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

Verbraak, F.D.

Citation for published version (APA):
Verbraak, F. D. (1999). CMV retinitis in HIV-positive patients in the pre-HAART era

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
CHAPTER VI

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

Frank D Verbraak, René Boom, Pauline M. E. Wertheim-van Dillen, Gerardus J. van den Horn, Aize Kijlstra, Marc D. de Smet

ABSTRACT

Aims/background: In the pre-HAART era, HIV positive patients with CD4+ cell counts below 50 cells/mm³, and those with detectable CMV DNA in their peripheral blood, were considered to be at high risk for the development of CMV disease. With the start of HAART, a restoration of immune function occurred in these patients, and as a consequence patients become less vulnerable to CMV disease. Since it is not exactly known how HAART influences CMV viral load in peripheral blood and the incidence of CMV disease in high risk HIV positive patients we followed a group of patients before and after initiation of HAART.

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

Results: In the pre-HAART period 3 patients developed CMV disease, and 4 patients died (without clinical manifest CMV disease). After the start of HAART not one patient developed CMV disease, or died. With HAART, mean CD4+ cell counts
increased from 32 cells / mm$^3$, to 190 cells / mm$^3$ at the end of follow-up. CMV DNA could be detected in blood of 11 patients. Quantification showed a decline in the amount of detectable DNA during follow-up. At the last examination only one patient showed a positive PCR assay. This was the only patient with a CD4$^+$ cell count remaining below 100 cells / mm$^3$.

**Conclusion:** In HIV positive patients at high risk of CMV retinitis, either with a positive CMV PCR assay in blood and/or with CD4$^+$ cell counts below 50 cell / mm$^3$, HAART causes a dramatic decrease in occurrence of CMV disease. This decrease is paralleled by an increase in CD4$^+$ cell count, and a decrease in the amount of CMV DNA in the blood, which decreases below detection levels in all patients with CD4$^+$ cell counts above 100 cells / mm$^3$.

**INTRODUCTION**

The presence of CMV DNA either in whole blood or in cell free samples has been recognized as an important risk factor, in addition to low CD4$^+$ cell counts, for the development of CMV retinitis in HIV positive patients. Studies on serum or plasma samples reported useful statistical parameters for CMV DNA PCR assays in predicting CMV retinitis (sensitivity between 75 and 90%; specificity between 60 and 85%; positive predictive value between 60 and 70%; the negative predictive value between 80 and 98%). Overall incidence of CMV retinitis in these studies during a follow-up period of 12 months was between 25 to 35%. Spector et al. reported a 12-month Kaplan-Meier CMV disease event rate of 14% in PCR CMV negative patients and of 43% in the PCR positive patients, corresponding to a 3.4-fold increased risk of developing CMV disease. In over 90% of cases CMV disease manifested itself as retinitis.

The use of triple combination therapy, two reverse transcriptase inhibitors and one protease inhibitor, often called Highly Active Anti-Retroviral Therapy (HAART), has resulted in a dramatic change in the morbidity associated with HIV. A significant decline in the incidence of CMV disease has been reported in patients receiving this combination antiretroviral therapy. Van den Horn et al. reported that patients, with CMV retinitis, treated with HAART showed no recurrences during a follow-up period of 42 to 52 weeks provided that the CD4$^+$ cell count remained above 100 cells / mm$^3$. HAART induces a rapid redistribution and eventually a restoration of the immune system, and as a result, patients, normally expected to be at high risk for developing CMV retinitis or recurrences of already present CMV retinitis, are again able to...
suppress active CMV infection.

Since it is not exactly known how HAART influences CMV viral load in peripheral blood and the incidence of CMV disease in high risk HIV positive patients, we assessed a group of patients before and after the start of HAART.

**PATIENTS AND METHODS**

*Patient selection*

Patients were selected from a group of 100 consecutive HIV positive patients seen in March and April of 1996 at the AIDS clinic of the Academic Medical Center of the University of Amsterdam. Eligible patients either tested positive by CMV PCR in blood (n=18), or had a CD4 count below 50 cells / mm$^3$ (n=15). Patients with a CMV disease diagnosed before the start of the cohort study were excluded (n=4). Additionally 4 patients refused to participate. All patients underwent a full ophthalmologic examination, including fundoscopy in mydriasis at base line and every other month thereafter.

