

Diagnostic tests for Cytomegalovirus in renal transplantation

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GUIDELINES

No recommendations possible based on Level I or II evidence

SUGGESTIONS FOR CLINICAL CARE

(Suggestions are based on Level III and IV evidence)

Serology should be used pre-transplant to define Cytomegalovirus (CMV) serological status and thereby stratify the risk of CMV infection post-transplant. [1]

- 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 antigenemia 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 and depend on the particular assay used. [2]
- There is marked variability in CMV DNA levels between different PCR assays.
- There is high inter-laboratory variability in CMV DNA levels.
- Some CMV PCR assays are unable to detect CMV at low viral loads.
- If treatment for CMV does not result in clinical improvement and a reduction in viral load, testing for ganciclovir resistance is recommended.
- Optimal cut-offs for both antigenemia and quantitative PCR testing have been proposed, however, this is dependent on each laboratory and there is no consistency between different test assays.

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 antigenemia assay or a quantitative nucleic acid assay.
- 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.

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.

BACKGROUND

A variety of diagnostic tests for 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-Ig G 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. [1-4]

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

Currently, there remain 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 transplantation. 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?
- What is the optimal viral load level at which accurate pre-emptive diagnosis of CMV can be made?
- Which quantitative assay is the most accurate for the rapid diagnosis of CMV?

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 – January Week 1, 2009)

Date of search: 7 January 2009.

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. [1-4]

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.

In order to provide rapid treatment for CMV, an accurate and rapid test is required. Cytomegalovirus cultures are too slow and insensitive for timely institution of treatment. Similarly, serologic tests, while useful to define the risk for CMV infection, are not helpful in the diagnosis of acute infection.

The most common tests in current use are quantitative assays. These consist of antigenemia testing, usually for the CMV pp65 antigen. This test is a semi-quantitative fluorescence assay in which circulating leukocytes are stained for CMV antigen. This method of testing is rapid but labour intensive. The other commonly used test is quantification of the CMV viral load using nucleic acid-based testing with PCR.

There are a number of papers examining the sensitivity and specificity of antigenemia and quantitative PCR testing. One paper examining Receiver Operating Characteristic (ROC) curve analysis found that the sensitivity of antigenemia testing for CMV syndrome was 64% with a specificity of 81%. [5]

Similarly, analyses using quantitative PCR testing on plasma samples with a commercial test in patients using prophylaxis, found a sensitivity of only 38% with a positive predictive value of 17% for the development of CMV disease using a positive cut-off value of >400 copies/mL. [6] A key issue, which is thus far unresolved, is the optimal setting for viral load detection in terms of pre-emptive

disease prevention. If a lower limit of detection (LOD) for the viral load is too low, unnecessary interventions may occur, while if the LOD is set too high, intervention may be delayed, potentially resulting in CMV disease.

Other studies have compared quantitative PCR with pp65 antigenemia testing and found a variety in the concordance of results, depending on which assays were used. [7-9] Optimal cut-offs for both antigenemia and quantitative PCR testing have been proposed, [10] however, this is dependent on each laboratory and there is no consistency between different test assays.

Some studies have found that CMV infection can be diagnosed earlier with PCR testing compared with pp65 antigenemia. [7,11]

A recent study examined a panel of samples (2 negative, 7 samples constructed from CMV nucleocapsids, 3 clinical plasma samples) sent to 33 laboratories in the USA, Canada and Europe. [12] All laboratories performed quantitative nucleic acid testing using PCR. Laboratory-developed assays were used for 18 sample sets, while commercial assays were used for 17 sample sets including 9 data sets for the Roche COBAS® Amplicor kit. There was large variability in the viral load reported, especially at low viral loads. Assuming an acceptable variability of $\pm 0.5 \log_{10}$, only 57.6% of results fell into the acceptable range.

This paper found that the use of commercially available reagents and procedures was associated with significantly less variability compared with laboratory-based assays. Importantly, there was marked inter-laboratory variability on replicate samples, especially in samples with low viral loads. This result reflects a need for a standard for CMV viral load calibration.

SUMMARY OF THE EVIDENCE

No systematic reviews or randomised controlled trials are available on this topic. The evidence is largely comprised of cohort studies which are variable in methodology and participant characteristics. Most are small, single-centre studies. Also, a number of diagnostic test accuracy studies comparing antigenemia and quantitative PCR testing are available.

WHAT DO THE OTHER GUIDELINES SAY?

Kidney Disease: Improving Global Outcomes, 2009: [13]

- In patients with CMV disease, we suggest weekly monitoring of CMV by NAT or pp65 antigenemia. (2D)
- In patients being treated for CMV, we suggest continuing therapy until CMV is no longer detectable by plasma NAT or pp65 antigenemia. (2D)

UK Renal Association: No recommendation.

Canadian Society of Nephrology: No recommendation.

European Best Practice Guidelines: No recommendation.

International Guidelines:

International Herpes Management Forum Workshop Guidelines (IHMF, 2007): [14]

- Assay of whole blood is most sensitive for detection of CMV viraemia in polymerase chain reaction (PCR) assays and so should be used in preference to alternative samples such as plasma.
- Formalised studies are required to determine if a standardised assay for CMV can provide

more reliable results than local tests.

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 hybridisation 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.

CONFLICT OF INTEREST

Helen Pilmore has no relevant financial affiliations that would cause a conflict of interest according to the conflict of interest statement set down by KHA-CARI.

