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Quantiferon-Cytomegalovirus assay: A potentially useful tool in the evaluation of CMV-specific CD8+ T-cell reconstitution in pediatric hematopoietic stem cell transplant patients

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Abstract

Pediatric HSCT recipients are at high risk for CMV reactivation due to their immature immune system and therapy following transplantation. Reconstitution of CMV-specific T-cell immunity is associated with control and protection against CMV. The clinical utility of monitoring CMV-specific CMI to predict CMV viremia in pediatric HSCT patients using the Quantiferon-CMV (QIAGEN®) test was investigated prospectively. Thirty-seven pediatric allogeneic HSCT recipients were enrolled from 3/2010-6/2012. CMV viremia was detected via weekly real-time PCR. The Quantiferon-CMV test was conducted pretransplant, early after transplantation, 30, 90, 180, 270, and 360 days post-transplantation. The incidence of CMV viremia was 51% (19/37) with half of the episodes within ≤30 days post-transplant. Fifteen patients showed CMV-specific immunity (average of 82 days). The cumulative incidence of CMV reactivation in patients who developed CMV-specific immunity was lower than those who did not (15% vs 53%; $P = .023$). The ROC statistical analysis showed that the AUC was 0.725 in predicting viremia, for Quantiferon-CMV test. In this cohort, the Quantiferon-CMV assay was a valuable method for identifying pediatric HSCT patients at high risk for CMV viremia, suggesting potential clinical utility to individualize patient's management post-transplant.

KEYWORDS
immune reconstitution, pediatric hematopoietic stem cell transplantation, Quantiferon-CMV

1 | INTRODUCTION

Allogeneic HSCT is a life-saving procedure for children suffering from malignant and non-malignant diseases. Due to impaired T-cell reconstitution and immunosuppressive therapy following HSCT, pediatric patients are at high risk of human CMV reactivation, an infection associated with considerable morbidity and mortality. Indeed not only can CMV infection cause severe and multiorgan disease (retinitis, gastroenteritis, encephalitis, hepatitis, pneumonitis, myocarditis), but it is also associated with the development of bacterial or fungal infections, graft rejection, or GVHD.[1,2]

Among these patients, regular virological surveillance, prophylactic, and preemptive antiviral therapies have effectively reduced

Abbreviations: ATG, antithymocyte immunoglobulin; AUC, area under the curve; BMT, bone marrow transplantation; CMI, cell-mediated immunity; CMV, cytomegalovirus; D, donor; GVHD, graft-vs-host disease; HHV-6, human herpesvirus 6; HHV-7, human herpesvirus 7; HSCT, hematopoietic stem cell transplant; ICS, intracellular cytokine staining; IFN-γ, interferon−γ; ROC, receiver operating characteristic; R, recipient; SOT, solid organ transplantation; TBI, total body irradiation.

the incidence and impact of symptomatic CMV infection. However, prolonged usage of antiviral drugs increases the risk of late symptomatic CMV infections, drug toxicity, and induces drug-resistant CMV strains.[3]

Reconstitution of CMI, in particular CMV-specific T-lymphocyte responses, is associated with protection against viral reactivation and disease.[4] Therefore, measuring a patient's CMI response to CMV may help determine his/her risk of CMV infection as well as individualize management strategies.[5,6] At present, several immunological assays are available for monitoring CMV-specific T cell–mediated immune responses in transplant recipients. However, they are expensive, often require laboratory expertise, and are neither widely available nor standardized.[7]

The Quantiferon-CMV assay is an ELISA-based functional assay, equivalent to a commonly used diagnostic test for *Mycobacterium tuberculosis*. It measures the amount of IFN-γ secreted in whole blood from CMV-specific T cells in response to peptides simulating 23 CD8+-specific epitopes of CMV proteins, collectively representing over 95% of the general population. IFN-γ levels may correlate with the patient's level of CMI.[8]

The aim of this prospective study was to investigate the clinical utility of monitoring CMV-specific CMI to predict CMV reactivation and disease in pediatric HSCT patients using the Quantiferon-CMV (QIAGEN®) assay.

