CMVR diagnoses and progression of CD4 cell counts and HIV viral load measurements in HIV patients on HAART

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Abstract
Aim—To assess the impact of highly active antiretroviral therapy (HAART) on the prevalence and progression of CMV retinitis (CMVR) among AIDS patients with baseline CD4 cell counts <100 cells × 10^6/l.

Methods—A longitudinal cohort study of 1292 patients. CD4 cell counts and HIV viral load measurements were obtained before commencing therapy, at 3 months, 1 year, 2 years, and at last follow up. The CMVR prevalence rate was measured for the subgroup with baseline CD4 cell counts <100 cells × 10^6/l. CMVR adverse event (AE) rates per 100 person days at risk were calculated for the subgroup with CMVR and baseline CD4 cell counts <100 cells × 10^6/l.

Results—1292 patients were started on HAART. 8% of patients had CD4 counts <50 cells × 10^6/l and 40% had detectable HIV viral load at last follow up. The prevalence of CMVR for the subgroup with baseline CD4 <100 cells × 10^6/l was 10%. For those with baseline CD4 <100 cells × 10^6/l, the mean CMVR AE rate was greatest during the first 6 months of follow up after HAART commencement (p <0.003). The mean AE rate per 100 person days at risk was 0.36 (95% CI 0.167 to 0.551) before starting HAART, and 0.14 (95% CI 0.085 to 0.199) after starting HAART (p = 0.03).

Conclusions—HAART significantly prolongs the disease-free intervals in patients with pre-existing disease but recurrences persist within the first 6 months of starting therapy. AE were absent beyond 18 months of follow up in all patients including those with persistently low CD4 counts and detectable HIV viral load indicating clinical immunorestoration. New methods for monitoring the response to therapy are needed to identify those at risk.

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Cytomegalovirus retinitis (CMVR) is the most common intraocular infection in patients with AIDS and occurs primarily in patients with an absolute CD4 count of 50 cells × 10^6/l or less.[1–3] The resolution of CMVR rarely if ever occurs in immunosuppressed patients without anti-CMV therapy, with a median time to progression of approximately 2 weeks.[2]

Highly active antiretroviral therapy (HAART) including protease inhibitors (PI) induces a dramatic rise in the CD4 cell count and reduced HIV viral load measurements.[4 5] The lack of CMVR reactivation after stopping anti-CMV maintenance therapy has been attributed to the use of HAART.[6–9] Initial reports questioned the initial benefits of HAART in CMVR patients.[10 11] Subsequent studies, however, demonstrated significant benefit indicating that patients may no longer require lifelong anti-CMV therapy, although the safety of long term withdrawal of anti-CMV therapy remains unknown.[8 12 13] Furthermore, the early immune benefits of HAART may not necessarily provide sufficient protection against the development of CMVR[10 11] and the extent of immune reconstitution of the CD4+ repertoire has produced conflicting results.[14–16]

The purpose of this study was to measure the prevalence of CMVR in the HAART era in the light of CD4 cell counts and HIV viral load measurements. The impact of HAART on CMVR adverse events (AE) was assessed in the subgroup with baseline CD4 < 100 cells × 10^6/l.

Patients and methods

This was a longitudinal study of 1292 HIV patients attending one tertiary referral centre. All patients received HAART including one or more of the following four PI—nelfinavir, indinavir, saquinavir, and ritonavir—between June 1994 and February 1998. A rigorously validated computer database, into which all HIV diagnoses had been entered prospectively, was used to retrieve demographic data, CD4 cell counts, HIV viral load measurements, and CMVR diagnoses.

The most recent CD4 cell counts and HIV viral load measurements before starting HAART (baseline), at 3 months (within 2 months), 1 year (within 3 months), 2 years (within 6 months), and at last follow up were recorded. Patients with CMVR received appropriate anti-CMV therapy during the study including intravenous ganciclovir, foscanet, and cidofovir; intravitreal ganciclovir, foscarnet, and vitravene; and oral ganciclovir.