A CMV event could be either a CMV retinitis, defined as a necrotizing retinitis with characteristic “cheese like” appearance with or without hemorhages, as observed by an experienced ophthalmologist, or extra-ocular CMV disease, for which diagnosis immuno-histologic proof had to be present.

| Table 1 Effect of HAART on occurrence of CMV events and survival in HIV positive patients at high risk for developing CMV disease. |
|---|---|---|---|---|
| Before HAART (n = 25) | After HAART (n = 18) |
| PCR + | PCR - | Time to event or death (months)* | PCR + | PCR - |
| n=13 | n=12 | n=7 | n=11 |
| CMV events, | 2,2,3 | - | - |
| n=3 | 1,2,2,4 | - | - |
| Death, | 3 | 7 | 11 |
| Living without | - | - | - |
| CMV, | 11 | 7 | 11 |
| n=7 | 5 # | 18.6 $ |
| Mean follow-up (months) | | |

HAART = highly active antiretroviral therapy  
PCR = polymerase chain reaction, + = positive result, - = negative result  
* time between start of study and occurrence of event / death  
# mean follow-up between start of study and start of HAART (whole group)  
$ mean follow-up following start of HAART (whole group)
Before the start of HAART 4 patients died, without clinical manifest CMV disease, after 4, 7, 9, and 15 weeks respectively. Mean follow-up period for the other patients, from start of the study to start of HAART, was 5 months (range 4 to 6 months). Three patients, all belonging to the CMV PCR positive patient group, developed a clinical manifest CMV disease in the pre-HAART period. All patients were put on HAART in the second trimester of 1996. HAART consisted of triple therapy, using a combination of two reverse transcriptase inhibitors and one protease inhibitor. Mean follow-up after the start of HAART for these patients, without previous diagnosis of CMV disease (n=18), was 15.1 months (range 9 to 18 months).

Between November 1996 and July 1998 the 18 CMV disease free remaining patients, underwent a full ophthalmologic examination, including fundoscopy in mydriasis, every month during the first 6 months, and every other month during the remaining part of follow-up. At the same time blood samples were taken for quantitative PCR analysis. At each visit, the treating physician from the department of internal medicine received a questionnaire and was explicitly asked for any signs of CMV disease elsewhere. CD4+ cell counts were performed every third month.

**PCR analysis**

CMV DNA was purified from 50–1 EDTA blood specimens together with 70 molecules of Internal Control (IC) DNA as described previously, using 20–1 of size-fractionated silica particles. DNA was eluted in 100–1 TE buffer (10 mM TRIS, 1 mM EDTA, pH 8.0). CMV DNA levels in blood were determined as described previously (Boom R, Sol C, Weel J, et al. A highly sensitive assay for detection and quantification of human cytomegalovirus DNA in serum and plasma by PCR and electro-chemiluminiscence. Submitted). In short, purified DNA (25–1) was subjected to a 35-cycle PCR with a single primer pair which amplifies a 578 bp DNA fragment from exon 4 of the major immediate early gene of CMV and a fragment of identical size and GC content from IC DNA. The amounts of CMV and IC PCR products were subsequently determined by electro-chemiluminescence (ECL) in the QPCR System 5000 (Perkin Elmer) after hybridization with {Tris (2,2’-bipyridine) ruthenium (II) chelate} (TBR)-labeled probes specific for either CMV or IC amplimers. The viral load (expressed as copies CMV / ml blood) was calculated from the ratio (R) of CMV over IC ECL signals (after background correction) by the algorithm “copies CMV / ml blood = Rx 1400”.

147
Table 2
Effect of HAART on CD4+ cell counts and CMV viral load in 18 HIV positive patients, without previous CMV disease, at high risk for developing CMV disease.

