Clinical utility of cytomegalovirus urine cultures for ophthalmic care in patients with HIV

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Abstract

Background—The utility of cytomegalovirus (CMV) urine cultures was checked in patients with HIV (a) to identify those at risk for CMV retinitis and (b) to guide clinical decisions on treatment and prophylaxis of CMV retinitis.

Methods—HIV infected patients were tested for CMVuria by shell vial cell cultures. The prevalence of CMVuria was related to CD4 count, HIV risk group, and time before and after diagnosis of CMV retinitis

Results—A total of 639 shell vial cell cultures were obtained from 266 HIV infected ophthalmic patients. Only 4% of all patients with a CD4 count $>400 \times 10^6/l$ shed CMV in their urine compared with 42% with a CD4 count $\leq 50 \times 10^6/l$. Twenty three of 25 patients with CMV retinitis had a CD4 count $\leq 50 \times 10^6/l$. Among 130 patients with a CD4 count $\leq 50 \times 10^6/l$ (a) those who were CMVuric had a nearly sevenfold risk ($p < 0.0001$) of developing CMV retinitis (35%) compared with those who did not shed CMV in their urine (5%), and (b) CMVuria and CMV retinitis were more frequent in homosexuals (58%/25%) than in intravenous drug users (23%/15%). More than 1 year before diagnosis of CMV retinitis 18% of patients were CMVuric compared with 83% of patients who were CMV culture positive in the last 3 months. CMVuria under virustatic maintenance therapy is associated with worsening of retinitis in two thirds of cases.

Conclusion—Ophthalmic screening of patients with HIV should include those with a CD4 count $\leq 50 \times 10^6/l$ and focus on the subgroup with additional CMVuria. Screening of other patients can be dropped without undue risk in order to spare AIDS patients unnecessary hospital visits. CMVuria as a single finding, however, does not justify antiviral prophylaxis of CMV retinitis.

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Cytomegalovirus (CMV) retinitis is by far the most important disease of the eye in AIDS as it leads to blindness if not treated in time. The main task for the ophthalmologist in HIV infection is to recognise this blinding disease early and at least guide the antiviral treatment. Screening of AIDS patients for CMV retinitis would, therefore, be indicated.¹⁻⁵ AIDS patients, however, have to endure frequent visits

to different medical specialists during the final phase of their life. Thus, it would be helpful to know what factors indicate a high risk for developing CMV retinitis.⁶ This would not only be useful in reducing visits to the ophthalmologist but could also help in deciding whether to use antiviral prophylaxis. At present three markers seem to be candidates for recognising the risk of getting CMV retinitis: the number of CD4 lymphocytes in the blood (CD4 count), the presence of CMVuria,⁶ and the CMV pp65 antigen test.⁷

The CD4 count is a well established laboratory marker for the risk of developing opportunistic disease in HIV infection.⁸ Thus, chemoprophylaxis for *Pneumocystis carinii* pneumonia⁹ is recommended at CD4 counts lower than $200 \times 10^6/l$ and for cerebral toxoplasmosis at CD4 counts less than $100 \times 10^6/l$ with a positive test result for anti-toxoplasma IgG.¹⁰ CMV retinitis, however, occurs at much lower CD4 concentrations, usually below $50 \times 10^6/l$ ¹¹; from our clinical experience with many intravenous drug users among our HIV patients we felt that a CD4 count below $50 \times 10^6/l$ as the only criterion for starting primary CMV prophylaxis¹² would result in considerable overtreatment.

In this retrospective study we checked the usefulness of CMVuria in defining risk for CMV retinitis among those patients with CD4 counts lower than $50 \times 10^6/l$. Furthermore, we evaluated CMVuria as a factor for the effectiveness of secondary prophylaxis in patients with CMV retinitis.

Patients and methods

We analysed retrospectively the data from 294 HIV patients referred to our hospital by our medical clinic (outpatient department or infective ward) between January 1990 and April 1995 for complete ophthalmic examination. This included visual acuity testing and slit-lamp examination if possible but always funduscopy with dilated pupils. While all inpatients were screened routinely for eye involvement of HIV infection the basis for referral of outpatients was as follows: (1) all patients for an initial examination regardless of the stage of HIV infection; (2) all patients with ocular symptoms; and (3) all patients with CD4 counts less than $100 \times 10^6/l$ every 3 months.

The patients were staged according to the CDC classification¹³ and got regular CD4 counts (whole blood assay). Since 1991 they have also been checked routinely for cytomega-

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Figure 1 CD4 count and prevalence of CMVuria.

loviruria (CMVuria) by shell vial cell culture (SVCC) as described by Gleaves *et al.*¹⁴

We determined the prevalence of CMVuria among (1) all HIV patients as a function of the CD4 count, (2) all intravenous drug users and homosexuals with a CD4 count $\leq 50 \times 10^6/l$, and (3) 25 patients with CMV retinitis before and after the diagnosis of retinitis.

