CONSIDERATIONS REGARDING THE DIAGNOSIS OF CMV INFECTION IN HIV INFECTED PATIENTS

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ABSTRACT

***Aims**: The objective of the study was to evaluate the accuracy of Quantiferon-CMV assay in the diagnosis of cytomegalovirus (CMV) infection in Human Immunodeficiency Virus (HIV) positive patients and compare it with the older Enzyme-linked immunosorbent assay (ELISA) method.*

***Material and method**: This is a prospective study on 48 HIV infected adult patients with CD4 lymphocytes (cluster of differentiation 4) less then 200 cells/mm³ who were tested with QuantiFERON (QF) -CMV assay (Cellestis) and with ELISA-CMV (Bio-Rad).*

***Results**: Sixteen women and thirty-two men were included in the study, with a median age of 32.8 years (range between 20-62 years), of whom three were antiretroviral naive patients and 45 were multiple experienced. Out of 48 patients, seven were asymptomatic and 41 had different clinical manifestations. IgM ELISA was negative in all cases, while IgG ELISA was positive in 45 patients (95.5%) and the QF-CMV assay was positive in 38 cases (79.1%). All patients with CD4 lymphocytes less then 50 cells/mm³ and clinical manifestations, where positive for both tests (14 patients).*

***Conclusions**: QF-CMV assay has increased sensitivity in detecting CMV reactivation induced by severe immunodepression (92.7%). There is a strong correlation ($p < 0.01$) between severe immunodepression (CD4 count less then 50 cells/mm³) and CMV reactivation detected by QF.*

***Key words**: Cytomegalovirus, QuantiFERON, ELISA, HIV.*

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Background

Cytomegalovirus is an important pathogen in immunocompromised hosts, including patients with advanced HIV infection, neonates, and solid-organ and stem-cell transplant recipients. The seroprevalence of CMV infection in HIV-infected patients is high. Many studies found a CMV seroprevalence above 90% in men sex with men (MSM) HIV-infected patients, approximately 75% in intravenous drug users (IDU) and a lower prevalence (60%) in heterosexual HIV-infected patients in concordance with HIV-negative population[1]. Asymptomatic excretion of CMV in saliva, respiratory secretions, urine, and semen explains the increasing risk of exposure and contact with contaminated environmental surfaces containing viable virus could be a route of transmission[2]. CMV can also be acquired through blood transfusion and organ transplantation from CMV-infected donors[3].

The risk of exposure to CMV increases with age (rises by 1% per year)[4] and the infection rate is also influenced by socioeconomic status and geographical location (higher rates in developing countries and economically depressed regions)[5,6].

Primary CMV infection occurs during the childhood and adolescence[7]. In immunocompetent individuals, primary CMV infection is generally asymptomatic or in some cases may be accompanied by generalized lymphadenopathy.

CMV remains latent in the infected host throughout life and is reactivated in immunocompromised patients, developing CMV diseases including chorioretinitis, esophagitis, colitis, pneumonia, and central nervous system disease[8].

In HIV infection, more data suggest a significant role for CMV in HIV-disease progression[9]. In patients with low CD4 count, progressive loss of immune function, and loss of cell-mediated immunity, permits CMV reactivation. CMV-seropositive HIV-infected patients progress approximately 2.5-times faster than CMV-seronegative patients, and HIV-infected patients with CD4 T-cell counts over 100 cells/mm3 who are seropositive for CMV have an increased risk for progression to AIDS (Acquired Immune Deficiency Syndrome) as compared with CMV-seronegative individuals[10,11].

These observations have generated interest in immune-based strategies for the management of CMV disease[12].

The QuantiFERON-CMV assay is a whole blood interferon-gamma release assay test for Cell Mediated Immune (CMI) responses to peptide antigens that simulate CMV proteins. Individuals infected with CMV usually have CD8+ lymphocytes (cluster of differentiation 8) in their blood that recognize these antigens. This recognition process involves the production and secretion of cytokines and interferon (IFN). The detection and subsequent quantification of IFN forms the principle of this test. Few studies have shown a correlation between a lack of detectable cell-mediated immunity measured by the QF-CMV assay and a higher incidence of CMV infection and disease in immunocompromised patients[13]. In individuals with a history of CMV disease, CMV-specific CD8(+) T cell responses are reduced even in the setting of CD4(+) T cell reconstitution[14]. Measurement of cell-mediated immunity against CMV by the QF appears to be a promising strategy to identify patients at highest risk for the development of CMV disease[13,14].

Considering all this information, we proposed to evaluate the accuracy of Quantiferon-CMV assay in the diagnosis of CMV infection in HIV positive patients and compare it with the ELISA method.

