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COMPARATIVE STUDY OF CYTOMEGALOVIRUS VIRAL LOAD *VERSUS* ANTIGENEMIA IN HOSPITALIZED PATIENTS FROM 2011 TO 2016: IN SEARCH OF AN EQUIVALENCE

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Abstract: Cytomegalovirus (CMV) infections are highly prevalent in Chile and the world and are relevant in immunosuppressed patients. The most widely used diagnostic and monitoring techniques are CMV antigenemia and viral load (CV). This study compared CMV antigenemia with real-time PCR (RT-PCR) viral load to establish numerical equivalence between both methods. **Material and method:** CMV antigenemia and viral load were retrospectively analyzed from 2011 to 2016 in 154 samples corresponding to 119 patients (75 men and 44 women) with HIV disease (28.0%), transplant (16.0%) and acute pneumonia (16.0%) and others. A Log10 correlation of the values of both tests was made. **Results:** The CV presented a higher positivity than the antigenemia (40.3 and 16.9%, respectively). Both methods were concordant in 77.9% and presented a significant correlation ($p<0.0001$). The numerical equivalence found was: 1) 0 nuclei/400,000 leukocytes = <2000 copies/mL of blood. 2) 1-10 nuclei/400,000 leukocytes = 2,000-4,000 copies/mL of blood. 3) 11-50 nuclei/400,000 leukocytes = 4,001-50,000 copies/mL of blood. 4) >50 nuclei/400,000 leukocytes = to >50,000 copies/mL of blood. Both methods presented a similar behavior against a CMV infection in different types of patients. **Conclusions:** Real-time PCR turned out to be more sensitive. It was possible to establish an equivalence between the number of nuclei/400,000 leukocytes and the number of CMV copies/mL of blood, which suggests that both methods can be used interchangeably to make clinical decisions.
Keywords: Cytomegalovirus, antigenemia, viral load, equivalence.

INTRODUCTION

Cytomegalovirus (CMV) infections are highly prevalent in the world. The virus, after primary infection, has the ability to remain in a latent state in humans and reactivate or produce reinfections under certain circumstances (1). In immunocompetent patients, infections can be asymptomatic or mild, while in immunocompromised patients it can cause severe conditions that can even end in the death of the patient (1). The patients at greatest risk are transplant recipients and those with human immunodeficiency virus (HIV) (1, 2, 3). It can also cause serious congenital infections and infections in the critically ill patient (1, 4).

Reactivation consists of the detection of CMV viremia or antigenemia in a patient with or without symptoms, while infection is the presence of antigenemia in an asymptomatic patient. On the other hand, the syndrome corresponds to a positive antigenemia in a febrile patient and the disease by CMV (ECMV) corresponds to the syndrome plus organic involvement (3).

Laboratory diagnosis of CMV infection can be serological, determination of the cellular immune response, culture, antigenemia, and molecular diagnosis using real-time PCR (RT-PCR). In immunocompromised patients with suspected infection, the most frequently used techniques are antigenemia and RT-PCR.

Antigenemia is a technique based on the detection of CMV nuclear phosphoprotein in polymorphonuclear neutrophils, when the virus replicates early. It is interpreted as follows: 1) infection is equivalent to the presence of a positive nucleus by antigenemia, 2) suspected ECMV in solid organ transplant patients (SOT), positive receptor is equivalent to the presence of 10 nuclei/100,000 leukocytes, and 3) suspected ECMV in hematopoietic stem cell transplant recipients (THP) and solid organ transplant recipients (TOS) receptor negative is equivalent to 1 nucleus/100,000 leukocytes. It is a laborious technique that can be processed only once a

day in a hospital laboratory and that presents some difficulties such as the lability of the sample and that it is affected by the leukocyte count (1). RT-PCR makes it possible to detect and quantify a CMV gene and does not present the drawbacks mentioned above, and in recent times this technique has been used in many laboratories for infection control in transplant patients (1, 2). Due to the lack of standardization of this technique, the World Health Organization (WHO) recently established the first international standard for CMV DNA quantification, which allows a more reproducible approximation for CMV viral load, in which results must be expressed in international units per mL (IU/mL) (5,6)

