. [193] Intraocular cryptococcal invasion most commonly leads to chorioretinitis. A few of these cases have been described in AIDS patients. [193; 220; 221] In one of these patients a white elevated chorioretinal cryptococcoma was present which responded well to treatment with fluconazol. [193]

Candida albicans can also cause a choroiditis or chorioretinitis with overlying vitritis, but as with al other infections of the eye in AIDS patients inflammatory signs are minimal to mild. Most patients had a history of intravenous drug use, or sepsis from an indwelling vascular catheter. Infection starts deep in the retina and progresses towards the vitreous cavity. Upon reaching the vitreous the inflammation becomes more intense. [184]

Disseminated histoplasmosis is one of the life-threatening opportunistic infections associated with AIDS. Ocular histoplasmosis has been reported in occasional AIDS patient with disseminated histoplasmosis. [196; 222] The retinal lesions are described as creamy white intraretinal lesions, 1 / 6 to 1 / 4 disc diameter in size, with sharp borders, and with subretinal infiltrates in both eyes. Vitreous inflammation was minimal in these patients. Histopathology demonstrated the presence of *Histoplasma capsulatum* in retina, optic nerve, and choroid. Patients lived in an endemic area of the US, no cases have been described outside these areas.

Despite the fact that over 90% of retinitis in HIV-positive patients is caused by CMV, one has to be aware of alternative pathogens, especially in atypical cases. In most cases the clinical appearance of the lesions after careful ophthalmologic examination is sufficient to make a correct diagnosis. The different pathogens differ in the clinical signs and symptoms, which enables the ophthalmologist to make a correct diagnosis after careful slitlamp examination and fundoscopy.

Especially in cases in which the classical pattern of CMV retinitis is absent,

history, and systemic findings become much more important in making a correct diagnosis. CD4+ lymphocyte counts above 100 cells / mm^3 must make one suspicious of alternative pathogens beside CMV, like toxoplasma, VZV, or HSV. HIV related vasculopathy has to be considered, and sometimes it is best to follow a patient carefully for signs of progression of the lesions before starting anti-CMV therapy, and commit the patient to life long maintenance therapy. Systemic disease status has to be taken into account, and discussed with the treating specialist of the internal department, because most of the more rarely occurring intraocular inflammations are part of a more generalised systemic disease, like syphilis, tuberculosis, or B-cell lymphoma. Additional examinations can give a clue towards the involved pathogen, like CT-scan or MRI to disclose cerebral involvement in patients with toxoplasma or non-Hodgkin lymphoma.

CMV retinitis can occur concurrently with other retinal infections. Dual infections have been reported with Toxoplasma *gondii*, *Cryptococcus neofermaus*, and HSV. [104; 185; 189; 202; 223-225] In atypical cases of retinitis, which do not respond as expected to therapy, one should always be suspicious of the possibility of a dual infection. It has been suggested that multiple infections occur more frequently than expected by chance alone. Some synergistic effect has been proposed to explain this phenomenon. Perhaps some pathogens facilitate infections with other organisms. Or perhaps CMV infection causes a more pronounced immune depression allowing other pathogens to gain entrance into the eye. Toxoplasma and CMV retinitis have been described in different parts of the same eye, so coincidental infection is also possible, because CMV retinitis is a frequent opportunistic disease in AIDS patients.

Laboratory investigations

Diagnosis of CMV retinitis relies on clinical findings, especially the fundus appearance, and the lack of inflammatory signs. In some patients the presence of a systemic disease makes other etiologies more probable like non-Hodgkin lymphoma or syphilis. 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. Results of any test should be interpreted in the context of the whole clinical impression of the patient and with exclusion of other possible causative pathogens. [226]

Viral culture

Cytomegalovirus can be isolated from blood, urine, saliva, semen, broncho-alveolar-lavage, and tissue biopsies. Classical culture techniques uses human fibroblasts. Within the cell culture a characteristic cytopathic effect can be observed which occurs usually within 2 to 3 weeks, but can take as long as 6 weeks to become positive. More rapid culture techniques have been developed, the most commonly used is the shell vial method. This technique combines the use of centrifugation, to increase the sensitivity of viral detection, with monoclonal antibodies to detect early CMV antigens, indicative of active CMV replication. Shell vial method is faster but less sensitive than the conventional method of culture. Results can be obtained within 8 to 32 hours of cell culture infection. Although viral culture is an important diagnostic technique, a clear relation between a positive culture out of blood or urine and the presence of tissue destructive disease anywhere in the body is missing. [227] Isolation of CMV from peripheral sites confirms active replication but is not diagnostic for target organ disease. Additionally, AIDS patients may have positive CMV cultures without evidence of ocular disease. [226 227] The likelihood of a positive blood or urine culture correlates with immunologic status of a HIV-positive patient and has little relation with the current or future clinical CMV status of the patient. [70; 227] At best a negative result of blood or urine culture suggests the absence of current CMV disease. [228]

Biopsy site is essential and the best method would be direct detection of CMV in retinal tissue, which for obvious reasons is not feasable. Viral culture of aqueous humour never yielded a positive result, possibly because of the minute volume of the sample and the presence of inhibitors like neutralising antibodies, hampering in vitro propagation of CMV. CMV has been cultured from vitreous samples, but samples were taken post mortem. [202; 226]

Serology

Serologic tests for antibodies against CMV have little or no use in HIV-positive patients at risk for developing CMV disease. In this population the seropositivity for CMV is almost 100% [50; 202] Additionally changes in antibody titers may not occur in immunocompromized individuals. [202; 229] Anti CMV IgM antibodies may persist for many months after infection, or can become positive during virus reactivation. For this reasons serologic tests only have limited value in AIDS patients. Cytomegalovirus retinitis has even been described in a patient without a positive complement fixation test on serum. [171; 202]

Antigenemia test and PCR based DNA-emia

The CMV antigenemia test and the PCR based DNA-emia assays have also been used to confirm a diagnosis of a clinical manifest CMV disease. Most authors concluded the pp65-antigenemia test of limited value for diagnosis of concomitant CMV disease, because of its low positive predictive value. The correlation between a negative test and the absence of CMV disease, using a high level of pp65-antigenemia, is very strong, but a positive test is weakly correlated with the presence of CMV disease. [75; 76; 230-234]

Gerna et al. performed a study of 62 patients with a presumed CMV retinitis, before treatment, and compared pp65 antigenemia, CMV DNA emia, and results of viral culture. In 56 patients CMV DNA could be detected in PBL's of patients. The 6 negative patients were erroneously enrolled in the study, because all 6 proved to have only extensive cotton wool spots and haemorrhages, without active retinitis. The pp65-antigenemia test was positive, taking a cut-off level of 10 positive cells / 10^5 leukocytes, in 37 patients, and conventional viral culture was positive in 32 patients. [235]

A negative PCR or a negative pp65 antigenemia test is a very strong indication of the absence of CMV disease, but a positive test is only weakly correlated with active CMV disease. These tests can be positive in patients without retinitis, as has been shown in many studies to determine the predictive value of the tests for future development of CMV retinitis. For this reason alone the test can not be used in the diagnosis of CMV retinitis.

Additionally all these studies concentrate on HIV-positive patients with and without CMV disease. They do not compare patients with necrotizing retinitis caused by other pathogens, with patients with CMV retinitis. Therefore the clinical usefulness of these tests in differentiating between CMV and other etiologic factors of retinitis in AIDS patients still have to be established.

Testing of ocular fluids

PCR

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. [153; 202; 235-243] Once it was thought that the sensitivity of the method would yield too many false positive results, especially in HIV-positive patients, who will most of the time suffer from more than one opportunistic infection, active or latent. In fact the method proved to be both sensitive and specific. Control samples of patients without HIV infection, but with other ocular inflammations or vitreous haemorrhages, were seldom positive for the pathogens tested (see Table 2, page 39). More

importantly Danise et al. reported negative results in all aqueous humour control samples taken from 27 HIV-positive patients with uveitis, but without retinitis. Patients were tested for the presence of DNA from CMV, VZV, HSV, and toxoplasma. [243]

Most investigators prefer vitreous samples above aqueous humour samples. In aqueous humour the number of false negatives seems to be higher. [243] Mitchell et al demonstrated the presence of VZV DNA in the aqueous humour in two patients, and at the same time CMV DNA in the vitreous, both with a final clinical diagnosis of CMV retinitis. [153] At the time of sampling both patients showed signs of Zoster ophthalmicus inflammation, with a keratouveitis, characteristic of an anterior segment inflammation caused by VZV. These cases seem to be exceptions rather than the rule.

Verbraak et al. reported two patients with a clinical diagnosis of toxoplasma retinitis with a positive PCR assay for CMV in aqueous humour. One of these patients was thought to have a dual infection, but excessive breakdown of the blood aqueous barrier was the only explanation in the second patient. As such a breakdown will be present in all cases of necrotizing retinitis to some extent, the authors conclude that results of PCR testing must be interpreted with caution and can never replace clinical judgement. [202]

Different investigators used different methods of DNA extraction, and also different primers. Not all pathogens were tested in each sample, so one is not allowed to simply add all data. Nevertheless, with this in mind, to get an overall impression, the totals are given in table 2 of those studies, which tested for different pathogens. In aqueous humour the PCR assay used, demonstrated the causative pathogen in accordance with the final clinical diagnosis in 42 of the 47 samples tested. Three samples were false-negative, and two samples were false-positive. In vitreous samples agreement between the test result and final diagnosis was present in all 127 samples tested. Toxoplasma was not tested in vitreous samples. In none of the 47 control aqueous humour samples, DNA of one of the tested pathogens could be detected, 27 of these controls were HIV-positive patients with uveitis without retinitis at the time of sampling. In the 125 vitreous control samples, only two tested false-positive. One of these patients, with an old previously confirmed toxocara granuloma in the tested eye, developed CMV retinitis 6 weeks after the sample was taken. [153]

The procedure to take a vitreous biopsy has become relatively easy and uncomplicated, but is still far more complicated compared to a paracentesis. Both procedures have in common that the eye needs to be perforated, and both theoretically can cause an endophthalmits. This has never been reported after paracentesis or vitreous biopsy, but has been reported following intravitreal treatment of retinitis. A vitreous biopsy can cause a vitreal haemorrhage and is an additional risk factor for retinal detachment

in a patient with a necrotizing retinitis, who is already at high risk for the development of a retinal detachment. Detachments have never been attributed to the sampling procedure, but one can never be completely sure that the biopsy did not play some role in the development of a later occurring detachment. Complications are practically never seen following a paracentesis, the largest series reported only one complication, secondary cataract following puncture of the lens, in over 300 procedures. [244] The advantage of an aqueous humour tap, as an easy procedure without complications, outweighs the slightly higher accuracy of the vitreous biopsy. Perhaps the only exceptions are patients with obvious anterior segment inflammation due to VZV or HSV, like those reported by Mitchel, where aqueous humour tested erroneously positive for VZV and the vitreous sample proved to test positive for CMV which was considered to be the real pathogen involved.

