Cytomegalovirus viral load and mortality after haemopoietic stem cell transplantation in the era of pre-emptive therapy: a retrospective cohort study

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Summary

Background **Although cytomegalovirus viral load is commonly used to guide pre-emptive therapy in the post-transplantation setting, few data are available correlating viraemia with clinical endpoints. We therefore investigated the association between cytomegalovirus viral load and mortality in the first year after haemopoietic stem cell transplantation.**

Methods **In this retrospective cohort study, we included patients from the Fred Hutchinson Cancer Research Center, WA, USA, who received an allogeneic haemopoietic stem cell transplantation between Jan 1, 2007, and Feb 28, 2013, were cytomegalovirus seropositive or had a seropositive donor, and underwent weekly plasma cytomegalovirus monitoring by PCR through to day 100 post-transplantation. Cox proportional hazards models were used to estimate the association of cytomegalovirus viral load at different thresholds with overall mortality by 1 year post-transplantation, adjusting for the use of pre-emptive therapy and other factors such as neutropenia, and graft-versus-host disease.**

Findings **Of the 1037 patients initially selected for inclusion in this cohort, 87 (8%) patients were excluded because of missing cytomegalovirus testing and 24 (2%) were excluded because of their participation in cytomegalovirus prophylaxis trials. In the remaining 926 patients included in this study, the cumulative overall mortality was 30·0% (95% CI 26·9–33·0) 1 year after haemopoietic stem cell transplantation. 95 patients developed cytomegalovirus disease; death was directly attributable to cytomegalovirus disease in three (1%) of 263 patients who died in the first year after transplantation. A cytomegalovirus viral load of 250 IU/mL or greater was associated with increased risk of early (day 0–60 post-transplantation) death (adjusted hazard ratio [HR] 19·8, 95% CI 9·6–41·1). The risk was attenuated after day 60 (adjusted HR 1·8, 95% CI 1·3–2·3). Similar associations were noted for higher cytomegalovirus viral load thresholds.**

Interpretation **Cytomegalovirus viraemia is associated with an increased risk of overall mortality in the first year after haemopoietic stem cell transplantation, independent of the use of pre-emptive therapy, and with evidence of a positive dose-response relationship. These data indicate the suitability of viral load as a surrogate clinical endpoint for clinical trials for cytomegalovirus vaccines, biologics, and drugs.**

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Introduction

Cytomegalovirus is a highly prevalent herpesvirus that is an important cause of morbidity and mortality in immunocompromised patients such as those undergoing haemopoietic stem cell transplantation. Current prevention strategies that use antiviral drugs, such as ganciclovir or foscarnet at the onset of viraemia (pre-emptive therapy), have successfully limited the incidence of cytomegalovirus end-organ disease to 3–6% in the first 3 months after haemopoietic stem cell transplantation.[1–3] Yet, these therapies have clinically significant toxic effects, viral resistance does occur, and cytomegalovirus pneumonia remains a deadly disease. Clinicians caring for immunocompromised patients are in need of better preventive therapies; however, without an accepted virological endpoint, clinical trials powered to prevent cytomegalovirus end-organ disease are probably too costly and time consuming.[1]

Although DNA viral load testing by quantitative PCR is increasingly used to guide pre-emptive therapy, data linking specific viral load thresholds with clinical outcomes are scarce.[4–6] Several studies have described the early kinetics of viral replication in bone marrow transplant recipients and the association of viral load with cytomegalovirus disease, but few of these patients received pre-emptive therapy.[7–9] Furthermore, the development of a standard method of quantitation (WHO standard IU/mL) has reduced the heterogeneity in the performance of different assays, making comparison of viral load values between different laboratories feasible.[10] The aim of this study was to estimate the association between cytomegalovirus viral load measured with the new international standard and non-relapse mortality and overall mortality during the first year after haemopoietic stem cell transplantation. Mortality was chosen for the endpoint not only because of the now low incidence of

