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ORIGINAL PAPER

Cytomegalovirus Diagnosis in Lung Transplantation: Validation of a qPCR Cutoff in a Reference Transplant Center in Brazil

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ABSTRACT

Objectives: Cytomegalovirus (CMV) infection is a significant complication in lung transplant recipients. Although quantitative polymerase chain reaction (qPCR) is internationally recommended for CMV monitoring, pp65 antigenemia remains widely used in Brazil due to economic and structural constraints within the public healthcare system. Establishing a locally validated qPCR cutoff aligned with current institutional practice is essential to support a safe and sustainable transition to molecular diagnostics in this setting. Methods: This retrospective study included 80 adult lung transplant recipients at Santa Casa de Porto Alegre, a major transplant center in southern Brazil, from April to December 2023. All patients underwent both pp65 antigenemia and qPCR testing. Receiver operating characteristic (ROC) curve analysis was performed using antigenemia (> 3 cells/100,000 granulocytes) as the reference method to determine the optimal qPCR cutoff for positivity. Results: ROC analysis identified an optimal qPCR cutoff of 3.75 log IU/mL (≈ 3,162 IU/mL), with an area under the curve of 0.91, 100% sensitivity, and 82.6% specificity. The proposed threshold demonstrated excellent discriminatory performance for CMV detection in this population. Conclusion: The locally defined cutoff of 3.75 log IU/mL provides an objective reference for interpreting qPCR results in Brazilian lung transplant programs where antigenemia remains in use in many centers. These findings offer practical, real-world evidence to guide institutional CMV surveillance strategies and support the gradual and economically sustainable integration of molecular monitoring in low- and middle-income countries.

Descriptors: Cytomegalovirus; Lung Transplantation; Quantitative PCR; Antigenemia; Brazil.

Diagnóstico de Citomegalovírus no Transplante Pulmonar: Validação de um Ponto de Corte de qPCR em um Centro de Referência em Transplante no Brasil

RESUMO

Objetivos: A infecção por citomegalovírus (CMV) é uma complicação significativa em receptores de transplante de pulmão. Embora a reação em cadeia da polimerase quantitativa (qPCR) seja recomendada internacionalmente para o monitoramento do CMV, a antigenemia pp65 permanece amplamente utilizada no Brasil devido a restrições econômicas e estruturais no sistema público de saúde. Estabelecer um ponto de corte (*cutoff*) de qPCR validado localmente e alinhado com a prática institucional atual é essencial para apoiar uma transição segura e sustentável para o diagnóstico molecular neste cenário. **Métodos:** Este estudo retrospectivo incluiu 80 receptores adultos de transplante de pulmão na Santa Casa de Porto Alegre, um importante centro de transplantes no sul do Brasil, de abril a dezembro de 2023. Todos os pacientes foram submetidos tanto à antigenemia pp65 quanto ao teste de qPCR. A análise da curva ROC (*receiver operating characteristic*) foi realizada utilizando a antigenemia (> 3 células/100.000 granulócitos)

como método de referência para determinar o ponto de corte ideal da qPCR. **Resultados:** A análise ROC identificou um ponto de corte ideal de qPCR de 3,75 log UI/mL (≈ 3.162 UI/mL), com uma área sob a curva de 0,91, sensibilidade de 100% e especificidade de 82,6%. O limiar proposto demonstrou excelente desempenho discriminatório para a detecção de CMV nesta população. **Conclusão:** O ponto de corte definido localmente de 3,75 log UI/mL fornece uma referência objetiva para interpretar os resultados de qPCR em programas brasileiros de transplante de pulmão onde a antigenemia permanece em uso rotineiro. Esses achados oferecem evidências práticas do mundo real para orientar as estratégias institucionais de vigilância do CMV e apoiar a integração gradual e economicamente sustentável do monitoramento molecular no contexto de países em desenvolvimento.

Descritores: Citomegalovírus; Transplante de Pulmão; PCR Quantitativo; Antigenemia; Brasil.

INTRODUCTION

Cytomegalovirus (CMV) infection is a significant complication in immunocompromised patients, particularly in lung transplant recipients.[1,2] Early and accurate diagnosis is crucial for timely intervention.[3] Historically, pp65 antigenemia has been the diagnostic standard, detecting CMV antigens in blood leukocytes.[4] However, this method is being replaced by quantitative polymerase chain reaction (qPCR), which offers higher sensitivity and faster results.[5] Despite being the international standard, the widespread adoption of qPCR is still limited in many developing countries due to financial and infrastructural constraints. As a result, pp65 antigenemia remains in routine use in Brazil and other resource-limited settings, not due to superior diagnostic accuracy but because of lower implementation costs, existing laboratory infrastructure, and reimbursement limitations within the public healthcare system. In this context, antigenemia represents a pragmatic and structurally embedded strategy in transplant programs operating under financial constraints. Therefore, establishing local evidence to define reliable qPCR cutoff values becomes essential to guide clinical decision-making and support a gradual, economically sustainable transition to molecular diagnostics.

