Is cytomegalovirus a significant co-factor in HIV infection and disease?

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Disclosure

No conflicting interests

I have read and understood ICMJE policy on declaration of interests and declare that I have no conflicting interests
Why searching a Co-factor in HIV?

- Long natural history of HIV disease
- Variety of natural history (rapid, slow, normal progressors)
- HIV itself is not enough!
- Low direct pathogenic activity of HIV, except for CNS
- HIV-Morbidity is mediated by immune damage (CD4 depletion, immune activation)
- Stopping HIV replication efficiently (by HAART), HIV mediated morbidity persists

Which Co-factor in HIV?

- **Socio demographic:**
  - Gender
  - Black
- **Life style:**
  - Illicit drugs
  - Alcohol
- **Host cofactors**
  - HLA
  - CCR5
  - Restriction factors

**Microbial:**
- Micoplasma penetrans
- Syphilis
- HCV/HBV
- TB/malaria
....an old story

Mycoplasmas as cofactors in infection due to the human immunodeficiency virus.
Montagnier L, Blanchard A.


A longitudinal study of seroreactivity against Mycoplasma penetrans in HIV-infected homosexual men: association with disease progression.
Grau O1, Tuppin P, Slizewicz B, Launay V, Goujard C, Bahraoui E, Delfraissy JF, Montagnier L.


Why is hCMV a good candidate as cofactor?

- “Very old” (80 million years) human DNA virus which has probably co-evolved with its host since the beginning of human life (Mc Geoch 1995 J Mol Biol)
- Very common virus but not everybody is infected (!)
- Never eradicable virus from the infected subjects with several ESCAPE mechanisms
- High immune-regulatory capacity that could REMODEL human immune system
- Mostly Asymptomatic but potentially very dangerous virus, that in very certain conditions is able to cause HIGH MORTALITY and MORBIDITY:
  - In foetus: abortus, sensorineural hearing loss
  - In solid organ recipient (SOT): fever, GI involvement etc..
  - Stem cell transplant recipient (SCT): lethal pneumonia, etc......
  - In AIDS subjects: retinitis with eye loss, fatal encephalitis, esophagitis etc..
**hCMV characteristics**

- β-Herpes virus DNA
- the largest herpes virus (120 – 200 nm in Diameter)
- Complex structure:
  - 1 double stranded DNA per virion with a large genome of ~235 kb encoding ~165 genes
  - A Capsid
  - Envelope
  - Tegument
- Human specific (other models: murine and simian: MCMV, ...,)
- lifelong infection latent in endothelial, renal, pulmonary tissues as well myeloid cells

FIG. 3. Life cycle of HCMV in a human cell. HCMV enters human cells either through direct fusion or through the endocytic pathway. The virus attaches to the cell via interactions between viral glycoproteins (e.g., gB and gH) and a specific surface receptor(s) (e.g., prion-derived growth factor α), followed by the fusion of the envelope with the cellular membrane to release nucleocapsids into the cytoplasm. These nucleocapsids are translocated into the nucleus, where viral DNA is released. This initiates the expression of IE-1/IE-2 genes. Viral replication and maturation follow the stimulation and parallel accumulation of viral synthesis function. This process involves the encapsidation of replicated viral DNA as capsids, which are then transported from the nucleus to the cytoplasm. Secondary envelopment occurs in the cytoplasm at the endoplasmic reticulum (ER)-Golgi intermediate compartment. This is followed by a complex two-stage final envelopment and egress process that leads to viral release by exocytosis at the plasma membrane.

Typical owl’s eye inclusion seen on HE stain.

Seroprevalence

Between 30% and 90% in developed countries
• 15% childhood
• 40% 30 y.o.
• 60 - 70% > 65 y.o. industrialized country

1/3 of CMV + seems to be reinfected in their life by other CMV strains (Ross JID 2010)
hCMV: a spectrum of diseases

TABLE 2. Clinical features of HCMV infection

<table>
<thead>
<tr>
<th>Patient type</th>
<th>Clinical feature(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy individual</td>
<td>Usually asymptomatic; infrequently mononucleosis with fever, myalgia, adenopathy, splenomegaly</td>
</tr>
<tr>
<td>Fetus/infant with congenital infection</td>
<td>Jaundice, hepatosplenomegaly, petechiae, microcephaly, hypotonia, seizures, lethargy</td>
</tr>
<tr>
<td>SOT recipient</td>
<td>Febrile illness with leukopenia and malaise; pneumonitis; enterocolitis, esophagitis, or gastritis; hepatitis; retinitis; other tissue-invasive disease (nephritis, cystitis, myocarditis, pancreatitis)</td>
</tr>
<tr>
<td>Hematopoietic SCT recipient</td>
<td>Pneumonitis; enterocolitis, esophagitis, or gastritis; less commonly retinitis, encephalitis, hepatitis</td>
</tr>
<tr>
<td>HIV/AIDS patient</td>
<td>Retinitis; enterocolitis, esophagitis, or gastritis; immune recovery vitritis-posterior segment inflammation; pneumonitis; hepatitis</td>
</tr>
</tbody>
</table>

ASYMPOMATIC

Neonatologists/gynecologists

Transplantologist/Hematologists

CMV

Gerontologist/Cardiologist

Herpes virologist

Immunologists

HIV experts
A continuous balance between hCMV and host

The International Workshop on “CMV & Immunosence”, held in Parma, Italy, 25–27th March, 2013, (local organizer Paolo Santoni)

CMV-mediated immune remodelling activity

MAIN PRIMARY TARGET: Endothelial cells

"Antigenic storm"

production of > 700 protein open reading frames VERY STABLE but MANY (Science 2012)

Induction of HIGH T and B cell response:

- CD8 (classical cytolytic activity) mainly against non structural proteins
- CD4 (more recent rediscovered) mainly against latent associated antigens and structural proteins
- iTreg
- γδ T cells (recognize stress antigen)
- B cells (production of neutralizing antibody)