Research in context

Evidence before this study

We did a systematic search of PubMed to explore the association between cytomegalovirus viral load and mortality in haemopoietic stem cell transplantation recipients. Search terms included "cytomegalovirus", AND "hematopoietic cell transplant", AND "viral load", AND "mortality". The search was limited to studies published in English up to Oct 1, 2015. Several studies have shown an association between cytomegalovirus viral load, assessed as a binary event, and overall mortality. However, no studies were identified that examined the association between cytomegalovirus viraemia as a quantitative measure and mortality. There have been a few studies, not identified in this search, that have very elegantly assessed cytomegalovirus viral load kinetics in transplantation patients and described the correlation between viral load and risk of cytomegalovirus disease. However, these studies were done in a population of patients where pre-emptive antiviral therapy was quite rare and they did not examine mortality as an endpoint.

Added value of this study

In this large contemporary cohort study, we present data that cytomegalovirus viraemia after haemopoietic stem cell transplantation is associated with overall and non-relapse mortality, independent of the use of pre-emptive antiviral therapy to prevent cytomegalovirus and organ disease and other relevant risk factors. To our knowledge, this is the first report to examine the association between different viral load thresholds (using the new WHO standard) and mortality. The principal results of the study are that cytomegalovirus viraemia after haemopoietic stem cell transplantation is associated with an increased risk of death despite the efficacy of antiviral pre-emptive therapy in preventing cytomegalovirus disease; viraemia occurring early after transplantation is associated with a higher risk of death; and that higher viral loads are associated with an increased risk of death. Furthermore, our use of the WHO standard for quantitative cytomegalovirus PCR allows validation studies with direct comparison of viral loads.

Implications of all the available evidence

The association of cytomegalovirus viral load with overall mortality after haemopoietic stem cell transplantation establishes the suitability of using cytomegalovirus viral load as a surrogate clinical endpoint for clinical trials assessing new vaccines, drugs, or biologics in transplantation patients. This is crucially important for the development of new treatments because cytomegalovirus disease is now a rare event when pre-emptive therapy is used. It is possible that these data will affect international guidelines for cytomegalovirus pre-emptive therapy in haemopoietic stem cell transplantation patients and provide an important link for regulatory authorities worldwide.

cytomegalovirus disease but also to account for the indirect effects of cytomegalovirus infection and its treatment, such as neutropenia, and death from fungal infection and Gram-negative bacteraemia.[6,11]

Methods

Study design and participants

In this retrospective non-interventional cohort study of previously collected cytomegalovirus viraemia and clinical outcome measures, we enrolled patients receiving their first allogeneic haemopoietic stem cell transplantation at the Fred Hutchinson Cancer Research Center, WA, USA, between Jan 1, 2007, and Feb 28, 2013. Eligible patients were cytomegalovirus seropositive or had a seropositive donor and consented to have their clinical data used for retrospective research. Patients were excluded from the study if more than 40% of the expected weekly cytomegalovirus surveillance tests were missed, more than two consecutive weekly tests were missed, or if they received cytomegalovirus prophylaxis as part of a clinical trial. The institutional review board at Fred Hutchinson Cancer Research Center approved this protocol for accessing and analysing these data.

Procedures

Patients were closely followed for 1 year post-transplantation for cytomegalovirus-associated disease and death. As part of the study, a detailed chart review was done to confirm disease status and extract additional information regarding end-organ involvement and other important clinical details. Since 2007, allogeneic haemopoietic stem cell transplantation recipients underwent weekly cytomegalovirus testing by quantitative PCR measured in blood plasma at least until day 100 post-transplantation (using BioRad master mix [Hercules, CA, USA] and Roche MP96 instrument for extraction mix [Indianapolis, IN, USA]).[12] The lower limit of detection for this assay (95% reproducibility limit) is 20 IU/mL. The conversion factor to the WHO standard is four copies=1 IU.

Pre-emptive antiviral therapy was initiated once the viral load reached 125 IU/mL for most patients.[6] Patients thought to be high-risk, such as those receiving 1 mg/kg bodyweight of prednisone or more, or cord blood transplantation recipients, were started at any positive viral load. After day 100 post-transplantation, patients who were thought to be at risk of late cytomegalovirus disease were recommended to continue weekly cytomegalovirus PCR testing and to start pre-emptive therapy if the viral load was 250 IU/mL or greater.

