Open Forum Infectious Diseases

BRIEF REPORT

Absolute Lymphocyte Count Thresholds: A Simple, Readily Available Tool to Predict the Risk of Cytomegalovirus Infection After Transplantation

Atibordee Meesing[1,2] **and Raymund R. Razonable**[1,3]

[1]Division of Infectious Diseases, Department of Medicine, Mayo Clinic, Rochester, Minnesota; [2]Division of Infectious Disease and Tropical Medicine, Department of Medicine, Khon Kaen University, Khonkean, Thailand; [3]William J von Liebig Center for Transplantation and Clinical Regeneration, Mayo Clinic College of Medicine and Science, Rochester, Minnesota

This study of 64 solid organ and hematopoietic stem cell transplant recipients found that peripheral blood absolute lymphocyte count of <610 and <830/μL, respectively, correlated with cytomegalovirus infection. In an era when sophisticated measures of CMV-specific T cells are emerging, we emphasize the utility of the inexpensive and readily-available absolute lymphocyte count.

Keywords. infection; cytomegalovirus; lymphocyte.

Effective control of cytomegalovirus (CMV), a common infection after solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT) [1, 2], requires functional CMV-specific T cells [3–5]. There is increasing interest in utilizing sophisticated CMV-specific T-cell assays for CMV prognostication after transplantation, but these are expensive and are not readily available. Based on observations that the risk of CMV infection and relapse is associated with lymphopenia [6], we aimed to define the peripheral blood absolute lymphocyte count (PBALC) threshold that correlates with CMV infection in SOT and HSCT patients.

METHODS

Study Population

This observational study was conducted between July 1, 2017, and March 31, 2018. After Mayo Clinic Institutional Review Board approval, we enrolled 43 consecutive patients with CMV DNA detected in peripheral blood (COBAS Ampliprep/Taqman CMV Monitor, Roche Diagnostics). In parallel, we randomly selected 21 transplant patients undergoing CMV surveillance who did not develop CMV infection.

CMV-seropositive (CMV R+) HSCT recipients underwent weekly CMV surveillance, and any quantifiable CMV (>137 IU/mL of plasma) was treated with intravenous ganciclovir or oral valganciclovir. Valganciclovir prophylaxis was given for 3 months to moderate-risk CMV R+ kidney, pancreas, and heart recipients; 6 months to high-risk CMV D+/R- kidney, liver, heart, and pancreas recipients and CMV R+ lung recipients; and 12 months for CMV D+/R- lung recipients. CMV R+ liver recipients underwent CMV surveillance, and preemptive valganciclovir therapy was initiated when a viral load of 1000 IU/mL of plasma was reached. All D+/R- SOT patients underwent CMV surveillance for 3 months after completing antiviral prophylaxis [7].

Clinical and laboratory data were recorded, including viral load and PBALC (differential count). PBALC values were collected from pretransplant, pre-CMV (2–4 weeks before CMV infection or testing), time of CMV infection (or CMV PCR testing), and virologic clearance (for those with CMV). PBALC was correlated with CMV infection, which was defined as detection of CMV by nucleic acid testing of blood (CMV DNAemia) or other specimens, or demonstration of CMV-associated cytopathic changes on histopathology [8]. CMV nucleic acid testing was performed using COBAS Ampliprep/Taqman (Roche Diagnostics), as previously described [9]. If accompanied by symptoms and signs, CMV infection was further categorized as a CMV syndrome (for SOT) or tissue-invasive CMV disease [10]. Patients without signs and symptoms were categorized simply as having CMV infection.

Statistical Analysis

The chi-square test or Fisher exact test was used to compare categorical variables. Both CMV infection and disease were considered outcome variables of the study. Bivariate correlation analyses were performed using the Pearson or Spearman test for nonparametric variables. The 2-tailed statistical significance level was $P < .05$. Receiver operating characteristic curve analysis was used to determine the PBALC cutoff associated with CMV infection and disease. Associations were expressed as hazard ratio (HR) and 95% confidence interval (CI). All analyses were performed with JMP software.