Some patients had their urine tested more than once for excretion of CMV while they were still in the same CD4 class (Fig 1, table 2) or in the same time interval (Fig 2). In order to reduce the possible distortion of results by such multiple testing of single patients a class mean was always calculated as the mean of all patients' percentage of positive cultures in that CD4 class or that time interval.

For example, if 24 urine cultures were obtained from 23 patients with a CD4 count from 600 to $401 \times 10^6/l$ (see Fig 1, second column) and 21 patients were negative, one patient was positive, and from one patient a positive and a negative culture were obtained our calculation for the patients with CMVuria in that CD4 class is:

$$(21 \times 0 + 1 \times 1 + 1 \times 0.5) : 23 = 6.5\%$$

Statistical analysis was done using the χ^2 test.

Results

CMVURIA AND CD4 COUNT

In 266 of 294 HIV patients seen at our clinic between January 1990 and April 1995 at least one urine culture for CMV was obtained (HIV

Figure 2 Prevalence of CMVuria before the diagnosis of CMV retinitis.

Table 1 Risk groups of HIV patients in Freiburg (n=266)

Risk group	Patients	
	n	%
Homosexuals	103	39
Drug addicts	86	32
Heterosexuals	31	12
Africans	11	4
Haemophiliacs	14	5
Transfusion	4	2
Unknown*	17	6
Total	266	100

*No information available on risk group.

risk groups see Table 1). While 639 shell vial cultures could be included in our study there were 208 ophthalmic examinations without a corresponding testing for CMVuria. The commonest reasons for cultures not being done were (1) ophthalmic examination before 1991 (when regular CMV testing was established), (2) fundus control within a short time (CMVuria was tested usually every 3 months), and (3) inability of outpatients to produce urine on that particular occasion.

The prevalence of CMVuria for different CD4 classes in patients not treated for CMV infection is shown in Figure 1 (266 patients, 525 urine cultures). All patients with a CD4 count $>600 \times 10^6/l$ tested negative. Only 4% of all patients with a CD4 count $>400 \times 10^6/l$ shed CMV in the urine compared with 21% of all patients with a CD4 count from 400 to $101 \times 10^6/l$. There is, however, no great difference among patients with a CD4 count from 100 to $51 \times 10^6/l$ and $\leq 50 \times 10^6/l$ with 41% and 42%, respectively, being positive for CMV in urine culture.

CMVURIA AND CMV RETINITIS

The prevalence of CMVuria and CMV retinitis in patients with a CD4 count $\leq 50 \times 10^6/l$ is shown in Table 2. The rate of retinitis was 18% in these patients with a higher but not significantly different percentage in homosexuals (25%) than in intravenous drug users (15%). CMVuria, on the other hand, was more frequent ($p < 0.001$) in homosexuals (58%) than in intravenous drug users (23%). Interestingly only two of 25 patients with CMV retinitis had a CD4 count $>50 \times 10^6/l$ and 21 had CMVuria. Among all patients with a CD4 count $\leq 50 \times 10^6/l$ those with CMVuria have a nearly sevenfold risk for CMV retinitis ($19/54 = 35\%$) compared with patients with a CD4 count $\leq 50 \times 10^6/l$ without CMVuria ($4/76 = 5\%$, $p < 0.0001$). Among the subgroups of homo-

Table 2 Prevalence of CMVuria and CMV retinitis in different risk groups

	Patients with CD4 count $\leq 50 \times 10^6/l$		
	All patients n (%)	Homosexuals n (%)	Drug misusers n (%)
All	130	59	39
+ CMV retinitis	23 (18)	15 (25)	6 (15) ^{ns}
+ CMVuria	54 (42)	34 (58)	9 (23)*
+ CMVuria + CMV retinitis	19 (35)	13 (38)	4 (44) [†]

ns= $p \geq 0.05$; * $p < 0.001$ homosexuals compared with drug misusers; †percentage of patients with CMVuria.

Table 3 CMV retinitis patients with CMVuria under intravenous virustatic therapy

	AS	KC	SW
Time after diagnosis	14 weeks	29 weeks	11 weeks
Present weekly therapy	Foscarnet discontinued	Ganciclovir 3×10 mg/kg	Ganciclovir 3×10 mg/kg
Course of retinitis	No progression	New lesion: paramacular	Marked progression on both eyes

sexuals and intravenous drug users with a CD4 count $\leq 50 \times 10^6/l$ the relative risk for CMV retinitis is still nearly five- and sevenfold, respectively ($p < 0.01$).