Demographic, clinical, and laboratory data for this study were accessed from an ongoing research database. The records also included communications and reports from referring providers. Patients remained at the transplant centre until about day 100 post-transplantation, after which they were often referred back to their primary oncologists. The long-term follow-up clinic remained in contact with patients and their providers as needed. Chart review was done to identify all cases of cytomegalovirus

end-organ disease as typically defined,[13] record all episodes of pre-emptive therapy occurring in the first year after haemopoietic stem cell transplantation, and determine the cause of death. Any death occurring after relapse was classified as due to relapse.[14] Death without relapse was further classified as due to graft-versus-host disease, organ failure, infection, or other; these classifications were not mutually exclusive.

Statistical analysis

Kaplan-Meier and cumulative incidence estimation methods were used to initially estimate the incidence of overall and non-relapse mortality, initiation of pre-emptive therapy, and of cytomegalovirus reactivation and disease after transplantation. The cumulative incidence of cytomegalovirus disease, initiation of pre-emptive therapy, and cytomegalovirus reactivation was estimated treating death as a competing risk event. For non-relapse mortality, relapse was a competing risk event. Cox proportional hazards were used to estimate the association of cytomegalovirus plasma viral load after transplantation with overall and non-relapse mortality by 1 year post-transplantation and with cytomegalovirus disease by day 100. The proportional hazards assumption was tested and variables that violated the assumption were stratified by time. Models for cytomegalovirus disease and non-relapse mortality were also fitted using the methods of Fine and Gray.