CD4 cell counts were grouped into one of the following four categories: <50 cells × 10^6/l, 51–199 cells × 10^6/l, 200–499 cells × 10^6/l, >500 cells × 10^6/l. HIV viral load measurements were all converted into a bDNA equivalent (Chiron assay) using the mean estimate of the bias and were recorded as detectable or undetectable using a detectability threshold of 500 copies/ml.[17]

Table 1 CD4 cell counts are grouped in one of four categories. HIV viral loads are classified as detectable or undetectable. The number of patients (and percentages) in each group is given

	CD4 cell counts				HIV viral load measurements	
	< 50	50–199	200–499	>500	Undetectable	Detectable
Baseline	342 (27.94%)	409 (33.42%)	408 (33.33%)	65 (5.31%)	69 (9.14%)	686 (90.86%)
3 Months	113 (15.50%)	302 (41.43%)	252 (34.57%)	62 (8.50%)	232 (54.21%)	196 (45.79%)
1 Year	45 (6.39%)	252 (35.80%)	336 (47.73%)	71 (10.09%)	360 (61.22%)	228 (38.78%)
2 Years	37 (7.55%)	142 (28.98%)	239 (48.78%)	72 (14.69%)	261 (59.32%)	179 (40.68%)
Last follow up	18 (8.26%)	61 (27.98%)	102 (46.79%)	37 (16.97%)	110 (56.99%)	83 (43.01%)

The prevalence of CMVR was measured. The subgroup of patients with baseline CD4 count <100 cells $\times$ 10^6/l and CMVR (diagnosed either before or after commencing HAART) was identified. Completeness of case finding was verified by cross referencing pharmacy records for prescriptions of foscarnet, ganciclovir, and cidofovir. A retrospective case note analysis was performed and the number and timing of CMVR adverse events (AE) were recorded. An AE was defined as one of the following:

- CMVR progression or reactivation in either eye
- The development of further, that is a new area of, CMVR in either eye
- Failure to respond to induction therapy within 4 weeks.

For the purpose of the analysis, the first CMVR diagnosis was not called an AE; however, the development of CMVR in the second eye was considered as an AE. Only one AE was counted if both eyes reactivated or progressed simultaneously.

Patient days at risk for developing AE were measured. Linear regression analysis with robust standard errors clustered on patients was used to estimate the rates of AE per 100 patient days at risk using STATA statistical software version 5. The regressions were calculated using the number of events as outcome variable and the number of hundreds of patient days at risk as predictor variable. The follow up of patients with CMVR was analysed in blocks of 180 days (6 month blocks). Statistical significance was achieved if p <0.05.

Results

CD4 AND HIV VL FOLLOW UP DATA

A total of 1292 patients received HAART including one PI. The patients' mean age was 37.28 years (SD 8.22 years) and the range was 20–77 years. CD4 cell counts and HIV viral load are given (Table 1, Figs 1 and 2). The numbers of patients available for analysis at each time point are given in Table 2.

CMVR PREVALENCE

Sixty six patients of 1292 developed CMVR. Fifty three patients (4%) developed CMVR before starting HAART, of whom five had a baseline CD4 count >100 cells $\times$ 10^6/l and 48 had a baseline CD4 <100 cells $\times$ 10^6/l. Thirteen patients (1%) developed CMVR after starting HAART of whom four had a baseline CD4 count >100 cells $\times$ 10^6/l and nine had a baseline CD4 <100 cells $\times$ 10^6/l.