2 | PATIENTS AND METHODS

Thirty-seven allogeneic HSCT pediatric patients (23 boys and 14 girls), aged 1-17 years old, were enrolled in a longitudinal prospective study from March 2010 to June 2012. All patients were recruited from the Pediatric Bone Marrow Transplantation (BMT) Unit of the "Aghia Sofia" Children's Hospital of Athens housed in the "Marianna V. Vardinogiannis – Elpida" Children's Oncology Unit. The study was approved by the institutional review board of the hospital. Written informed parental consent was obtained prior to participation. Patients were followed up for 12 months post-transplantation.

2.1 | Evaluation of CMV DNAemia and serology

CMV viremia was considered the detection of human CMV (h-CMV) in blood, in the absence of clinical manifestations or organ function abnormalities, while CMV disease was defined as either systemic or local h-CMV infection, associated with clinical symptoms and/or functional abnormalities.[9]

Routine surveillance for viremia comprised of weekly determination of CMV, Epstein-Barr virus, adenovirus, BK virus, HHV-6, and HHV-7during the first 100 days post-HSCT and thereafter if clinically indicated. CMV viremia was evaluated by the Hellenic Pasteur Institute using in-house PCR according to WHO International Standard for h-CMV. The lowest limit of detection for the assay was 500 copies/mL. CMV serology of both donors and recipients was determined using chemiluminescence assay (Abbott Diagnostics).

2.2 | Viral infection prophylaxis and preemptive CMV therapy

All HSCT patients received viral prophylaxis with low-dose acyclovir (250 mg/m^2 3 times/d) irrespective of CMV serostatus. If CMV DNAemia was detected, all patients were treated with either ganciclovir (2 × 5 mg/kg/d) or foscarnet (2 × 60 mg/kg/d) for patients prior to engraftment. Duration of prophylaxis for hematological malignancies was 6 months and for non-malignant conditions 12 months. Preemptive treatment was discontinued when 2 consecutive negative PCR results were obtained.

2.3 | GVHD prophylaxis and treatment

GVHD prophylaxis consisted of cyclosporine A and 4 doses of methotrexate. First-line therapy for acute GVHD was the administration of prednisone (2 mg/kg/d) with slow tapering depending on clinical response. Chronic GVHD was treated with prednisone at 1-2 mg/kg/d with slow tapering.

2.4 | Immunological monitoring

Surveillance for CMV T-cell immune recovery was performed at the following time points: pretransplant, early after transplantation (within 3 days), 30, 90, 180, 270, and 360 days post-transplantation. Patients were divided into different risk groups according to the donor/recipient (D/R) CMV serostatus and subsequent risk for CMV disease. Donor-positive serostatus defined intermediate risk independent of recipient serostatus (D+/R+, D+/R−). Donor-negative serostatus could preclude high risk for positive serostatus recipients (D−/R+) and low risk for negative (D−/R−).

Evaluation of the immune response was performed using the Quantiferon-CMV assay (QIAGEN®). The CMV peptide pool included 23 peptides derived predominantly from CMV pp65 and IE1, as well as epitopes from pp50, IE2, and g B. The HLA alleles represented were HLA-A1, A2, A3, A11, A23, A24, A26, B7, B8, B27, B35, B40/60, B41, B44, B52, B57, and B58.

The assay was performed according to the manufacturer's instructions. Briefly, 1 mL of heparinized whole blood was directly placed into each of 3 specialized blood collection tubes: CMV peptide pool (CMV), negative control (nil), and positive control containing phytohemagglutinin (mitogen). Following incubation at 37°C for 15-24 hours, the plasma was harvested. Plasma IFN-γ levels were measured using a standard ELISA assay and the concentrations calculated using software provided by the manufacturer. The IFN-γ response was interpreted as follows: "Positive" if CMV ≥0.2 IU/mL, "Negative" if CMV < 0.2 IU/mL and mitogen ≥0.5 IU/mL, and "Indeterminate" if CMV < 0.2 IU/mL and mitogen <0.5 IU/mL.