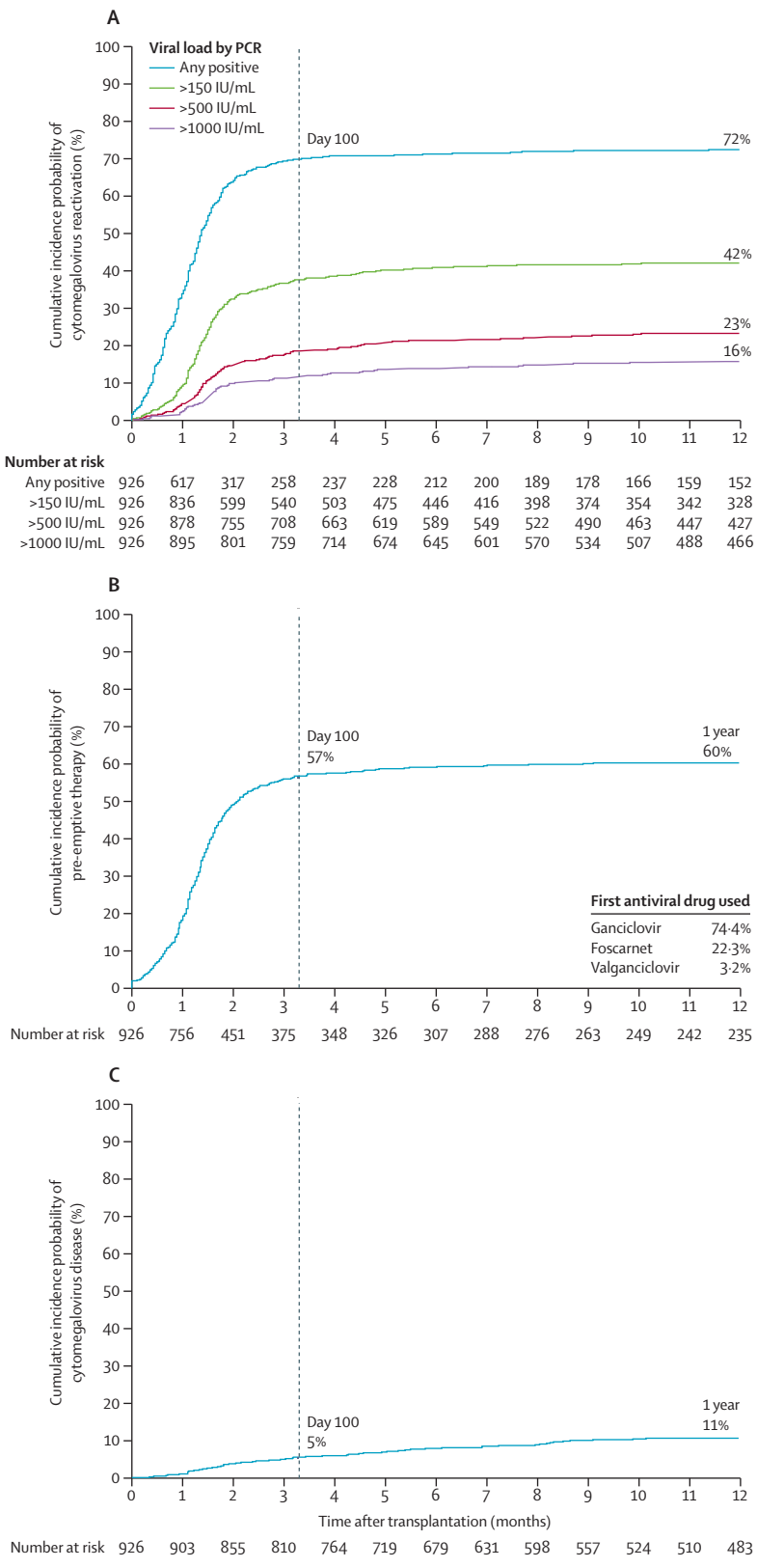
Viral load was assessed both as a categorical variable to assess for a dose-response association (non-viraemic, >0–500 IU/mL, 501–1000 IU/mL, and >1000 IU/mL) and as a series of binary variables based on viral load cutoff points selected a priori (any positive, >150 IU/mL, >250 IU/mL, >500 IU/mL, >750 IU/mL, and >1000 IU/mL). The lowest threshold of 150 IU/mL was chosen as this is roughly the lower limit of quantification of commercial assays (eg, Roche COBAS AmpliPrep/COBAS TaqMan, Roche Diagnostics, Indianapolis, IN, USA). The upper threshold of 1000 IU/mL was chosen because many other centres use this threshold to initiate pre-emptive therapy. In both the categorical and cutoff point metrics, viral load was treated as time dependent but such that it could only increase. For the categorical variable, if the viral load increased to the next category threshold, the patient would then be assigned to the next risk stratum going forward, otherwise they would remain at the highest category attained. For the binary cutoff points, at the time of the first detectable viral load level above a given cutoff point, the variable was fixed as "above" throughout follow-up. This was done to allow for long-term effects of viral load on mortality. If a cytomegalovirus PCR test was missing, the value from the previous week's test was carried over for up to 3 weeks.

Demographic and clinical factors assessed as potential covariates in each of the models were age at haemopoietic stem cell transplantation, donor age, race, donor race, sex, donor sex, HLA matching, underlying disease risk, haemopoietic stem cell transplantation specific comorbidity index, conditioning regimen, cell source, year of transplantation, graft-versus-host-disease prophylaxis regimen, peak acute graft-versus-host-disease grade

	Number of patients (n=926)
Age (years)	
0–18	129 (14%)
18–40	195 (21%)
≥41	602 (65%)
Sex	
Male	506 (55%)
Female	420 (45%)
Race	
White	620 (67%)
Other	248 (27%)
Unknown	58 (6%)
Underlying disease	
Acute leukaemia	415 (45%)
Chronic leukaemia	100 (11%)
Lymphoma	110 (12%)
Other*	301 (33%)
Disease risk	
High	343 (37%)
Intermediate	76 (8%)
Low	507 (55%)
Previous autologous HCT	151 (16%)
HCT-CI score	
Low (0)	154 (17%)
Intermediate (1–2)	289 (31%)
High (≥3)	483 (52%)
Cytomegalovirus serological status	
Donor positive, recipient positive	333 (36%)
Donor negative, recipient positive	459 (50%)
Donor positive, recipient negative	134 (14%)
Conditioning regimen	
Myeloablative	559 (60%)
Reduced intensity conditioning	367 (40%)
Cell source	
Bone marrow	194 (21%)
Peripheral blood stem cells	630 (68%)
Cord blood	102 (11%)
HLA matching	
Matched, related	288 (31%)
Matched, unrelated	424 (46%)
Mismatched	62 (7%)
Haploidentical	50 (5%)
Cord blood	102 (11%)