Control of Virus spread BUT

Viral Latency

Chronic immune system stimulation
Primary infection
CD8+ CD28+ 
CD4+CD25+ 
CD8+ 

Polyclonal
Cytotoxic
Effector T cells
- CD8+ 
- CD4+ 
- Multiple TCR Vβ families

Selection into Memory
CD4+CD25+ 
CD4+CD25- 

Long term memory
Contains clonotypes originally selected in primary infection 
New clonotypes may arise 

Memory inflation
CD4+ CD28- 
CD8+ 

(Waller, 2008)

Virus neutralization
Activated T cells
- Cytolysis
- Endothelial cells
- HCMV

Latent infection
CD8+ 
- Monocytes
- Restricted viral gene expression

Activated NK cells
- Activated T cells

E

B

C

D

F

Activated B cells
- Activated CD8+ T cells

(IFN-γ, IL-12, IL-15, IL-18)

(Crough, 2009)
Mechanism of Induction of HIGH T and B cell response:

- Non constant viral shedding (plasma, genital fluids, saliva)
- Cross-presentation by DC
- Production of only “packaged” antigens (dense bodies)

**viral replication is not required necessarily**

Pepperl S J virol 2000

**CMV as cofactor in the pre-HAART era**
Results from Haemophilic cohort of HIV infected subjects (HCV+)

- Individuals CMV IgG positive were 2.5 times as likely to progress to an AIDS-defining disease, compared with CMV-seronegative individuals. Survival analysis revealed that the risk increased approximately 2 years after seroconversion (Webster, ..Griffiths PD; Lancet 1989)
- The same cohort was studied after 13 ys of FU and CMV/HIV presented an increased risk not only for AIDS progression but also for survival (Sabin, ..Griffiths PD Epidemiol Infect 1995)
- After 20 ys FU CMV seropositivity continues to be associated with a more rapid rate of progression to AIDS and death. This effect does not appear to be mediated by the changing HIV RNA level or CD4 cell suggesting that some other mechanism is responsible (Sabin, ..Griffiths PD JID 2000)

Results from intervention trials using anti-herpes agents

- A double-blind, placebo controlled study of high-dose acyclovir (800 mg 4 times daily) in patients with AIDS who had CD4+ T-cell counts of <150 cells/µL no effect on the incidence of CMV disease between the arms, found a significant survival benefit to those patients exposed to acyclovir (the odds ratio decreased from 0.39 to 0.23; P = .018) (Youle AIDS 1994)
- A meta analysis of 8 trials of high dose acyclovir reported an effect in the setting of deep immuno-depression and high risk of herpetic reactivation (Ioannidis JP, JID 1998)
- The effect of Valacyclovir as preventive therapy in high immunosuppressed patients without evidence of CMV disease at enrollment, showed an higher anti-CMV effect in comparison with acyclovir (fig). Authors showed that high plasma (urine) CMV viral load at baseline was associated to an increased risk of CMV disease and progression to death (fig) (Emery JID 1999)

Figure 1. Kaplan-Meier plot shows progression to human cytomegalovirus disease stratified by treatment group.

- The effect of Valacyclovir as preventive therapy in high immunosuppressed patients without evidence of CMV disease at enrollment, showed an higher anti-CMV effect in comparison with acyclovir (fig). Authors showed that high plasma (urine) CMV viral load at baseline was associated to an increased risk of CMV disease and progression to death (fig) (Emery JID 1999)
Evidence from MTC HIV/CMV transmission

- The infants born to HIV infected women, that aquired both CMV and HIV had a more rapid progression to AIDS and showed CNS diseases (Kovacs NEJM 1999) (fig)

- More recently, Jennifer Slyker demonstrated that maternal CMV load was an independent factor associated to mortality in HIV infected infants (AIDS 2009) (fig)

- The same group described that CMV replication in breast milk and cervical fluids are higher than in plasma and are associated with CMV transmission to the HIV exposed uninfected infants, toghether with maternal CD4 count. Valaciclovir treatment seemed to be not able to inhibit CMV transmission (Slyker CID 2014) (fig)

CMV as cofactor in HAART era:

..... learning from HIV negative population
Patients with high levels of inflammatory markers such as IL-6 or CRP showed a significant increased risk in cardiac death if they had positive CMV serology.
This is the first study known to report a relation between high human CMV antibody levels and mortality. Analyses included 1,468 of 1,789 participants. For individuals with CMV IgG antibody titers in the highest quartile compared with lower quartiles, fully adjusted models showed that all-cause mortality was 1.43 times (95% confidence interval: 1.14, 1.79) higher over 9 years. In fully adjusted models, the hazard of CVD mortality was also elevated (hazard ratio: 1.35, 95% confidence interval: 1.01, 1.80).

A composite measure of tumor necrosis factor and interleukin-6 mediated a substantial proportion of the association between CMV and all-cause (18.9%, P < 0.001) and CVD (29.0%, P = 0.02) mortality.

This study is the first known to show that high CMV IgG antibody levels are significantly related to mortality and that the relation is largely mediated by interleukin-6 and tumor necrosis factor.
CVD related mortality

In a cohort of 511 individuals aged at least 65 years who were followed up for 18 years.

- The mean age of the participants was 74 years
- 70% were CMV-seropositive.
- CMV was strongly linked to socioeconomic status
- CMV infection increased the annual mortality rate by 42% (Hazard ratio = 1.42, 95% CI: 1.11–1.76 after adjusting for age, sex and baseline socio-economic and health variables)

- 3.7 years lower life expectancy from age 65.
- Infection was associated with a near doubling of cardiovascular deaths, whereas there was no increase in mortality from other causes.
CMV has also been implicated to cause restenosis of coronary arteries following coronary angioplasty and to interact with p53 to promote vascular smooth muscle cell proliferation.