Clínica Dávila is a private health institution that has medical specialties and intensive and intermediate treatment units, for adults, pediatrics and neonatology. It attends 550,000 consultations and 38,000 hospitalizations per year. At the moment, it has 550 beds, 16 surgical wards and 6 maternity wards. Given the complexity of the institution, CMV infection, syndrome, and disease are frequently seen in both transplant patients and other patients at risk. Due to the limitations of antigenemia, and the availability of RT-PCR for CMV viral load, a comparison between CMV antigenemia and viral load by RT-PCR was proposed in order to establish a numerical equivalence between both methods to that can be used interchangeably in patient monitoring.

MATERIALS AND METHODS

This study contemplated a retrospective comparative analysis of CMV antigenemia and viral load from 2011 to the first semester of 2016. Both tests were taken at the same time. 154 samples were analyzed, coming from 119 patients, 75 men and 44 women, whose clinical diagnoses and evolution were obtained from the institutional clinical records. The most frequent diagnoses were: HIV disease, transplantation and acute pneumonia (Table 1). The kits used for antigenemia were Argene, Cinakit® (Biomérieux) and for CMV viral load: Tib Mol Biol® (Roche).

STATISTICS

A correlation study was made using the Log10 of the values of the determinations both for antigenemia (number of positive nuclei/400,000 leukocytes) and for CMV viral load (number of copies/mL of blood).

RESULTS

When analyzing the percentage of positivity, it was observed that the HIV viral load presented a higher positivity than antigenemia, corresponding to 40.3% versus 16.9%. (Table 2). The agreement percentage of both methods was 77.92% (120/154 samples). Only one patient had positive antigenemia (1 nucleus/400,000 cells) and negative PCR (Table 3). A statistically significant positive correlation was observed ($p < 0.0001$), which is shown in Figure 1.

The evolution of the results of both tests was analyzed in 14 patients with both methodologies, reflecting the evolution of the results over time between both methods (Figure 2).

Finally, to establish an equivalence, the distribution of net values with both methods was plotted and a correlation line R=1 was drawn, based on which, the equivalence between both methods was established (Figure 3). The equivalence values are shown in Table 4.

DISCUSSION

The results of this study indicate that both methods are concordant in 77.92%, and the discrepancies were given by samples that were mainly positive by real-time PCR and negative for antigenemia (33/154). The latter correlates

with a higher sensitivity of real-time PCR found in this study and in previous works (2, 5, 6, 7). In one case, there was a positive antigenemia, with negative simultaneous and consecutive PCRs, which could be a false positive (Table 3).

Both methods presented a statistically significant positive correlation, therefore it is verified that they have a similar behavior against CMV infection in different types of patients. The correlation coefficient obtained in this study was 0.6169 (Figure 1), being higher than that found in previous studies (2, 5, 7). In addition, both methodologies presented a similar evolution over time in 14 patients who were monitored at different times (Figure 2).

Despite the fact that antigenemia is a good marker for predicting CMV disease and has cut-off points for different groups of patients, it is a laborious technique and patients must have an acceptable leukocyte count to perform the test. In addition, it requires operator experience and the processing time is long (6 hours on average). The lability of the sample must be taken into account, which implies that it must be processed within a few hours of being taken (5, 7). The viral load, on the other hand, can be used in patients with low leukocyte counts, the processing time is shorter and the stability of the sample is greater (5).

Taking the above into account, the objective of this study was to establish an equivalence between both methods so that the treating physician uses the CMV viral load to assess the infectious status of the patient and response to treatment. These results support the use of real-time PCR and it is expected that in the future, this latter methodology will replace antigenemia. There is currently a WHO standard for CMV viral load, which allows intercomparison of methods to measure CMV viral load, allowing standardization in intercomparison (5, 6).

In this study, it was possible to establish an equivalence based on the number of nuclei/400,000 leukocytes and the number of CMV copies/mL of blood (Table 4), which suggests that both methods can be used interchangeably to make clinical decisions.

CONFLICTS OF INTEREST: NONE TO DECLARE.