Materials and methods

A total of 96 ELISA (IgM and IgG) and QF-CMV tests were performed on 48 HIV-1 positive patients with a median age of 32,8 (age range 20-62 years), of which 16 were females and 32 males, enrolled in the study from November 2013 to March 2014. Other criteria for inclusion were a CD4 count < 200 cells/mm^3, asymptomatic or with suggestive clinical manifestations: neurological (central or peripheral), ocular, gastrointestinal, pancreatic or hepatic symptoms (Table 2). The patients with other associated opportunistic infections were excluded from the study[15].

This study was approved by the Ethical Committee of the Clinical Infectious Diseases Hospital Constanta and was carried in accordance with standard operation procedures ensuring Good Clinical Practice Guides. Written informed consent was obtained from all the subjects before admission to the study.

Both the QF-CMV (Cellestis) and ELISA-CMV (Bio-Rad) tests were performed on freshly isolated blood, the harvesting entailed collection of 3 (3x1ml) peripheral blood QF (Cellestis) tubes (for the positive control, negative control and CMV stimulus) and 10ml of peripheral blood in sodium citrate tubes for ELISA-CMV (IgM and IgG) testing. The QF-CMV tubes were incubated overnight at 37^0 C and processed according to the manufacturer's protocol and the ELISA samples were centrifuged at 3000 rpm for 10 minutes, after which the plasma was aliquoted and used for testing.

QF-CMV and ELISA tests were performed separately in a double-blind fashion and the results were read according to the specifications of the manufacturer; QF test was considered positive at a value ≥ 0,2 UI/ml, ELISA CMV IgM was negative when the raport optical density/cut-off ≤ 0,90 and ELISA CMV IgG was positive at a value ≥0,5 UA/ml.

Statistical analysis

For statistical biomedical analysis, Excel Analysis ToolPack and MedCalc programs were used. The receiver operating characteristic (ROC) curve was a fundamental tool for diagnostic tests evaluation. The area under the ROC curve (AUC) was calculated for each diagnostic method. For all other statistical comparisons, p value of less than 0.05 ($p< 0.05$) was considered statistically significant.

Results

Sixteen women and thirty-two men were included in the study, with a median age of 32.8 years (range between 20-62 years), of whom three

were antiretroviral naive patients and 45 were multiple experienced (Table 1).

Characteristics	Number of patients (pts)	
Gender	32 male	16 female
Age	20-62 years (median age - 32.8)	
Heterosexual/MSM	48/0	
IDU yes/no	1/47	
HVB/HCV	2/0	
Malignancies yes/no	0/48	
CD4	5-182 (median -92cells/mm^3)	
ARN-HIV <50 copies/ml	36	
> 400copies/ml	12	
ARV treatment	45 experienced	3 naive
	- 36 compliant	
	- 9 non-compliant	
Clinical manifestation	41 symptomatics	7 asymptomatics
Total	48 pts	

Table 1: Characteristics of the HIV infected patients.

All patients were heterosexual, with a median CD4 count of 92 cells/mm^3 (ranging between 5-182 cells/mm^3), only 36 patients had HIV RNA <50 copies / ml.

Only one patient was a known intravenous drug user, two of the experienced patients were known with virus B coinfection; no malignancies were detected.

Out of 48 patients, seven were asymptomatic and 41 had different clinical manifestations: pancreatitis (12 patients), esophagitis (3 patients), gastritis (2 patients) colitis (6 patients), liver involvement (9 patients), cholecystitis (7 patients), central and peripheral nervous system involvement (8 patients), retinitis (4 patients); eight cases we considered immune reconstitution inflammatory syndrome (IRIS) (Table 2).

IgM ELISA was negative in all cases, while IgG ELISA was positive in 45 patients (95.5%) and the Quantiferon-CMV assay was positive in 38 cases (79.1%).

The patients who were treated with gancyclovir for CMV retinitis showed positive ELISA IgG, but negative Quantiferon-CMV assay (two cases with CMV retinitis and one with neurological symptoms) (Table 3).

Clinical manifestations	Number of patients
pancreatitis	12 (IRIS - 2)
esophagitis	3
gastritis	2
colitis	6
liver involvement	9 (IRIS- 2)
cholecystitis	7 (IRIS – 2)
nervous system involvement	8 (5 peripheral, 3 central, IRIS - 2)
retinitis	4 (2 patients under gancyclovir treatment)
Total	41 pts

Table 2: Clinical manifestation of CMV infection.

QF -CMV	**positive**	**positive**	**negative**	**negative**
ELISA-CMV IgG	**positive**	**negative**	**positive**	**negative**
No. patients	35	3	10	0

Table 3: The results of the laboratory tests QF assay and ELISA-CMV IgG.

All patients with CD4 < 50 cells/mm3 and clinical manifestations, where positive for both tests (14 patients) and we observed a strong correlation ($p < 0.01$) between severe immunodepression (CD4 < 50 cells/mm3) and CMV reactivation detected by Quantiferon. (Table 4).

CD4 (cells/mm^3)	< 50	50 -100	100 -200
QF-CMV positive /negative	14/0	4-Sep	15/6
Total patients	14pts	13pts	21pts

Table 4: Correlations between QF and CD4 count.