Animal model experiments involving murine CMV or rat CMV have also shown the development of atherosclerotic lesions in blood vessels (Vliegen, Microbes Infect. 2004).

In a recent study of a model of mice fed a high-fat diet and infected with murine CMV, an increase in arterial blood pressure was observed during a 6-week period. This increase was enhanced in infected mice fed a high-cholesterol diet, and the murine-CMV-infected mice had a significant increase in proinflammatory cytokines, tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and MCP-1 (Cheng J. 2009).

Induction of proinflammatory cytokines by CMV infection may also sequentially up-regulate expression of intracellular adhesion molecules in uninfected neighboring cells through paracrine action (Dengler TJ., 2000).

Hemodynamic factors modulate HCMV infection of endothelial cells, thus providing new insights into the induction/acceleration of atherosclerosis by HCMV (DuRose, J virol 2012).

CMV-specific iTregs were studied in 131 older (65-85) and 57 young recognized the same antigens as conventional CD4+ T cells and were significantly more frequent at older ages.

They suppressed antigen-specific and nonspecific proliferation and in large part expressed Foxp3.

Frequencies of CMV-specific iTregs and CD8+ T cells (summated response) were significantly associated with diastolic and mean arterial pressures.

A novel CMV-induced regulatory-type CD4+ T-cell subset is readily detectable in CMV-infected people and, like the aggregate CD8+ T-cell response to the most dominant CMV antigens, is quantitatively associated with arterial stiffness in older life.

Whereas CD8+ effector T cells might directly cause vascular injury, iTregs may attenuate this response.
CMV and cognitive impairment and depression and frailty

- Consistent associations between measures of psychological stress and CMV antibody levels in a large (N=887, mean age=44, 88% male) occupational sample (Rector JL 2014)
- Cytomegalovirus (CMV) and herpes simplex virus 1 (HSV-1) seropositivity were associated with cognitive impairment using data from the National Health and Nutrition Examination Survey (NHANES) III (Tarter, Simanek, Aiello JID 2014)
- Compared with those who were CMV seronegative, women in the highest quartile of CMV antibody concentration had a greater incidence of frailty (hazard ratio: 3.46, 95% confidence interval: 1.45, 8.27) and mortality (hazard ratio: 3.81, 95% confidence interval: 1.64, 8.83) (Wang 2010)

CMV and Immune Risk Phenotype: a milestone with some limitations

The enhanced prevalence of CD28− T cells in elderly, together with other parameters, such as a disturbed CD4/CD8 ratio and CMV-seropositivity, has led to the definition of the so-called ‘immune risk phenotype’ (IRP) predicting a higher 2-year mortality in a longitudinal study of Swedish octogenarian and nonagenarian humans. (Wikby et al., 2005)

- CMV-IgG
- Decrease naive T cells
- Increase CD8+ T cells
- CD4/CD8 ratio < 1
- CD8+CD28−CD45RA+ expansion
- Increase proinflammatory cytokines (TNF, IFN, IL6)

Pawelec, Tubingen
CMV as cofactor in HAART era:

- CMV viraemia increase the risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy. (Deayton, Sabin 2004)
- CMV specific IFNg CD8+ response has been linked to increased IMT in men. (Hsue, 2006)
- Valganciclovir treatment has been proposed to decrease immune activation in individuals with incomplete ARV–response. (Hunt, 2010)
- Stronger CMV-specific CD8 response in HIV treated subjects. (Naeger, 2010)
- Under ART age as well as the mounting of robust anti-CMV T-cell responses independently alter T-cell reconstitution in treated patients. (Appay 2011)
- CMV/IgG antibody have been associated with subclinical atherosclerosis in HIV aviremic women. (Parinello, 2012)
Importance of cytomegalovirus viraemia in risk of disease progression and death in HIV-infected patients receiving highly active antiretroviral therapy

Deayton JR1, Prof Sabin CA, Johnson MA, Emery VC, Wilson P, Griffiths PD. Lancet 2004

- 374 patients were enrolled in a prospective study.
- Serial blood samples were tested for cytomegalovirus by PCR.
- Rates of new cytomegalovirus disease, new AIDS-defining disorders, and death were calculated over a median follow-up of 37 months after stratification according to baseline and most recent cytomegalovirus PCR status at any point during follow-up.
- Of 2969 PCR assays, 12.6% were positive for cytomegalovirus DNA. 259 (69.3%) patients were persistently negative for cytomegalovirus by PCR; 15 were persistently positive; and 100 were intermittently positive and negative.
- In multivariate models, cytomegalovirus PCR-positive status as a time-updated covariate was significantly associated with increased relative rates of progression to a new AIDS-defining disorder (2.22 \( p=0.005 \)) and death (4.14 \( p=0.0002 \)).

CMV elicits massive immune responses even in asymptomatic HIV- individuals

Sylwester/Picker, JEM, 2005
Higher CMV-specific CD8+ IFN-γ Production Associated with More Atherosclerosis

- 93 HIV-infected subjects and 37 uninfected controls.
- The mean age was 48 years and 85 (91%) were male.
- CMV-specific T-cell responses, but not hs-CRP and T-cell activation, were independently associated with higher carotid IMT (P = 0.001).

Hsue et al, AIDS, 2006

The proportion of CMV-specific CD8+ T cells was consistently higher in the HIV-seropositive subjects compared to the HIV-seronegative subjects.

David M. Naeger, Jeffrey N. Martin, Elizabeth Sinclair, Peter W. Hart, David R. Bangsberg, Frederick Hecht, Priscilla Hsue, Joseph M. McCune, Steven G. Deeks.


Proportion of CD4+ and CD8+ T cells responding to CMV pp65 and IE proteins was measured using flow cytometry in 685 unique HIV seronegative and seropositive individuals.