<table>
<thead>
<tr>
<th>Pat No</th>
<th>Exam Before CD4+</th>
<th>HAART After CD4+</th>
<th>Exam1 CD4+</th>
<th>Exam1 PCR</th>
<th>Exam2 CD4+</th>
<th>Exam2 PCR</th>
<th>Exam3 CD4+</th>
<th>Exam3 PCR</th>
<th>Exam4 CD4+</th>
<th>Exam4 PCR</th>
<th>Exam5 CD4+</th>
<th>Exam5 PCR</th>
<th>Exam6 CD4+</th>
<th>Exam6 PCR</th>
<th>Exam7 CD4+</th>
<th>Exam7 PCR</th>
<th>Last exam After n Mths</th>
<th>PCR</th>
<th>CD4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1469</td>
<td>70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>110</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>230</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>130</td>
</tr>
<tr>
<td>2</td>
<td>150</td>
<td>9/4</td>
<td>150</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>140</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>180</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>115</td>
<td>1136</td>
<td>210</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>250</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>250</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>170</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>562</td>
<td>90</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>460</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>1529</td>
<td>160</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>130</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>170</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>382</td>
<td>30</td>
<td>2071</td>
<td>1474</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16#</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>180</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>2355</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>130</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>120</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>180</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>-</td>
<td>60</td>
<td>6488</td>
<td>1464</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>120</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>186</td>
<td>875</td>
<td>40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>140</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>-</td>
<td>110</td>
<td>-</td>
<td>260</td>
<td>-</td>
<td>-</td>
<td>346</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16#</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>-</td>
<td>60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>90</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>120</td>
</tr>
<tr>
<td>13</td>
<td>50</td>
<td>-</td>
<td>190</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>130</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>120</td>
</tr>
<tr>
<td>14</td>
<td>30</td>
<td>-</td>
<td>70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>150</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>180</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>-</td>
<td>230</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>110</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>250</td>
</tr>
<tr>
<td>17</td>
<td>30</td>
<td>-</td>
<td>140</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>130</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>190</td>
</tr>
<tr>
<td>18</td>
<td>30</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>130</td>
</tr>
</tbody>
</table>

CD4+ = CD4+ cell count in cells / mm³  
HAART = highly active antiretroviral therapy  
PCR = quantitative polymerase chain reaction, in copies CMV / ml  
- = negative result, ND = not done  
Exam 0 = first examination, at intake, before the start of HAART  
Exam 1 - 7 = examinations, monthly, after the start of HAART  
After the 7th examination, follow-up scheduled every other month, results not shown.  
# patient 7 one positive test 12 months after start HAART (280 copies / ml)  
* patient 11 one positive test 14 months after start HAART (98 copies / ml)  
Last exam After n Mths = last examination of the patient at n months after the start of HAART
Influence of HAART.

Statistical analysis

For statistical analysis we compared the CMV event rate, during follow-up before the start of HAART, with CMV event rate following the start of HAART, using the Kaplan-Meier method and the log rank test.  

RESULTS

During a mean follow-up of 5 months, range 4 to 6 months, before the start of HAART, 3 patients developed CMV disease. Two patients were diagnosed with CMV retinitis after 7 and 9 weeks respectively, and one case of biopsy proven CMV colitis occurred after 12 weeks of follow-up. Additionally 4 patients died before the start of HAART, without a clinical manifest CMV disease, after 4, 7, 8, and 17 weeks respectively (table 1). Following the start of HAART not one patient developed a clinical manifest CMV disease, during a mean follow-up of 15.1 months (range 9 to 18 months), and not one of these patients died.

Statistical analysis comparing the incidence of CMV disease in patients before and after the start of HAART using the Kaplan-Meier method and the log rank test resulted in a p-value of .10, indicative of a trend, but not statistically significant.

Most patients responded to HAART with a steady increase in their CD4+ cell counts (table 2). The mean CD4 positive count increased from 32 cells / mm$^3$ (range 10 to 150) at the start of follow-up, through 136 cells / mm$^3$ (range 40 to 260) halfway, to 150 cells / mm$^3$ (range 20 to 250) after 6 months, and 190 cells / mm$^3$ (range 60 to 460) at the last examination. With the exception of one patient (pat.nr. 10), all CD4+ cell counts were over 100 cells / mm$^3$ at the last examination.