The standard for comparison is the clinical diagnosis in all studies reported. This may be not reliable enough to assess sensitivity and specificity of a laboratory method. A definite diagnosis can only be established with the histopathologically proven presence of the causative pathogen in the involved retina. [239] This is, for obvious reasons, impossible to obtain in patients. Fox has described results of DNA detection in aqueous humour and vitreous samples in 4 patients post mortem. Samples were obtained within 24 hours after death. Two eyes, one patient, showed histologic evidence for CMV retinitis, and tested positive for CMV DNA in both aqueous humour and in vitreous. The 6 eyes of 3 patients, without histologic proof of CMV retinitis, all tested negative in aqueous humour, and positive in only one vitreous sample. [238]

Comparing the results of both sample types, vitreous fluid 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.

Intraocular antibody production

Measurement of intraocular antibody production by analysing aqueous humour samples has been proven to be a useful adjunct in the differential diagnosis of aspecific retinitis in immunocompetent patients. [245-248] Intraocular antibody production was determined by calculation of the Goldmann-Witmer coefficient. The ratio of anti-herpes virus or anti-toxoplasma antibody level in serum and aqueous humour was compared to

Table 2 PCR based assay of ocular fluid samples for differential diagnosis of necrotizing retinitis in AIDS patients

Author (year)	Sample Type *	Sample Number	PCR CMV	VZV	HSV	Toxo	Definite Diagnosis
Fenner (1991)	AH	13	12	nd	nd	nd	CMV
	AH	3	-	nd	nd	nd	Toxo/VZV
	AH	12	-	nd	nd	nd	Control (non HIV +)
Fox (1991)	VF	9	9	nd	-	nd	CMV
	AH	3	3	nd	-	nd	CMV
	SRF	5	5	nd	-	nd	CMV
	VF	8	-	nd	-	nd	Control (non HIV +)
	AH	18	-	nd	-	nd	Control (non HIV +)
Garweg (1993)	VF	5	4	-	-	nd	CMV
	AH	9	8	-	1	nd	CMV (1 double CMV+HSV)
Stewart (1993)	VF	2	2	nd	-	nd	CMV
Mitchel (1994)	VF	41	40	-	-	nd	CMV (2 weakly pos)
	VF	5	-	5	-	nd	ARN
	VF	2	-	-	-	nd	Toxo
	AH	2	-	2	-	nd	CMV (both typical VZV Anterior Segment Keratouveitis)
	VF	50	1	-	-	nd	Control (non HIV +)
Gerna (1994)	AH	15	15	nd	nd	nd	CMV, untreated patients
	AH	12	8	nd	nd	nd	CMV, treated patients
McCann (1995)	VF	19	18	-	-	nd	CMV, untreated patients
	VF	40	19	-	-	nd	CMV, treated patients
	VF	3	-	-	3	nd	ARN / HSV
	VF	4	-	4	-	nd	ARN / HSV
	VF	6	-	-	-	nd	other (HIV + , non viral ocular disease)
	VF	54	-	-	-	nd	Control (non HIV +)
Doornenbal (1995)	AH	2	2	-	-	nd	CMV
	VF	3	3	-	-	nd	CMV
	VF	1	1	1	-	nd	CMV
	VF	1	-	1	-	nd	ARN
	SRF	1	1	-	-	nd	CMV
	AH	2	-	-	-	nd	Control (non HIV +)
	VF	7	-	-	-	nd	Control (non HIV +)
Verbraak (1996)	AH	13	13	-	-	-	CMV
	AH	2	2	-	-	1	Toxo (1 double pos)
Short (1997)	VF	14	-	11	-	nd	PORN (3 neg, heavily treated)
Danise (1997)	AH	5	5	-	-	-	CMV
	AH	3	-	3	-	-	ARN
	AH	7	-	-	-	6	Toxo
	AH	2	-	-	-	-	ARN/Toxo
	AH	27	-	-	-	-	Control (HIV +)
Total	AH	32	31	-	-	-	CMV
	AH	2	-	2	-	-	CMV
	AH	4	-	4	-	-	ARN
	AH	9	1	-	-	7	Toxo (1 double pos)
Total	VF	89	86	-	-	-	CMV
	VF	32	-	29	-	-	VZV
	VF	6	-	-	6	-	HSV

AH = Aqueous Humour, VF = Vitreous Fluid, SRF = Sub Retinal Fluid, For additional comment see text.

ratio of total IgG in serum and aqueous humour. A coefficient exceeding the value of 3 was considered to be positive. Verbraak et al. reported a positive Goldmann-Witmer coefficient in 11 out 28 of tested patients with a necrotizing retinitis indicating local antibody production against the involved pathogen. The high rate of negative results (60%) were explained by antibody production below detection levels, or by factors masking a measurable normal antibody production. Abnormal or deficient B-cell function in AIDS patients could contribute to the low sensitivity of serologic diagnosis in these patients. [249;250] An impaired antibody response has been described in the late stages of AIDS both in quantity and in quality. [251] Doornenbal et al. found no confirmation of the diagnosis of CMV retinitis in the 9 patients tested. In this study one of the patients tested positive for CMV, VZV, and HSV, most probably due to aspecific polyclonal stimulation.[236]

Detection of local antibody production by determination of the Goldmann-Witmer coefficient in aqueous humour samples is by far not as sensitive as the PCR assay and probably also not as specific in AIDS patients with a necrotizing retinitis. Because of a notoriously variable IgG response the test is not useful in this group of patients. [226]

THERAPY

In patients with a iatrogenic immunosuppression the best way to treat CMV retinitis is by discontinuation or reducing the dose of immunosuppresive drugs. In case treatment cannot be stopped or reduced, antiviral treatment becomes necessary until sufficient restoration of the immune-response has occurred. In AIDS patients immune-response deteriorates progressively. Additionally, response to anti-viral treatment in patients with HIV infection was generally worse than in patients immunosuppressed by other causes.[252; 253] Without therapy CMV retinitis in AIDS patients inevitably leads to blindness in the course of 6 months. [254; 255] The HIV epidemic made CMV retinitis in a short period of time the most common intraocular infection, and stimulated the search for effective anti-viral therapy enormously. After proving to be effective in uncontrolled studies, conducted since 1980, ganciclovir was approved by the FDA in June 1989 for the use in treating CMV disease, based on a retrospective randomised study. [17] Followed by foscavir, which was licensed for treatment of CMV retinitis in September 1991, based on a prospective randomised study by Palestine et al..[13] With the advent of ganciclovir and foscavir the first effective anti-CMV drugs to be registered, two very potent antiviral drugs became available. Both drugs are only virustatic and can not eradicate CMV from the infected retina. Discontinuation of therapy inevitably leads to relapse of retinitis in a short period of time. This necessitates lifelong

secondary prophylaxis or maintenance therapy for CMV retinitis in AIDS patients.

Assessment of extent and progression of CMV retinitis

At the start of the AIDS epidemic reports about CMV retinitis, its treatment and its progression were difficult to compare due to lack of consistency in the description of the disease and the treatment outcome measurements. Visual field determinations have been used to monitor patients with CMV retinitis.[89;256] Serial measurements can indeed detect loss of visual field corresponding to enlargement of the lesions, but the method lacks sensitivity to detect the early changes of reactivation of retinitis. For patients with central lesions in zone 1, extending 20 degrees from the fovea, Amsler grids can be used by the patients to self-monitor progression. Generally there is a sharp demarcation of the scotomata caused by the lesion which enables patients to detect progression if it occurs.

A system for assessment of disease outcome was developed by Holland et al., that uses retinal photographs and three factors: development of new lesions, enlargement of pre-existing lesions, and change in retinal opacification of lesion borders.[17] Progression of retinitis was defined as any new lesion, or an enlargement of a pre-existing lesion with more than 750 μm. Change in disease activity observed as an increase in opacification of the edge of a pre-existing lesion was also considered to be a sign of impending progression. The border of a pre-existing lesion can suggest disease activity, but without actual progression, showing a whitish border, which represents gliosis with calcification, or conversely can look innocent, while slowly progressing, a phenomenon which is called smouldering retinitis.[114]

To describe the extent of the retinitis the retina was divided into three zones. Zone 1 consisted of the central macular area, within the major vascular arcade, and the retina around the optic disk (1 disc diameter, 1500 μm, from the disc and 2 disc diameter, 3000 μm around the fovea). Zone 2 extended from the edge of zone 1 to the equator of the eye. Zone 3 extended from the equator to the ora serata. Lesions in zone 1 were considered to be immediately sight threatening, and lesions in zone 3, lying directly under the vitreous base, would predispose to retinal detachment by vitreoretinal traction. The extent of the involved retina was measured in a semi quantitative way: 10% of retinal area involvement, between 10 and 25%, between 25 and 50% , and more than 50% involvement. The area within the macular vascular arcade was used as reference and consisted of 5% of retinal surface.