RESULTS

Sixty-four SOT (56%) and HSCT (44%) patients were enrolled; 43 (67.2%) had CMV infection or disease. Before transplant, the

Received 29 June 2018; editorial decision 5 September 2018; accepted 7 September 2018.
Correspondence: R. R. Razonable, MD, Division of Infectious Diseases, Mayo Clinic, 200 First Street SW, Rochester, MN 55905 (razonable.raymund@mayo.edu).

Open Forum Infectious Diseases®
© The Author(s) 2018. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
DOI: 10.1093/ofid/ofy230

median PBALC for all 64 patients (interquartile range [IQR]) was 965/μL (750–1515). Among 43 patients who developed CMV infection or disease, the median PBALC (IQR) declined from a pretransplant value of 1050/μL (810–1520) to 450/μL (330–640; $P < .0001$) at onset of infection; the median PBALC (IQR) 2–4 weeks before onset of CMV infection was 490/μL (170–950). The 10 patients with CMV disease had a significantly lower median PBALC (315/μL; IQR, 155–520) than the 33 patients with CMV infection (450/μL; IQR, 365–670; $P = .03$). In contrast, the median PBALC of 21 transplant recipients who did not develop CMV infection or disease increased from a pretransplant baseline (IQR) of 870/μL (635–1430) to 1060/μL (630–2215; $P = .07$). Overall, a PBALC <830/μL after transplantation correlated with the development of CMV infection or disease (sensitivity, 95%; specificity, 71%). A PBALC <830/μL conferred a higher risk of CMV infection or disease compared with ≥830/μL (HR, 7.5; 95% CI, 2.69–31.03; $P < .0001$).

PBALC and Risk of CMV in SOT Patients

The SOT cohort had a median age (IQR) of 52 (40–62) years and was mostly male (72%) (Table 1). CMV risk statuses were D+/R- (58%), D+/R+ (30%), and D-/R+ (11%). The most common types of organ transplant were liver (47%), kidney with or without pancreas (22%), and lung or heart-lung (14%).

Table 1. Baseline Characteristics of 64 Solid Organ and Hematopoietic Stem Cell Transplant Recipients

	SOT (n = 36)	HSCT (n = 28)
Age (IQR), y	52 (40–62)	60 (37–66)
Male, No. (%)	26 (72.2)	15 (53.4)
White, No. (%)	33 (91.7)	28 (100)
CMV status, No. (%)		
D+R+	11 (30.6)	10 (35.7)
D+R-	21 (58.3)	2 (7.1)
D-R+	4 (11.1)	12 (42.9)
Type of transplant (SOT), No. (%)		
Lung	2 (5.6)	
Heart/lung	3 (8.3)	
Kidney	5 (13.9)	
Kidney/pancreas	3 (8.3)	
Liver	17 (47.2)	
Liver/heart	1 (2.8)	
Pancreas	2 (5.6)	
Heart	1 (2.8)	
Heart/kidney	2 (5.6)	
Type of transplant (HSCT), No. (%)		
Matched related		9 (32.1)
Matched unrelated		16 (57.1)
Haploidentical		1 (3.6)
Autologous		2 (7.1)
Onset to CMV infection and disease (IQR), mo	7 (2.4–9.3)	1.6 (1.4–6.1)

Abbreviations, CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; PBALC, peripheral blood absolute lymphocyte count; SOT, solid organ transplantation.

Twenty-five of 36 patients developed CMV (infection, 72%; disease, 28%) at a median (IQR) of 7 (2.4–9.3) months after transplantation. Among SOT recipients, a PBALC <610/μL correlated with CMV infection and disease (sensitivity, 80%; specificity, 73%). Patients with a PBALC <610/μL had a higher risk of CMV compared with those with a PBALC ≥610/μL (HR, 2.2; 95% CI, 0.91–6.97; $P = .08$). The median PBALC of SOT patients with CMV infection or disease was lower than patients without CMV, whereas the median PBALC was lower among patients with CMV disease than those with CMV infection (Table 2).

