Impact of detectable human cytomegalovirus DNAemia on viro-immunological effectiveness of HAART in HIV-infected patients naïve to antiretroviral therapy

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SUMMARY

Our objective was to explore whether positive human cytomegalovirus (HCMV) DNAemia at baseline impaired CD4+ T-cell increase after 1 year of HAART. A sub-study of a randomized clinical trial in selected patients with <200 cell/mm^3 CD4+ at baseline was conducted. Six out of 30 patients had detectable HCMV DNAemia at baseline, all reaching HCMV suppression at week 52 after HAART (only 1 of them was treated with valgancyclovir). No significant differences were found between patients with detectable or undetectable HCMV DNAemia in terms of CD4+ T-cell increase and HIV RNA response to HAART. Although some data may favor HCVM pre-emptive therapy to decrease immune activation, our results do not indicate that this practice may increase CD4+ T-cell count after HAART. At the same time, HAART proved effective in reducing HCMV DNAemia without the need for a specific therapy.

KEY WORDS: HIV, HCMV, HAART, Immune recovery

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In the pre-HAART era, human cytomegalovirus (HCMV) was a frequent cause of major opportunistic diseases in HIV-infected patients. Up to 40% of HIV-infected patients developed HCMV retinitis, the risk being greatest when CD4+ T-cell count fell to 50-100 cells/mm^3 (Pertel *et al., 1992*). Moreover, active HCMV infection was associated with rapid progression to AIDS and death (Wohl *et al.,* 2005).

Even in the HAART era, detection of HCMV DNAemia was associated with unfavorable outcomes, including death for any causes (Deayton *et al.,* 2004; El Amari *et al.,* 2011). However, according to the American guidelines for prevention and treatment of opportunistic infections in HIV-positive patients, HCMV DNAemia without signs of organ diseases does not represent an indication for specific anti-HCMV therapy (Kaplan *et al.,* 2009). Nevertheless, prescription of specific antiviral therapy is fairly common in clinical practice for HIV-infected patients with detectable HCMV DNAemia even though there is no evidence of retinitis or end-organ diseases.

Hunt *et al.* (2011) studied a cohort of patients under virologically effective HAART who did not respond optimally from an immunological point of view and found that HCMV DNAemia persisted notwithstanding HAART, while specific treatment with valgancyclovir helped to reduce T-cell hyperactivation. Since immune hyperactivation has been associated with premature immune-senescence and incomplete immune recovery (Anthony *et al.*, 2003; Hazenberg *et al.*, 2000), the observation by Hunt *et al.* may pave the way to specific HCMV treatment to facilitate immune recovery. However, the same authors did not find any effect on absolute CD4+ T-cell count increase, they stud-

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ied a specific category of non immunological responders, and persistent immune activation does not entirely explain immune damage during the natural history of HIV infection or incomplete immune recovery after HAART (Goicoechea *et al.,* 2006). Therefore, the evolution active HCMV infection and its effect of immunological response to HAART merits further studies.

To our best knowledge, there are no data correlating active HCMV infection and blunted CD4+ T-cell increase after HAART in patients who did not have any experience of antiretroviral drugs (so-called *naïve* patients). Only one study (Panagiotakis *et al.,* 2007) explored the relationship between HCMV infection and CD4+ T-cell count but the cohort included patients either *naïve* or experienced to antiretroviral drugs and patients with active or latent HCMV infections were merged in the same category because intracellular HCMV genome was quantified. Our study, therefore, aimed to:

1) explore whether HCMV DNAemia had an impact on immune recovery in a group of HIV-infected patients *naïve* to antiretroviral treatment;
2) assess the evolution of HCMV DNAemia during the first year of treatment.

A sub-analysis of the multicentre SISTHER trial (Torti *et al.,* 2008) was conducted. Patients followed at the coordinating Centre (University of Brescia, Brescia, Italy) with baseline CD4+ T-cell count <200/mm^3 were included in the analysis. CD4+ T-cell counts and HIV RNA were assessed at baseline, week 28 and week 52 of treatment; HIV RNA was measured using a standard method (branched chain DNA-enhanced label amplification assay, Quantuplex 2-0, Chiron Diagnostics, Monza, Italy, with cut-off of 50 copies/ml). HCMV DNAemia was assessed by real-time polymerase chain reaction, Q-Cytomegalovirus RT-PCR™, Nanogene, Turin, Italy, with a limit of detection of 316 copies/mL on sera collected at baseline, week 28 and week 52 of treatment and stored at -70°C. All analyses were performed in the local laboratory (Chair of Microbiology, University of Brescia).