Data are n (%). HCT=haemopoietic stem cell transplantation. HCT-CI=haemopoietic stem cell transplantation-specific comorbidity index.[16] *Other diseases include myelodysplastic syndrome (n=106), multiple myeloma (n=55), myelofibrosis (n=37), aplastic anaemia (n=26), and others each with a frequency of less than 20 patients (n=77).

Table 1: **Cohort demographic and clinical characteristics**



(time dependent), National Institutes of Health chronic graft-versus-host disease (time dependent), and neutropenia (time dependent). High-risk disease was classified as acute myeloid leukaemia evolved from myelodysplastic syndrome, high-grade non-Hodgkin lymphoma not in complete remission, Hodgkin's lymphoma, secondary myelodysplastic syndrome, acute myeloid leukaemia not in complete remission, chronic myeloid leukaemia in second chronic phase or accelerated phase or blast crisis, or acute lymphoblastic leukaemia not in first complete remission. Intermediate-risk disease was classified as chronic lymphocytic leukaemia not in complete remission, multiple myeloma not in complete remission, or acute myeloid leukaemia in complete remission. Low-risk disease included lymphocytic leukaemia in complete remission, low-grade non-Hodgkin lymphoma, high-grade non-Hodgkin lymphoma in complete remission, multiple myeloma in complete remission, chronic myeloid leukaemia in first chronic phase, or acute lymphoblastic leukaemia in first complete remission.[15] Factors were included in the final models if they were significant themselves ($p<0·05$) or if their inclusion in the model markedly modified the association between cytomegalovirus viraemia and disease (>10%). Karnofsky scores were not consistently collected for the patients, and duration of underlying disease was also not assessed.

Landmark analyses were also done in patients who survived to day 100 post-transplantation to estimate the association between maximum cytomegalovirus viral load and the viral area under a curve (AUC) before day 100 on non-relapse mortality and overall mortality by 1 year after transplantation. All analyses were done with SAS version 9.4.

Role of the funding source

TCM, YC, and MAM are employed by Merck and Co and participated in the design of the study, data interpretation, and revision of the report. However, neither Merck and Co nor National Institutes of Health had a role in data collection, data analysis, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of the 1037 patients initially selected for inclusion in this cohort, 87 (8%) patients were excluded because of missing cytomegalovirus testing and 24 (2%) were

Figure 1: **Cumulative incidence of cytomegalovirus reactivation (A), initiation of pre-emptive therapy (B), and cytomegalovirus disease (C) 1 year after haemopoietic cell transplantation for all patients**
Pre-emptive antiviral therapy was initiated with either induction-dose ganciclovir (5 mg/kg intravenously every 12 h), foscarnet (90 mg/kg intravenously every 12 h), or valganciclovir (900 mg orally every 12 h). Induction therapy was continued for at least 1 week followed by at least 2 weeks of maintenance (once daily) therapy until cessation of viraemia.

excluded because of their participation in cytomegalovirus prophylaxis trials. The demographic and clinical characteristics of the 926 patients in the analytic cohort are presented (table 1).[16] Follow-up was terminated on May 3, 2014. Median time of follow-up was 483 days (IQR 209–1110) post-transplantation.