METHODS

This retrospective study was conducted at Santa Casa de Porto Alegre, a leading transplant center in southern Brazil. Adult lung transplant recipients (> 18 years) who underwent CMV antigenemia testing during the study period were consecutively included, particularly those within the first 6 months post-transplant or requiring intensified immunosuppression. The median time from transplantation to the first suspected infection was 45 days (interquartile range: 30-60 days), reflecting the critical risk period. CMV infection was defined as antigenemia > 3 cells/100,000 granulocytes, while CMV disease required viral replication and symptoms. Antigenemia was the standard diagnostic test for CMV infection in the center. To determine a qPCR cutoff, all included patients underwent both pp65 antigenemia and qPCR during the study period.

Procedures

Clinical and laboratory data were retrospectively collected from electronic medical records and institutional laboratory databases. Variables included age, sex, transplant type, episodes of acute rejection, CMV donor-recipient serostatus, use of antiviral therapy, and 90-day survival.

A pp65 antigenemia was performed using indirect immunofluorescence (CMV Brite Turbo Kit, IQ Products), with a predefined institutional cutoff of > 3 cells per 100,000 granulocytes considered positive. This threshold represents the standard reference for initiating clinical management at the study center.

The qPCR testing was performed on whole blood samples using World Health Organization (WHO)-recommended controls, xPrimer/probe CMV reagents (ThermoFisher Scientific), and TaqMan™ Comprehensive Microbiota Control (Applied Biosystems). Amplification was conducted in a real-time PCR thermocycler, and results were validated according to cycle threshold values and standard amplification curves. Viral load values were initially expressed in copies/mL and converted to IU/mL using a correction factor of 1.69 derived from comparisons with the WHO International Standard for CMV.

CMV surveillance followed institutional practice adapted to the logistical realities of a public Brazilian transplant center serving patients from multiple regions of the country. CMV prevention was predominantly based on a risk-stratified strategy. Universal prophylaxis for at least 6 months was administered to donor-recipient serological mismatch (D+/R–) patients. For seropositive recipients (R+), a preemptive strategy was adopted, and CMV laboratory monitoring was typically performed weekly during the first post-transplant month, biweekly up to 4 months, bimonthly until 1 year, and quarterly during the 2nd year. Antiviral therapy was initiated upon detection of significant viral replication according to institutional criteria (antigenemia > 3 cells per 100,000 granulocytes) or in the presence of symptomatic disease. Intravenous ganciclovir was administered according to

institutional practice, with dosing adjusted for renal function and clinical response. In patients managed with a preemptive strategy who required augmented immunosuppression, such as corticosteroid pulse therapy, ganciclovir was administered for 8 weeks. Routine surveillance bronchoscopy is not systematically performed at our center. In cases of tissue-invasive suspicion, immunohistochemistry and histological evaluation are performed from a transbronchial biopsy.

Maintenance immunosuppressive regimens reflected institutional and national protocols, as well as drug availability during the study period. Although tacrolimus-based regimens are often preferred internationally due to a lower incidence of acute cellular rejection and bronchiolitis obliterans syndrome (BOS), cyclosporine and azathioprine are the primary drugs provided by the Brazilian public health system. Therefore, a cyclosporine-based regimen was initially preferred at our center. However, tacrolimus was prioritized for patients with higher immunological risk (e.g., donor-specific antibodies) or those who developed recurrent acute rejection or early signs of BOS. Regarding therapeutic drug monitoring, during the first 6 months post-transplant, cyclosporine C0 levels were maintained between 250-350 ng/mL, with dose adjustments individualized based on clinical status.

Despite geographic and socioeconomic barriers, CMV laboratory monitoring was consistently integrated into routine post-transplant care.

Statistical analysis

Qualitative variables were analyzed using the chi-square or Fisher's exact test, while quantitative variables were compared using Student's *t*-test or the Mann-Whitney U test as appropriate. Logistic regression was used to assess associations between variables and complications. The comparison between qPCR and antigenemia was performed using a receiver operating characteristic (ROC) curve, considering antigenemia > 3 cells/100,000 granulocytes as positive. Antigenemia was considered the reference method according to institutional practice during the study period. Analyses were conducted using SPSS software version 22.0.