In 7 patients a positive CMV PCR test was obtained during follow-up after the start of HAART (table 2). Quantification of the PCR test showed a decrease of the amount of CMV DNA detectable in the peripheral blood of all these patients. At the 7th examination, longest follow-up 10 months after the start of HAART, none of the tested patients had detectable CMV DNA in their blood. However, in patient 7, 12 months following start of HAART, 280 CMV copies / ml could be measured, and in patient 11, 14 months following the start of HAART, 98 CMV copies / ml could be detected (not shown in table 2). At the last examination, after a mean follow up of 15.1 months, only one patient (pat.nr. 10) tested positive, with a CMV viral load of 727 copies / ml. This was also the only patient with a CD4+ cell count less than 100 cells / mm$^3$. 

149
DISCUSSION

In this study we present data showing that in 18 patients, previously considered to be at extremely high risk for developing CMV disease, not one new case of CMV disease manifested itself during a mean follow-up of 15.1 months (range 9 - 18 months). HAART resulted in a gradual rise in CD4+ lymphocyte counts and a gradual drop in CMV viral load in the peripheral blood. At the last examination CMV DNA became undetectable, with the exception of one patient whose CD4+ cell count remained less than 100 cells / mm$^3$. No patient died during follow-up.

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

During the years 1995 and 1996 the estimated annual incidence of CMV disease in HIV positive patients with CD4+ cell counts less than 50 cells / mm$^3$ was 17% in our center. The difference between this pre-HAART event rate of CMV disease and the observed absence of events in the patients in this study following HAART is significant ($X^2$-test, p<.02). Additionally we compared our results, with the CMV event rate of 27%, observed during a 12 month period, in 201 placebo assigned patients, reported in a study by Spector et al. Both patient groups are comparable with respect to baseline characteristics, including the additional risk of a positive result of CMV DNA PCR assay in 45% of patients reported by Spector et al., and in 55% (10 / 18) of our patients. Again the difference in CMV event rate was significant ($X^2$-test, p<.02).

The fact that no clinical manifest CMV disease occurred in our group of HIV positive patients can only be explained by the success of the HAART treatment. Others have also reported about the decreased incidence of CMV disease in HIV positive patients with favorable responses to the installation of HAART treatment. The decrease of CMV viral load found in this study confirms the restoration of the immune system enabling the patients to successfully suppress reactivation from their latent CMV infection.

All three patients with a clinical manifest CMV disease in the pre-HAART period, belonged to the group of patients with a CMV PCR positive blood test. This observation confirms the predictive value of the used PCR assay, even though patient number is small, and pre-HAART follow-up short (sensitivity 100%, specificity 55%, positive predictive value 23%, negative predictive value 100%). The test results compare favorably with those reported in the literature, suggesting at least an equal sensitivity.
Although HAART is considered very effective in treating HIV infection, with a sometimes dramatic improvement in the clinical manifestations of opportunistic infections, CMV retinitis has been reported in HIV positive patients after the start of HAART, even after a rise of CD4⁺ lymphocyte count above 100 cells / mm³. Diagnosis was made very shortly after the initiation of HAART, within a 4 to 8 week period. During the rest of follow-up after these first 2 months, not one new case of CMV disease occurred in these studies.

After the start of HAART, uveitis or vitritis has been described in some patients with CMV retinitis. This was believed to be due to restoration of the previously deficient immune response in these patients, leading to an intraocular inflammatory response against the virus. In our study neither a new case of CMV retinitis developed after the start of HAART, nor did any patient showed signs of uveitis.

We conclude that in HIV positive patients at high risk for development of CMV retinitis, either with a positive CMV PCR assay in blood and / or with CD4⁺ lymphocyte counts below 50 cells / mm³, HAART causes a dramatic decrease in the occurrence of CMV disease. This decrease is paralleled by an increase in CD4⁺ lymphocyte count, and a decrease in the amount of CMV DNA in the blood, which becomes undetectable in all patients with CD4⁺ cell counts above 100 cells / mm³.

REFERENCES

Chapter VI