TABLE 1 Characteristics of HSCT patients. Data are presented as actual number of patients (percentage proportion)

Total number of patients	N = 37
Gender	
Male	23 (62%)
Female	14 (38%)
Median age (years)	8.5 (1-17)
Disease	
Malignant	21 (57%)
Non-malignant	16 (43%)
Stem cell source	
Bone marrow	26 (70%)
Peripheral blood	6 (16%)
Cord blood	3 (8%)
Bone marrow + cord blood	2 (6%)
Donor type	
Related	19 (51%)
Unrelated	18 (49%)
Donor/Recipient serostatus	
D−/R+	12 (32%)
D+/R+	16 (43%)
D−/R−	7 (19%)
D+/R−	2 (6%)
Conditioning regimen	
TBI	
Yes	1 (3%)
No	36 (97%)
ATG	
Yes	32 (86%)
No	5 (4%)
Acute graft vs host disease (aGVHD)	14 (100%)
D−/R+	6 (43%)
D+/R+	4 (28%)
D−/R−	3 (21%)
D+/R−	1 (8%)
Chronic graft vs host disease (cGVHD)	1 (3%)
CMV viremia	19 (51%)
CMV disease	2 (5%)
Number of deaths	8 (21%)

2.5 | Statistical analysis

All statistical analyses and data management were performed using STATA for Windows v 8.5, (StataCorp, College Station, TX, USA). The incidence of CMV viremia was estimated using cumulative incidence estimates, and differences between groups were calculated using log-rank test. $P < .05$ was considered statistically significant.

We examined the optimal cutoff value of Quantiferon-CMV assay in predicting the absence of an episode of CMV viremia in the group of patients with previous CMV reactivation using ROC analysis.

3 | RESULTS

The demographic and clinical characteristics of the patients are shown in Table 1. Thirty-seven children suffering from malignant and non-malignant diseases who underwent HSCT were prospectively recruited. The monitoring period for CMV CMI using Quantiferon assay was 12 months in 25 patients. Twelve of 37 patients were monitored less (1-6 months) due to death (8 patients) or graft rejection (4 patients).

A total of 207 Quantiferon-CMV samples were collected (mean: 5 samples per patient; range 2-7). During the study period, 19 patients experienced mostly asymptomatic CMV reactivation. Nine of these had multiple episodes of CMV reactivation (mean: 3 episodes per patient; range 2-5). Two patients had evidence of CMV disease (5%): One had an indeterminate CMI test prior to the onset of disease and the other had a transient-positive CMI test 30 days post-transplantation but was negative in all measurements that followed. The distribution of patients' viremia according to donor/recipient serostatus is shown in Table 2.

The cumulative incidence of CMV reactivation was 57% for the high- and intermediate-risk group (Figure 1). The median viral load was 3720 copies/mL (interquartile range [IQR] 2137.5-13000 copies/mL).

There were 7 D−/R− patients that were excluded from all further analyses as a low-risk group. Only 1 D−/R− patient experienced CMV viremia 40 days after HSCT at the cutoff level of detection for the PCR assay (500 copies/mL) that was treated with foscarnet. This patient did not develop CMV disease, detectable CMV-specific T-cell immunity at any time point analyzed or CMV seroconversion. The comparison of viremia among the serological status-stratified groups by Fisher's exact test was statistically significant ($P = .016$).

Pretransplant measurements of Quantiferon-CMV were conducted in 34 patients. Of these, 17 were negative (46%), 5 were positive (13.5%), and 12 were indeterminate (32.4%). There was no correlation between pretransplant CMV CMI and reactivation within 30 days ($P = .72$).

The percentage of indeterminate Quantiferon-CMV results in the total collected sample was 27% (56/207), and more than half (58.9%) were measured 1-3 days after transplant (33/56). Within the 12 months of study, 15 patients (40.5%) showed stable CMV-specific immune reconstitution at an average time of 82 days. The cumulative incidence of positive Quantiferon-CMV results was 46.7% (Figure 2). In this group, only 1 patient had a recurrent episode of CMV reactivation while more than 1 episode was observed in the group of indeterminate/negative patients. Interestingly, 2 patients developed stable CMV-specific immunity without previous CMV viremia. The serostatus of these 2 patients was D+/R+ and D−/R+. The mean duration of viremia for those who had positive Quantiferon-CMV results was 14 days and for those patients with

CMV serostatus	D−/R− (7)	D+/R+ (16)	D+/R− (2)	ΔD−/R+ (12)
Patients with CMV viremia at 30 d	0	5 (31%)	0	6 (50%)
Patients with CMV viremia at 100 d	1 (14%)	4 (25%)	1 (50%)	1 (8%)
Patients with CMV viremia at 180 d	0	1 (6%)	0	0
Patients with CMV disease	0	0	0	2 (16%)

