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Original Article

Clinical Application of Variation in Replication Kinetics During Episodes of Post-transplant Cytomegalovirus Infections

I.P. Lodding [a,*], H. Sengeløv [b], C. da Cunha-Bang [a,b], M. Iversen [c], A. Rasmussen [d], F. Gustafsson [c], J.G. Downing [a], J. Grarup [a], N. Kirkby [e], C.M. Frederiksen [a], A. Mocroft [f], S.S. Sørensen [g], J.D. Lundgren [a], On behalf of the MATCH Programme Study Group

[a] Centre for Health and Infectious Disease Research (CHIP), Department of Infectious Diseases and Rheumatology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[b] Department of Hematology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[c] Department of Cardiology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[d] Department of Surgery C, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[e] Department of Clinical Microbiology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
[f] Research Department of Infection and Population Health, University College of London (UCL), Royal Free Hospital Rowland Hill Street London NW3 2PF, United Kingdom
[g] Department of Nephrology, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark

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ABSTRACT

Background: Cytomegalovirus (CMV) infection in transplant recipients is reported to replicate with a doubling time of 1.2–2 days, and weekly screening is recommended for early diagnosis. We re-evaluated these features in our cohort of transplant recipients.
Methods: The CMV doubling time of the first CMV infection in the first year post-transplant could be calculated for 193 recipients of haematopoietic stem cell or solid organ transplantation. Factors determining the proportion of recipients with a high diagnostic CMV viral load (≥18,200 IU/mL) were explored using mathematical simulation.
Findings: The overall median doubling time was 4.3 days (IQR 2.5–7.8) and was not influenced by prior CMV immunity, or type of transplantation ($p > 0.4$). Assuming a fixed doubling time of 1.3 days and screening intervals of 7 or 10 days, 11.1% and 33.3% were projected to have a high CMV viral load at diagnosis, compared to 1.4% and 4.3% if the doubling time varies as observed in our cohort. Consistently, 1.9% of recipients screened weekly had a high diagnostic virus load.
Interpretation: Screening intervals can be extended to 10 days in cohorts with comparable CMV doubling time, whereas shorter than 7 days is required in cohorts with shorter doubling times to maintain pre-emptive screening quality.

1. Introduction

Cytomegalovirus (CMV) infection is an important cause of complications in transplant recipients. If untreated, it may progress to CMV disease, a condition associated with increased morbidity and mortality. A pre-emptive strategy, comprising of regular screening with CMV PCR to detect and treat CMV infection before it causes clinical disease, has therefore become generally accepted (Kotton et al., 2013; Razonable and Humar, 2013; Andrews et al., 2011; Tomblyn et al., 2009).

A series of longitudinal studies, using measurements of CMV viral load with PCR in transplant recipients, have described the viral dynamics of CMV in vivo (Bowen et al., 1998; Gor et al., 1998; Cope et al., 1997; Hassan-Walker et al., 1999; Ghisetti et al., 2004). Subsequent studies established CMV as a rapidly replicating virus in the human host, with a doubling time ranging from 1 (Emery et al., 1999, 2000, 2002) to 2.3 days (Mattes et al., 2005; Nebbia et al., 2007; Atabani et al., 2012; Funk et al., 2007; Buyck et al., 2010; Munoz-Cobo et al., 2011). Based on the assumption of a rapid doubling time, current guidelines recommend — based on empiric evidence — weekly screening with CMV PCR when recipients are managed pre-emptively (Kotton et al., 2013; Razonable and Humar, 2013; Andrews et al., 2011).

"The Management of Post-Transplant Infections in Collaborating Hospitals" (MATCH) programme was introduced at Rigshospitalet in Copenhagen, Denmark in 2011, with the aim to reduce the risk of severe viral diseases in transplant recipients (unpublished data; da Cunha-Bang, C. et al.). MATCH constitutes a platform for collaboration between the transplantation units and the Department of Infectious Diseases, and the associated database contains data on a large cohort of consecutive transplant recipients of both solid organ transplantation (SOT) and haematopoietic stem cell transplantation (HSCT). Consistent with the current guidelines, weekly screening intervals for CMV were applied

* Corresponding author.
E-mail address: paula.isabelle.lodding@regionh.dk (I.P. Lodding).

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in MATCH. However, when using this screening approach, very few recipients with high viral load at time of diagnosis of the CMV infection were detected. This raised the question whether the previously determined CMV doubling time estimates were valid in our cohort.

In this study, the reproducibility of the published doubling time estimates was investigated. Furthermore, the rationale of a weekly screening interval with CMV PCR measurements in transplant recipients, managed pre-emptively, was evaluated.

2. Recipients and Methods

2.1. Recipients and Definition of CMV Infection

Consecutive recipients in the MATCH database transplanted between January 1 2003 and August 27 2013 (n = 2344), who developed a first CMV infectious episode within the first 12 months following transplantation, were eligible for inclusion (n = 329). All applicable regulatory and ethical approvals related to the project are obtained in accordance with the national legislation.