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TABLES AND FIGURES

Diagnosis	n
HIV disease	28
Trasplant	16
Acute pneumonia	16
Lymphoma	6
Ulcerative colitis, Crohn's disease	5
Leukemia	4
Febrile syndrome	4
Systemic lupus erythematosus	4
Decompensated chronic lung disease	3
Myositis and myopathies under treatment	3
Tumor	3
Aplastic anemia, agranulocytosis	3
Multiple myeloma	2
Cytomegalovirus disease	2
Acute hepatitis	2
Others (miscellaneous)	18
Total	119

Table 1. Diagnosis of patients.

Test	Positive (n)	Negative (n)	Positivity (%)
Antigenemia	27	128	16,9
Viral load	62	93	40,3

Table 2. Percentage of CMV antigenemia and viral load positivity by PCR.

	Antigenemia CMV (+)	Antigenemia CMV (-)	Total
PCR CMV (+)	1	96	97
PCR CMV (-)	24	33	57
Total	25	129	154

Table 3. Table of contingency antigenemia versus PCR of cytomegalovirus.

Antigenemia (nuclei/400,000 leukocytes)	Viral load - CMV (copies/mL blood)
0	<2000
1-10	2.000-4.000
10-50	4001-50.000
>50	>50.000

Table 4. Table of equivalence between antigenemia and CMV viral load

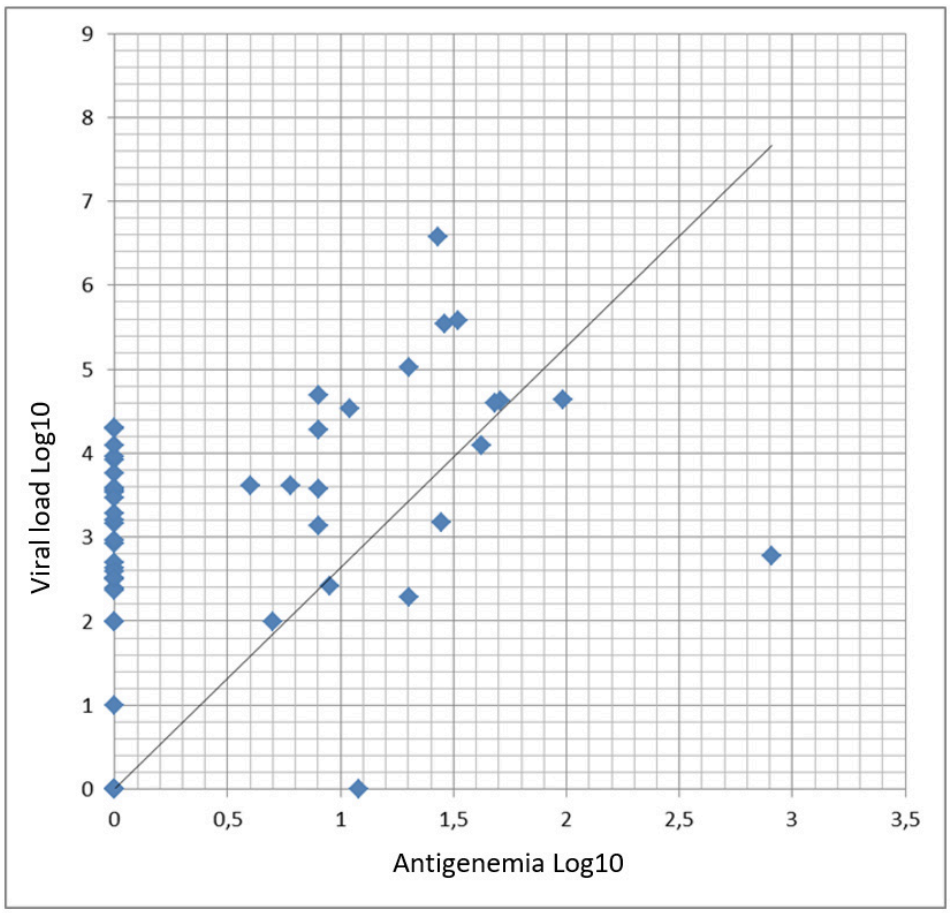

Figure 1. Antigenemia correlation graph (Log10 of positive nuclei /400,000 leukocytes) and CMV viral load (Log10 of copies/mL of blood). Pearson correlation: p=0.6169, r2=0.3805. Significance of the correlation p<0.0001).