Using statistical analysis, we calculated the sensitivity of the Quantiferon-CMV test for patients with reactivation of CMV and specific clinical manifestations. We found that the sensitivity of the method was 92.7%.

Applying the same method for ELISA CMV reaction we obtained a sensitivity of 87,8% (Fig 1.).

According to our data, Quantiferon-CMV Assay obtained an AUC of 77.9% compared to the ELISA CMV IgG test (Figure 1), which had an

AUC of 51.2%, the difference between the two being 26.7% and statistically significant (p=0.03).

Thus, it is obvious that the Quantiferon-CMV assay is superior to ELISA in the diagnosis of CMV reactivation in HIV-infected patients.

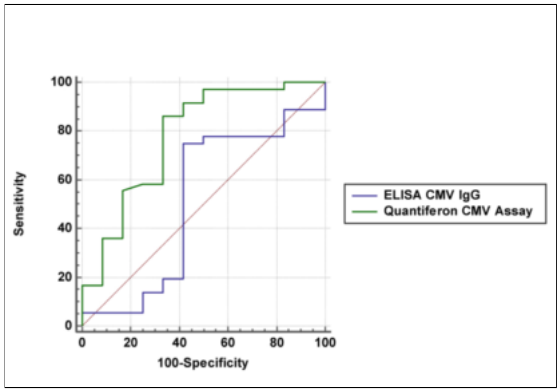

Fig. 1: ROC curve comparison between ELISA CMV IgG and Quantiferon CMV Assay.

Discussion

The Quantiferon-CMV assay targets the Cell Mediated Immune (CMI) responses to peptide antigens that simulate CMV proteins. The CMV peptides are designed to target CD8+ T cells and react with human leukocyte antigen (HLA) Class I haplotypes, including A1, A2, A3, A11, A23, A24, A26, B7, B8, B27, B35, B40, B41, B44, B51, B52, B57, B58 and B60[13]. Individuals infected with CMV usually have CD8+ lymphocytes in their bloodstream that recognise these antigens. This recognition process involves the production and secretion of the cytokine, IFN. The detection and subsequent quantification of IFN represents the principle of this test[16].

Preliminary studies have shown a correlation between the lack of detectable cell-mediated immunity measured by the QF-CMV assay and a higher incidence of CMV infection and disease in immunocompromised patients. Measurement of cell-mediated immunity against CMV appears to be a promising strategy to identify patients at highest risk for the development of CMV disease[17].

Few studies have shown that the level of agreement between IFN responses to CMV peptides, as measured by Quantiferon-CMV, and anti-CMV serology information is 97% in healthy individuals. All seronegative individuals were negative by Quantiferon-CMV and 94% of seropositive individuals were positive by Quantiferon-CMV. Similar results have been obtained by testing samples from solid organ transplantation patients[18,19].

Few literature data about the use of Quantiferon-CMV in HIV-infected patients was found. In 2007, Singh KP published the results of a case-control (1:2) study that was performed with cases defined as having a history of CMV end-organ disease (n=15) and controls (n=30) matched by current CD4(+) T cell count. CMV-specific CD8(+) T cells responses were quantified using the high throughput Quantiferon-CMV test. 40/44 (91%) had a positive Quantiferon-CMV test and the magnitude of response to CMV peptides correlated significantly with the response to mitogen ($p<0.0001$) but not with CD4(+) T cell count at the time of testing, CD4(+) T cell nadir or HIV viral load[14]. Our research group (Dumitru IM, et al) published in 2011, seven cases of HIV infected patients with CMV cholecystitis, cases diagnosed by QF-CMV test[20]. Also in 2011, a group of researchers from France announced that CMV-related immune response appeared as a major contributor to chronic immune activation, and together with CD8+ T cells inflation, lower CD4/CD8 and age participate to the immunosenescence profile observed despite persistent viral suppression[21].

In our study, the Quantiferon-CMV assay has increased sensitivity and detection of CMV reactivation induced by severe immunodepression (92.7%) in HIV infected patients and was superior to the ELISA method.

Conclusion

Quantiferon-CMV assay has increased sensitivity in detecting CMV reactivation induced by severe immunodepression (92.7%). There is a strong correlation ($p < 0.01$) between severe immunodepression (CD4 < 50 cells/mm^3) and CMV reactivation detected by Quantiferon. This study recommends using the Quantiferon-CMV test in the initial assessment package of the HIV infected patient, before the commencement of HAART (Highly Active Antiretroviral Therapy) in both naïve patients and experienced, with severe immunosuppression and any suspicion of cytomegalovirus reactivation.

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Authorship

1. Irina Magdalena Dumitru - substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, final approval of the version to be published.

2. Sorin Rugina - substantial contributions to conception and design, acquisition of data, reviewing the manuscript, final approval of the version to be published.

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7. Eugen Dumitru - involvement in clinical diagnosis, final approval of the version to be published.

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