This HIV effect was observed even in patients who lacked measurable immunodeficiency.

Among the HIV seropositive subjects, CMV-specific CD8+ T cell responses were proportionately lower during recent infection, higher during chronic untreated infection and higher still during long-term antiretroviral treated infection.

The CD8+ T cell response to just two CMV proteins (pp65 and IE) was approximately 6% during long-term therapy, which is over twice that seen in HIV-seronegative persons.

CMV-specific CD4+ T cell responses followed the same trends, but the magnitude of the effect was smaller.
Pts were grouped into higher or lower CMV responders
we observed that higher CMV responders presented significantly lower CD4+ T cell counts and recovery compared to lower CMV responders (P=0.019)

Inverse correlations between percentages of pp65-specific CD4 T cells and counts of naive CD4 T cells

Loss of naive T cells with CMV-mediated memory inflation may provide potential mechanistic insights underlying the association between CMV infection and more rapid HIV disease progression as described previously.

Note: Valacyclovir, which has activity against HSV1/2 but not CMV, failed to decrease immune activation (Yi et al, CID, 2013).
CMV/IgG antibody have been associated with subclinical atherosclerosis in HIV aviremic women (Parinello JID 2012)

601 HIV-infected women (226 treated and aviremic; 148 treated and viremic; and 227 untreated) and 90 HIV-uninfected women.

Subjects had a mean age >40 years, were mostly African American/black (64%), and had a prevalence of smoking near 50%.

Among HIV-infected women, higher CMV IgG levels were associated with carotid artery stiffness and was independent of other factors including age, race/ethnicity, and smoking.

We not found evidence for an association among untreated or viremic.

Finally, no associations were observed in an HIV-uninfected

Increased carotid intima-media thickness is not associated with T-cell activation nor with cytomegalovirus in HIV-infected never-smoker patients.


In 59 HIV-infected individuals [n=30 undergoing ≥4 years of antiretroviral therapy (ART); n=29 never treated with ART] and 30 age-matched HIV-negative controls, the level of activation and senescence, as well as the frequency of CMV-specific T cells, on peripheral blood mononuclear cells, was measured while examining their association with carotid intima-media thickness.

Partial correlations were adjusted for age, systolic blood pressure, and nadir CD4 cell count.

The previously described roles of T-cell activation, CMV, and immunosenescence in the atherosclerotic risk of HIV-infected patients, as assessed by carotid intima-media thickness, were not apparent in our cohort of particularly ‘healthy’ HIV-infected never-smokers.

In HIV-infected individuals at low cardiovascular disease risk, our data show that the increased risk of carotid atherosclerosis is not associated with immunological markers described to be associated with HIV disease progression.
Aim:
To evaluate the prevalence and predictors of CMV coinfection in a cohort of HIV-positive patients

To assess the impact of CMV/HIV coinfection on the risk of AIDS and non-AIDS event/death.

Study population

10129 HIV + persons enrolled in ICONA (1/10/12)

90% of pts were CMV tested at enrollment
10% have a median time from enrollment and CMV test 17 (IQR 6-45) months

6111 (60.3% of all ICONA cohort)

5119 CMV IgG Pos (83.8%)

992 CMV IgG Neg (16.2%)

INCLUSION CRITERIA
Patients with ≥1 follow-up visit and ≥1 CMV Ig G test available without active CMV disease were included
Statistical Analysis

Cross-sectional

- Baseline characteristics
  CMV+ vs. CMV-
  (chi-square/Wilcoxon tests)

- Factors associated with CMV seropositivity
  (logistic regression)

Survival

- End points:
  AIDS event/death
  non-AIDS event/death

- Time to event was estimated by Kaplan-Meier curves and Cox regression

Predictive factors of CMV+ at baseline: results from multivariable logistic regression

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Adjusted odds ratio</th>
<th>CI95%</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female vs. male</td>
<td>1.07</td>
<td>0.90</td>
<td>1.27</td>
</tr>
<tr>
<td>Age, +10 year</td>
<td><strong>1.38</strong></td>
<td><strong>1.26</strong></td>
<td><strong>1.51</strong></td>
</tr>
<tr>
<td>Caucasian vs. other</td>
<td>0.61</td>
<td>0.46</td>
<td>0.82</td>
</tr>
<tr>
<td>HCVAb+ vs. HCVAb-</td>
<td>0.90</td>
<td>0.73</td>
<td>1.13</td>
</tr>
<tr>
<td>HBsAg+ vs. HBsAg-</td>
<td>1.13</td>
<td>0.81</td>
<td>1.56</td>
</tr>
<tr>
<td>Hetero vs. IVDU</td>
<td>0.92</td>
<td>0.73</td>
<td>1.17</td>
</tr>
<tr>
<td>MSM vs. IVDU</td>
<td><strong>1.36</strong></td>
<td><strong>1.04</strong></td>
<td><strong>1.78</strong></td>
</tr>
<tr>
<td>AIDS</td>
<td>0.86</td>
<td>0.68</td>
<td>1.07</td>
</tr>
<tr>
<td>CD4, +100 cells/µL higher</td>
<td><strong>1.03</strong></td>
<td><strong>1.00</strong></td>
<td><strong>1.06</strong></td>
</tr>
<tr>
<td>On ART vs. off ART</td>
<td>0.96</td>
<td>0.77</td>
<td>1.20</td>
</tr>
</tbody>
</table>
Results from prospective analysis:

1) end point: AIDS event/AIDS death

2) end point: non AIDS event/non AIDS death

Number of events and deaths reported for the 2 end points:

AIDS event/death
- AIDS death: 77 (1%)
- AIDS event: 5621 (92%)
- non event/death: 413 (7%)

NON AIDS event/death
- non AIDS death: 374 (6%)
- non AIDS event: 5725 (94%)
- non event/death: 12 (0%)

Median follow-up was 4.4 (IQR 1.1-8.9) years
The 10 year estimate was 10.9% (95%CI 8.3-13.5) for CMV negative and 12.4% (95%CI 11.1-13.6) for CMV positive.