TABLE 2 CMV viremia and disease in pediatric HSCT patients. Data are presented as actual number of patients (percentage proportion)

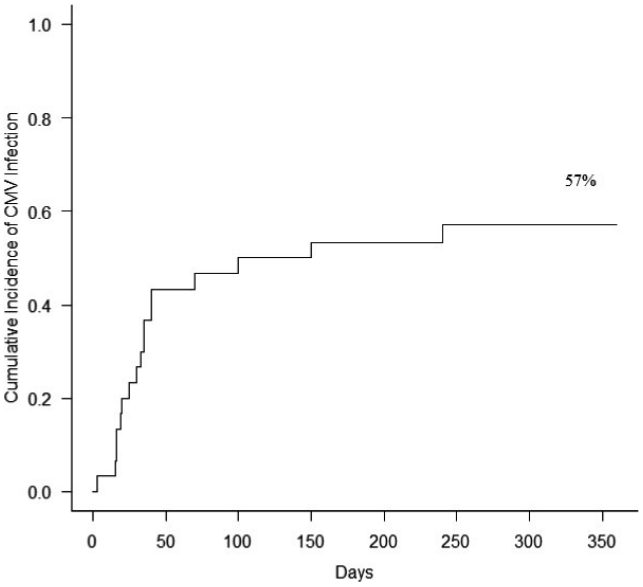

FIGURE 1 Cumulative incidence of CMV infection within 360 d post-transplant. The cumulative incidence of CMV infection was 57% for the high- and intermediate-risk group. CMV, cytomegalovirus

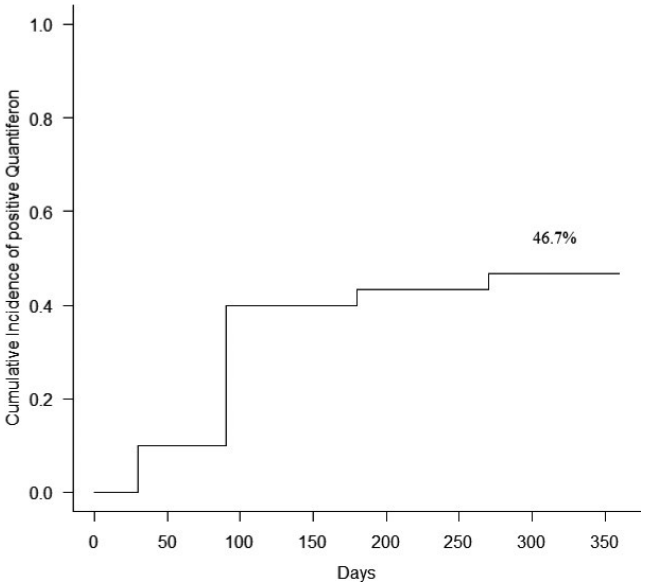

FIGURE 2 Cumulative incidence of specific CMV immunity within 360 d post-transplant cumulative incidence of positive Quantiferon-CMV results is 46.7%. CMV, cytomegalovirus

negative Quantiferon-CMV results 23 days (P = .24), and the median duration of viremia assessed with the Mann-Whitney U test was 21 days (7, 56) for patients with negative Quantiferon-CMV results and 21 days (7, 49) for those with positive Quantiferon-CMV results (P = .43).

Among patients with CMV reactivation, the most commonly detected virus was Epstein-Barr virus (in 5 patients). Other viruses detected were BK virus in 2 patients and adenovirus in one.

The cumulative incidence of CMV reactivation in patients after they developed CMV-specific immunity was lower than those who did not (15% vs 53%; P = .023) (Figure 3).

Two representative cases are shown in Figure 4 to illustrate the pattern of CMV-specific immunity in relation with CMV reactivation. The first patient (D+/R+) exhibited negative results pretransplantation and early post-transplantation, but from the 90th day post-transplantation, he had repeatedly positive Quantiferon-CMV results and did not experience any further CMV reactivation. The second patient (D−/R+) had persistent Quantiferon-CMV-negative results, except for a single positive result at 30th day post-transplantation and suffered from recurrent episodes of CMV reactivation.