The prevalence of CMVR among the subgroup of patients with baseline CD4 <100

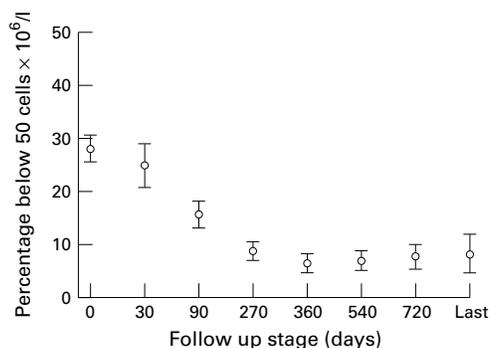*Figure 1   Percentage of patients with CD4 cell counts <50 cells $\times$ 10^6/l. The mean and 95% confidence intervals are given. An initial lag phase is observed and a plateau of about 8% of patients with CD4 cell counts <50 cells $\times$ 10^6/l is reached.*
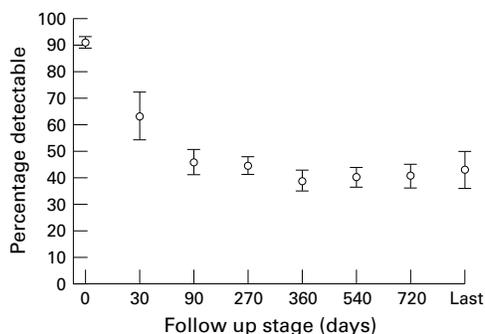

Figure 2   Percentage of patients with detectable HIV viral load. The mean and 95% confidence intervals are given. A rapid initial reduction in the percentage of patients with detectable HIV viral load is observed until a plateau of about 40% detectability is reached.

cells $\times$ 10^6/l was 10.37% (baseline CD4 count was available in 1224 patients of whom 511 had a baseline CD4 <100 cells $\times$ 10^6/l, and of those 53 developed CMVR). Complete follow up data were available in 47 patients (43 pre-HAART and four post-HAART commencement).

CMVR AE RATES AFTER STARTING HAART

Forty seven patients with CMVR and a baseline CD4 count <100 cells $\times$ 10^6/l were analysed. The number of patients with AE and the mean AE rates (per 100 patient days at risk) for each follow up block after the

Table 2   The total number of patients (and percentage of the total) analysed at each time point is given

	CD4 counts	HIV viral load counts
Baseline	1224 (94.75%)	755 (58.44%)
3 Months	729 (56.43%)	428 (33.13%)
1 Year	704 (54.49%)	588 (45.51%)
2 Years	490 (37.93%)	440 (34.06%)
Last follow up	218 (16.88%)	193 (14.94%)

Table 3   The number of patients with CMVR adverse events (AE), the mean AE rates (per 100 patient days at risk) and 95% confidence intervals for each follow up block are given. The maximum number of AE in any follow up block is 5

180 day block from HAART commencement	0 AE	1 AE	2 AE	3 AE	4 AE	5 AE	Mean AE rate (and 95% CI)
1	28	10	6	1	1	1	0.413 (0.222 to 0.604)
2	39	5	1	0	0	0	0.089 (0.014 to 0.164)
3	37	2	2	0	0	0	0.079 (−0.010 to 0.170)
4	31	1	0	0	0	0	0.021 (−0.022 to 0.064)
5	23	0	0	0	0	0	No adverse events
6	4	0	0	0	0	0	No adverse events

commencement of HAART are given in Table 3. We found a significantly greater rate of AE during the first 180 day block compared with each subsequent follow up block (p <0.003, Fig 3).

CMVR AE RATES BEFORE AND AFTER STARTING HAART

The number of patients who developed AE before and after starting HAART is given in Table 4. Thirty seven AE occurred before HAART commencement (0.79 AE per patient) and 47 AE occurred after HAART commencement (one AE per patient). The total number of days at risk before HAART commencement was 10 006 days (mean 212.89, SD 233.29) and the total number of days at risk after HAART commencement was 29 878 days (mean 635.70, SD 234.44).