In several large trials assessment of progression was performed by a central reading centre using a masked grading of fundus photographs, and by the participating clinicians.[257;258] The movement of the border of retinitis

was detected sooner, and activity of the border was considered to have increased more often, when evaluated by the fundus photograph reading centre than by the clinician. Disagreement between observations was mainly caused by difficulty to detect progression by clinicians in the absence of an obvious increase of border activity, and by small border advancement in the first 3 to 5 weeks, after initiation of therapy, surpassing the threshold for border movement of the reading centre, while retinitis responded favourably to anti-viral treatment. [259; 260]

Side-by-side comparison of good-quality photographs from the current visit (as soon as they are available) with photographs from previous visits may be superior in detecting progression accurately, but the ability to detect progression by photographs depends entirely on the ability to capture a clear image of the retinal area of interest. For peripheral lesions, in case of media opacities, or a camera out of focus, inaccurate assessments are inevitable. Besides in some patients with advanced AIDS who require bedside examination it is not practical to perform photography. For practical reasons clinical assessments usually involve comparisons between current ophthalmoscopic appearances and photographs (or diagrams) from previous visits. In this way decisions about patient management can be made immediately. Perhaps a combined approach provides the most accurate information. For central lesions retinal photographs are more accurate, whereas for diffuse and peripheral lesions fundoscopy is preferable. [261]

Monitoring therapy with PCR or antigenemia

Patients with CMV retinitis have been followed by pp65-antigenemia and PCR assays to determine the viral load and the effect of treatment on viral load. Conclusions that can be drawn from these studies are the following. In all patients with a favourable response to therapy there is a dramatic decrease of pp65-antigenemia and of PCR based DNA-emia. [69; 79; 235; 262-267] Not all patients become negative for these tests following initiation of therapy, even though clinical response is sufficient (around 13% remain positive). Patients with the highest base-line levels of viral load, remain positive and have a shorter time to relapse (21 versus 72 days) and a shorter time to death (difference in median survival of 121 days). [235; 263; 264] Patients with CMV retinitis have significantly lower levels of CMV viral load, compared to patients with an extra-ocular CMV disease. The CMV viral load increases with development of a relapse of CMV disease, and this increase precedes the recurrence in most, but not all patients. Patients have been reported with negative or low level viral load at the time of recurrence.

Systemic therapy

Whatever systemic drug is chosen for the treatment of CMV retinitis the treatment scheduling 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. Longer induction periods in these cases are not warranted. Generally a full response can be seen 4 weeks after initiation of therapy. [255; 268]

Present antiviral drugs suppress CMV replication but are unable to eliminate the virus from the eye. Viral particles are still present at the borders of lesions as shown by electronmicroscopic studies of eyes treated with ganciclovir. [269] After discontinuation of treatment a relapse will generally occur after a 3 week period. [270] As a consequence secondary prophylaxis has to be given for the rest of the patient's life.

a. Ganciclovir

Ganciclovir is a nucleoside analogue that is taken up into viral infected cells, triphosphorylated, and then inhibits viral DNA replication. After approval by the FDA in 1989 a randomised controlled trial in patients with small peripheral lesions proved efficacy of ganciclovir as initial treatment. [271] In a previous study ganciclovir was shown to significantly prolong the time to relapse in patients receiving ganciclovir as maintenance therapy. [270] Intravenous ganciclovir treatment is initiated with an induction course of ganciclovir 5 mg / kg / twice a day, followed by life-long maintenance therapy of ganciclovir 5 mg / kg / day for 7 days, or 6 mg / kg / day for 5 out of 7 days, to prevent reactivation. The 5 out of 7 days maintenance was developed for convenience of the patients. Comparisons between both maintenance schedules have never been studied.

The most important toxic side effect of ganciclovir is bone marrow suppression, which is reversible when ganciclovir is discontinued. In the first years of administration of ganciclovir 38% of patients developed dose-limiting neutropenia.[272] In 1991, in a closely monitored group of patients treated with ganciclovir, 16% were reported to have dose limiting neutropenia (defined as an absolute neutrophil count < 500 cells / μL) and 5% to have dose limiting thrombocytopenia (defined as platelet count $<$ 20000 / μL). [273] Few patients were able to tolerate combined treatment

of full doses of ganciclovir and zidovudine.[274] Much of this complication can now be counteracted by the concurrent use of granulocyte-monocyte colony stimulating factor (GM-CSF) or granulocyte colony stimulating factor (G-CSF).[275; 276]

Oral maintenance with ganciclovir

In 1995 two large studies independently showed the efficacy of oral ganciclovir as maintenance therapy. Time to progression, assessed by photographic evaluation, was compared between an oral dosage of 3 gr / day and intravenous ganciclovir in standard dose (5 mg / kg / day). In the European / Australian study mean time to progression in the oral maintenance group was 51 days and in the intravenous group 62 days.[275; 277] In the American study time to progression was 57 days in the oral ganciclovir maintenance group and 62 days in the intravenous group.[275; 278] Both studies found these differences to be not significant. The conclusion of both studies was that the advantage of oral therapy outweighed the slightly shorter time to progression found in the oral treated group. It justified the choice of an oral dose of 3 gr / day of ganciclovir as first line maintenance treatment in those patients without immediately sight threatening lesions.[275;279] Toxic side effects were less frequently reported in the oral compared to the intravenous maintenance therapy assigned group. A big advantage of oral over intravenous maintenance therapy is that an indwelling catheter can be avoided. Besides the fact that these catheters can become infected resulting in sepsis (at a rate of 2 per 1000 catheter days), patients experienced the placement of such a catheter as an enormously negative stigma.

b. Foscavir

Foscavir is a pyrophosphate analogue inhibiting CMV replication. In contrast with ganciclovir, foscavir does not need a first phosphorylation. After FDA approval in 1991, foscavir was given intravenously in induction dosages of 60 mg / kg / three times a day. More recently it has been shown that induction with 90 mg / kg / twice a day is equally effective.[280] Maintenance therapy used dosages of foscavir of 90 to 120 mg / kg / day.[281; 282]

Foscavir is nephrotoxic and can impair renal function. Because foscavir is excreted by the kidney an impairment of renal function will lead to higher foscavir levels, which again will result in increasing nephrotoxicity and can, in a short period of time, end in renal insufficiency. Close monitoring of creatinine levels and prompt adjustments of the dose in each patient is necessary. Concomitant saline hydration of 500 ml with induction dose,

and 1000 ml with each maintenance dose lessens the probability of nephrotoxicity. Using extra hydration and dose adjustments for renal function, the 6 month period prevalence of nephrotoxicity was still 13%. [283] Hypocalcemia, hypomagnesemia, and hypokalemia, were each reported in around 20% of patients receiving foscavir maintenance therapy. [283] Other side effects were dysuria, genital ulcers, infusion related nausea, and paraesthesias. [13;283;284] In contrast to previous observations seizures do not occur more frequently in patients treated with foscavir. [283]

Comparison between ganciclovir and foscavir

The SOCA (Studies of Ocular Complications of AIDS) research group conducted a large prospective randomised trial, the Foscarnet-Ganciclovir Cytomegalovirus Retinitis Trial (FGCRT), to compare both drugs. [257] Ganciclovir and foscavir are equally effective in treating CMV retinitis. As first line treatment both drugs bring active CMV retinitis under control after an induction course of 2 to 3 weeks. The median time to disease progression was 47 days for the ganciclovir assigned group and 53 days for the foscavir group. [258-260] At 120 days 85% of patients experienced one or more relapses of CMV retinitis. It was observed that with each recurrence the time interval to the next relapse decreased. Increased risk of progression was present in patients with bilateral disease from the start, and in patients with a CD4+ cell count less then 14 cells / mm^3 . Not associated with increased risk of progression were: location of lesions, size of lesions, appearance of lesions, interval from diagnosis of AIDS to diagnosis of retinitis, and the Karnofsky score. [259;260] A positive blood culture of CMV was another risk factor for a shorter time to progression (50% chance of recurrence of 45 days for CMV culture negative patients and 27 days for culture positive patients) . [285]

Survival

A much debated finding of the study by the SOCA group was the longer survival of patients treated with foscavir compared to those assigned to ganciclovir. The foscavir receiving group of patients had a median survival 4 months longer than the ganciclovir group. The exact cause of this difference has never been elucidated, but one of the possible explanations is the reported antiretroviral activity of foscavir. [268;286] One argument in favour of this assumption is the fact that in patients receiving the highest doses of foscavir maintenance therapy prolongation of survival was the longest. [287] The anti-HIV effects of both drugs were analysed by comparing the effect of the drugs on HIV p24 antigen levels in treated patients. [288] Each drug had a suppressive effect on circulating p24 antigen, which was predictive of improved survival, but no significant difference

between this suppressive effect could be demonstrated between both drugs. Polis et al. confirmed the increased survival in foscavir treated patients and even suggested that for this reason foscavir should become the initial treatment of choice in patients with CMV retinitis.[286] However other factors not controlled for in the study might play an important role, like differences in the anti-retroviral medications used by the patients. For example many patients treated with ganciclovir could not tolerate additional treatment with zidovudine, because both can cause bone marrow suppression.[274] Moyle et al. were not able to show a difference in survival between patients treated with ganciclovir versus foscarnet treated patients, and found that the longest survival was present in those patients who could tolerate zidovudine therapy, irrespective of their anti-CMV therapy.[284]

Clinical practice did not change despite the possible higher survival rate in the foscavir treated patients.[289] The gained survival has to be balanced against a higher rate of complications reported in the foscavir group (20% of patients on foscavir had to switch to ganciclovir due to drug toxicity versus an 8% switch to foscavir in the ganciclovir group) and a loss of quality of life due to the long infusion times necessary for foscavir (1 hour infusion time for ganciclovir versus 1 hour pre-infusion and 1 hour infusion for foscavir).[257;258]

c. Cidofovir

Cidofovir is a nucleotide analogue with a broad spectrum of activity against DNA viruses including CMV. Initial phosphorylation by viral kinases is not necessary and cidofovir is active in infected and uninfected cells. It has a long intracellular half life and for this reason administration can be infrequent, allowing the drug to be given intravenously, without the need for an indwelling catheter. FDA approval was provided in 1996 based on two randomised controlled trials comparing treatment of peripheral lesions with deferral.[19;20] Both studies showed treatment with weekly induction treatment of 5 mg / kg cidofovir, followed by maintenance therapy once every other week, with 3 or 5 mg / kg to be highly effective. Time to progression in patients with a first episode of CMV retinitis was 120 days in the treatment arm versus 22 days in the deferral arm of the study. An important dose limiting nephrotoxicity exists, which is partly counteracted by the concurrent use of probenecide and saline hydration with the cidofovir infusions. Patients have to be monitored very closely for development of proteinuria and a rise in creatinine due to destruction of the proximal tubule of the glomerulus occurring with cidofovir treatment. In the randomised trials mentioned, in 24% of patients treatment was stopped due to treatment limiting nephrotoxicity (2+

proteinuria or serum creatinine between 2-3 mg / dl). A second adverse effect was neutropenia which developed in 15% of patients during therapy. In addition around 20% of patients exhibited probenecide intolerance, manifesting as chills, headaches, high fevers, rash, or nausea, resolving after 12 hours to 3 days.