**KM analysis for AIDS event/death**

![KM analysis for AIDS event/death](image)

- **Time to AIDS event/death**
- **Percent survival** (log rank p=0.67)

<table>
<thead>
<tr>
<th>Years from first available CMV test</th>
<th>N pts CMV-</th>
<th>992</th>
<th>501</th>
<th>248</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>N pts CMV+</td>
<td>5119</td>
<td>2413</td>
<td>1116</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

**Predictor factors for AIDS event/death by Cox regression**

- **AHR 95% CI**
  - Age, <10 year: 1.02, 0.74, 0.92
  - Female vs. male: 1.02
  - Caucasian vs. other: 1.02
  - MSM vs. IDU: 1.02
  - Hetero vs. IDU: 1.02
  - HCV Ab+ vs. HCV Ab-: 1.02
  - HBsAg+ vs. HBsAg-: 1.02
  - CD4 count (100 cells/μl): 1.02
  - HIV RNA (1 log10 cp/ml): 1.02
  - Years from HIV diagnosis, <5: 1.02
  - ART experienced vs. naive: 1.02
Causes of Non AIDS related event/death (n=386)

### Neurologic events

<table>
<thead>
<tr>
<th>Event</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningitis</td>
<td>79</td>
</tr>
<tr>
<td>Meningoencephalitis</td>
<td>3</td>
</tr>
<tr>
<td>Cerebral Atrophy</td>
<td>4</td>
</tr>
<tr>
<td>Encephalopathy not ABC</td>
<td>4</td>
</tr>
<tr>
<td>Other specific neurological symptoms</td>
<td>9</td>
</tr>
<tr>
<td>Other neurological disorders</td>
<td>11</td>
</tr>
<tr>
<td>TOT</td>
<td>115</td>
</tr>
</tbody>
</table>

### Cardiovascular disease

- Neoplasia: 165 (44%)
- Cardiac failure: 11 (31%)
- Other cardiovascular diseases: 13 (31%)

### Neoplasia

- In situ uveal melanoma: 12 (31.4)
- In situ renal carcinoma: 12 (31.4)
- In situ lung: 15 (37.9)
- In situ breast: 7 (17.5)
- In situ bladder: 10 (25.6)
- In situ colorectal: 7 (17.5)
- In situ liver: 16 (40.0)
- In situ gallbladder: 12 (31.4)
- In situ stomach: 3 (7.9)
- In situ small intestine: 3 (7.9)
- In situ lymph nodes: 3 (7.9)
- In situ skin: 3 (7.9)
- In situ pancreas: 3 (7.9)
- In situ ovary: 3 (7.9)
- In situ testis: 3 (7.9)
- In situ prostate: 2 (5.1)
- Other neoplasia: 30 (75.6)
- TOT: 165 (100)

### KM analysis for non AIDS event/death

- CMV IgG positive
- CMV IgG negative

Log rank p = 0.0058

The 10 year estimate was 7.4% (95%CI 5.9-7.9) for CMV negative and 10.3% (95%CI 9.0-11.6) for CMV positive.
Cox regression analysis showed that CMV positivity was an independent predictor of NON AIDS event/death with an Adjusted Hazard Ratio of 1.52. Other factors that predict were age and female sex and also AIDS presentation at baseline.

Which kind of NON AIDS event/death was associated with CMV coinfection?

Multivariable analysis for different kind of SnAEs:

- Association between CMV seropositivity and non-AIDS-related malignancies (adjusted HR, 1.98 [95% CI, .73–5.36]; P = .17)
- nonvascular neurological diseases (adjusted HR, 0.94 [95% CI, .54–1.62; P = .82)
- cardiovascular and cerebrovascular disease, with an adjusted HR of 2.27 (95% CI, .97–5.32; P = .05)
What about smoking?

- We analysed information about smoking habits at baseline, which was available for 3,470 of 6,111 patients (56.8%).
- The proportions of cigarette smokers among CMVpositive and CMV-negative individuals were comparable (51.2% vs 50.1%; P = .64).
- After adjustment for smoking status, CMV coinfection remained associated with a higher hazard of severe non–AIDS-defining events/non–AIDS-related death (adjusted HR, 1.77 [95% CI, 0.92–3.40]; P = .08).
- The marginally non-significant association was probably due to the smaller sample size, as suggested by the increase in the CI.
- Further, being a cigarette smoker at baseline, was independently associated with severe non–AIDS-defining events/non–AIDS-related death (adjusted HR, 1.53 [95% CI, 0.07–2.21]; P = .02);

Conclusions

- 83.8% of the pts enrolled in the Icona cohort was CMV+ at baseline

- CMV seropositivity is associated with: older age, MSM, non-caucasian origin, higher CD4 count

- CMV/HIV coinfection was associated with 50% higher risk of non AIDS event/death after controlling for known confounding
Conclusions

We suppose that the increased risk may be due to CMV-chronic immune activation in addition to that caused by HIV itself.

A follow-up study using stored plasma samples has been designed to test this hypothesis.

sCD163 increase in HIV/CMV coinfected subjects included in ICONA cohort
Serena Vita, M Lichtner, G Marchetti, C Mascia, E Merlino, P Cicconi, V Vullo, PL Viale, A Costantini, A d’Arminio Monforte
for ICONA Foundation Study Group

- To evaluate the role of CMV chronic infection in sustaining residual immune activation in HIV infected subjects undergoing suppressive ART.

- To study the differences in plasma markers of myeloid immune activation, related to cardio cerebro vascular diseases in a matched population of HIV monoinfected and HIV/CMV coinfected patients.

