

1. Diagnostic tests for Cytomegalovirus in renal transplantation

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<p style="text-align: center;">Guidelines</p> <p style="text-align: center;">No recommendations possible based on Level I or II evidence</p>

Suggestions for clinical care

(Suggestions are based on Level III and IV evidence)

With the use of CMV diagnostic tests in renal transplant:

- Serology should be used pre-transplant to define CMV serological status and thereby stratify the risk of CMV infection post-transplant.
- Quantitation of viral load may enable the prediction of likelihood of progression to disease based on absolute value and the rate of rise of viral load. Hence, a pp65 antigenaemia or a quantitative DNA test is preferred over a qualitative DNA test. The threshold levels for various tests at which to initiate pre-emptive therapy have not been critically defined in renal transplant.

If pre-emptive treatment is to be used:

- Patients at risk of CMV infection should be monitored for evidence of infection by a validated and preferably standardised detection method. In practical terms, this currently means either the pp65 antigenaemia assay or a molecular diagnostic test.
- Monitoring of patients not receiving prophylaxis should be regular but there is no evidence to guide the frequency of testing based on outcome data or cost-effectiveness. Less than fortnightly testing is unlikely to be effective for pre-emptive treatment strategies.

Background

A variety of diagnostic tests for cytomegalovirus (CMV) are in common clinical use for the detection of CMV infection and disease following renal and other solid organ transplantation.

The diagnosis of CMV infection can be made serologically, based on seroconversion or a four-fold rise in CMV-IgG titre. More specifically, infection is diagnosed by the

demonstration of viral replication directly by viral culture, or indirectly, by the detection of viral antigen or nucleic acid.

Infection may be asymptomatic or cause disease – either the CMV syndrome or end-organ disease. A diagnosis of end-organ CMV disease can be confirmed by demonstrating tissue involvement by typical histological findings, or by the culture of CMV from tissue specimens.

Culture-based techniques, both conventional and shell vial assay culture, have poor sensitivity. Although they remain the gold standard for the diagnosis of infection, they are no longer considered appropriate tests when there is a requirement for rapid and early detection to guide pre-emptive therapy.

Antigen detection and qualitative PCR have enhanced sensitivity compared with culture techniques in the detection of infection and this also appears to be the case for quantitative PCR and the newer nucleic acid-based techniques (Razonable et al 2002, Sia & Patel 2000, Boeckh & Boivin 1998, de la Hoz et al 2002).

The comparison of different techniques between centres is made difficult by the heterogeneity of populations studied and the non-standardization of methodology for similar tests.

Currently, there are many unresolved issues regarding the most appropriate application of available diagnostic tests for CMV in renal and other transplant settings.

The current clinical applications of available tests include:

- evaluation of serostatus pre-transplant
- diagnosis of established disease
- detection of CMV infection
- quantitation of viral replication (viral load) to predict progression to disease and allow selection of individuals who should receive pre-emptive therapy, and
- quantitation of viral replication (viral load) during treatment of CMV disease to monitor response and determine treatment duration.

To achieve the latter three goals, a test for CMV should be sensitive and specific with high negative predictive value for infection and a high positive predictive value for progression to disease. It should also have the capacity to be quantified, have a short turnaround time and a high degree of reproducibility.

The purpose of the following section has been to review the literature on diagnostic tests for CMV in solid organ transplantation with an emphasis on renal transplant. The outcome sought was the production of guidelines for the use of currently available tests for CMV in the setting of renal transplantation.

In particular, the questions considered were:

- which test should be recommended for the detection of CMV infection following renal transplantation?

- can viral load quantitation predict those individuals with CMV infection who are at high risk of disease and therefore might benefit from pre-emptive treatment?
- what is the optimal frequency of testing for patients being monitored for the presence of CMV infection and being considered for pre-emptive therapy?, and
- can the duration of treatment of CMV infection be determined by monitoring the viral load in response to treatment?

Search strategy

Databases searched: MeSH terms and text words for CMV were combined with MeSH terms and text words for solid organ transplantation, including kidney transplantation, which were then combined with MeSH terms and text words for diagnostic tests for CMV. The search was carried out in Medline (1966 – June Week 1 2002).

Date of search: 28 June 2002.

What is the evidence?

There are no published randomised controlled trials (RCTs) designed to evaluate the performance of diagnostic tests for CMV in solid organ transplantation using clinically relevant endpoints.

There are many cohort studies of varying size and quality that attempt to address specific features and characteristics of the available diagnostic tests and a number of studies that compare different tests. Most of these studies are small, single-centre cohort studies, which have employed a variety of methodologies and study subjects. Current statements about the use of CMV diagnostic tests in solid organ transplant are based on this level of evidence. There have been a number of narrative reviews of the subject published recently by acknowledged experts in the field but no systematic reviews or meta-analyses (Boeckh & Boivin 1998, de la Hoz et al 2002, Razonable et al 2002, Sia & Patel 2000).

It is not currently possible, on the basis of existing evidence, to produce guidelines for the use of CMV diagnostic tests in renal transplant based on level I and II evidence.

What do the other guidelines say?

Kidney Disease Outcomes Quality Initiative: No recommendation.

British Renal Association: No recommendation.

Canadian Society of Nephrology: No recommendation.

European Best Practice Guidelines:

The screening procedure for CMV infection should include virus detection in blood leukocytes using the pp65 antigenaemia technique or a more sensitive technique.