Surprisingly blood cultures and even urine cultures in which cidofovir is concentrated stay CMV culture positive during therapy. Base line blood cultures were positive in 41% and remained positive in 30% of patients at 11 weeks, and urine cultures at base line were positive in 87% and remained so in 40% of cases. This is in sharp contrast with culture results in ganciclovir or foscavir treated patients, which become negative in a short period of time in all responsive cases. Nevertheless extraocular CMV disease or involvement of the second eye in unilateral cases is not more frequent in cidofovir treated patients.

Local therapy

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 and foscavir have been successfully applied. The most common serial intravitreous schedule for ganciclovir is to inject 400 μg of drug 2 times weekly as induction therapy, followed by 400 μg injections once every other week as maintenance. [290-293] For foscavir, induction therapy consists of a 1200μg intravitreal injection every 3 days, followed by weekly injections of the same dose as maintenance. [294-296] Injections are given after topical anaesthetics and following 15 minutes of 30 mmHg decompression to the eye. That way the intraocular pressure is lowered before injecting the volume of 0.1 mL, adding to patient comfort, preventing temporary loss of vision, and avoiding reflux of injected fluid at the injection site. [297]

Recent studies have addressed the safety and efficacy of higher doses of the injected drug. Ganciclovir has been given in a dose of 2000 μg. [298; 299] It was shown that intravitreal concentrations remained above the inhibitory concentration (ID_{50}) for 7 days. Weekly administration did not result in accumulation in the vitreous. For this reasons an intravitreal dose of 2000 μg was recommended. Foscavir has been given in an intravitreal dose of 2400 μg, which did not result in local complications or intraocular drug toxicity, and seemed more effective in controlling retinitis. No conclusions can be drawn based on only limited observations of these higher intravitreal doses of ganciclovir or foscavir since no comparative studies have been performed.

The observation that direct delivery of the drug to the eye seemed to be more effective in halting CMV retinitis progression, lead to the use of

intravitreal therapy as adjuvant therapy to improve efficacy of systemic therapy. The use of this treatment modality in all patients with direct vision threatening lesions in zone I, in addition to systemic therapy, is recommended by many physicians. Another treatment strategy is the combination of adjuvant intravitreal injections in patients using oral maintenance therapy. This combination of therapies avoids at the same time the disadvantage of strictly local therapy, which does not treat extra-ocular CMV disease.

All patients with untreated CMV retinitis have evidence of visceral non-ocular CMV infection at autopsy, but it is not known at which rate these infections can cause clinically manifest CMV disease.[100] Autopsy reports have shown CMV inflammation to be present in a variety of organs. In a consecutive post-mortem examination of 48 AIDS patients Seregard found histopathologic evidence of CMV retinitis in 15 patients (31%).[300] In 14 patients a diagnosis of CMV retinitis was made before death and patients were treated with anti-viral therapy. In 10 of these patients associated non-ocular active CMV infections were present, most often in the brain and the adrenal glands, compared to 5 of 33 patients without retinitis. Patients have been reported with lethal CMV encephalitis, while they received adequate maintenance therapy for their CMV retinitis.[301]

Nevertheless systemic therapy for CMV retinitis has been shown to reduce the incidence of extra-ocular CMV disease.[302] Additionally survival appears to be prolonged in patients receiving systemic anti CMV therapy.[40;287] However, the possible benefit of early treatment of extra-ocular CMV infection versus treatment at the time disease becomes clinically manifest, has not been investigated.

Cidofovir can also be given intravitreally. A 20 μg injection of cidofovir was initially found to be both safe and effective. In a follow-up study of treatment of 24 eyes of 17 patients with intravitreal injections of 20 μg median time to progression after a single injection was 55 days. Eight patients received a second injection after which median time to progression was 63 days, rejecting early occurrence of resistance.[303-305] Not one patient showed a relapse within a period of 35 days and for maintenance therapy repeated injections every 5 - 6 weeks seems to be sufficient.[306] Patients did not receive additional systemic anti-viral therapy.

Two types of adverse effect can occur after intravitreal injections with cidofovir: hypotony and iritis. After a first injection with 20 μgr cidofovir intraocular pressure decreased by 20% within 2-3 weeks following injection, and recovered partially at week 5 or 6. After a second injection intraocular pressure dropped significantly without recovery at 5 - 6 weeks. After a third injection intraocular pressure again dropped at week 2-3 and tended to recover again at week 5-6.[307] Fluorophotometry showed a decrease in aqueous flow rate, and results of ultrasound biomicroscopy and histopathology showed damage of the nonpigmented epithelium of the pars plicata in a

manner analogous to the tubular damage when cidofovir is given systemically.[308] Vision of patients is not affected by the decrease in intraocular pressure and the clinical importance of it is not yet known.

A mild to moderate anterior uveitis can be seen after intravitreal injections with 20 μg cidofovir, between 3 and 12 days following injection. The use of probenecid (2 g orally 3 hours prior to injection, then 1 g orally each at 2 hours and 8 hours following injection) reduced the incidence of iritis from 70% to 18%. Uveitis responded promptly to local drops of mydriatics and corticosteroids and no permanent sequelae were noted.
Lower doses of intravitreally administered cidofovir (10 μg) did show fewer side effects, but at the same time were not as effective in controlling retinitis.[309; 310] Early relapse was especially seen in patients treated with the 10 μg dose, allowing for development of drug resistance.

Frequent intravitreal injections are a burden to the patient and to the treating ophthalmologist. Complications can occur like scleral induration, vitreous haze, iritis, vitreous haemorrhage, infectious endophthalmitis.[290-292; 311] Retinal detachments have been reported following intravitreal treatment, but were always ascribed to the always present risk of developing a detachment in an eye with necrotizing retinitis. Nevertheless a single needle perforation of the pars plana can trigger local granulation formation, traction at the vitreous base, and lead to retinal detachment.[312]

Intraocular device

The problems with serial local injections stimulated interest in an intraocular device for sustained release of ganciclovir, which was introduced in 1992.[313] Martin et al. conducted a trial comparing the intraocular device with deferred treatment in 26 patients with lesions in zone 2 or 3. Median time to progression was 15 days in the deferral group and 226 days in the group assigned to get the device implanted.[21] The 226 days before disease progressed was substantially longer compared to the median 47 days reported in the SOCA study in the patients receiving intravenous ganciclovir maintenance therapy. A second randomised study compared two types of intraocular devices, one with a 1-μg-per-hour release, and one with a 2-μg-per-hour release, with intravenous ganciclovir treatment. Median time to progression of retinitis was 221 days in the 1-μg-per-hour implant assigned patients, 191 days in the 2-μg-per-hour implant group of patients, and 71 days for the intravenously treated patients.[314] The observed large difference in time to progression probably reflects a real difference in efficacy of both treatments in controlling CMV retinitis. Intraocular concentration of ganciclovir is higher in eyes treated with an intraocular device compared with patients treated intravenously (4.1 μg/ml versus 0.93 μg/ml) Intraocular device treated eyes will not show drug level fluctuations, as will be present

in eyes of patients receiving intermittently administered systemic maintenance therapy. Drug concentration fluctuations might facilitate the emergence of viral drug resistance. Vitreous humour concentrations of ganciclovir has been shown to be subtherapeutic in patients using intravenous ganciclovir maintenance therapy. [315] However in CMV retinitis, being a retinal infection, intravitreal levels of anti-viral drugs are perhaps less important. Breakdown of blood retinal barrier in retinitis will allow high retinal levels of intravenously administered drugs. Subretinal fluid samples taken from eyes operated upon because of retinal detachment did show ganciclovir levels equal to plasma concentrations of the drug in patients intravenously treated with ganciclovir. [316]

The surgical procedure to place the intraocular device into the eye is a risk factor for the development of retinal detachment. CMV retinitis by itself is, in the absence of intraocular procedures, already associated with a high rate of retinal detachment. For this reason it is difficult to establish the real additive effect of perforating procedures upon the normally occurring rate of retinal detachment. The study by Musch et al. comparing treatment with the implant and intravenous ganciclovir demonstrated a 12 % occurrence of retinal detachment in the implant treated patients, compared to 5% in the systemically treated patients. Surprisingly in contrast with the first impressions, there was no significant difference in time to retinal detachment between both groups. [314] In the SOCA study the risk of development of detachment was 27% at 6 months. [89] In the study by Martin et al., detachment or retinal tear occurred in 18% of patients, and 5 of the 7 retinal detachments occurred sooner than 65 days after device implantation.

The strictly local therapy of an intraocular device will not prevent CMV retinitis in the second eye in patients starting with involvement of only one eye. Martin et al. reported a 50% involvement of the second eye in those patients with unilateral disease at 6 months. [21] The SOCA study reported a 27% cumulative risk of bilateral involvement at 6 months in systemically treated patients. [89] In the study by Musch et al. treatment with an intraocular device as sole treatment doubled the risk of second eye involvement compared to intravenous treatment. [314]

As stated before, extra-ocular CMV disease is left untreated with local therapy. Martin et al reported non-ocular disease developing in 31% of patients treated with an intraocular device. [21] Musch et al. found a 10% occurrence of extra retinal CMV disease in the implant treated group of patients, and not one case in the intravenously treated patients. [314]

Transient intravitreal haemorrhages were seen in 7.8% of eyes receiving an implant, endophthalmitis attributable to the implant surgery was observed in 2 patients (1.5%). [314] In addition to the complications already mentioned for invasive local therapies, the surgical procedure of implantation

of the device diminishes visual acuity due to surgical induced astigmatism for a period of 28 days.

Long term treatment

Long term treatment preventing progression in patients with CMV retinitis is the most difficult part of the management of patients. Present antiviral therapy brings a first episode of CMV retinitis in almost all patients under control. Median survival of patients treated with systemic maintenance therapy has been reported to be between 8.5 and 12.6 months. Because the period between relapses may be as short as 7 - 8 weeks, a significantly number of relapses can be anticipated. [317] Disease progression has been related to the occurrence of more resistant strains, but such a relationship has not been shown in all patients. [318] Approximately 10% of patients treated with systemic therapy harbour viral strains with increased resistance against the used drug, rendering current therapy inadequate. [172;318] The progressively shorter intervals between successive reactivations of CMV retinitis and the increased difficulty to bring recurrences under control can either be explained by the emergence of viral resistance or merely reflect an ongoing deterioration of the immune-system of the patient. [91] Median times to first, second, and third progressions were 47, 42, and 35 days and 53, 35, and 33 days for ganciclovir and foscavir, respectively. [89]

Several strategies have been advocated in treating recurrences: increasing the dose of the drug, switching to another drug, using a combination of drugs, or supplementing systemic therapy with local therapy.