REFERENCES

1. Razonable RR, Paya CV, Smith TF. Role of the laboratory in diagnosis and management of cytomegalovirus infection in haematopoietic stem cell and solid organ transplant recipients. *J Clin Microbiol* 2002; **40**: 746-52.
2. Sia IG, Patel R. New strategies for prevention and therapy of cytomegalovirus infection and disease in solid-organ transplant recipients. *Clin Microbiol Rev* 2000; **13**: 83-121.
3. Boeckh M, Boivin G. Quantitation of cytomegalovirus: methodologic aspects and clinical applications. *Clin Microbiol Rev* 1998; **11**: 533-54.
4. De la Hoz RE, Stephens G, Sherlock C. Diagnosis and treatment approaches to CMV infections in adult patients. *J Clin Virol* 2002; **25** (Suppl 2): S1-S12.
5. Greanya ED, Partovi N, Yoshida EM *et al*. The role of the cytomegalovirus antigenemia assay in the detection and prevention of cytomegalovirus syndrome and disease in solid organ transplant recipients: a review of the British Columbia experience. *Can J Infectious Dis Med Microbiol* 2005; **16**: 335-41.
6. Humar A, Paya C, Pescovitz MD *et al*. Clinical utility of cytomegalovirus viral load testing for predicting CMV disease in d+/r- solid organ transplant recipients. *Am J Transplant* 2004; **4**: 644-49.
7. Gouarin S, Vabret A, Gault E *et al*. Quantitative analysis of HCMV DNA load in whole blood of renal transplant patients using real-time PCR assay. *J Clin Virol* 2004; **29**: 194-201.
8. Ksouri H, Lakhal A, Ben Amor R *et al*. Prognosis value of the pp65 antigenemia and semi-quantitative PCR in the detection of the CMV reactivation in bone marrow grafted patients. *Bulletin de la Société de Pathologie Exotique* 2006; **99**: 155-59.
9. Gentile G, Picardi A, Capobianchi A *et al*. A prospective study comparing quantitative Cytomegalovirus (CMV) polymerase chain reaction in plasma and pp65 antigenemia assay in monitoring patients after allogeneic stem cell transplantation. *BMC Infect Dis* 2006; **6**: 167.
10. Kim DJ, Kim SJ, Park J *et al*. Real-time PCR assay compared with antigenemia assay for detecting cytomegalovirus infection in kidney transplant recipients. *Transplant Proc* 2007; **39**: 1458-60.
11. Sanghavi SK, Abu-Elmagd K, Keightley MC *et al*. Relationship of cytomegalovirus load assessed by real-time PCR to pp65 antigenemia in organ transplant recipients. *J Clin Virol* 2008; **42**: 335-42.
12. Pang L, Fox JD, Fenton JM *et al*. Interlaboratory Comparison of Cytomegalovirus Viral Load Assays. American Society of Transplantation Infectious Diseases Community of Practice. Canadian Society of Transplantation. *Am J Transplant* 2009; **9**: 258-68.
13. Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9**(Suppl 3): S1-S157.
14. Griffiths P, Whitley R, Snyderman DR, *et al*. International Herpes Management Forum. Contemporary management of cytomegalovirus infection in transplant recipients: guidelines from an IHMF workshop, 2007. *Herpes* 2008; **15**(1): 4-12.

APPENDICES

Table 1. Characteristics of included studies

Study ID (author, year)	N	Study design	Setting	Participants	Diagnostic test	Results	Sensitivity (%)	Specificity (%)	PPV*	NPV†
Greanya et al 2005	134	Retrospective review	Canada	Kidney, liver, lung, kidney-pancreas transplant recipients	Antigenaemia testing		64	81	76	71
Humar et al 2004	364	Prospective study	Not stated	Transplant recipients receiving prophylaxis	Serial viral load determinations by quantitative PCR		38	60	17	82
Gouarin et al 2004	21	Prospective monitoring with HCMV, retrospective analysis of specimens using real-time PCR	France	Kidney transplant recipients	Real-time PCR on whole blood vs HCMV pp65 antigenaemia assay	Spearman rank tests r=0.77	NA	NA	NA	NA
Gentile et al 2006	38	Prospective study	Italy	Allogeneic stem cell patients at risk of CMV infection	Quantitative CMV-PCR in plasma		64	100	100	94
					pp65 antigenaemia		73	100	100	95
Sanghavi et al 2008	12	Prospective study	US	Organ transplant recipients	Quantitative real-time PCR assay targeting US17 and UL54 regions of the CMV genome vs pp65 antigenaemia (reference test)		US17 = 96 UL54 = 94	US17 = 97 UL54 = 97	US17 = 44 UL54 = 45	US17 = 100 UL54 = 100

*PPV = positive predictive value; †NPV = negative predictive value

Table 2. 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 hybrid capture	Viral RNA Viral DNA	Blood Potentially any	17 -	97 -	1-2 < 1	Active - potential to discriminate active from past infections
bDNA antigenaemia assay	Viral DNA Viral pp65 antigen	Blood Blood	- 50-83	- 71-80	1-2 1	Active and past infection
Histopathology/immunostaining	Early CMV antigen	Tissue Liver	- 84	- 97	-	Active

Table has been compiled from reviews by Sia and Patel 2000, Razonable et al 2002.

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