In this study, the occurrence of aGVHD did not influence the immunological recovery against CMV (30% vs 55.5%; P = .22). ROC analysis of the Quantiferon-CMV assay in predicting CMV viremia in all at-risk patients exhibited an AUC of 0.725. The cutoff value of IFN-γ >0.2 IU had a sensitivity of 45% and specificity 100% in predicting patients at risk for recurrent CMV viremia (Figure 5).

4 | DISCUSSION

In this study, we monitored 37 patients post-HSCT to demonstrate that the Quantiferon-CMV assay can be used in pediatric HSCT patients to evaluate the risk of CMV reactivation. Indeed, in our cohort, once stable CMV-specific CD8+ T-cell reconstitution was measured by the assay, the cumulative incidence of CMV viremia was significantly lower.

CMV reactivation was observed in more than half of our patients (19/37), who were mostly seropositive recipients (R+). Indeed 9 of

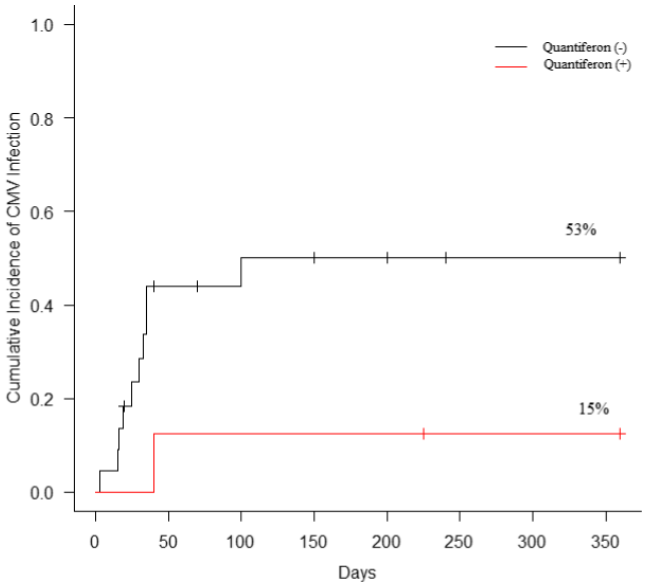

FIGURE 3 Relationship between stable CMV-specific immunity and CMV infection. The cumulative incidence of CMV infection in patients after they developed CMV-specific immunity was lower than those who did not (15% vs 53%). CMV, cytomegalovirus

them suffered multiple episodes of viremia, whereas only 2 patients developed CMV disease. A higher incidence of viral reactivation was observed in R+ patients irrespective of the donor serologic status. As they all received routine preemptive therapy and cleared viremia following antiviral treatment, we were not able to predict virus-associated complications. Considering that subclinical CMV viremia was common in transplant recipients, it is possible that patients with low-level viral load could clear viremia without treatment after establishing CMV CMI.[10] In our cohort, the unexpected single episode of CMV viremia, at the cutoff level of detection, in a D−/R− patient was retrospectively considered a marginal result without clinical consequences, therefore a false-positive result. This patient never developed CMV CMI. In contrast, the 2 selected examples of patients illustrate the usefulness of the Quantiferon-CMV assay. The patient with consecutive positive results had no recurrent episodes of CMV viremia, whereas the patient with the negative results continued to have recurrent episodes of CMV viremia.

As expected, based on Quantiferon-CMV assay, we observed that CMV-specific immunity is dynamic in early stages post-HSCT (one or more negative or indeterminate results). Specifically, in our study, most indeterminate samples (58.9%) were collected early post-transplantation. In HSCT patients, the reconstitution of the T-cell compartment is mediated by 2 pathways: a thymus-dependent (thymopoiesis) and a thymus-independent (graft-derived naïve T cells).[11] Studies have shown that compared to adults, children have better thymic function and CMV-positive recipients demonstrate faster CD8(+) T-cell recovery post-transplant.[12] Therefore, the immunocompromise seen in children after HSCT is less severe than in adults. In addition, the overall incidence of CMV infection in pediatric HSCT patients is significantly lower than in their adult counterparts.[13]