In order to calculate the CMV doubling time, the episodes needed to be recorded with ≥2 quantifiable and increasing CMV PCR measurements taken within 14 days of each other (see section Calculation of CMV Doubling Time and Adjustment for Anti-CMV Treatment) (n = 193). Although the MATCH programme was initiated in 2011, it was possible to reconstruct course of events including relevant laboratory assessments for all recipients since 2003 stored electronically into the MATCH database. A CMV infectious episode was defined as two consecutive quantifiable CMV PCR values ≥273 IU/mL (i.e. 300 copies/mL) taken within 2 weeks of each other, or one measurement ≥2730 IU/mL (da Cunha-Bang et al., 2011). The first of two subsequent consecutive negative CMV PCRs following an infectious episode defines the end of that episode. Only the first CMV episode was eligible for inclusion, i.e. the number of included recipients equals the CMV infectious episodes.

2.2. CMV IgG Serostatus

The CMV IgG serostatus for donor and recipient was determined pre-transplant, and the eligible combinations for inclusion were D+/R−, D+/R+ or D−/R+. The recipients were stratified according to risk of CMV infection depending on donor (D)/recipient (R) CMV IgG serostatus (positive (+)/negative (−)) prior to transplantation, as either high-, intermediary- or low risk. The high risk group constituted of D+/R− for SOT recipients, and D−/R+ for HSCT recipients. D+/R+ constituted the intermediary risk group for both types of transplantations, and the low risk group D−/R+ for SOT and D+/R− for HSCT. Due to the small number of recipients in our cohort with low risk serostatus (n = 13), these recipients were analysed together with the recipients at intermediary risk.

2.3. CMV DNA Surveillance and Anti-CMV Treatment

This study is based on measurements of CMV in plasma by PCR, performed on a semi-regular basis as a part of surveillance of CMV in the MATCH programme. The COBAS Amplicor kit (DiDomenico et al., 1996) was used until 2011, and since 2011 the COBAS AmpliPrep/COBAS TaqMan has been used. The Department of Clinical Microbiology simultaneously tested the two PCR kits, and determined the conversion factor between the COBAS Amplicor kit and the COBAS AmpliPrep/COBAS TaqMan to be a factor 1:1. Thus, to make our results more widely applicable we have converted our virus loads into IU/mL using the conversion factor for the COBAS AmpliPrep/COBAS TaqMan (1 copy/mL corresponding to 0.91 IU/mL).

The SOT recipients received (val)ganciclovir prophylaxis for 3 months following transplantation and were subsequently treated pre-emptively with (val)ganciclovir in case of CMV infection. In general, the SOT recipients with serostatus D+/R−, D+/R+ or D−/R+ were screened monthly in the prophylaxis phase (month one to three post-transplant), and then weekly in months four to six post-transplant. Hereafter the recipients are tested monthly until month 12 post-transplant.

The HSCT recipients were monitored weekly by CMV PCR from week four to week 17 post-transplant and then at week 19, 26 and 52, except in case of graft-versus-host disease where weekly monitoring was continued.

In case of CMV infection (see definition) treatment with (val)ganciclovir, or in case of resistance, foscovir (Cunha-Bang et al., 2013) was initiated.

2.4. Calculation of CMV Doubling Time and Adjustment for Anti-CMV Treatment

An algorithm was constructed to detect the first positive sample of the infectious episode. This sample is termed V_1, and corresponds to the time t_1 (Fig. 1). The algorithm was then constructed to find the highest positive sample within 14 days of the V_1 sample; this sample is termed V_{peak} and the time at which it occurs is termed t_{peak}. The doubling time is calculated as previously described (Emery et al., 1999; Atabani et al., 2012). First the CMV growth rate is determined from the slope of virus over time:

$$Growth\ \ rate = \frac{\Delta \ln Virus\ \ load}{\Delta time}.$$

The doubling time can then be calculated using the standard exponential function:

$$Doubling\ \ time = \frac{ln2}{Growth\ \ rate}.$$

Out of the 329 infection episodes, 193 had ≥2 increasing CMV PCR measurements taken within 14 days of each other. Thus, this formula was applied to these episodes and the doubling time was calculated.

When calculating the CMV doubling time, it is necessary to adjust for any administration of anti-CMV treatment. Information on anti-CMV treatment was systematically collected for the included CMV infections from patient files. For each infectious episode, the proportion of time on which the calculation of doubling time was based on and that was covered with anti-CMV treatment, was determined (Fig. 1). Thus this variable can be between 0% (recipients who didn't receive any anti-CMV treatment during the time used for calculation for doubling time) and 100% (recipients who were initiated in anti-CMV treatment the before or the same day as the V_1 sample).

2.5. Modelling CMV Screening Intervals

A mathematical simulation model was constructed to determine factors that influence the optimal screening interval for preemptive treatment. A diagnostic viral load ≥18,200 IU/mL was defined as undesirably high, based on previous clinical experiences and observations of the prevalence of CMV disease at diagnosis of CMV infection (unpublished data; Lodding, I. et al.). We decided a priori that any monitoring strategy had to result in ≤5% of the newly developed CMV infections to be diagnosed at or above this undesirable level. The lower limit of detection for the CMV PCR assay was set at 273 IU/mL, and the results were available 24 h after blood draw. The CMV infection was assumed to emerge randomly within the screening interval, and the doubling time for the infection was either set at 1.3 days (as reported in the literature Funk et al., 2007) or allowed to vary as observed in our cohort (see Resultssection). The observed distribution of doubling time was either fitted as observed or from best Chi Square fit (five degrees of freedom). Based on observations from our cohort, CMV replication was most likely to occur during the first 3 months after transplantation,