EVALUATION OF THE CMV RNA ELITe MGB KIT FOR MONITORING CMV INFECTIONS IN PEDIATRIC SOLID ORGAN TRANSPLANT

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INTRODUCTION

The introduction of antiviral drugs that specifically target CMV without altering viral DNA replication has raised concerns about the reliability of CMV-DNA as a marker for monitoring infection progression.
However, in its tegument, CMV also contains mRNAs that can be traced in the plasma being associated with mature viral particles (virions). ELITechGroup has recently launched the CMV-RNA ELITe MGB kit, which is a new One-Step Real-Time PCR assay that detects and quantifies the CMV-specific virion mRNA UL 21.5 .

RESEARCH

This kit was used to monitor liver-transplanted children in a study aiming to evaluate whether CMV RNA could provide an accurate marker for monitoring CMV active infections in solid organ transplant recipients.

MATERIAL AND METHODS

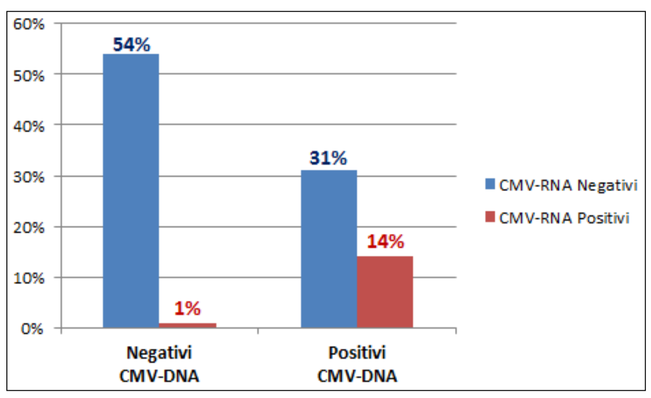
Upon the parent's informed consent, samples were collected from 6 children, (mean age 8), who underwent liver transplants at the Papa Giovanni XXIII Hospital in Bergamo. For approximately 12 weeks, CMV DNA and RNA were weekly monitored in paired whole blood and plasma samples using ELITechGroup CMV DNA ELITe MGB® Kit and CMV RNA ELITe MGB® Kit, respectively (Figure 1). The assays were performed with ELITe InGenius instrument (ELITechGroup) (Figure 2). CMV-specific T-cell immunity was tracked using the IGRA ELISpot CMV test, performed before transplantation, 2 weeks and 4 weeks after transplantation. In total, 72 whole blood and plasma samples were processed. The results were analyzed using MedCalc® software.

Figure 1. CMV DNA ELITe MGB® Kit

Figure 2. ELITe InGenius instrument

RESULTS

A "pre-emptive therapy" approach was used to monitor CMV infection and antivirals were administered at a viral load of 10^5 cp/mL. Overall, 11 samples (15.3%) were positive for CMV-RNA in plasma and 32 (44.4%) for CMV-DNA in whole blood (Table 1). Only 14% were positive for both CMV-RNA and CMV-DNA, while 1% were positive for CMV-RNA only and 31% were positive for CMV-DNA only. The remaining 54% tested negative for both (Table 1 and Graph 1). Consequently, diagnostic concordance between markers was poor (K=0.3; 95% CI=0.13-0.5).

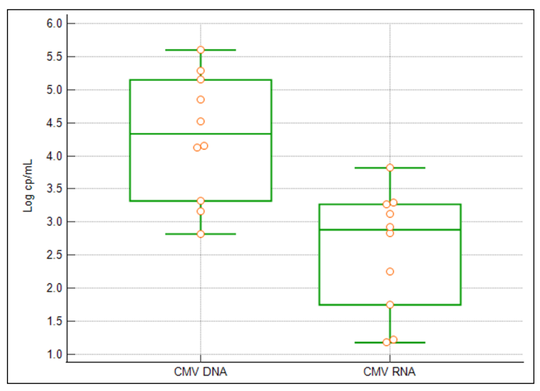


Graph 1. CMV-RNA and CMV-DNA results stratified according to positivity and negativity results.

CMV_RNA	CMV_DNA neg	CMV_DNA pos	
neg	39	22	61 (84.7%)
pos	1	10	11 (15.3%)
	40 (55.6%)	32 (44.4%)	72

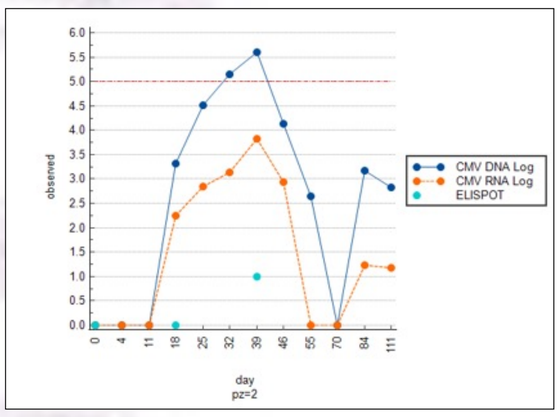
Table 1. CMV-RNA and CMV-DNA results stratified according to positivity and negativity.