All 25 patients with CMV retinitis had been checked once or several times preceding the occurrence of retinitis. In Figure 2 the percentage of patients who shed CMV in their urine is shown at the time before the diagnosis of CMV retinitis (109 urine samples, 25 patients). More than 1 year before the diagnosis positive cultures of CMV could be obtained in only 18% of the patients, while during the last 3 months before clinical manifestation of retinitis CMVuria was found in 83% of patients (19/23).

CMVURIA UNDER VIRUSTATIC THERAPY

Twenty three of 25 patients with CMV retinitis were started on intravenous therapy with either foscarnet or ganciclovir. One patient refused treatment and one patient who had peripheral retinal involvement in only one eye and was CMV negative in the urine only received intraocular injections of ganciclovir. In 21 patients 114 urine cultures for CMV were obtained after the initiation of intravenous antiviral therapy. At the beginning of treatment 19/21 patients were CMV positive in the urine (90%). During the first month of induction treatment 47% of urine cultures (14/30 cultures in 8/16 patients) were still positive for CMV, while thereafter this was found in only 5% (4/84 cultures in 3/18 patients).

In two of these three patients the occurrence of CMVuria was associated with progression of retinitis under maintenance therapy with ganciclovir (Table 3). They received the reinduction dose whereafter retinitis stabilised again and CMV was eliminated from the urine.

Discussion

LIMITATIONS OF THE STUDY

Apart from the fact that our group of HIV patients is small in comparison with larger centres some other limitations of our study need to be discussed. Since our investigation is retrospective in nature there is no close monitoring of HIV patients for CMVuria. Thus, 208 examinations could not be related to a culture result while 639 urine cultures could be included in our study. There are, however, fewer than 10% of patients (28/294) without even one urine examination for CMV in the study period.

Selection bias probably plays no major role for inpatients as they were nearly all examined ophthalmically. There was, however, a trend for outpatients to be sent more readily if they had positive urine cultures for CMV. Thus, the true prevalence for CMVuria in the different

CD4 classes might be slightly lower. This would, however, still increase the statistical risk for patients with CMVuria to develop CMV retinitis.

CD4 COUNT AS INDICATOR FOR CMV RETINITIS SCREENING

Often a devastating course of CMV retinitis can be prevented by screening of asymptomatic subjects and by early therapy.^{1 2 5} Several screening concepts have been offered so far although the efficacy of most of them has not been proved. While Fabricius³ suggests ophthalmic screening for all patients with AIDS, others advise regular examinations only for patients with CD4 counts $< 200 \text{ cells} \times 10^6/l^4$ or $< 50 \text{ cells} \times 10^6/l^{15}$.

We feel that even this is a rather low threshold prompting many unnecessary visits of AIDS patients to the ophthalmologist. Indeed, 92% of our patients with CMV retinitis had CD4 counts $\leq 50 \times 10^6/l$ which confirms the findings of others^{11 16} that these patients are at particular risk. However, in our study only every fifth patient had a CMV retinitis (every fourth in a homosexual population), if one only takes into account the very low CD4 count of $\leq 50 \times 10^6/l$. Also we saw no patient with CMV retinitis having a CD4 count $> 200 \times 10^6/l$ as has been reported.¹⁷ Based upon our data, we believe one should not rely only on the CD4 count as single indicator for screening.

CMVURIA AS AN INDICATOR FOR THE RISK OF CMV RETINITIS

In our study 83% of AIDS patients with CMV retinitis shed CMV in their urine during the last 3 months before diagnosis of retinitis. These data are similar to those of Zurlo *et al*⁶ who even found 19/19 patients to have positive urine cultures during the 7 days before diagnosis of retinitis. In our series CMV retinitis occurred in 35% of all patients with both a CD4 count $\leq 50 \times 10^6/l$ and CMVuria, indicating a nearly sevenfold risk of patients with CMVuria developing CMV retinitis in comparison with those without CMVuria.

If primary prophylaxis with oral ganciclovir is considered at all for HIV patients¹² it seems worth while mainly to address the high risk group with a CD4 count $\leq 50 \times 10^6/l$ and additional CMVuria. If we had screened only these patients, in 19 of 25 cases (76%) CMV retinitis would have been diagnosed early. Of the six remaining cases two had higher CD4 counts and four were not CMV positive in the urine. Interestingly, these latter four patients had a more benign course of retinitis with no loss of central vision and no involvement of an eye which was primarily free of retinitis (3/8). Thus we agree with Zurlo *et al*⁶ who stated that viruria is sensitive in identifying a population

that could have CMV disease, but we do not agree with their conclusion that CMVuria is not a helpful factor for clinical decisions.

