In the name of God

The Diagnostic Protocol of Cytomegalovirus (CMV) infection in immunocompromised hosts

Protocol developed by:

Gholamreza Pouladfar M.D.

Mazyar Ziyaeyan PhD.

Marzieh Jamalidoust PhD.

Professor Alborzi Clinical Microbiology Research Center

Shiraz University of Medical Science

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1-Introduction:

Cytomegalovirus (CMV) is a common infection, and, although serious disease is rare in immunocompetent individuals, CMV is a major pathogen for immunocompromised patients, including:

- solid organ transplant recipients

- hematopoietic cell transplant recipients

-HIV-infected patients

-patients treated with immunomodulating drugs.

The range of clinical disease due to CMV in immunocompromised patients is broad and includes febrile syndromes (eg, fever, malaise, leukopenia, neutropenia, atypical lymphocytosis, thrombocytopenia), hepatitis, pneumonitis, retinitis, encephalitis, esophagitis, colitis.

Since the signs and symptoms of CMV disease often overlap with other infectious processes and rejection, the diagnosis is made by integrating the clinical history, clinical presentation, and laboratory data. Because CMV produces lifelong latent infection, distinguishing active disease from latent infection and asymptomatic reactivation presents an additional diagnostic challenge.

In this protocol, we emphasis on the role of molecular study and its strengths and limitations in the diagnosis and monitoring of CMV infection and tissue invasive disease.

2- Approach to diagnosis:

Appropriate diagnostic tests are essential for the management of CMV infection and disease in immunocompromised patients. There have been numerous studies supporting the clinical utility of CMV replication assays. Two techniques detect CMV replication including CMV pp65 antigenemia and DNA assays (quantitative PCR assays) (table 1) [3]. Quantitative PCR assays offer several advantages over the antigenemia assay, including better assay

standardization, increased stability of the specimen, smaller specimen volume, and the ability to test patients with leukopenia. For these reasons, quantitative PCR assays are more widely used than the antigenemia test, and we prefer them for the diagnosis and monitoring of immunocompromised patients with CMV infection and disease [4,5] Because viral replication can occur in the setting of asymptomatic reactivation, qualitative CMV DNA testing has limited clinical utility.

Table 1. Quantitative PCR§ for for cytomegalovirus

No	Diagnosis of disease	Specimens
1	Viral syndrome	Plasma or whole blood
2	Pneumonitis	Plasma or whole blood; consider BAL§
3	Gastrointestinal disease	Plasma or whole blood
4	Central nervous system disease	CSF§, plasma, whole blood
5	Retinitis	Vitreous or aqueous fluid
6	Decisions regarding preemptive therapy	Plasma or whole blood
7	Monitoring response to therapy	Plasma or whole blood
8	Treatment failure	Plasma or whole blood

§PCR: polymerase chain reaction; BAL: bronchoalveolar lavage; CMV: cytomegalovirus; CSF: cerebrospinal fluid;

3- Role in initial diagnosis:

Studies of high risk patients using quantitative PCR assays developed in individual laboratories have shown a higher viral load in patients with active CMV disease compared with those with asymptomatic infection [5]. However, CMV DNA values were not comparable among the assays because of differences in assay design and quantification standards.

A natural history study of CMV disease in liver transplant recipients showed that the optimal cutoff for predicting CMV disease was in the range of 2000 to 5000 copies/mL of plasma; at a cutoff of >5000 copies/mL, the sensitivity was 86 percent and the specificity was 87 percent [6]. All patients with a viral load >20,000 copies/mL developed CMV disease. In a more recent study of solid organ transplant recipients at lower risk for CMV infection, a cutoff of approximately 4000 international units/mL was established for initiating preemptive

therapy [7]. Not only is the level of CMV load predictive of symptomatic disease, but the rate of increase can also be used to predict which patients are at risk for CMV disease. [8].

4- Monitoring response to treatment:

Viral load assays are useful in monitoring responses to antiviral therapy in immunocompromised patients. The following principles should be considered when monitoring the response to treatment:

1- Viral load values among different CMV tests are not comparable [9]. Patients should therefore be monitored using the same assay.

2- Viral load values in most patients are one log10 higher in whole blood specimens compared with plasma, so the same specimen type should be used when monitoring response to therapy [10]

3- When monitoring patients, a baseline viral load should be obtained the day that antiviral therapy is initiated and should be **repeated weekly**. Based on the half-life of CMV in plasma, testing should be performed no more frequently than every five to seven days [11, 1].

4- The reproducibility of viral load tests is such that changes in viral load need to **exceed three-to fivefold** to represent meaningful changes in viral replication [11].

5- It is advised that treatment doses of an antiviral agent should be given until the CMV load is negative, followed by maintenance doses [9]. Resolution of viremia is proven with one or two consecutive negative viral load assays, ideally drawn one week apart. Viral load values usually become undetectable several weeks after initiating therapy, although clearance of DNA will take longer for higher initial viral load values and when testing is done on whole blood samples compared with plasma [12, 13]

6- A persistently detectable CMV load following **two weeks** of therapy has been associated with relapsing CMV infection [14], leading many experts to recommend treatment until CMV DNA is no longer detected in plasma or whole blood [9].

5- Indications for investigation:

1- The initial diagnosis of following diseases in immunocompromised patients

-febrile syndromes

- hepatitis

- pneumonitis

- retinitis

- encephalitis, myelitis, or polyradiculopathy

-esophagitis

- colitis.

2- The monitoring of responses to antiviral therapy (please refer to the points mentioned in the section of "4- Monitoring response to treatment"**.** For monitoring of treatment, specimens should be send weekly, **not earlier.**

6- How to assess CMV infection?

-Quantitative PCR assay (TaqMan real-Time PCR assay)

7-Sampling:

- 3 to 5 mL of clotted blood (needed for molecular CMV tests), CSF or bronchoalveolar lavage specimen

- Samples are sent to Prof. Alborzi Clinical Microbiology Research Center every day, 8 a.m-10 p.m, including weekends and holidays.

8- Tests results:

- Results are ready on Sunday and Wednesday delivered 10 a.m – 2 p.m

9- References

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