A first recurrence can be managed in most patients by reinduction therapy, a second course of induction therapy of the same anti-viral drug used for maintenance. [91] Intraocular levels of the drug, reached by maintenance therapy, are probably not high enough to stop viral replication and prevent reactivation. Vitreous concentrations of ganciclovir and of foscavir have been measured in patients, receiving standard intravenous maintenance, treated for retinal detachment. Intravitreal concentrations of both drugs were higher compared to paired plasma sample concentration, but still resulted in borderline or progressively subtherapeutic intraocular concentrations. [315;319] The prolonged use of ganciclovir at induction levels has become possible in patients with the concurrent use of granulocyte-monocyte colony stimulating factor (GM-CSF) or granulocyt colony stimulating factor (G-CSF). [320]

The genes conferring resistance to ganciclovir and to foscavir can be different and are located in separate regions of the viral genome. For this reason switching of therapy can be effective in controlling a relapse. However the clinical effect of switching is not as dramatic as one would

expect. The ability to switch from ganciclovir to foscavir is limited due to the greater toxicity of foscavir.[317] With cidofovir a very potent alternative treatment for recurrent CMV retinitis became available. In a study to the efficacy of cidofovir in treatment of relapsing CMV retinitis in 100 patients, extensively treated with a median of 4 anti-CMV courses in the pre-trial period, median time to progression was 49 days for the 3 mg kg, and an upper-limit of 115 days was reported for the 5 mg /kg treatment arm of the study (median not reached during study).[321] Unfortunately 60% of the patients in the 5 mg / kg arm experienced treatment limiting events necessitating discontinuation of cidofovir.

Ganciclovir and foscavir did show an in vitro synergistic anti-CMV effect.[322;323] In refractory cases a combination of both drugs has shown superior efficacy in controlling disease progression than either drug alone.[324-328] In a randomised trial treatment of relapsing CMV retinitis was compared between standard re-induction with the anti-viral the patient already used, followed by maintenance with the same drug and continuation of the previous maintenance with induction of the second drug followed by combined maintenance with daily standard doses of both drugs. Median time to progression in the mono-therapy groups were 1.3 months for foscavir, and 2.0 months for ganciclovir, while it was 4.3 months for the combination therapy. However, although combined treatment was found to be more successful in the treatment of recurrences, it was also associated with the greatest negative impact of treatment on quality-of-life measures.[329] Another approach is the use of a combination of a lower dose of ganciclovir and foscavir as maintenance therapy, either both combined daily or each drug on alternating days.[325] Combination of ganciclovir and cidofovir is another option that could be considered, because in vitro studies suggest synergy between both drugs. Studies on this subject have not been reported yet.

Many clinicians advocate the use of local therapy, intravitreal injections with ganciclovir or foscavir, as a supplement to clinically inadequate systemic therapy, especially in treating active sight threatening lesions. Comparative studies however have not been reported. The efficacy of the implant in patients with recurrent CMV retinitis also seems very high. Two recent studies reported inactive CMV retinitis within one month postoperatively in 76% and 86% respectively of the eyes treated.[330;331] Median time to progression was 7 months in those eyes with a favourable initial response. Most patients (84%) received systemic antiviral medication in addition to the implant.[330] The most common complication was retinal detachment occurring in 12 (21%) out of 56 eyes treated. Self-limiting complications were vitreous haemorhage in three eyes and hypotony maculopathy in two eyes.[331]

Drug resistance

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 relatively resistant to ganciclovir and / or foscavir have been reported in patients treated for CMV retinitis. [318; 324; 332-335] 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.

Ganciclovir has to be phosphorylated intracellularly to become an active anti-CMV agent. The first step in phosphorylation is initiated by a virally induced enzyme, a phosphotransferase , encoded by the UL97 gene. The subsequent steps of phosphorylation into a triphosphate is performed by cellular enzymes. The triphosphate actively binds to viral polymerase and forms the basis for inhibition of viral DNA replication. Clinical resistance against ganciclovir is largely induced by mutations in UL97. These mutations have been analysed on the molecular level, and around 10 to 15 mutations have been described. [336]

Cidofovir mimics a monophosphate form and avoids the need for the viral induced UL97 enzyme action. It is phosphorylated in both infected and uninfected cells. The anti-viral activity is due to the ability of its metabolite, cidofovir diphosphate, to preferentially inhibit viral polymerase, and also to serve as an alternative substrate with respect to dCTP. [337]

Foscavir acts in a non-competitive way by blocking the cleavage of diphosphate bonds during the incorporation of molecules into the DNA polymerase, aborting production of viral DNA. Resistance against cidofovir or foscavir is caused by mutations in the viral DNA polymerase, gene region UL54.

Determination of susceptibility and resistance in viral isolates requires viral growth in tissue culture. Increasing levels of the tested drug are added to a series of culture wells. After a week the cultures are stained and the number of plaques in the control well is compared to the plaques in the wells with different drug concentrations. The concentration of the drug in the well with a reduction of 50% of the number of plaques is considered the IC_{50}. This value can differ between different laboratories due to the techniques used. [336] The plaques reduction assay is a labour-intensive and time-consuming activity, because after the virus is grown from blood or urine, it still takes several passages to yield enough virus to inoculate the test wells. Each passage requires at least a week, and results of this test require at least 4 to 6 weeks. This means that plaques reduction assays can not be of help in managing individual patients. Other antiviral susceptibility assays

have been developed like the DNA hybridisation assay, or the in situ enzyme linked immunoadsorbent assay, but these assays still require virus isolation and several passages to obtain sufficient virus.[310;338] A more rapid screening for resistance to ganciclovir and foscavir of primary isolates of CMV from blood has been described using single test doses of each drug and an immediate early antigen plaque reduction assay. [339] This test provides results within 4 to 6 days, but still requires virus isolation from the tested sample.

In a prospective study to the incidence of CMV resistance against ganciclovir, foscavir , and cidofovir, 122 patients with CMV retinitis had regular CMV cultures performed. Positive cultures were tested for the presence of viral resistance. Resistance was defined using the following thresholds for IC_{50} : for ganciclovir 6 μM, for foscavir 400 μM, and for cidofovir 2 μM. Around 80% of patients had a pre-treatment positive culture from either blood or urine. In 0.9% of the blood samples tested ganciclovir resistance was present, and in 2.7% of urine samples. Overall in 4% of the patients a blood or urine culture isolate was resistant to foscavir. [340; 341] Results of pre-treatment cidofovir resistance were not mentioned. Lifetable analysis of the probability to develop drug resistance at 3, 6, and 9 months of therapy was: ganciclovir 7%, 12%, 27%, foscavir 9%, 26%, 37%, and cidofovir 29%, 29%, 29%. [341; 342]

In a comparative study isolates of patients, who had received ganciclovir and / or foscavir, were analysed for resistance against cidofovir. Isolates with high level resistance against ganciclovir were also resistant against cidofovir, while those with low level resistance were not. Interestingly isolates with foscavir resistance, without or with only low level resistance against ganciclovir, were not cross-resistant against cidofovir, even though it is assumed that a single mutation in the viral DNA polymerase could be responsible for resistance to all DNA polymerase inhibitors. [343] Isolates with foscavir resistance and high level ganciclovir resistance were also resistant against cidofovir. These results suggest that low level ganciclovir resistance, mediated by mutations in the UL97 gene, is not associated with resistance against foscavir or cidofovir. However high level ganciclovir resistance, due to mutations in the viral polymerase gene, can result in cross-resistance against foscavir and cidofovir.[26] In another study addressing this subject it was shown that all ganciclovir sensitive strains were not resistant against cidofovir, ganciclovir resistant but not foscavir resistant strains were in 15-20% also resistant against cidofovir, and strains resistant against both ganciclovir and foscavir were in 80% resistant against cidofovir. [344] The sequential treatment of patients with ganciclovir followed by foscavir can progressively select viruses with multiple mutations in the viral DNA polymerase, generating multiple drug resistant CMV strains.

The clinical relevance of anti-CMV drug resistance detected in an isolate

out of blood or urine is another point to consider. Clinicians tend to assume that viral resistance has developed when a patient with CMV retinitis shows progression during therapy. In recent studies it has been shown that only 6 out of 117 patients (5%) were CMV culture positive at the time of retinitis progression. One explanation for this discrepancy could be that the resistant CMV strains were more difficult to isolate. In only 1 out of 9 patients (11%), where virus susceptibility testing was performed at the moment of retinitis progression, a resistant isolate was found. [278] Progression of CMV retinitis while patients are on therapy is likely the result of many other factors as well, like blood and tissue concentrations of the drug. The host immune response probably plays another important role. In vitro testing of isolated CMV may not reflect the spectrum of susceptibility of the strains in the infected host. Strains shed from one tissue may not necessarily be identical to those in other locations of the body. Different strains have been demonstrated at different sites, and also the presence of multiple strains in the blood compartment has been detected. [174; 345] The presence of different CMV strains in blood and the eye has been demonstrated in patients at the time of diagnosis of CMV retinitis. [177; 346] In situ susceptibility testing could be the answer with nucleic acid hybridisation techniques detecting resistant genotypes.