The characteristics of patients with a detectable or undetectable HCMV DNAemia at baseline were compared by chi-squared test or Student's t-test as appropriate. Between and within groups analysis of variance were performed with Bartlett's test for equal variances. Statistical analyses were performed with STATA software (StataCorp, College Station, TX).

Thirty patients were included in the analysis, selected from a population randomized in a 1:1 ratio to receive zidovudine/lamivudine (AZT/3TC)

TABLE 1 - *Patient characteristics at baseline.*

Variables	*Group A (HCMV DNA -) N. (%)*	*Group B (HCMV DNA+) N. (%)*	*p value*
Males	17 (70.8)	6 (100)	0.131
Age, years (mean, SD)	40.6 (6.7)	43.3 (8.7)	0.580
Risk factor for HIV			
• IVDU	4 (16.7)	0 (0)	
• Heterosex	15 (62.5)	4 (66.7)	
• MSM	3 (12.5)	1 (16.7)	
• Other/UK	2 (8.3)	1 (16.7)	0.703
HIV RNA, copies/mL (mean, SD)	146,197 (193,486)	315,210 (229,204)	0.195
CD4+ T-cell count, /mm^3 (mean, SD)	110.8 (58.3)	57.2 (63.3)	0.057
Anchor drug			
• LPV/r	12 (50)	5 (83.3)	
• TDF	12 (50)	1 (16.7)	0.141

HCMV = Human Cytomegalovirus; SD = standard deviation; IVDU= intravenous drug users; MSM = males who have sex with males; UK = unknown; LPV/r = lopinavir/ritonavir; TDF = tenofovir.

plus either tenofovir (TDF) or lopinavir/ritonavir (LPV/r). At baseline, 24 (84%) of these patients had undetectable HCMV DNAemia (group A), while 6 (16%) had detectable HCMV DNAemia (group B). In these six patients, HCMV DNAemia were (copies/mL): 8,766 (patient #1), 1,564 (patient #2), 70,211 (patient #3) 2,118 (patient #4), 4,152 (patient #5), 108,122 (patient #6). Among these patients, only patient #4 received specific treatment with valgancyclovir. Patients' characteristics at baseline are shown in Table 1.

There were no significant differences for mean baseline HIV RNA between groups A and B (mean HIV RNA: 146,197 and 316,210 copies/mL, for groups A and B, respectively; p=0.096). CD4+ T-cell counts at baseline were <100/mm^3 in 10/24 (41.7%) patients in group A -versus- 5/6 patients in group B, without significant differences in mean T-cell count between the two groups (110.9/mm^3 in group A -versus- 57.8/mm^3 in group B; p=0.057).

All 6 patients with detectable HCMV DNAemia at baseline reached undetectability (i.e., <316 HCMV DNA copies/mL) by W28, sustained up to W52. Only one of these patients received specific therapy for HCMV (valgancyclovir 900 mg/die) for disseminated HCMV disease. All patients with undetectable HCMV DNAemia at baseline maintained undetectable HCMV DNAemia throughout the follow-up.

Among patients in group A, the proportions of HIV RNA suppression <50 copies/ml were: 95.5% at week 28 and 87.5% at week 52; for group B, they were: 80% at week 28 and 60% at week 52. Overall, there were only 5 patients with detectable HIV RNA at week 52 but this was due to values just above the cut-off limit (61, 97 and 106 copies/mL, respectively, in group A; 54 and 57 copies/mL, respectively, in group B). Moreover, these values were both preceded and followed by undetectable HIV RNA, corresponding to so-called "blips". Figure 1 shows that CD4+ T-cell count of patients belonging to group B was always above the lowest values of the range of the group A at each time-point, apart from the CD4+ T-cell count of patient 4 (the only one who re-