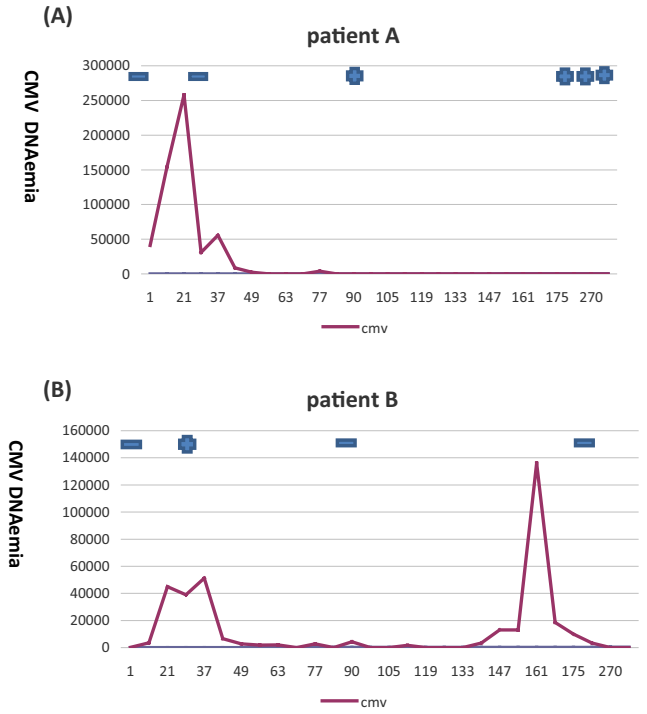

FIGURE 4 A, A 5-month-old boy with Wiskott-Aldrich syndrome who did not develop CMV viremia once consecutive positive Quantiferon-CMV results were obtained. +, positive result; −, negative result; CMV, cytomegalovirus. B, A 17-month-old boy with immunodeficiency with recurrent episodes of CMV viremia in the face of persistent negative Quantiferon-CMV result (except for a single transient positive Quantiferon result early post-transplantation). At 175th day, he experienced graft rejection. +, positive result; −, negative result; CMV, cytomegalovirus

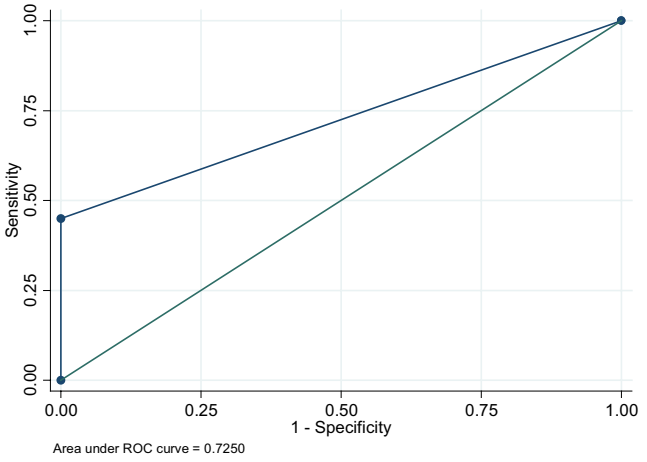

FIGURE 5 Receiver operating characteristic (ROC) analysis of the Quantiferon-CMV assay in predicting CMV viremia in all at-risk patients. Area under the curve = 0.725, SE 0.057, asymptotic normal distribution (95% CI) 0.613-0.836. The cutoff value of IFN-γ >0.2 IU had a sensitivity of 45% and specificity 100% in predicting patients at risk for CMV viremia. CMV, cytomegalovirus

Although immune reconstitution after transplantation can be delayed by acute or chronic GVHD,[14] in the pediatric population studied, GVHD did not impair CMV-specific immunity. This is in

accordance with the study of Hakki et al, where delayed recovery of T-cell immunity was only associated with low absolute counts of $CD4^+$ and $CD8^+$, using bone marrow as the source of stem cells and the use of high-dose steroids.[15]

To our knowledge, there are a limited number of studies evaluating T-cell responses using the Quantiferon-CMV assay. Lisboa et al measured CMI shortly after the onset of CMV viremia in adult SOT patients using Quantiferon-CMV assay and found that in patients with positive CMI test, the incidence of subsequent viral clearance was higher compared with those with a negative test at onset.[16] In the study of Kumar et al, Quantiferon-CMV assay was performed at baseline and every month during 3 months post-transplant in 108 adult SOT patients at high risk for CMV disease. Patients with a positive response at the end of prophylaxis had a lower incidence of late CMV disease than patients with a negative.[17] This assay was also evaluated in a clinical study of 41 HSCT adult patients who underwent weekly immunological monitoring from day 21 post-transplant. The median time to stable CMV-specific immune reconstitution was 59 days, and the incidence of CMV reactivation was lower in patients who developed CMV-specific immunity than those who did not.[18] A recent pilot study in pediatric allogeneic HSCT recipients showed no recurrent CMV infections among patients with positive Quantiferon-CMV results following an initial CMV infection.[19]