The determination of the most frequent mutations in the UL97 gene leading to ganciclovir resistance has allowed the development of molecular techniques to detect ganciclovir resistance directly in clinical samples. A combination of PCR followed by restriction endonuclease digestion can detect 5 of these mutations (at codon 460, 520, 594, F595m, and S595). [347; 348] More recently Bowen et al. has developed a rapid point mutation assay to screen for the mutations M460V, M460I, H520Q, A594V, L595S, and L595F. [349] This allows the rapid identification of resistant virus in clinical samples, and provides at the same time quantitative information of the prevalence of these mutations within the viral population. This way avoiding the problem of a mixed viral population, where the biologic features of the resistant strain can always be masked by the drug-sensitive phenotype. There is no need for viral passage. It can also detect different wild-type and / or mutant-type CMV strains, from samples of different body-compartments of the same patient. In the study by Bowen et al. for example a difference was detected in one patient between the blood and the urine samples. [349]

The power of this method lies in the possibility to detect the mutations in the UL97 gene leading to the emergence of ganciclovir resistance before a recurrence develops. In that way therapy can be changed accordingly and relapse prevented. In a prospective study of 19 patients treated with intravenous ganciclovir the occurrence of mutations was associated with a considerable increase in CMV viral load in the blood as well with progression of CMV retinitis. In patients without mutations viral load did not increase and no progression was seen. [350]

However more mutations do exist and a negative test would leave the clinician with the decision whether to test further. Additionally a comparable molecular analysis of the possible mutations of the viral DNA polymerase gene, located in the gene region UL54, associated with resistance against foscavir or cidofovir, has not been developed yet. Single aminoacid change in conserved domains of the gene has been detected in viral isolates resistant to foscavir. [351; 352]

For the clinician a more practical means to test for the emergence of resistance may be to serially quantitate CMV viral load by PCR or antigenemia test. The finding of an increase in viral load suggests a virologic problem. The test does not define resistance, but if drug levels are adequate and compliance has been good, it is reasonable to assume that resistance is beginning to evolve. [336; 353]

Other modalities, new drugs

Intravitreal treatment with an antisense oligonucleotide

Fomivirsen, ISIS 2922, a 21-base oligonucleotide targeting CMV immediate early 2 (IE2) m-RNA, via an antisense mechanism exhibits a potent and specific anti-CMV activity. In vitro experiments showed ISIS 2922 activity in CMV strains resistant to ganciclovir and/or foscavir. [22] Activity seems to be accomplished by three mechanisms: anti-sense mediated inhibition of target gene expression, sequence dependent inhibition of virus replication, and sequence independent inhibition of virusadsorption to host cells. [354] The drug has to be administered intravitreally, injections of 0.05 ml of a 3mg / ml concentration are given weekly for 3 weeks as induction therapy, followed by the same dosage bi-monthly. Results of phase III randomised clinical trials for CMV retinitis have recently been reported. [355] Median time to progression in the treatment group was 71 days, versus 14 days in the placebo group. Adverse effects of treatment were mild anterior chamber inflammation and vitritis in 15% of patients and transient intraocular pressure rise in 18%. Inflammation responded well to topical corticosteroid therapy and intraocular pressure normalised with topical anti glaucomatous therapy.

In vitro sequence dependent resistance to ISIS 2922 has been demonstrated, implying that the drug acts, at least in part via a virus-specific process rather than by rendering the host-cell incapable of supporting virusreplication. [356]

Transscleral iontoforesis

Transscleral iontoforesis is a local, non-invasive procedure to administer antiviral drugs to eyes with CMV retinitis. The procedure uses a low-ampère

current to drive molecules of the drug through the tissues of the eye. Foscavir is an ideal candidate for transscleral iontoforesis because at the pH level of the eye foscavir is a charged compound. Animal studies investigating the pharmacokinetics of foscarnet after transscleral iontoforesis demonstrated a therapeutic drug concentration in vitreous of 60 hours, without local complications. [357] Transscleral iontoforesis can also supplement systemic therapy in case of recurrence during maintenance therapy, which could be due to an insufficient intraocular drug level accomplished with systemic therapy. [358]

Liposome encapsulation

Ganciclovir has been encapsulated in liposomes to increase the intravitreal retention of the drug, thereby decreasing the frequency of injections. In a study of one patient of the intravitreal concentration following a single injection of liposome encapsulated ganciclovir it was shown that 24 days after injection the level of ganciclovir was still above the minimal inhibitory concentration (MIC_{50}). [359] Vision was temporarily decreased due to vitreal opacification in the period directly after the injection. No clinical trials have been reported since.

Cidofovir is a highly water soluble and polar drug and as such especially suitable for liposome encapsulation which would result in sustained release of the drug. In rabbit models using a multivesicular liposome system for intravitreal drug administration it was demonstrated that a safe sustained release is possible. [360]

Lobucavir

Lobucavir is a new nucleoside analogue with a broad antiviral activity in vitro. The drug has a similar anti-CMV activity as ganciclovir. However the drug has a better bioavailablity than ganciclovir. It is believed that lobucavir acts as a nonobligate DNA chain terminator. At present lobucavir is still in the phase of clinical trials for the management of CMV disease in HIV-positive patients. [22]

Laser coagulation

Laser coagulation of peripheral lesions to form a barrier against progression of CMV retinitis has been proven to be ineffective in halting the process. [117] In a study to the efficacy of prophylactic laser treatment to prevent retinal detachment in over 50% of the treated eyes reactivated retinitis crossed the laser scars (double or triple row of argon green laser coagulations, 500µm, 500 mW, 0.2 sec, gray-white lesion).[131]

Passive immunisation

No vaccine against CMV is available for general use. Passive immunisation

with normal or hyperimmune gamma globulin has had some success in reducing the severity of CMV disease in transplant patients. In combination with antiviral drugs an increased survival rate was reported in transplant patients with CMV pneumonitis and gastrointestinal disease. This indicated that there was some potential use for prophylaxis or therapy of CMV disease with a specific antibody against CMV.[361] Rasmussen et al showed antibody to CMV gH to be high in HIV-positive patients with CD4+ cell counts above 100 cells / mm^3, but low in patients with CD4+ cell counts less than 100, either with or without CMV retinitis.[169] Adjuvant treatment with human monoclonal antibody (MSL-109) of the immunoglobulin G - kappa subclass recognizing gH, appeared to be ineffective for treatment of CMV retinitis, in a large randomised trial of 209 AIDS patients with active retinitis. Patients received either 60 mg of MSL-109 intravenously once every week or placebo. Median time to progression was 67 in the treated versus 65 in the placebo assigned group.[362] Boppana et al. did not observe a specific deficiency in the antibody response of patients with CMV retinitis, but did show a more favourable clinical outcome in patients with higher neutralising antibody levels.[170] With the development of humanised antibodies against HCMV gH glycoprotein, new potential agents were provided for prevention or treatment of CMV infections. No clinical trials have been reported with these newly developed antibodies in HIV-positive patients with CMV retinitis so far. Hyperimmune CMV immunoglobulin has not proved useful in preventing CMV disease in HIV infected patients or as adjunctive therapy in the treatment of CMV disease.[363]

Desferrioxamine

In 1995 desferrioxamine (DFO), an iron chelator, was tested in vitro for its inhibiting properties of CMV replication. The ID_{50} of DFO for clinical isolates ranged from 3.1 to 4.9 μM. Inhibition of ribonecleotidereductase is the probable way the drug is effective. One patient suffering from progressive CMV retinitis, despite treatment with combination therapy ganciclovir / foscavir was treated with 1 gr of DFO intravenously each day. Retinitis became quiet one month of treatment, and no relapse of CMV retinitis was seen during a period of 3 month. No side effects were seen.[364]

Closing remarks on therapy

Few studies have addressed the pharmacokinetics of used drugs. The concentration of the drug at the site of action and / or in plasma as a function of time, is not exactly known. CMV isolates of patients, who have not been treated with anti-CMV medications, are inhibited in vitro by 50%, with drug concentrations of 1.5 μg / ml ganciclovir, 120 μg / ml foscavir, and 0.6 μg / ml cidofovir. This is the median effective inhibitory dose of each drug

(ED_{50}). The in vivo maximal, and minimal drug concentrations after various routes of administration and the in vitro measured ED_{50} have been the basis of current dosing regimes, see Table 3, page 60 .[255; 273; 295; 316; 318; 319; 337; 365-372]

Inter-patient variability seems to be large, resulting in a wide range of plasma concentrations in different patients using the same dosage. Large population differences have been demonstrated in the ganciclovir clearance, with possible important implications for ganciclovir dosing.[373] Regimes are based on empirical data from clinical trials, constructing dosing regimes that may be suboptimal, but nevertheless provide therapeutic benefit.[282; 374] No studies have correlated direct measurements of intraocular drug concentrations with the clinical outcome.[375]

Different treatment modalities, their indications, and complications are summarised in Table 4, page 60/61 .

Now that more drugs and different modes of administration have been developed the choice of ideal therapy for each patient has to be individualised. Patients with centrally located sight threatening lesions will need high drug concentrations directly at the site of infection. This can now be achieved with local therapy. Intravitreal injections will give the highest intraocular concentrations, and can be repeated in case of clinical suspicion of reactivation. The intraocular implant will result in high local levels, delivered at a steady state for a long period of time. Local therapy is associated with a small risk of developing endophthalmitis and a relatively high risk for developing a retinal detachment. These are serious complications with a high risk of direct permanent loss of vision. Local therapy does not protect the other eye in patients with unilateral involvement, and does not treat extra-ocular CMV disease. Systemic therapy has been shown to be effective in almost all cases and will treat non ocular disease and will protect the second eye, but dose-limiting side effects can occur: bone marrow suppression associated with ganciclovir, and nephrotoxicity associated with foscavir and cidofovir. Systemic maintenance with either ganciclovir or foscavir demands placement of an indwelling catheter, with a risk of infection. For that reason oral maintenance with ganciclovir could be the preferred therapy, but at the cost of lower efficacy and shorter time to relapse. Because of the infrequent administration needed for intravenous cidofovir, an indwelling catheter is also not necessary. The very narrow therapeutic index of cidofovir and the serious nephrotoxicity that can develop, limits the use of cidofovir. The additional use of probenecid reduces the toxic side effects of cidofovir, but adds its own toxicity profile. Treatment of relapsing CMV retinitis with a combination of intravenous ganciclovir and foscavir is more effective than monotherapy with either drug, but the majority of patients is unable to continue this treatment for a long period of time.

Table 3 Reported pharmacokinetic characteristics of ganciclovir, foscavir, and cidofovir.*

Drug	Regime	ED_{50}	Plasmaconcentration maximal	Plasmaconcentration minimal	Intravitrealconcentration maximal	Intravitrealconcentration minimal	Half-life plasma	Half-life intracellular	Half-life intravitreal
ganciclovir	5mg/kg/2xday IV 1gr 3xday oral 200µg intavitreally 4.5mg implant	1.5µg/ml	8.2µg/ml 1.2µg/ml	0.05µg/ml 0.23µg/ml	0.6-1.8µg/ml 17.9µg/ml 0.69-7.4µg/ml	0.2µg/ml not available 0.69-7.4µg/ml	2.9hr	16.5hr	13.3hr
foscavir	90mg/kg/2xday IV 2400µg intavitreally	120µg/ml	181µg/ml	16µg/ml	not available 269µg/ml	57µg/ml not available	3.4hr		32hr
cidofovir	5mg/kg/every 2 weeks IV		0.63µg/ml	25µg/ml	not available			3.2hr	65hr

*after M.A.Jacobson, Treatment of cytomegalovirus retinitis in patients with the acquired immunodeficiency syndrome. The New Ergeland Journal of Medicine, 1997, 337:105-114.
For additional comment see text.