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of Santa Casa de Porto Alegre (protocol number 6,780,371), with informed consent waived due to the retrospective nature of the study and patient data anonymized at all times to ensure confidentiality.

RESULTS

During the study period, 80 lung transplant recipients were analyzed. Baseline demographic and clinical characteristics are summarized in Table 1. Most patients were male (60%), with a mean age of 49.0 ± 16.9 years. Bilateral transplantation was performed in 73.7% of cases. Acute rejection occurred in 35 patients (45%), whereas no cases of chronic rejection were recorded.

Table 1. Baseline characteristics of lung transplant recipients (n =80).

Characteristics	
Age, years (mean ± SD)	49.0 ± 16.9
Male sex, n (%)	48 (60.0)
Transplant characteristics	
Bilateral lung transplantation, n (%)	59 (73.7)
Acute rejection, n (%)	35 (45.0)
Chronic rejection, n (%)	0 (0.0)
CMV serostatus (donor/recipient), n (%)	
D+/R+	37 (46.3)
D-/R+	34 (42.5)
D-/R-	7 (8.8)
D+/R-	2 (2.5)
Underlying diseases, n (%)	
Pulmonary fibrosis	28 (35.0)
COPD	25 (31.3)
Cystic fibrosis	15 (18.8)
Lymphangioleiomyomatosis	4 (5.0)
Bronchiectasis	4 (5.0)

Continue...

Table 1. Continuation.

Characteristics	
Underlying diseases, n (%)	
Hypersensitivity pneumonitis	2 (2.5)
Childhood interstitial lung disease	1 (1.3)
Obliterative bronchiolitis	1 (1.3)
Immunosuppressive regimens, n (%)	
Cyclosporine + prednisone + azathioprine	64 (80.0)
Tacrolimus + prednisone + azathioprine	7 (8.8)
Tacrolimus + prednisone + mycophenolate	5 (6.3)
Cyclosporine + prednisone + mycophenolate	4 (5.0)

Source: Elaborated by the authors. COPD = chronic obstructive pulmonary disease; D = donor; R = receptor; SD = standard deviation.

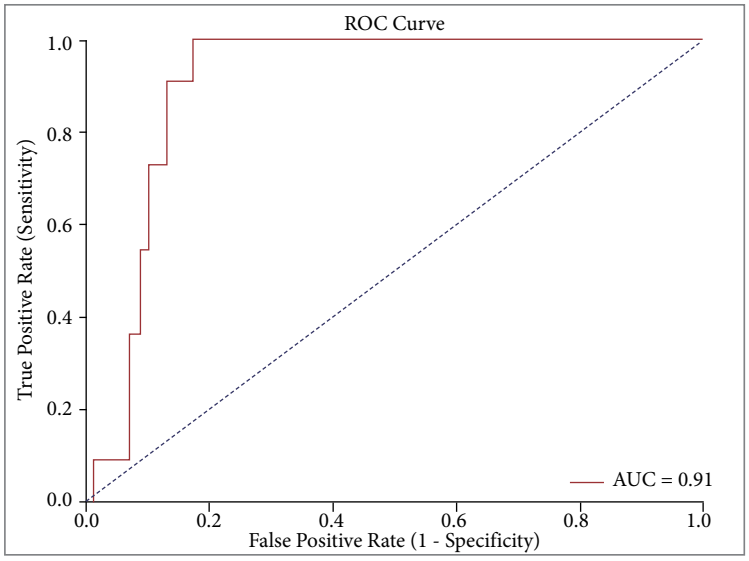
Pre-transplant serology showed that 90% of donors were CMV IgG positive. Regarding ganciclovir use, 80% of patients were under preemptive therapy, 3.7% received it for diagnosed CMV infection, and 15% were under surveillance without antiviral therapy. The 90-day survival rate was 97.5%.

The diagnostic performance of qPCR compared to antigenemia is presented in Table 2. ROC curve analysis (Fig. 1) identified an optimal qPCR cutoff of 3.75 log IU/mL (≈ 3,162 IU/mL or 5,344.25 copies/mL) with 100% sensitivity and 82.6% specificity (area under the curve [AUC] = 0.91).

Table 2. Diagnostic performance of qPCR for CMV detection using pp65 antigenemia as reference (cutoff: 3.75 log IU/mL).

Parameter	Value (%)
Sensitivity	100.00
Specificity	82.60
Positive predictive value	47.80
Negative predictive value	100.00
AUC	0.91

Source: Elaborated by the authors. pp65 antigenemia (>3 cells/100,000 granulocytes) was considered the reference method.



Source: Elaborated by the authors.

Figure 1. ROC curve of qPCR for CMV detection in lung transplant recipients using pp65 antigenemia as the reference method (AUC = 0.91).