In our pediatric cohort, most CMV viremia occurred between the 30th and 90th day post-transplantation. Our study design included measurements of Quantiferon-CMV at 30 and 90 days, and none in between. In addition, we observed that patients who had developed stable Quantiferon-CMV were at low risk of reactivation. Finally, there was no CMV viremia detected past 180th day post-transplantation (late CMV reactivation) found in this group. Therefore, closer monitoring of Quantiferon-CMV starting at 30 days post-transplantation (for example every 14 days), until three positive results have been obtained, might be useful. Thereafter, as stable CMV immunity is established, Quantiferon-CMV measurements, CMV surveillance and prophylactic treatment plans could become less intense in pediatric HSCT patients.

Alternative tests to assess CMV CMI include ICS, ELISPOT, and MHC-peptide tetramers. They measure IFN-γ production after stimulation with CMV antigens or other cytokines such as IL-2 and TNF-α. However, it is possible to obtain different results, depending on the choice of laboratory assay.[20] Abate et al used ELISPOT test for immunological monitoring in a cohort of 31 pediatric allogeneic HSCT recipients, with good performance in determining the risk or protection level against CMV viremia (ROC analysis, AUC 0.82).[21] In a different study, the ROC analysis of the Quantiferon-CMV and ELISPOT in predicting detectable viremia in a cohort of 120 adult kidney recipients was AUC 0.66 and AUC 0.62 for using every possible cutoff, respectively.[22] In this report, the sensitivity and specificity of the Quantiferon-CMV assay for all at-risk patients according to ROC analysis was better (0.725) using only the cutoff proposed by the manufacturer. This is in correlation with the above-mentioned study of Abate et al,[21] who used ELISPOT in a similar population, suggesting improved performance of the assay in pediatric HSCT patients.

Additionally, the Quantiferon-CMV assay is well standardized and easy to perform as necessary equipment is available in most laboratories. The evaluation of CD8+ but not CD4+ CMI and HLA dependence is among its disadvantages.[23]

The current study is potentially limited by the relatively small sample size, partly due to the fact that all patients were recruited from a single pediatric transplantation center. This could be overcome by multicenter studies and prospective long-term recruitment of participants. Furthermore, due to the inclusion of a fair number of patients suffering from non-malignant diseases, there is heterogeneity in the study group concerning the overall impairment of cellular immunity, for example, pretransplant immunosuppression. Another limitation is the heterogeneity of grafts because of the potential different dynamics of the reconstitution of CMV cell-mediated immune response. In addition, 12 of 37 patients were monitored less than 12 months (1-6 months) due to death (8 patients) or graft rejection (4 patients). Finally, no healthy controls were used to make baseline, prior to transplantation, comparisons.

5 | CONCLUSIONS

In this cohort of HSCT pediatric patients, positive Quantiferon-CMV was associated with a low risk of recurrent CMV viremia, accurately reflecting CMV CMI recovery. Quantiferon-CMV may help assess the risk of recurrent CMV viremia and complement the monitoring of pediatric patients post-transplantation. Therefore, pediatric patients with positive CMV CMI tests might benefit from less-intense surveillance and individualized prophylactic treatment plans.

AUTHORS' CONTRIBUTIONS

Bilio Paouri: Data collection, data analysis and interpretation, critical revision, drafting, and approval of article. Alexandra Soldatou: Research design, drafting article, critical revision, and approval of article. Eftihia Petrakou, Maria Theodosaki, Katerina Kaisari, Christina Oikonomopoulou: Data collection, critical revision, and approval of article. Charalampos Tsentidis: Statistics, data analysis, critical revision, and approval of article. Minos Matsas: Data collection, critical revision, and approval of article. Eugenios Goussetis: Concept/design, statistical analysis, critical revision of article, and approval of article.

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REFERENCES

1. Hebart H, Ensele H. Clinical aspects of CMV infection after stem cell transplantation. *Hum Immunol*. 2004;65:432-436.