PBALC and Risk of CMV in HSCT Patients

The 28 HSCT recipients had a median age (IQR) of 60 (37–66) years, with equal gender distribution. The majority were CMV-seropositive (D+/R+, 36%; D-/R+, 43%) and recipients of matched unrelated transplants (57%) (Table 1). Eighteen of 28 patients developed CMV (infection, 83%; pneumonia or enterocolitis, 17%) at a median (IQR) of 1.6 (1.4–6.1) months after transplantation. A PBALC <830/μL correlated with CMV (sensitivity, 100%; specificity, 80%). Patients with a PBALC <830/μL had a higher risk of CMV compared with a PBALC ≥830/μL (HR, 10.1; 95% CI, 2.05–181.88; $P = .002$). The median PBALC of HSCT patients with CMV infection or disease was significantly lower than in patients without CMV, but there was no difference in median PBALC between patients with CMV infection and disease (Table 2).

PBALC and the Duration of CMV DNAemia and Risk of Relapse

The median duration of CMV DNAemia (IQR) was 19 (14–32) days and was similar between patients with CMV disease (22 days; IQR, 14–45) and CMV infection (19 days; IQR, 14–33; $P = .86$), and those with a PBALC ≥830/μL (22 days; IQR, 14–28) and <830/μL (19 days; IQR, 14–34; $P = .92$).

Recurrent CMV infection (11.6%; 3 SOT and 2 HSCT) occurred at median (IQR) of 3.6 (2.8–4.1) months after initial infection. A PBALC <390/μL at the time of viral clearance correlated with CMV recurrence (sensitivity, 80%; specificity, 90%). Patients with a PBALC <390/μL upon viral clearance had a higher risk of recurrence than those with a PBALC ≥390/μL (HR, 6.4; 95% CI, 1.05–49.49; $P = .04$). Notably, 5 patients with recurrence had a nonsignificant decline in median PBALC during antiviral treatment (450; IQR, 215–575; to 220/μL; IQR, 7–390). In contrast, the median PBALC in 38 patients without CMV recurrence significantly increased from a pretreatment value (IQR) of 445/μL (328–663) to 985/μL (675–1365; $P < .0001$) at the time of viral clearance.

DISCUSSION

Lymphocytopenia is a major risk factor for CMV after transplantation [6, 11]. The PBALC was low among transplant recipients who developed CMV infection, especially transplant recipients with CMV disease, and those patients who had recurrent CMV infection. Herein, we propose PBALC values of <830/μL for

Table 2. Peripheral Blood Lymphocyte Subsets Among Transplant Patients With CMV Disease, CMV Infection, and Those Who Did Not Develop CMV Infection

	CMV Disease	CMV Infection	No CMV Infection	*P* Value
SOT (n = 36)	n = 7	n = 18	n = 11	
Pretransplant median PBALC (IQR), /μL	1140 (800–1790)	1260 (855–1595)	890 (660–1590)	.55
Median PBALC at 1st CMV (IQR), /μL	270 (140–460)	450 (388–675)	1120[a] (590–1400)	.001
HSCT (n = 28)	n = 3	n = 15	n = 10	
Pretransplant median PBALC (IQR), /μL	560 (16–1060)	970 (730–1080)	780 (483–1355)	.39
Median PBALC at 1st CMV (IQR), /μL	520 (300–560)	510 (330–670)	1020[a] (795–3308)	.03

Abbreviations, CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; PBALC, peripheral blood absolute lymphocyte count; SOT, solid organ transplantation.

[a]Use PBALC on the first day of CMV virological testing.

HSCT and <610/μL for SOT as clinically relevant thresholds for predicting the risk of CMV infection and disease after transplantation. Moreover, we also observed that a PBALC of <390/μL (and lack of lymphocyte recovery) at the time of virologic clearance correlated with subsequent CMV recurrence.