- To correlate CMV IgG levels with soluble markers

Personal data presented at CROI 2015, unpublished
**Study Population**

**Inclusion Criteria**
- ≥1 CMV IgG test available
- plasma sample after ≥1 yr of successful ART

**Matching criteria**

<table>
<thead>
<tr>
<th>HIV/CMV</th>
<th>HIV/CMV</th>
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</thead>
<tbody>
<tr>
<td>- (23 pts)</td>
<td>+ (46 pts)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td><strong>Age</strong> (Mdn, min-max)</td>
</tr>
<tr>
<td>2F; 21M</td>
<td>4F; 42M</td>
</tr>
<tr>
<td>40 (34-52)</td>
<td>42 (31-54)</td>
</tr>
<tr>
<td><strong>CD4 nadir</strong> (mm³, Mdn, min-max)</td>
<td><strong>HBV</strong></td>
</tr>
<tr>
<td>281 (2-598)</td>
<td>0</td>
</tr>
<tr>
<td>207 (2-611)</td>
<td>0</td>
</tr>
<tr>
<td><strong>HCV</strong></td>
<td><strong>CD14</strong> (ng/ml)</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>R&amp;D Systems</td>
<td></td>
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</tbody>
</table>
| CMV ELISA Quantitation Kit (GenWay Biotech) used to retest all samples and in the CMV positive populations to quantify Ab levels in duplicate.

**Exclusion criteria**
- previous or current CMV organ diseases, organ transplantation, use of immunosuppressive or immunomodulant drugs in the last year, cancer or treatment for cancer in the previous 5 years, insulin dependent diabetes mellitus, glomerular filtration rate < 39 ml/min, severe liver disease, endocrine disorders, autoimmune disease.

**Materials and Methods**

**Immunological Assays**
- Plasma levels in duplicate of
  - IL6 (pg/ml) eBIOSENCE
  - TNFalpha (pg/ml) eBIOSENCE
  - CD163: (ng/ml) R&D Systems
  - sCD14: (ng/ml) R&D Systems

CMV ELISA Quantitation Kit (GenWay Biotech) used to retest all samples and in the CMV positive populations to quantify Ab levels in duplicate.

**Statistical analysis**
- Mann-Whitney Test, Spearman correlation analysis (Prima 6.0 software)
sCD163 levels in CMV neg vs. CMV pos subjects

CD163 R expression (labile bound):
- macrophages, monocytes (especially CD14++CD16low), CD16 low DC, hepatic macrophages, HIV-infected macrophages and microglia

CD163R functions:
- scavenger receptor cysteine-rich (SRCR) molecule for Haptoglobin-Haemoglobin complex and free Hb
- co-receptor for TLR
- Induce apoptosis by TWEAK
- sCD163 production: (intracellular storing and release and cleavage by MMP)
- After ligation, by stimulated macrophages
- By steroid and IL-10 stimulated myeloid cells

sCD163 functions:
- primary function:
  - Terminal control of myeloid inflammation
  - Inhibition of IL-2 production and PMA-T cell proliferation

Without HCV chronic patients the sCD163 increase is still significant and evident

HIV+ monoinfected pts showed similar value to HD
**TNFα and sCD14 plasma levels**  
in CMV neg vs. CMV pos subjects

Regarding IL-6 no differences were found, suggesting that CMV immune activation are more linked to monocyte/macrophage in stead of T cells in this settings.
Correlation between CMV Ab levels and sCD163 and IL-6 in CMV+ pts (n=46)

![Graphs showing correlation between CMV Ab levels and sCD163, IL-6, and TNF levels.]

Significant correlation among the different immune markers only in CMV Ab+ subjects

![Graphs showing correlation between different immune markers such as sCD163, IL-6, and TNF levels.]
Conclusions

• CMV chronic infection appears to be related to an increase in soluble markers of myeloid activation in HIV infected subjects under successful ARV with similar biological (age and sex) and HIV related (HIV suppression, CD4 nadir and CD4 recovery) factors.

• This persistent activation of monocytes and macrophage that has been associated to cardio-vascular and neurological damage in general population, may explain the increased risk of non AIDS events found in CMV/HIV coinfected subjects.

Hypothesis
Conclusions:

Is cytomegalovirus a significant co-factor in HIV infection and disease?

- hCMV is a good candidate to be a co-factor due to its high impact on host immune response
- hCMV has an independent role in accelerating cardiovascular disease in general population
- hCMV contributes to progression to AIDS events and AIDS death in the natural history of HIV infection and disease
- hCMV seems to increase the risk of non AIDS events and death in ART treated HIV infected patients, especially cardiovascular disease
- hCMV could sustain residual immune activation by triggering myeloid cells despite ARV
CMV/HIV coinfection: Open questions

1) We need precise measurement of
   ✓ Viral burden (where? Plasma, genital tract, saliva, lymph nodes)
   ✓ Viral Latency or reactivation status definition
   ✓ Type of immune surveillance:
     ✓ Antibodies
     ✓ Intracellular cytokines T production:
       ✓ Which cytokines
       ✓ Which antigens

2) We need larger studies considering CMV negative HIV population and different age ranges

Clinical prospective

✓ CMV coinfection as a **marker for better monitoring** CV and neurological disease

✓ Possibility to **treat CMV infected** patients at high risk:
  ✓ Acyclovir
  ✓ Valganciclovir
  ✓ New drugs:
    ➞ Maribavir (CMV viral DNA assembly inhibitor from ViroPharma – Phase III discontinued)
    ➞ Brincidofovir (CMV DNA polymerase inhibitor from Chimerix/Marty – Phase III)
    ➞ Letermovir (CMV terminase complex inhibitor from Merck/AiCuris – Phase III)

✓ Possibility to **prevent in CMV neg**:
  ✓ VACCINE DEVELOPMENT: TransVax, GlaxoSmithKline and Sanofi Pasteur ➞ phase II trials.
  ✓ ➞ gB/MF59 vaccine exhibited a vaccine efficacy of 50% in healthy postpartum females or kidney transplant (reviewed in Rieder, CMI 2014)
  ✓ Hand Hygiene
  ✓ Condom
THANKS!