Among the positive cases, more than 10-fold difference in the quantification was observed ($p<0.0015$). However, a fixed ratio was not observed between the two markers (Graph 2).

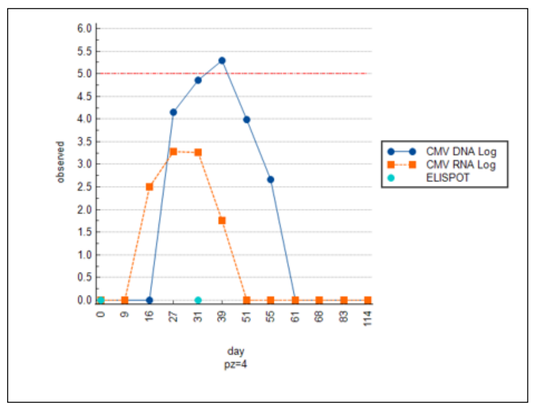


Graph 2. Distribution of CMV DNA and RNA quantifications.

Considering the viral kinetics, in two patients with clinically relevant CMV-DNA load (≥10^5 cp/mL), CMV-RNA appeared in the plasma around day 18 post-transplant, along with CMV-DNA in the blood, and after a similar ascending phase it turned negative approximately 10-15 days earlier than for CMV-DNA, following treatment with Valganciclovir. Only one of them was positive at CMV ELISpot (Graph 3-4).

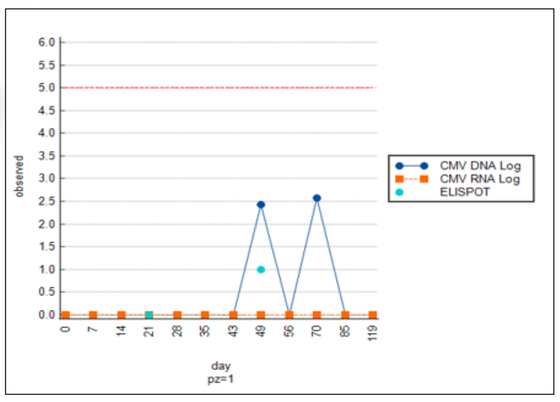


Graph 3. CMV DNA pos/CMV RNA pos/ ELISPOT pos.

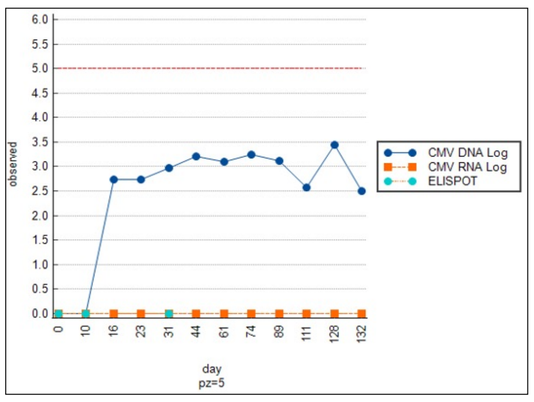


Graph 4. CMV DNA pos/CMV RNA pos/ ELISPOT neg.

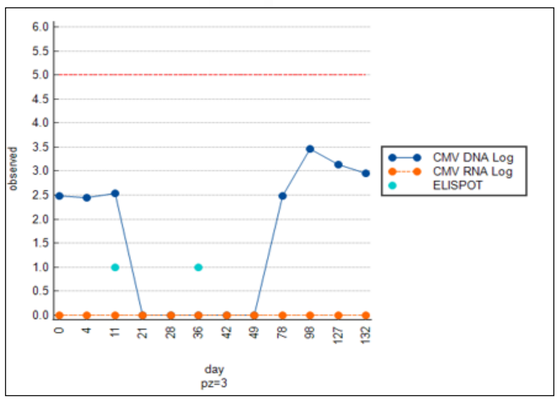
CMV RNA was found to be negative in three patients who had CMV-DNA subclinical reactivation (<3200 cp/ml) and in one patient, who was persistently negative CMV-DNA (graph 5,6,7,8).



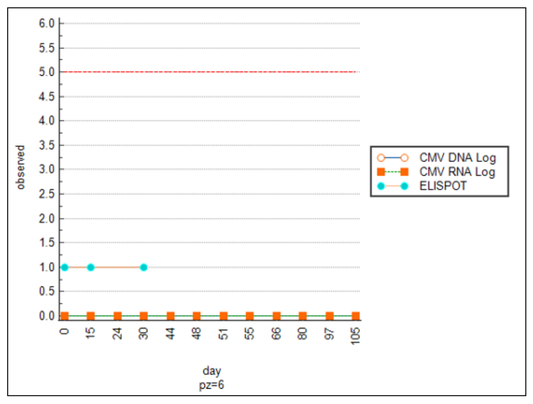
Graph 5. Patient 1



Graph 6. Patient 5



Graph 7. Patient 3



Graph 8. Patient 6

DISCUSSION

In liver-transplanted recipients, the CMV RNA in plasma is observed only in clinically relevant infections and becomes undetectable earlier than CMV DNA in whole blood. CMV RNA and CMV DNA describe different aspects of viral replication and should be monitored in parallel.

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