Therefore, 0.359 AE per 100 patient days at risk (standard error 0.095; 95% CI 0.167 to 0.551) occurred before commencing HAART, and 0.142 (standard error 0.085; 95% CI 0.085 to 0.199) after starting HAART (p = 0.03, Fig 4). The mean time between AE was 278 days (95% CI 181 to 597 days) before HAART and 703 days (95% CI 502 to 1174 days) after HAART.
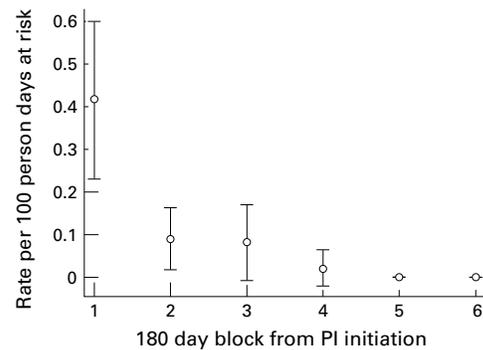

Figure 3   Rates (in 100 patients days at risk) of CMVR AE (mean and 95% CI) after commencing HAART. No AE were observed during blocks 5 and 6.

Table 4   The numbers of patients who developed AE before and after HAART commencement are given. The maximum number of AE per patient was 5. The total numbers of AE before and after HAART commencement were 37 and 47 respectively

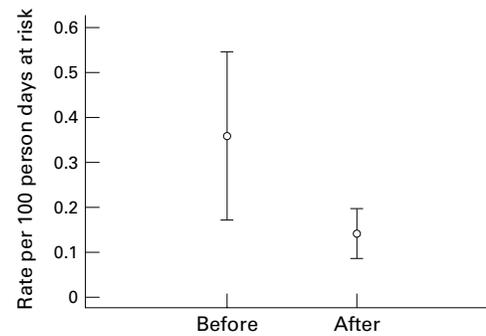| Numbers of AE	Numbers of patients with AE before HAART commencement	Numbers of patients with AE after HAART commencement
0	31	24
1	6	11
2	3	6
3	4	2
4	2	2
5	1	2
Total number of AE	37	47

Figure 4   Rates (in 100 patients days at risk) of CMVR AE (mean and 95% CI) before and after commencing HAART.

Discussion

The proportion of patients with CD4 cell counts <50 cells × 10^6/l decreased in response to HAART and the rate of change was greatest between 1 and 3 months after commencing treatment (Fig 1). However, the reduction in patients with a detectable HIV viral load was seen almost immediately after the start of HAART (Fig 2). This trend tapers out around 9 months for CD4 counts and around 3 months for HIV viral load measurements. The above changes demonstrate a good response to treatment although some patients have persistently low CD4 cell counts and detectable HIV viral load (Table 1). From a clinical standpoint, only five AE were observed during the second year of follow up and none during the last year of follow up (Table 3), indicating significant clinical immunorestoration beyond 12–18 months for all patients. Other clinical and laboratory studies also suggest significant immunorestoration to CMV in response to HAART.[12][18][19] Some patients, however, have a persistently low CD4 cell count (<20) following triple therapy and may remain at relatively high risk of developing AIDS diseases.[20] It would therefore seem logical to monitor closely patients with very low CD4 counts after PI initiation and to continue with anti-CMV therapy until CD4 cell counts have been greater than 100 for at least 6 months.

In addition, measuring detectable HIV viral load is markedly dependent on the assay used. Recent improvements in our laboratory assay, for instance, have reduced the detectability threshold to 50 copies/ml. Undetectable viraemia therefore probably indicates the limitations of the assays used as opposed to the absence of HIV-1 replication.[21] Our data suggest that in the HAART era, the sensitivity of a CD4 <50 cells × 10^6/l or a detectable viral load as markers for identifying patients at risk of CMV disease is reduced. Different means of

quantifying CMV specific CD4+ lymphocyte responses using flow cytometry are now available and should help identify patients at risk.[22][23]

In the pre-HAART era, CMVR developed in patients with a CD4 count ranging from 13 to 38 cells × 10^6/l.[24][25] In this study, we used a CD4 cut-off of 100 cells × 10^6/l because CMVR can develop at CD4 counts >50 in patients on HAART.[10][11][26][27] The subgroup on HAART with a baseline CD4 <100 cells × 10^6/l was therefore identified. Rigorous statistics were used to document significantly prolonged disease-free intervals in patients on HAART with useful quantitative estimates. The same group of patients studied served as their own controls. AE rates were inversely related to the number of days at risk. AE were 2.5 times greater before HAART and the number of days at risk were three times greater after HAART. This is associated with the increased survival of patients on PI therapy.[28][29] Furthermore, CMVR patients are increasingly likely to be started on HAART as CMVR indicates advanced AIDS. Ten patients (out of 47 with complete follow up) had been started on HAART within 1 month of developing first CMVR. In this subgroup, 0.24 AE were observed per 100 person days at risk. The small number of patients in this subgroup, however, precludes drawing any firm conclusions.