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Back slide

- 108 HIV type 1 (HIV-1)–infected hemophiliac men whose date of HIV seroconversion was accurately known;
- the men were then stratified according to their CMV immunoglobulin G (IgG) status.
- Individuals with prior CMV infection, as evidenced by serological findings, were 2.5 times as likely to progress to an AIDS-defining disease, compared with CMV-seronegative individuals.
- Survival analysis revealed that the risk increased approximately 2 years after seroconversion.

Webster A1, Lee CA, Cook DG, Grundy JE, Emery VC, Kernoff PB, Griffiths PD

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The effect of CMV infection on progression of human immunodeficiency virus disease in a cohort of haemophilic men followed for up to 13 years from seroconversion

C. A. SABIN1, A. N. PHILLIPS1, C. A. LEE2, G. JANOSSY3, V. EMERY4

AND P. D. GRIFFITHS5

- 111 HIV infected hemophilic men after 13 years of FU

**Effect on AIDS development**

**Effect on Survival**

The effect was age and CD4 dependent


- A double-blind, placebo controlled study of high-dose acyclovir (800 mg 4 times daily) in patients with AIDS who had CD4+ T-cell counts of <150 cells/µL
- no effect on the incidence of CMV disease between the arms
- found a **significant survival benefit** to those patients exposed to acyclovir (the odds ratio decreased from 0.39 to 0.23; P = .018)

Youle MS, Gazzard BG, Johnson MA, et al.


This survival effect was also, in part observed in a meta analysis of 8 trials of high dose acyclovir patient/years of follow-up. The effect was reported in the setting of deep immuno-depression and high risk of herpetic reactivation.

Ioannidis JP, Collier AC, Cooper DA, et al.
310 patients from 15 European or Australian centers were enrolled prospectively into this virology substudy. All were HIV-antibody positive, HCMV IgG–antibody positive, had <100 CD4 cells at screening, and had no evidence of HCMV end-organ disease. Were randomized to receive oral prophylaxis with valacyclovir (2 g 43/day), or low-dose acyclovir (400 mg 23/day) in a 3:2:2 ratio.

Progression to CMV disease according to CMV viral load in urine

Progression to death according to CMV viral load in urine

The Journal of Infectious Diseases 1999;180:695–701
Valanciclovir is more efficent than Acyclovir, even if a decrease in CMV viral load has been observed in the first 8 weeks of therapy.
The detection of cytomegalovirus DNA in maternal plasma is associated with mortality in HIV-1 infected women and their infants

AIDS. 2009 January 2; 23(1): 117–124

Jennifer A. Slyker1,2, Barbara L. Lohman-Payne2,3, Sarah L. Rowland-Jones1, Phelgona Otieno2, Elizabeth Maleche-Obimbo3, Barbra Richardson4, Carey Farquhar2,3, Dorothy Mbiori-Ngacha4, Vincent C. Emery5, and Grace C. John-Stewart2,3

1 MRC Human Immunology Unit, Oxford University, Oxford, UK

- A longitudinal study examined the relationship between maternal CMV DNAemia and maternal-infant mortality during two years postpartum in Kenyan women
- Maternal CMV DNAemia remained a significant risk factor for mortality in HIV-1 infected infants after adjusting for maternal CD4 cells/ mm3 or maternal death
- Maternal CMV DNA + was also associated to higher maternal mortality at 24 months (p=0.0006)
CMV as cofactor in other diseases

Inflammation in common variable immunodeficiency is associated with a distinct CD8(+) response to cytomegalovirus.

CMV-T cell populations > 20% of circulating CD4 and/or CD8 memory T cells repertoire

+ CD8 CMV specific T cells frequency

- naive and non CMV specific memory CD8 T cells

The detection of cytomegalovirus DNA in maternal plasma is associated with mortality in HIV-1 infected women and their infants

Jennifer A. Slyker1,2, Barbara L. Lohman-Payne2,3, Sarah L. Rowland-Jones1, Phoebea Dixon1, Elizabeth Mactche-Obispo1,2, Barbara Richardson2, Cyrus Panwar1,2, Dorothy Mwini-Migachi1, Vincent C. Emery4, and Grace C. John-Stewart2,5

1 MRC Human Immunology Unit, Oxford University, Oxford, UK
2 Department of Pediatrics, University of Washington, Seattle, WA
3 Department of Pathology, University of Washington, Seattle, WA
4 Institute of Infection and Global Health, University of Liverpool, Liverpool, UK
5 Department of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, NC

OBJECTIVE—Cytomegalovirus (CMV) is an important pathogen in healthy mucocutaneous and individuals with human immunodeficiency virus (HIV-1). The objective of this study was to determine whether the detection of CMV DNA (CMV DNAemia) in maternal plasma was associated with mortality in HIV-1 infected women or their infants.

Methods—A longitudinal study was designed to examine the relationship between maternal CMV DNAemia and maternal-infant mortality during two years postpartum. Sixty-four HIV-1 infected women and their infants were studied. CMV DNA loads were quantified in plasma from the mothers near the time of delivery. Baseline maternal CD4 counts, CD4%, HIV-1 RNA, and CMV DNAemia were evaluated as covariates of subsequent maternal or infant mortality in univariate and multivariate Cox regression.