2. Bueno J, Ramil C, Green M. Current management strategies for the prevention and treatment of cytomegalovirus infection in pediatric transplant recipients. *Pediatr Drugs*. 2002;4:279-290.
3. Ljungman P. CMV infection after hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2008;42(suppl 1):S70.
4. Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. *Clin Microbiol Rev*. 2009;22:76-98.
5. Gerna G, Lilleri D. Monitoring transplant patients for human cytomegalovirus: diagnostic update. *Herpes*. 2006;13:4.
6. Ljungman P. Would monitoring CMV immune responses allow improved control of CMV in stem cell transplant patients. *J Clin Virol*. 2006;35:493.
7. Danziger-Isakov L, Heeger PS. Clinical utility of measuring T-cell immunity to CMV transplant recipients. *Am J Transplant*. 2009;9:987-988.
8. Giuleri S, Manuel O. Quantiferon-CMV assay for the assessment of cytomegalovirus cell mediated immunity. *Expert Rev Mol Diagn*. 2011;11:17-25.
9. Ljungman P, Griffiths P, Paya C. Definition of HCMV infection and disease in transplant recipients. *Clin Infec Dis*. 2002;34:1094-1097.
10. Lilleri D, Gerna G, Fornara C, et al. Prospective simultaneous quantification of human cytomegalovirus-specific $CD4^+$ and $CD8^+$ T-cell reconstitution in young recipients of allogeneic hematopoietic stem cell transplants. *Blood*. 2006;108:1406-1412.
11. Surh CD, Sprent J. Homeostasis of naïve and memory T cells. *Immunity*. 2008;29:848-862.
12. Renard C, Barlogis V, Mialou V, et al. Lymphocyte subset reconstitution after unrelated cord blood or bone marrow transplantation in children. *Br J Haematol*. 2011;152:322-330.
13. Yoon HS, Lee JH, Choi ES, et al. Cytomegalovirus infection in children who underwent hematopoietic stem cell transplantation at a single center: a retrospective study of the risk factors. *Pediatr Transplant*. 2009;13:898-905.
14. Seggewiss R, Einsele H. Immune reconstitution after allogeneic transplantation and expanding options for immunomodulation: an update. *Blood*. 2010;115:3861-3868.
15. Hakki M, Riddell RS, Storek J, et al. Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: impact of host factors, drug therapy, and subclinical reactivation. *Blood*. 2003;102:3060-3067.
16. Lisboa L, Kumar D, Wilson LE, Humar A. Clinical utility of cytomegalovirus cell mediated immunity in transplant recipients with cytomegalovirus viremia. *Transplantation*. 2012;93:195-200.
17. Kumar D, Chernenko S, Moussa G, et al. Cell mediated immunity to predict cytomegalovirus disease in high risk solid organ transplant recipients. *Am J Transplant*. 2009;9:1214-1222.
18. Tey S-K, Kennedy GA, Cromer D, et al. Clinical assessment of antiviral $CD8^+$ T cell immune monitoring using Quantiferon-CMV® Assay to identify high risk allogeneic hematopoietic stem cell transplant patients with CMV infection complications. *PLoS ONE*. 2013;8:e74744.
19. Lee SM, Kim Y-J, Yoo KH, et al. Clinical usefulness of monitoring cytomegalovirus-specific immunity by quantiferon-CMV in pediatric allogeneic hematopoietic stem cell transplantation recipients. *Ann Lab Med*. 2017;37:277-281.
20. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation*. 2010;89:779-795.
21. Abate D, Cesaro S, Cofano S, et al. Diagnostic utility of human cytomegalovirus-specific T-cell response monitoring in predicting viremia in pediatric allogeneic stem-cell transplant recipients. *Transplantation*. 2012;93:536-542.
22. Abate D, Saldan A, Mengoli C, et al. Comparison of cytomegalovirus (CMV) enzyme-linked immunosorbent spot and CMV quantiferon gamma interferon-releasing assays in assessing risk of CMV infection in kidney transplant recipients. *J Clin Microbiol*. 2013;51:2501-2507.
23. Lochmanova A, Lochman I, Tomaskova H, et al. Quantiferon-CMV test in prediction of cytomegalovirus infection after kidney transplantation. *Transplant Proc*. 2010;42:3574-3577.

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