643 (69%) patients had cytomegalovirus reactivation at any level of viraemia by day 100 after haemopoietic stem cell transplantation. In the same period, 346 (37%) patients achieved a plasma viral load of more than 150 IU/mL, whereas a viral load of more than 1000 IU/mL was a rare event occurring in only 107 (12%) of patients (figure 1). Pre-emptive antiviral therapy was given to 558 (60%) patients; 512 patients received their first course before day 100 (figure 1). 95 patients were identified to have cytomegalovirus disease in the first year after transplantation (cumulative incidence 11%; figure 1). 59 (62%) patients had gastrointestinal tract disease, whereas cytomegalovirus pneumonia and retinitis were less common, occurring in 33 (35%) and three (3%) patients, respectively. Only 50 (53%) of patients with cytomegalovirus disease were diagnosed by day 100. As expected, cytomegalovirus viraemia was associated with an increased risk of cytomegalovirus disease, but only when patients were not receiving pre-emptive therapy for all our binary cutoffs (figure 2).

By day 100, 83 patients had died. 263 patients had died 1 year after transplantation. The cumulative overall mortality was 9·0% (95% CI 7·1–10·8) by day 100 and 30·0% (95% CI 26·9–33·0) 1 year after haemopoietic stem cell transplantation. Non-relapse mortality was 6·5% (95% CI 4·9–8·1) by day 100 and 18·0% (95% CI 15·5–20·6) 1 year after haemopoietic stem cell transplantation. In the 95 patients with cytomegalovirus disease, 35 (37%) died within the first year after haemopoietic stem cell transplantation. However, death was directly attributable to cytomegalovirus disease in only three (1%) of 263 patients who died in the first year after haemopoietic stem cell transplantation; two patients died of cytomegalovirus pneumonia and one had disseminated disease. Of the 263 deaths occurring 1 year after haemopoietic stem cell transplantation, 118 (45%) were due to relapse or disease progression (table 2). Of the 145 non-relapse deaths, infection was either the main or contributing cause in 109 (75%) cases and graft-versus-host disease was implicated in 84 (58%) deaths.

Due to lack of proportional hazards for overall mortality across the full timeframe, we evaluated several cutpoints and selected 60 days as the point where, in each of the intervals before and after it, the proportional hazards assumption held. In Cox models adjusted for the use of pre-emptive therapy, neutropenia, and other important risk factors, patients with cytomegalovirus viral loads of more than 500 IU/mL had a significantly increased risk of death from any cause in the first year after haemopoietic stem cell transplantation compared with patients who did not have cytomegalovirus reactivation (figure 3). The risk was highest in the first 60 days after transplantation (adjusted hazard ratio [HR] 21·3 [95% CI 5·9–76·6] for viral loads of 501–1000 IU/mL and 26·5 [10·3–68·0] for viral loads of >1000 IU/mL) compared with later periods (adjusted HR 1·7 [1·0–3·0] for viral loads of 501–1000 IU/mL and 3·1 [2·1–4·7] for viral loads >1000 IU/mL). The risk of death by day 60 in patients who had lower levels of viraemia (any positive viraemia to 500 IU/mL) was not significantly different from patients who did not reactivate (adjusted HR 1·6 [0·8–3·4]); however, after day 60, this comparison did reach statistical significance (adjusted HR 1·4 [1·0–2·0]).

Similar results were noted in the multivariable models assessing specific viral load thresholds as a risk factor for overall mortality (figure 3). In each model,