DISCUSSION

This study adds to the growing body of evidence supporting qPCR as a valuable diagnostic tool for CMV infection in transplant recipients, particularly in lung transplantation.[3,4] In our cohort, qPCR demonstrated excellent discriminatory performance for CMV detection, with an area under the ROC curve (AUC) of 0.91 and an optimal cutoff of 3.75 log IU/mL. These findings support the high analytical sensitivity of qPCR for detecting circulating viral DNA and reinforce its role in CMV surveillance strategies.

Although pp65 antigenemia has historically been widely used for CMV monitoring, important methodological limitations should be considered. Antigenemia relies on the detection of viral proteins within circulating leukocytes, inherently linking its diagnostic performance to peripheral blood leukocyte counts. In patients with leukopenia or neutropenia, common conditions among lung transplant recipients receiving intensive immunosuppressive or antiviral therapy, the sensitivity of antigenemia may be reduced.[6,7] In contrast, molecular assays such as qPCR quantify circulating viral DNA and are less dependent on leukocyte count, providing more consistent performance in cytopenic patients.[7]

Additionally, antigenemia may have reduced sensitivity in cases of tissue-invasive CMV disease, particularly gastrointestinal involvement, where viral replication can remain predominantly localized with limited peripheral dissemination.[7,8] In such scenarios, blood-based assays may underestimate the extent of organ involvement, and diagnosis frequently requires histopathological confirmation. These biological and technical considerations further support the growing preference for molecular monitoring in transplant populations.

Despite these limitations, antigenemia remains in use in several low- and middle-income settings due to lower direct costs and established laboratory familiarity. In this context, our findings are particularly relevant. By establishing a locally validated qPCR cutoff aligned with international standardization in IU/mL, this study provides objective data that may inform institutional CMV monitoring protocols in Brazilian transplant centers, as well as in other low- and middle-income countries.[6] Rather than proposing an abrupt methodological transition, our results support the gradual integration of standardized molecular monitoring strategies in settings where antigenemia continues to be employed.

Taken together, our data demonstrate the practical applicability of qPCR within a real-world public transplant program and contribute locally derived evidence to a field largely informed by studies from high-income countries. These findings may assist transplant centers in optimizing CMV surveillance strategies according to available resources while maintaining alignment with international recommendations.

This study has limitations that should be considered. It was conducted at a single center with a relatively limited sample size, which may restrict the generalizability of the proposed cutoff to other transplant populations. In addition, the analysis focused on laboratory diagnostic performance and did not evaluate clinical outcomes such as CMV disease severity or graft survival. Finally, the absence of systematic histopathological correlation limits the comprehensive assessment of potential discordance between blood-based assays and tissue-invasive disease.

CONCLUSION

This study demonstrates that qPCR exhibits excellent discriminatory performance for CMV detection in lung transplant recipients, with an optimal cutoff of 3.75 log IU/mL. Although molecular monitoring is internationally established, pp65 antigenemia remains widely used in Brazil due to economic and structural constraints within the public healthcare system. In this context, the locally validated cutoff provides practical, real-world evidence to support a gradual and economically sustainable transition from antigenemia to standardized molecular diagnostics in Brazilian transplant centers. Rather than proposing an abrupt replacement, our findings contribute objective data to guide institutional protocols in middle-income healthcare systems where modernization must be aligned with financial feasibility. Further studies evaluating clinical outcomes and cost-effectiveness are warranted to optimize CMV surveillance strategies in Brazil and comparable settings.

CONFLICT OF INTEREST

The author A.C. Pasqualotto has received research grants from Gilead, Pfizer, MSD, Mundipharma, IMMY, and MiraVista. He has consulted and/or given paid talks on behalf of Gilead, Knight (United Medical), Pfizer, MSD, PAHO, IMMY, Mundipharma, Sandoz, bioMérieux, Teva, Sanofi, Astra-Zeneca, and Astellas Pharma. All other authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTION

Substantive scientific and intellectual contributions to the study: Pasqualotto AC, Nascimento DZ. **Conception and design:** Pasqualotto AC. **Data analysis and interpretation:** Castelo ACG, Barbosa LMO, Nascimento DZ, Pinheiro CB. **Article writing:** Castelo ACG, Barbosa LMO, Pinheiro CB, Nascimento DZ, and Pasqualotto AC. **Critical revision:** Nascimento DZ, and Pasqualotto AC. **Final approval**: Pasqualotto AC.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

FUNDING

Not applicable.

DECLARATION OF USE OF ARTIFICIAL INTELIGENCE TOOLS

Artificial intelligence tools were used only for linguistic revision.

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