Serial CMV serology tests should be performed to detect seroconversion from negative to positive and from IgM to IgG production (Berthoux et al 2000).

Comment: This guideline reflects the lack of evidence in this area. No RCT evidence is available. Use of pp65 antigenaemia is based on open trials and experience. Nucleic acid tests emerging at this time and noted to be showing promise but experience is limited and most work has been done on leukocytes rather than plasma.

International Guidelines: International Herpes Management Forum (IHMF), 2000:

Pre-emptive therapy thresholds:

For the pp65 antigenaemia assay, a threshold of more than 10 positive cells/ 2×10^5 PBL has been used to guide pre-emptive therapy for solid organ transplant recipients.

For the Roche Amplicor™ CMV Monitor test using plasma, the optimal threshold is 1000-5000 copies/mL in solid organ transplant (Griffiths & Whitley 2000).

Frequency of testing for guiding pre-emptive therapy:

Weekly monitoring of CMV viraemia using the pp65 antigenaemia or a molecular assay during the first 3 months after transplantation (Griffiths & Whitley 2000).

Comment: These guidelines were determined using level III and IV evidence. No RCT evidence was identified to assist in the formulation of guidelines relating to the use of diagnostic tests.

American Society of Transplantation: Clinical Practice Guidelines, 2000:

Diagnostic tests:

Techniques that use specific monoclonal antibodies against CMV and techniques that quantify CMV DNA levels in peripheral blood leukocytes appear to be sensitive and specific. They have not been compared in large prospective trials that accurately assess their positive and negative predictive values for populations with different risks for CMV.

Cost-effectiveness of tests in identifying patients for pre-emptive therapy have not been adequately studied.

Insufficient data exists to justify the widespread clinical application of any post-transplant CMV screening (Kasiske et al 2000).

Immunocompromised Host Society Consensus Conference Recommendations, 2000:

Serostatus should be determined before transplantation.

CMV antigenaemia or DNA/RNA detection are methods of choice for diagnosis and monitoring of active CMV infection after organ transplantation.

CMV surveillance is not necessary when CMV prophylaxis is given to all high- and medium-risk transplant recipients.

CMV surveillance should be performed when the effectiveness of antiviral therapy is being monitored.

CMV surveillance should be performed with test methods that are sensitive enough to detect low levels of viral replication, e.g. antigenaemia or PCR (van der Bij & Speich 2001, Snydman 2001).

Comment: Guideline recommendations were determined by an expert group and published as a position paper. In the area of diagnostic tests, no RCT evidence was presented.

Implementation and audit

1. In the absence of evidence-based guidelines, each centre should seek to participate in randomised trials that will define the optimal use of CMV diagnostic tests.
2. All renal transplant units should regularly review the performance of CMV diagnostic tests based on the clinical outcomes of patients. Cases of CMV disease should be audited with attention to the performance of diagnostic tests, particularly when used as part of a pre-emptive strategy and for monitoring treatment endpoints.
3. Renal transplant units should have access to quantitative CMV tests and the tests used should be regularly subjected to quality control procedures. Preference should be given to using standardised tests, which can be compared with other centres rather than in-house assays.

4. Tests should be performed at regular intervals when screening patients not on prophylaxis (at least fortnightly and preferably weekly) and systems should be in place to ensure that testing takes place.

Suggestions for future research

There is a great need for further research into the optimal application of CMV diagnostic tests to transplant patients. Clinical outcomes should be the endpoints of RCTs that compare standardised diagnostic tests for CMV or define the best use of a given test.

1. Perform RCTs comparing standardised CMV diagnostic tests in their ability to detect CMV infection and predict disease development following renal transplantation. For example, pp65 antigen detection vs quantitative PCR or DNA hybridization to predict disease development.
2. Define viral load thresholds to guide pre-emptive therapy by performing an RCT of different thresholds for the institution of pre-emptive therapy.
3. Perform RCTs of the treatment of CMV infection comparing empiric treatment duration with treatment duration guided by the measurement of viral load suppression.
4. Perform RCTs comparing the monitoring of different blood compartments as a means of detecting infection and predicting progression to disease.

References

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Appendix

Table 1. Summary of diagnostic tests and their performance in the diagnosis of cytomegalovirus infection*

Test name	Detects	Specimen used	Sensitivity (%)	Specificity (%)	Time for result (days)	Disease state
Serology	IgG or IgM	Blood	-	-	< 1	Past infection
Conventional tube cell culture	CMV infected cells	Blood Tissue Urine	-	-	2-21	Active
Shell vial assay	Immediate early viral antigen	Blood	8-63	86-88	1-2	Active
PCR	Viral DNA	Serum/plasma PBC [†] Tissue Urine BAL [‡] CSF [§]	50-100 20-100 - - - -	45-63 35-91 - - - -	1-2	Does not discriminate active from past infection
Reverse PCR	Viral RNA	Blood	17	97	1-2	Active
Hybrid capture	Viral DNA	Potentially any	-	-	< 1	Potential to discriminate active from past infections
bDNA	Viral DNA	Blood	-	-	1-2	-
Anigenaemia assay	Viral pp65 antigen	Blood	50-83	71-80	1	Active and past infection
Histopathology/ Immunostaining	Early CMV antigen	Tissue Liver	- 84	- 97	-	Active

* From reviews by Sia and Patel 2000, Razonable et al 2002.

[†]PBC = peripheral blood collection; [‡]BAL = broncho-alveolar lavage; [§]CSF = cerebrospinal fluid.