Lymphocyte depletion during the post-transplant period is a recognized risk factor for CMV infection or disease, although the magnitude of clinically relevant decline is not fully defined. We observed that SOT and HSCT recipients who developed CMV infection had a significant decline in PBALC during the post-transplant period to a level <830/μL for HSCT and <610/μL for SOT. In contrast, the PBALC among patients who did not develop CMV infection increased. Moreover, we also observed that further decline (or lack of significant recovery) in lymphocyte count during antiviral treatment of CMV infection and disease may predict risk of relapse. In our cohort, a declining trend in PBALC during antiviral treatment correlated with CMV relapse, whereas recovery of lymphocyte count during antiviral treatment correlated with lack of recurrence.

The results of our study are limited by the relatively small size of a heterogenous cohort of SOT and HSCT recipients, and thus, we were unable to perform multivariate analysis. Accordingly, we encourage other clinician-scientists to perform similar studies to confirm our findings. In another study of a larger cohort of 130 SOT recipients, we also found a similar value: A PBALC of <630/μL was significantly correlated with CMV infection or disease [12]. Hence, we believe that our observations may be reproducible.

In conclusion, we highlight the importance of the simple, readily available PBALC as a prognostic indicator of CMV risk and outcome after transplantation. Specifically, a PBALC <830/μL in HSCT and <610/μL in SOT can serve as a marker of a transplant patient's heightened risk for CMV. Moreover, a declining trend over time may also serve a similar prognostic role. In an era when measuring CMV-specific T cells is emerging, our study emphasizes the clinical utility of an inexpensive, commonly ordered, and readily available PBALC—a component of routine differential of a complete blood count.

Acknowledgments

Financial support. This study did not have any financial support.

Potential conflicts of interest. All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Harvala H, Stewart C, Muller K, et al. High risk of cytomegalovirus infection following solid organ transplantation despite prophylactic therapy. J Med Virol **2013**; 85:893–8.
2. Camargo JF, Komanduri KV. Emerging concepts in cytomegalovirus infection following hematopoietic stem cell transplantation. Hematol Oncol Stem Cell Ther **2017**; 10:233–8.
3. Roddie C, Peggs KS. Immunotherapy for transplantation-associated viral infections. J Clin Invest **2017**; 127:2513–22.
4. van Rhee F, Barrett J. Adoptive transfer of Ag-specific T cells to prevent CMV disease after allogeneic stem-cell transplantation. Cytotherapy **2002**; 4:3–10.
5. Mui TS, Kapp M, Einsele H, Grigoleit GU. T-cell therapy for cytomegalovirus infection. Curr Opin Organ Transplant **2010**; 15:744–50.
6. Gardiner BJ, Nierenberg NE, Chow JK, et al. Absolute lymphocyte count: a predictor of recurrent cytomegalovirus disease in solid organ transplant recipients. Clin Infect Dis. **In press**.
7. Arthurs SK, Eid AJ, Deziel PJ, et al. The impact of invasive fungal diseases on survival after lung transplantation. Clin Transplant **2010**; 24:341–8.
8. Razonable RR, Humar A; AST Infectious Diseases Community of Practice. Cytomegalovirus in solid organ transplantation. Am J Transplant **2013**; 13(Suppl 4):93–106.
9. Razonable RR, Åsberg A, Rollag H, et al. Virologic suppression measured by a cytomegalovirus (CMV) DNA test calibrated to the World Health Organization international standard is predictive of CMV disease resolution in transplant recipients. Clin Infect Dis **2013**; 56:1546–53.
10. Ljungman P, Boeckh M, Hirsch HH, et al; Disease Definitions Working Group of the Cytomegalovirus Drug Development Forum. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. Clin Infect Dis **2017**; 64:87–91.
11. Nierenberg NE, Poutsiaka DD, Chow JK, et al. Pretransplant lymphopenia is a novel prognostic factor in cytomegalovirus and noncytomegalovirus invasive infections after liver transplantation. Liver Transpl **2014**; 20:1497–507.
12. Meesing A, Abraham R, Razonable RR. CMV-specific CD8+ T-cell immune competence score as predictor of outcome for CMV infection after transplantation. Am J Transplant **2018**; 18(Suppl 4):877–877.