Zurlo *et al*⁶ only looked at the possible use of CMVuria as a single predictor for clinical purposes. We could show, as did others,^{18, 19} that the prevalence of CMVuria increases with falling CD4 count. There are many HIV patients, however, with a CD4 count $>50 \times 10^6/l$ ⁶ and even healthy people without HIV infection who shed CMV in their urine.²⁰ Knowing that CMV retinitis occurs only in a few with higher CD4 counts¹⁷ we do not advise regular testing for CMVuria² until the CD4 count has dropped below $50 \times 10^6/l$. If a patient is tested positive we examine him every 6 weeks, if tested negative every 3 months.

With our screening concept we aim to reduce the frequency of visits of the AIDS patient in general without a substantial increase of the risk of an undetected CMV retinitis. Our concept results in different screening frequencies for different risk groups: homosexuals with a CD4 count $\leq 50 \times 10^6/l$ were 2.5 times more likely to shed CMV in their urine than intravenous drug users²⁰ and 1.7 times more likely to develop CMV retinitis. This difference among the risk groups cannot be attributed to greater immunosuppression in homosexuals²¹ since our comparison refers only to HIV patients with a CD4 count $\leq 50 \times 10^6/l$. It is obvious that, with our model, low risk groups for CMV retinitis will benefit most from a reduction in screening examinations. This is an important point in clinical work at our eye hospital with 32% intravenous drug users among our HIV patients, who are often difficult to motivate for follow up examinations.

Once intravenous therapy is started CMV is usually eliminated from the urine within a month (95% of urines becoming CMV negative). We presented three patients who became viruric again under intravenous therapy. In two of these patients there was considerable progression of retinitis making a new treatment regimen necessary. We therefore check retinitis patients under treatment at each visit for CMVuria and propose a change of therapy if they become positive again. Many of our patients, however, had progression of CMV retinitis but no CMV in the urine. Therefore, regular fundus controls in patients with retinitis may not be replaced by laboratory tests.

OTHER CMV ANTIGEN TESTS

Cytomegalovirus disease should be regarded as a systemic infection with different organ manifestations among which CMV retinitis is the commonest.²² From nine cultures of bronchoalveolar lavage (BAL) material which were done on our retinitis patients eight had results which agreed with urine cultures done at that time while only one patient was negative in urine culture and positive in BAL culture. Clearly from the ophthalmologist's point of view CMV cultures from urine have the advantage over other body fluids that they are easy to obtain and bear low HIV infectivity.²³ Also Zurlo *et al* pointed out that during the 7 days

before diagnosis of CMV retinitis 19/19 patients had a positive urine culture while only 14/23 had a positive blood culture.⁶

For reasons not yet understood the sensitivity of cultures for the diagnosis of extraocular CMV disease seems to be much lower. Zurlo *et al*⁶ found only 2/5 patients to be positive in urine and only 2/6 patients to be positive in blood during the last week before diagnosis of CMV pneumonitis or CMV colitis. Here the CMV pp65 antigen test may have advantages for the early diagnosis of CMV disease because it was found to be more sensitive than shell vial cultures for detection of CMV in polymorphonuclear leucocytes.²⁴ The CMV pp65 antigen test in contrast with conventional cultures gives quantitative information by counts of positive cells.⁷ A third sort of CMV antigen test, the polymerase chain reaction (PCR),⁷ was shown to be highly concordant with the CMV pp65 antigen test,²⁵ but there is still the disadvantage that PCR is less rapid and simple than the CMV pp65 antigen test.⁷ One might expect, however, that the latter two antigen tests are less negatively influenced by antiviral treatment than the CMV culture technique.

Ophthalmologists caring for HIV patients know that early diagnosis of CMV retinitis is the key to preservation of vision and that screening of patients at risk is worth while. We have learned that regular eye examinations of all patients with AIDS are less effective than introducing a CD4 margin—for example, 50 cells $\times 10^6/l$, below which screening should start. Clearly it is no longer sufficient to increase the screening frequency only in cases of extraocular manifestations of CMV disease.^{3, 5} Ophthalmologists should instead demand regular systemic testing for CMV antigen in all HIV patients with a CD4 count below 50 cells $\times 10^6/l$.²⁴

At present a study is under way in our clinic comparing the usefulness of CMV-PCR, the CMV pp65 antigen test in blood, and CMVuria for ophthalmological screening. But as long as none of the tests available has proved yet to be a better predictor of retinitis we will continue to use the urine culture for the reasons mentioned above. Holding to this concept ophthalmologists may not need primary chemoprophylaxis for CMV retinitis.

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