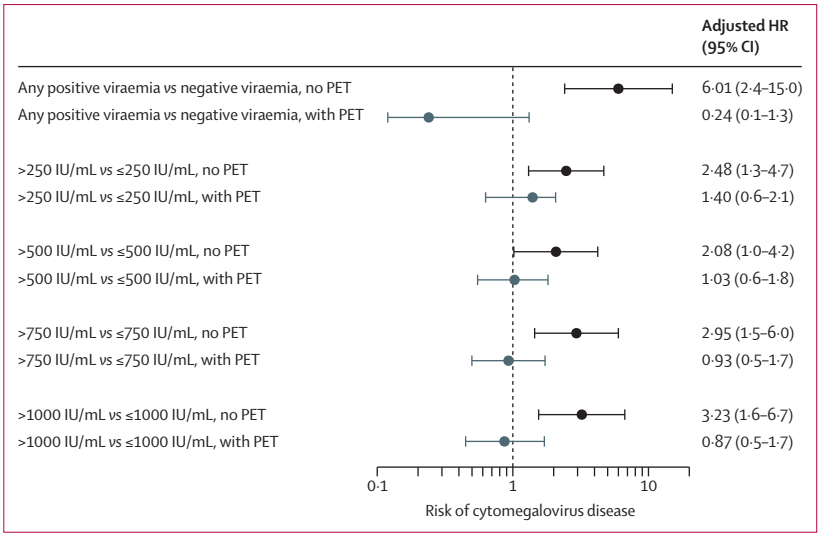


Figure 2: **Multivariable Cox proportional hazards models assessing cytomegalovirus viral load as a time-dependent risk factor for cytomegalovirus disease 1 year after haemopoietic cell transplantation, stratified by use of pre-emptive therapy (n=926)**

Points show adjusted HR and whiskers show 95% CI. Models each adjusted for cytomegalovirus serostatus, HLA matching, cell source, underlying disease, haemopoietic cell transplantation specific comorbidity index, and disease risk. HR=hazard ratio. PET=pre-emptive therapy.

	Deaths by day 100	Deaths by 1 year
Deaths from relapse or disease progression	33/83 (40%)	118/263 (45%)
Deaths from causes other than relapse or disease progression	50/83 (60%)	145/263 (55%)
Graft-versus-host disease alone	3/50 (6%)	14/145 (10%)
Infection alone	13/50 (26%)	29/145 (20%)
Organ failure alone	4/50 (8%)	10/145 (7%)
No information available	0 (0%)	5/145 (3%)
Multiple contributing causes (not mutually exclusive groups)	30/50 (60%)	87/145 (60%)
Graft-versus-host disease	19/30 (63%)	70/87 (80%)
Infection	24/30 (80%)	80/87 (92%)
Organ failure	15/30 (50%)	36/87 (41%)
Other	5/30 (17%)	11/87 (13%)

Data are n/N (%).

Table 2: **Causes of death in first year after haemopoietic stem cell transplantation**