Table 4 Overview of different anti CMV drugs, drug administrations, and drug scheduling.

Drug	Dosage / schedule induction	Dosage / schedule maintenance	Median time to progression, first	Median time to progression, >1	Advantages	Disadvantages	indications
ganciclovir intravenous	5mg/kg/ every 12 hr	5mg/kg/day, 7days or 6mg/kg/day, 5/7 days	62 days	progressively shorter	protects other eye and other organs	requires daily infusions, side effects: bone marrow suppression	lesions outside zone I, bilateral disease, systemic therapy required
ganciclovir oral	-	3 gr / day	51 days	-	protects other eye and other organs	lower intraocular and systemic drug levels	maintenance for not sight threatenin lesions, protection other eye and distant organs in patients with local therapy

Drug	Dosage / schedule induction	Dosage / schedule maintenance	Median time to progression, first	Median time to progression, >1	Advantages	Disadvantages	indications
ganciclovir intravitreal	400 µgr / 0.1 ml / twice weekly, untill response (2000 µgr also used)	400 µgr / 0.1 ml / once weekly	40% at 70 days	-	immediate high intraocular level of drug	requires frequent intravitreal injectons, no protection other eye or distant organs	immediate sight threatening lesions in zone I, systemic therapy contraindicated or ineffective
ganciclovir device	implantation of device	device	226 days	-	high level of drug intraocular for prolonged time	requires surgical procedure, risk of early retinal detachment, no protection other eye or distant organs	immediate sight threatening lesions in zone I, systemic therapy contraindicated or ineffective
foscavir intravenous	90 mg/ kg/ every 12 hr	90 -120 mg/kg/day	53 days	progressively shorter	protects other eye and other organs	requires daily infusions, side effects: nephrotoxicity, disturbance Ca,Na,K metabolism	lesions outside zone I, bilateral disease, systemic therapy required, ganciclovir contraindicated or ineffective
foscavir intravitreal	1200 µgr / 0.1 ml / twice weekly, 3weeks (2400 µgr also used)	1200 µgr / 0.1 ml / once weekly	33.3% at 140 days	-	immediate high intraocular level of drug	requires frequent intravitreal injectons, no protection other eye or distant organs	immediate sight threatening lesions in zone I, systemic therapy contraindicated or ineffective
combination i.v. therapy ganciclovir & foscavir	-	5 mg GCV / kg / day 90 -120 mg Fosc / kg / day	-	120 days	synergistic effect of both drugs	combined toxic side effects, long daily infusions	immediate sight threatening lesions in zone I, systemic therapy with monotherapy ineffective
cidofovir intravenous	5 mg / kg / once weekly	3 or 5 mg / kg / once every two weeks	120 days	3 mg: 49 days	infrequent infusions needed ,no need for indwelling catheter	narrow therapeutic index, nephrotoxicity frequent, probenecide related sickness	daily infusions contraindicated or poorly tolerated, systemic ganciclovir and foscavir contraindicated, or ineffective
cidofovir intravitreal*	20 µgr / 0.1 ml/ weekly	20 µgr / 0.1 ml/ once every 5-6 weeks	55 days	63 days	immediate high intraocular level of drug	frequent ocular hypotony, iritis	immediate sight threatening lesions in zone I, systemic therapy contraindicated or ineffective
ISIS 2922 intravitreal*	0.15 mg / 0.05ml weekly	0.15 mg / 0.05ml once every two weeks	71 days	-	immediate high total intraocular level of drug, different point of action	requires frequent intravitreal injectons, no protection other eye or distant organs, inflammation / high intraocular pressure	immediate sight threatening lesions in zone I, systemic therapy contraindicated or ineffective

* not yet approved by FDA For additional comment see text

Combinations of local therapy with the additional use of oral maintenance would theoretically seem to provide an optimal treatment modality, combining the advantages of each therapy, while reducing the complications associated with both. However, comparative studies have not been reported. Treatment choices should be individualised for each patient, taking into account the needed efficacy, the risk of specific toxic side effects, the risk of complications associated with drug delivery, and the underlying medical condition (including concomitant medications).

Treatment evaluation first and foremost emphasised enlargement of previous retinal lesions and/ or the occurrence of new lesions. This end point is useful in comparing the efficacy of a treatment versus no treatment or between different treatments. The loss of visual function is not taken into account, and the consequences of a second or third relapse of retinitis may have more serious implications towards visual functioning of a patient. Quantification of the rate of loss of functional retina, the loss of visual acuity and visual field should perhaps be part of therapy studies more often.

Besides visual function also the quality of life is as a rule not taken into account. Wu developed a questionnaire to access patient-reported visual function in CMV retinitis and found it to be a valid and reliable method to measure performance of vision related activities, visual symptoms, and the impact of treatment administration. Nevertheless to our knowledge no longitudinal studies have made use of such a questionnaire to evaluate therapeutic modalities.[376]

Treatment of CMV retinitis requires the active participation of several health care providers and last but not least the patient self. Patients should be encouraged to become involved in their own health care. The primary care physician (internist, infectious disease specialist), the ophthalmologist, family practitioner, nurses, social workers and the patient each with his / her expertise will be part of the management team. Assessment of retinitis progression, monitoring treatment and its side effects, the safe and effective delivery of the treatment regime, organisation of home care therapy, and the assistance in providing low-vision care in case of serious loss of vision are all integral parts of this treatment. Patients should be active participants in the decision making about which therapy to use, how aggressive therapy should be. Involving patients in their own care often confers a sense of empowerment over their disease. This is particularly important for HIV infected patients.

Prevention

Treatment of CMV retinitis requires lifelong administration of virustatic drugs that are toxic and expensive. Progression of disease despite

treatment is inevitable in most patients. For these reasons prevention of CMV disease has become a top priority.

The natural history of CMV according to most authors can be described in 3 phases. In phase 1 CMV is truly latent, the patient is anti-CMV-IgG seropositive, but pp65 antigenemia, CMV-DNA-emia, and viral cultures are negative. In phase 2, CMV is reactivated in the patient without overt disease, but antigenemia, PCR and cultures become positive. In phase 3 the patient suffers from clinical manifest visceral CMV disease. [377] To prevent CMV disease, therapy in phase 1 is prophylactic, treatment is given before active CMV replication is present. In this phase the risk of disease is low, and acceptable risk of drug related toxicity is also low. In phase 2 active CMV replication is present and therapy is called pre-emptive. This therapy should aim to restore the latent phase in a patient. CMV assays should become negative again. Risk of clinical manifest disease is higher and acceptable drug related toxicity increases likewise. In phase 3 a patient receives a full course of available treatment. To prevent blindness drug toxicity has to be, and will be accepted.

As has been stated in the section "Risk factors" the most sensitive method to discriminate between low and high risk HIV-positive patients for future development of CMV disease is the determination of CMV DNA in blood, which in the model implicates active CMV replication, and precedes visceral disease. This being true, still two considerable problems arise in translating this to an acceptable and successful prevention of CMV disease in the individual patient. First the time between a first positive test and disease is highly variable: mean time is in the order of 5 months and can be as long as 18 months, resulting in a very long period to use prophylaxis in perhaps to many patients. Secondly in 20% of patients at the time of diagnosis of CMV retinitis no CMV DNA could be detected in peripheral blood samples. [263; 377; 378] Perhaps, during a prior period of active CMV replication, CMV reaches the eye, remains latent intraocular, and can cause a local CMV retinitis some months later, without systemic detectable CMV DNA in the interlude, or even at the moment of diagnosis of retinitis. So a high number of patients would erroneously not receive prophylaxis, while actually they were at high risk for developing CMV disease.

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. Three studies report about the use of known anti-CMV drugs in preventing CMV disease in HIV-positive patients.

Valaciclovir, a valine ester of aciclovir, can achieve a 3- to 4 fold higher total plasma aciclovir exposure compared to oral aciclovir. With oral valaciclovir in a dosage of 2 gr 4 times a day, is total aciclovir exposure

comparable with 10 mg / kg / day of aciclovir given intravenously. This is considered to be efficient in many strains of CMV to inhibit replication.[379]

In a randomised double-blind trial, oral valaciclovir in the maximum tolerated dose, 2 gr. 4 times a day, was compared with two dose regimes of oral aciclovir, 800 mg 4 times a day, and 400 mg twice a day, as prophylaxis for CMV disease in 1227 HIV-positive patients, with CD4+ cell counts less than 100 cells / mm^3, without previous CMV disease, but seropositive for CMV. [380] CMV disease developed in 11.7% of patients receiving valaciclovir, and in 17.5% of aciclovir treated patients. A reduction of 33% comparing the valaciclovir group with the pooled data of the aciclovir groups. Time to CMV disease was significantly longer in the valaciclovir group, but toxicity and earlier medication discontinuation was also more common. Even a trend toward earlier mortality in the valaciclovir group was reported. CMV retinitis was the diagnosed end point in 80% of patients, while gastrointestinal disease accounted for 15%. Valaciclovir resulted in a proportional reduction in the different CMV diagnoses.

In a substudy the impact of base-line CMV DNA detection in whole blood samples were analysed on the effect of prophylactic therapy. For this analysis data of 310 patients were used, who fulfilled the above mentioned entry criteria, and of which in addition blood samples were available.[381] Patients, PCR positive in blood at baseline, were 2.57 times more likely to develop CMV disease at any time during follow-up, and time to CMV disease was significantly shorter. Comparing these data of the valaciclovir receiving group with the pooled data of the aciclovir groups, the greatest difference occurred in valaciclovir treated patients, who were PCR positive in blood at base line. The higher rate of CMV disease, and the earlier occurrence of CMV disease found in the PCR positive patients disappeared in those patients treated with valaciclovir. The authors conclude that the greatest effect of valaciclovir could be demonstrated in patients, who were PCR-positive in blood at trial entry, indicating that valaciclovir is most effective as pre-emptive therapy.