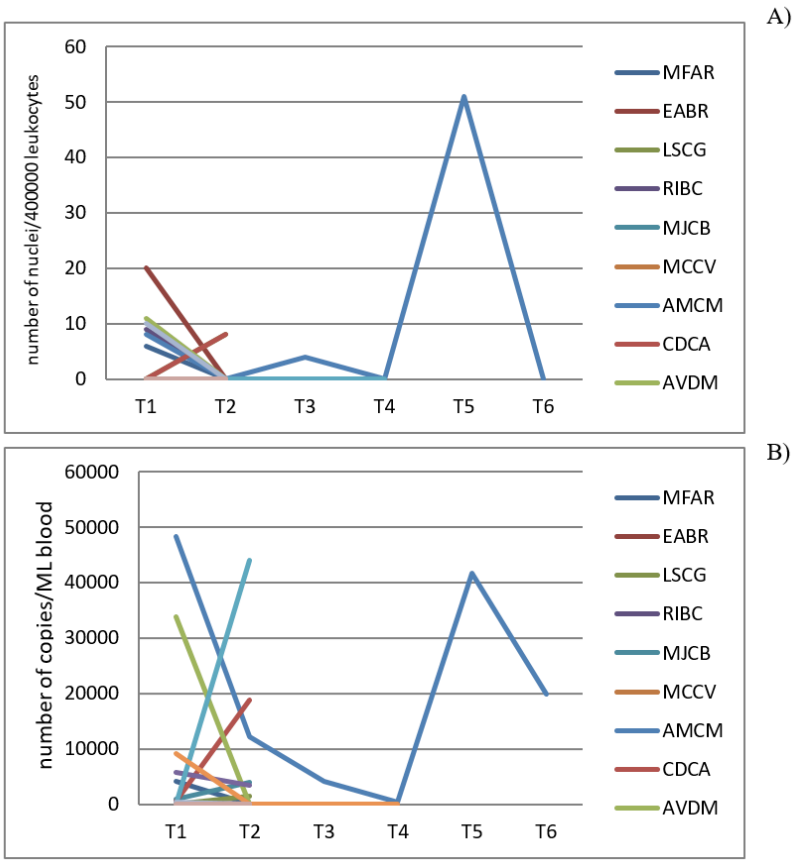

Figure 2. Evolution of the results over time of 14 patients in which A) antigenemia and B) CMV viral load were requested. T=time. T1-T6: time 1 to 6 of the exam.

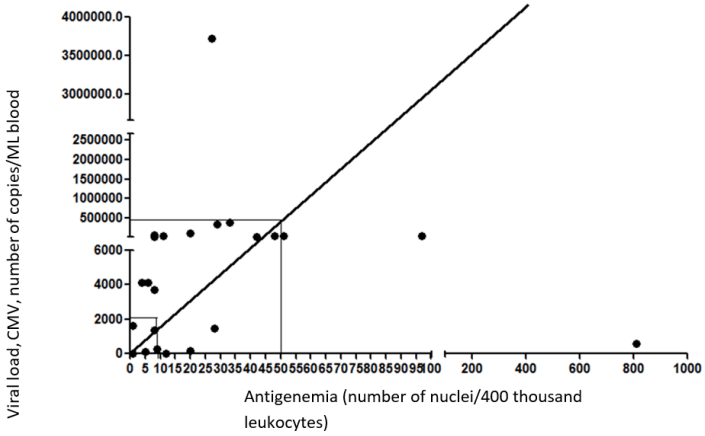

Figure 3. Graph equivalence in positive samples for antigenemia (positive nuclei /400,000 leukocytes) and CMV viral load (copies/mL of blood). Note that most of the samples presented values between 1 and 50 nuclei/400,000 leukocytes and between 2,000 and 500,000 copies/mL of blood.

AUTHORS' SIGNATURE

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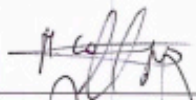
Marcia Campos M.		
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