Results—CMV DNA was detected in 11/64 (17%) of the HIV-1 infected women. HIV-1 and CMV viral load were strongly correlated in CMV DNAemic women (p=0.84, p=0.001). Detection of CMV DNAemia was associated with decreased maternal survival at 24 months postpartum (log-rank p=0.069). Additionally, HIV-1 infected infants born to CMV DNAemic women had a 4-fold increased risk of mortality during 24 months of follow-up. Maternal CMV DNAemia remained a significant risk factor for mortality in HIV-1 infected infants after adjusting for maternal CD4 cells/mm3 (adjusted HR=4.3, CI=1.4–13), CD4% (HR=1.2, CI=1.0–10), HIV-1 viral load (HR=4.1, CI=1.4–12) or maternal death (HR=3.7, CI=1.9–13).
Acyclovir Therapy Reduces the CD4+ T Cell Response against the Immunodominant pp65 Protein from Cytomegalovirus in Immune Competent Individuals
Characteristics of population according to CMV serology at baseline

<table>
<thead>
<tr>
<th></th>
<th>Tot n=6111 (100%)</th>
<th>Cmv pos n=5119 (83.8%)</th>
<th>CMV neg n=992 (16.2%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years median</td>
<td>36 (32-42)</td>
<td>36 (32-42)</td>
<td>35 (31-40)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>(IQR)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Ethnic group, n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>1745 (28.6)</td>
<td>1446 (28.3)</td>
<td>299 (30.1)</td>
<td>0.23*</td>
</tr>
<tr>
<td>Black</td>
<td>145 (4.7)</td>
<td>252 (4.9)</td>
<td>33 (3.3)</td>
<td>0.06*</td>
</tr>
<tr>
<td>Asian</td>
<td>33 (0.6)</td>
<td>30 (0.6)</td>
<td>3 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2.3</td>
<td>125 (2.4)</td>
<td>20 (2.0)</td>
<td></td>
</tr>
<tr>
<td>HCV pos, n(%)</td>
<td>2047 (33.5)</td>
<td>1674 (32.7)</td>
<td>373 (37.6)</td>
<td>0.0028*</td>
</tr>
<tr>
<td>HIV pos,n(%)</td>
<td>330 (5.4)</td>
<td>284 (5.6)</td>
<td>46 (4.7)</td>
<td>0.24</td>
</tr>
<tr>
<td>Mode of transmission</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>for HIV, n (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IVDU</td>
<td>1829 (29.9)</td>
<td>1495 (29.2)</td>
<td>334 (33.7)</td>
<td>0.0002*</td>
</tr>
<tr>
<td>MSM</td>
<td>1564 (25.6)</td>
<td>1363 (26.6)</td>
<td>201 (20.3)</td>
<td></td>
</tr>
<tr>
<td>Years from HIV diagnosis, median</td>
<td>1 (0-7)</td>
<td>1(0-7)</td>
<td>2 (0-7)</td>
<td>0.40*</td>
</tr>
<tr>
<td>(IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIDS, n (%)</td>
<td>683 (11.2)</td>
<td>551 (10.8)</td>
<td>132 (13.3)</td>
<td>0.02*</td>
</tr>
<tr>
<td>On ART, n (%)</td>
<td>755 (12.4)</td>
<td>637 (12.4)</td>
<td>118 (11.9)</td>
<td>0.63*</td>
</tr>
<tr>
<td>CD4+/μl, median</td>
<td>443 (270-634)</td>
<td>448(279-636)</td>
<td>417 (225-620)</td>
<td>0.0017*</td>
</tr>
<tr>
<td>HIV RNA, log10 sp/μl, median (IQR)</td>
<td>3.74 (2.62-4.60)</td>
<td>3.74 (2.62-4.59)</td>
<td>3.76 (2.65-4.66)</td>
<td>0.47*</td>
</tr>
</tbody>
</table>

*Wilcoxon test for independent variables  ° chi-square

Materials and Methods

Immunological Assays
Plasma levels in duplicate of

- IL-6 (pg/ml), eBiosciences
- TNF-α (pg/ml), eBiosciences
- sCD163 (ng/ml), R&D System
- sCD14 (μg/ml), R&D System

CMV ELISA Quantitation Kit, GenWayBiotech to retest all samples and in the CMV positive populations to quantify Ab levels in duplicate.

Statistical analysis

- Mann-Whitney Test
- Spearman correlation analysis (Prism 6.0)
Results

- Significantly higher plasma levels of sCD163 in CMV+ compared to CMV- group
- Moreover, an increase of sCD14 and TNFα levels was found even if marginally statistically significant
- No differences in IL-6 levels between the groups were observed

Correlation with CMV IgG levels in coinfected subjects

A significant correlation was found with sCD163 ($r=0.49$, $p=0.0006$), IL-6 ($r=0.42$, $p=0.0041$), TNFα ($r=0.34$, $p=0.021$) but not for sCD14 ($r=0.15$, $p=0.29$).
Conclusions

- CMV chronic infection appears to be related to an increase in soluble markers of myeloid activation in HIV infected subjects under successful ARV with similar biological (age and sex) and HIV related (HIV suppression, CD4 nadir and CD4 recovery) factors.

- This persistent activation of monocytes and macrophage that has been associated to cardio-vascular and neurological damage in general population, may explain the increased risk of non AIDS events found in CMV/HIV coinfection subjects.

Proposed Mechanisms for CMV as cofactor in HIV

Figure 1. Potential mechanisms by which cytomegalovirus (CMV) might enhance human immunodeficiency virus (HIV) replication. Direct coinfection in cells such as macrophages could lead to enhanced HIV replication, through transcriptional activation of the HIV long terminal repeat (LTR) or to enhanced viral tropism, through provirus formation. Alternatively, CMV infection can lead to the production of the CMV-encoding receptor (CCR5 or D6) expression, leading to entry of HIV into cells that are normally non permissive. The bottom panel captures the data presented by Johnson et al (submitted), whereby CMV infection of macrophages leads to the activation of resting and blood mononuclear cells, resulting in upregulation of CD5, enhanced HIV entry, and increased HIV replication. Abbreviation: CBMC, cord blood mononuclear cell.