Two randomised placebo controlled trials of the efficacy of oral ganciclovir in preventing CMV disease gave conflicting results.[35;48] In the first study, the Syntex study, patients were enrolled with CD4+ cell counts less than 50 cells / mm^3, or CD4+ cell count less than 100 cells / mm^3 and a diagnosis of an AIDS defining opportunistic infection. Median CD4+ cell count was 22 cells / mm^3, and 88% of patients had cell counts less than 50 cells / mm^3. Patients received either oral ganciclovir, 1000 mg 3 times a day, or placebo. Patients underwent an ophthalmologic examination, including fundoscopy in mydriasis, at entry and every other month during follow-up. Kaplan-Meier estimates of protocol defined CMV events at 12 months were 26% in the ganciclovir treated, and 14% in the placebo receiving group. Resulting in a reduction risk of 49%. Ganciclovir decreased

the risk of CMV disease irrespective of the anti-retroviral therapy the patients used. Concomitant use of acyclovir did not influence the risk of CMV disease. During the study period 19% of patients in the ganciclovir group, and 16% of patients in the placebo group, discontinued the medications because of adverse side effects. Granulocyte colony-stimulating factor was prescribed in 24% of patients in the ganciclovir, and in 9% of placebo receiving patients. Patients receiving ganciclovir did not show a longer overall survival compared to placebo, 12 months Kaplan-Meier death rate of 21% respectively 26%.

In the second study, the CPCRA study, patients were included, with CD4+ cell counts less than 100 cells / mm^3, without previous CMV disease, and received either oral ganciclovir, 1000 mg 3 times a day, or placebo. Median CD4+ cell count was 34 cells / mm^3. Neither at base line nor during follow-up ophthalmologic screening was included, and only performed in case of complaints by the patients. No difference could be found of CMV event rate between both groups (101 / 662 ganciclovir receiving patients versus 55 / 332 placebo assigned patients) Anti retroviral medication, especially didanoside, influenced the CMV event rate considerably. Subgroup analysis of patients without didanoside at study entry suggested a protective effect in this subgroup. Adverse effects were more frequently reported in patients with ganciclovir, especially serious neutropenia (neutrophils $< 750 \times 10^6$ / l).

CMV event rate was higher in the Syntex study 22%, compared to 16% in the CPCRA study. This could be due to the difference in CD4+ cell counts, median CD4+ cell counts in the Syntex 1654 study 22 cells / mm^3, versus 34 in the CPCRA study, but more likely is caused by different examination protocols. In the CPCRA study systematic ophthalmologic examination was not part of the protocol. CMV retinitis can be present without signs. Without fundoscopy in mydriasis, one can easily miss a substantial number of CMV events.

In the Syntex study the effect of a CMV DNA PCR assay in peripheral blood was analysed. Surprisingly the effect of prophylaxis resulted in a small reduction of CMV disease in those patients with high viral loads at base line, a 39% reduction in event rate in patients with medium level viral loads, and a 90% reduction in those who were PCR negative. Oral ganciclovir seems to be best in preventing clinical manifest CMV disease in patients belonging to phase I, CMV present but latent, and functions in this group of patients as a true prophylaxis. However the prevalence of CMV disease in patients, PCR negative at base line, was very low and the need for prophylaxis questionable.

A point of concern is the observation that both foscavir and ganciclovir given intravenously are capable of clearing circulating CMV for a relative short period of time. Within 2 to 3 weeks of treatment even with the most

sensitive method, the CMV DNA PCR assay, one cannot detect CMV in the peripheral blood. However 40% of patients again show a positive CMV DNA-emia after 1 month of cessation of therapy, rising to over 80% after 2 months.[377]

Other drugs such as cidofovir, or lobucavir have not been tested as prophylactic drug and perhaps will show some success in the prevention of CMV disease, but the working spectrum and the toxicity profile of these drugs is such that a real improvement seems unlikely.

Perhaps different strategies of prophylactic / pre-emptive therapy can be more effective. The use of a combination of oral therapies could be the answer. Or the use of pulsed therapy, using intravenous treatment when viral load is high, switching to oral therapy, when viral load is again low. Monitoring viral load will then become very important to anticipate development or relapse of visceral disease, and also to detect the occurrence of resistance.

New drugs and different strategies need to be evaluated and there is great need for a more reliable quantitative virologic marker, or markers, to differentiate between patients, at no or low risk for development of CMV disease, and those at high risk, who will benefit most from adequate prophylaxis.

Recently several studies have addressed the cost-effectiveness of CMV disease prevention in AIDS patients using oral ganciclovir and periodic plasma testing for CMV viral load. [382; 383] These studies were performed based on costs of health care in the USA. The studies showed a small benefit at great cost. The authors conclude that at the moment there are no cost-effective strategies for advanced HIV infection and positive CMV serology. Perhaps it would become potentially cost effective if it would become possible to target the prophylaxis to patients who are most likely to benefit.

INFLUENCE OF HAART

The immune system is attacked by HIV in an unprecedented way.[23] At the start of the infection there is a polyclonal activation of both T- and B cells, and an increase in the production of proinflammatory lymphokines, like interleukin 2, tumour necrosis factor alpha and interleukin 6. A threefold to fourfold increase in T cell turnover of both CD4+ and CD8+ cells has been noted. This process also augments the replication of HIV and ultimately leads to cell death of T lymphocytes. Especially the CD4+ lymphocytes become victim of this process. The CD4+ lymphocyte is the carrier of long term immune memory and plays a pivotal role in the concerted action of the immune system against an invading organism.

Each T-lymphocyte has a specific receptor that determines the antigen against which the T-cell will respond maximally. In healthy individuals there exists a large repertoire of antigen specificity due to the presence of a large number of different T-cells. The continuous loss of CD4+ lymphocytes will lead to a diminishing repertoire of antigens against which an effective immune response can be elicited. Important defence against opportunistic infections of T lymphocytes reside in the CD4+ subset. Ongoing loss of these cells will eventually put the patient at higher risk for developing an opportunistic infection like CMV retinitis. Besides this loss of T-cells, there is also an inability of the remaining cells to respond in a proper way, because of the presence of actively replicating HIV. The risk of CMV retinitis will become significant after the CD4+ cell count drops below 100 cells / mm^3. Not all patients will develop CMV retinitis, however, because the immune competence against CMV also depends on the anti-CMV repertoire of the remaining T-cells.

Protease inhibitors interfere with the enzymatic reaction that cuts long strands of HIV-encoded protein into usable strands. 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. However, will the increase in CD4+ cell counts also lead to a restoration of the immune-repertoire? Reconstitution of T-cell repertoire is age dependent. In patients immunocompromized due to chemotherapy it has been shown that beyond the age of 20 years, hardly any reconstitution takes place. [384] Studies of the T-cell receptor repertoire after the start of HAART have shown that in spite of a rise in CD4+ cell count, the repertoire stayed as restricted as before therapy. [385]

In those patients with an increase in the number of CD4+ cells and a sufficient extension of their repertoire, as a consequence of the restoration of the immune system, there is a drop in incidence of CMV retinitis and a better control of pre-existing CMV retinitis. [386; 387] Is it still necessary to screen patients with previously low CD4+ cell counts? Which patients will need continuation of maintenance, and which can stop?

Before HAART, screening of HIV-positive patients was scheduled according to their CD4+ cell counts. Patients with CD4+ cell counts above 100 cells / mm^3 were seen annually. Screening every 6 months started when CD4+ cell count fell between 50 and 100 cells / mm^3. When CD4+ cell count were less than 50 cells / mm^3 patients were seen every 2 to 3 months. This seemed prudent in light of the observation that 15% of patients with CD4+ cell counts less than 50 cells / mm^3 could harbour unsuspected CMV retinitis. The positive change in immune-responsiveness caused by HAART brings up the question which patient still has to be

screened. Patients have been reported with excellent response to HAART, with CD4+ cell counts above 100 cells / mm^3, who still developed CMV retinitis. [388-390] Almost all patients were diagnosed with retinitis in the first 2 months after the start of HAART. Three out of 4 patients who were diagnosed after this period belonged to the group of patients defined as poor responders to HAART, with either continuing high HIV viral loads, or no increase in CD4+ cell counts. [390] Most authors advise to screen all patients according to the lowest CD4+ cell count recorded before the start of HAART irrespective of the first favourable response to HAART. [386]

Another issue is the need for continuation of maintenance therapy in patients with a quiet CMV retinitis and a good response to HAART with a rise in CD4+ cell count of over 100 cells / mm^3. [391-394] Recurrences have been reported following HAART, but almost exclusively in those patients that did respond poorly to HAART. [387] HIV viral load, CD4+ cell count, presence or absence of activity of retinitis, localisation of the lesions, visual acuity, general condition and current medications, and last but not least the patient's own opinion, are all important in the decision to stop or to continue maintenance.

Interestingly, patients with CMV retinitis have been described with an increased inflammatory response in the eye following the start of HAART. [395] This increased vitreous inflammation could be the result of immune-restoration with recurrence of a more effective immune response, not seen in the pre-HAART era. [23]

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.

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.

SUMMARY

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. The incidence of clinical manifest CMV disease is 24% / year in HIV-

positive patients with CD4+ lymphocyte counts less than 50 cells / mm^3. In over 90% of these cases the eye is involved, showing a necrotizing retinitis. Patients with higher CD4+ cell counts and CMV retinitis have been reported, but were exceptional. Detection of CMV DNA by PCR, either in whole blood or in plasma / serum, and the quantitative pp65 antigenemia, allow a better discrimination between patients, with comparable CD4+ cell counts, who will and who will not develop CMV retinitis.

Initial 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. 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, and in spite of large areas of necrotic retina the inflammatory response is minimal.

The most frequent complication of CMV retinitis is a rhegmatogenous retinal detachment. Prevalence of retinal detachment varies between 15 and 35%. 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.

CMV probably 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.

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, and 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.

The treatment of CMV retinitis consists of an induction therapy to bring the retinitis under control, achieved with a higher dose of drug for 2 to 3 weeks, followed by lifelong maintenance therapy with a lower dose to prevent a relapse. 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. 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.

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. The most effective local therapy is delivered by the intraocular device. Median time to progression was 221 days in patients with the implant, versus 71 days with intravenous therapy. Several strategies have been advocated in treating frequent recurrences: increasing the dose of the drug, switching to another drug, using a combination of drugs and supplementing systemic therapy with local therapy. CMV strains resistant to ganciclovir and / or foscavir and / or cidofovir have been reported in patients treated for CMV retinitis. Although oral valaciclovir, and oral ganciclovir, significantly reduced CMV event rate in patients at risk for developing CMV disease, the unwanted toxic side effects, and their modest efficacy, limits the wide spread use of these drugs as primary prophylaxis.

As a result of Highly Active Anti-Retroviral Therapy (HAART) the CD4+ cell counts rise dramatically in most patients. As a consequence of the restoration of their immune system there is a drop in incidence of CMV retinitis and a better control of pre-existing 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.

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