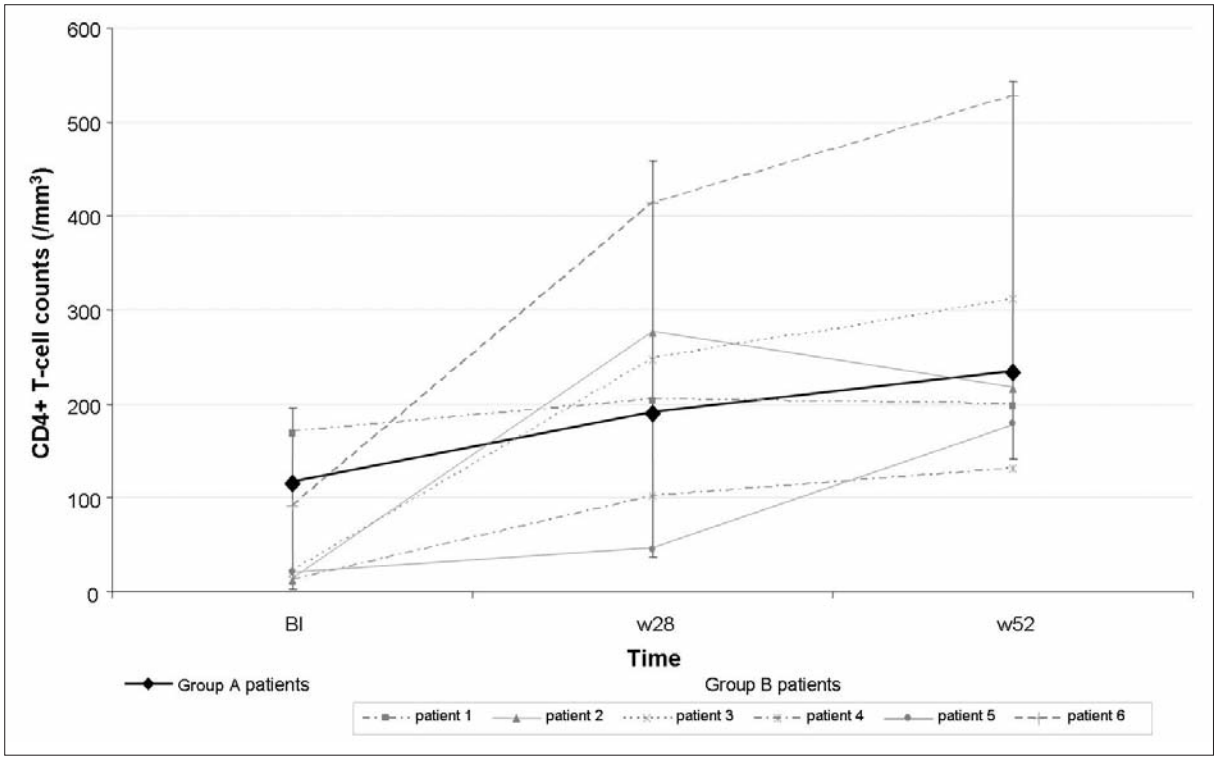

FIGURE 1 - *Trend in CD4+ T-cell counts for patients with undetectable (Group A) or detectable (Group B) human cytomegalovirus (HCMV) DNAemia. Bold line indicates the median of CD4+ T-cell counts of patients in Group A, with error bars representing range. Each patient with detectable HCMV DNAemia is represented as a single line.*

ceived valgancyclovir) at Week 52. Indeed, analysis of variance demonstrated that the differences in the CD4+ trends between the two groups were not statistically significant.

In conclusion, our data showed that in all 5 patients with replicating HCMV who did not undergo specific treatment, HAART alone was able to suppress this replication even in the presence of a low basal CD4+ T-cell count. Moreover, even though the number of patients is very small, HCMV replication at baseline did not appear to impair recovery of CD4+ T-cell count. Hunt *et al.* (2011) found that in immunological non responders under HAART anti-HCMV treatment reduced immune activation, but also in this category of patients no significant impact on CD4+ T-cell count was found. Therefore, our results concur to demonstrate, as already suggested (Wohl *et al.,* 2009), that there is no need to treat HCMV pre-emptively in patients initiating virologically effective HAART. This may also be true if the objective is to better reconstitute the absolute CD4+ T-cell count.

It is important to emphasize, however, that the number of patients studied and the duration of follow-up are still very limited both in Hunt's trial and in the present study, precluding the possibility to correlate HCMV DNAemia with the risk of clinical complications which might occur as a consequence of immunological hyper-activation notwithstanding a normal recovery of the absolute CD4+ T-cell count (Deayton *et al.,* 2004; El Amari *et al.,* 2011). Therefore, further studies are needed to clarify the mechanisms through which detectable HCMV DNAemia could increase non AIDS clinical complications during the course of HAART, including the relationships between immune activation, reconstitution of the CD4+ T-cell pool and clinical events.

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