Furthermore, four patients (out of 47 with complete follow up) developed first CMVR after HAART commencement. This is a particularly interesting subgroup in whom first CMVR developed between 3 weeks and 7 months after HAART commencement. Only one AE developed in one patient 5 months after the initial CMVR episode (the latter occurred 6 months after HAART commencement). At the time of the AE, the viral load was detectable and CD4 count was 7 cells × 10^6/l. No further AE were observed in this patient; the viral load was persistently undetectable and CD4 count >170 cells × 10^6/l from 1 year after HAART commencement. None of the other three patients developed any AE. Two patients died 2 and 3 years after HAART commencement. Of those who died, one had persistently detectable viral load (pre-HAART and post-HAART); the CD4 was 54 cells × 10^6/l at the time of commencing HAART, but remained <10 cells × 10^6/l on all subsequent measurements. The second patient who died had persistently detectable viral load (pre-HAART and post-HAART), his CD4 count was 49 cells × 10^6/l at the time of commencing HAART, remained <50 cells × 10^6/l for 3 months, then rose to 208 cells × 10^6/l and remained >130 cells × 10^6/l on all subsequent measurements. The fourth patient had a detectable viral load pre-HAART and post-HAART; the CD4 was 38 cells × 10^6/l before HAART commencement. A good CD4 response (but detectable viral load albeit reduced absolute value) was observed post HAART (202 and 403 cells × 10^6/l at 1 and 2 years respectively). The absence of a clear association between CD4, viral load and AE in patients on HAART

therefore suggests that newer methods for assessing immune reconstitution may be needed to identify patients at risk.

The apparent HAART benefit, was absent during the first 6 months of treatment as the rate of AE in block 1 was paradoxically greater than before starting HAART (0.413 and 0.359 events/100 person days respectively). This is consistent with other studies where CMVR acute events or progression occurred within the first 3 months of PI initiation.[10][11][26][30][31] AE rates were markedly lower during the second follow up block and virtually non-existent during the last 18 months of follow up (Table 3). The reasons for the continued progression of CMVR during the first few months of HAART therapy is not entirely clear but several possibilities exist. The initial rise in CD4 cells may simply indicate a partial immune restoration as the CMV specific CD4 repertoire may still be lacking or non-functional.[16] In addition, some evidence suggests that the initial increases in CD4 cells may result from the recirculation of lymphocytes.[18] This initial increase in CD4 cells may therefore be unable to prevent relapses because of loss of specific anti-CMV cells. Autran *et al* have suggested that immune reconstitution occurs in two stages.[15] Initially the redistribution of lymphoid cells result in a rise in CD4 cells but the new naïve cells are not produced until the second stage, which occurs 1 year or later.[15] This is consistent with the observed clinical changes of continued CMVR progression during the early stages of commencing HAART, and long term protection against further recurrences. Another possibility is that undiagnosed CMVR may have been present at the time of PI commencement, thus manifesting as new disease. Some patients however, had CMVR before HAART commencement and developed recurrences after the onset of HAART. For the latter group, it is possible that an alteration at the level of the blood-retinal barrier may prevent the passage of immune cells to the target retinal tissues.

In summary, this study demonstrates the efficacy of PI therapy in prolonging the CMVR-free intervals. A persistently elevated relapse rate occurs during the first 6 months of PI therapy but marked clinical immunorestoration is seen beyond 1 year despite a persistently low CD4 count and detectable HIV viral load in some patients. High risk patients should therefore be continued on anti-CMV prophylaxis until definite evidence of immunorestoration is observed.

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