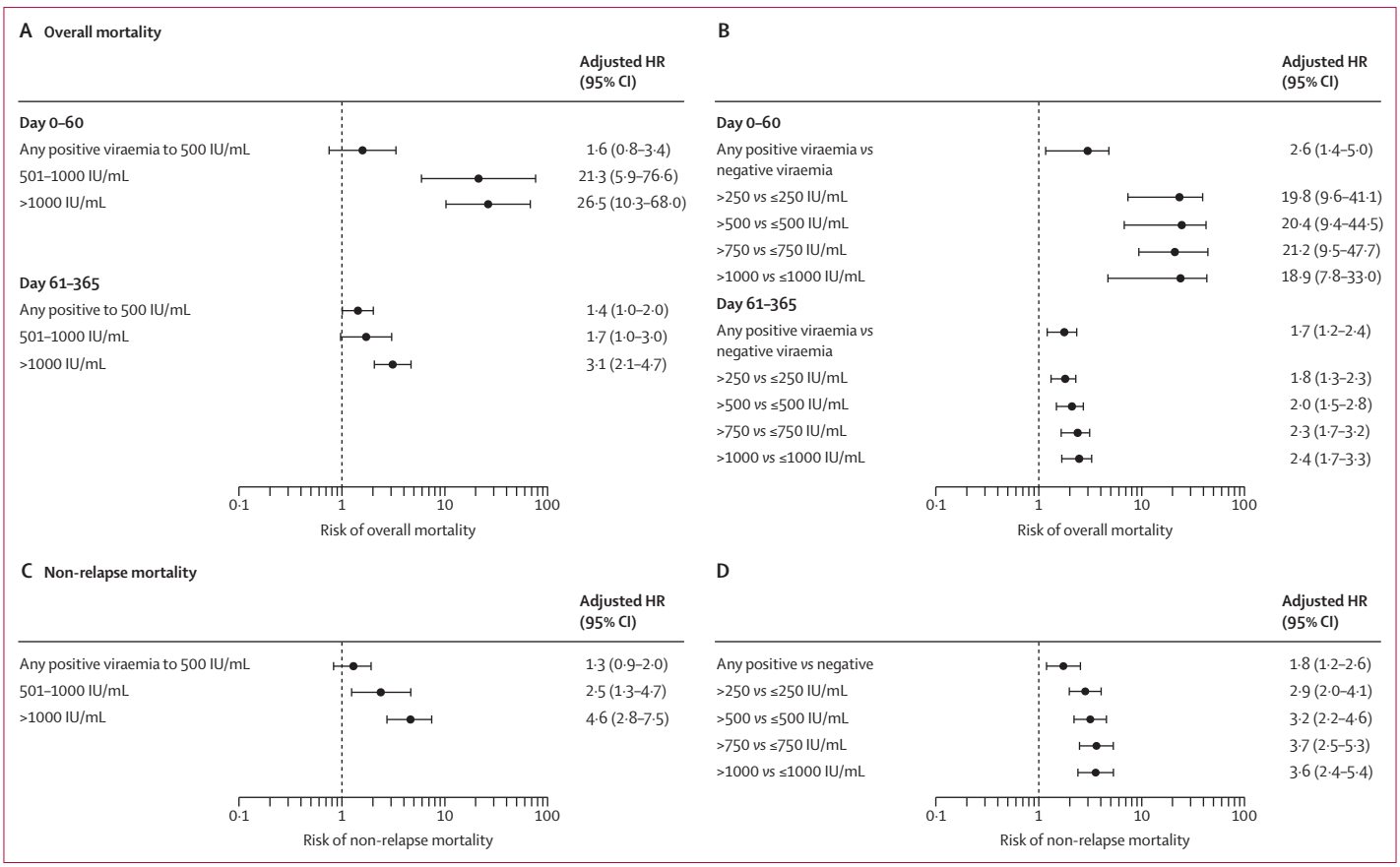


Figure 3: **Multivariable Cox proportional hazards models assessing cytomegalovirus viral load as a time-dependent risk factor for mortality 1 year after haemopoietic cell transplantation (n=926)**
Viral load as a risk factor for overall mortality, where viral load is a categorical variable (A) or a threshold variable (B). Viral load as a risk factor for non-relapse mortality, where viral load is a categorical variable (C) or a threshold variable (D). The comparator group for categorical models (A and C) is patients with no cytomegalovirus reactivation. Overall mortality models were adjusted for age, transplantation year, underlying disease, disease risk, haemopoietic stem cell transplantation specific comorbidity index score, acute graft-versus-host disease grade, neutropenia, and pre-emptive therapy. Non-relapse mortality models were adjusted for patient age, HLA-matching, disease risk, haemopoietic stem cell transplantation specific comorbidity index score, acute graft-versus-host disease, chronic graft-versus-host disease, neutropenia, and pre-emptive therapy. Results of the categorical model for all factors are available in the appendix.

having a viral load above the given threshold carried a significantly higher risk of death than having a viral load below the threshold. This was true even for the lowest threshold (any positive *vs* negative) where the adjusted HR was 2·6 (95% CI 1·4–5·0) until day 60 and 1·7 (1·2–2·4) after day 60. For the higher thresholds, as in the categorical models, the HRs were higher before day 60 compared with after day 60. These analyses were repeated, stratifying by cytomegalovirus serostatus (donor positive, recipient positive; donor negative, recipient positive; or donor positive, recipient negative), and the point estimates were consistent between stratum. However, because of the low incidence of cytomegalovirus viraemia in donor positive, recipient negative patients, the associations were not statistically significant in this group (appendix p 2).

See Online for appendix

In the 832 patients who survived to day 100, the 1 year cumulative overall mortality was 23·1% (95% CI 20·1–26·1). These estimates varied according to the maximum viral load achieved before day 100 (figure 4). The cumulative incidence estimates were 20·5% (95% CI 15·0–26·1) for patients with a viral load of less than 150 IU/mL, 27·1% (20·9–33·2) for a viral load of 151–1000 IU/mL, and 34·2% (24·3–44·2) for a viral load of more than 1000 IU/mL before day 100. In multivariate analysis, only a maximum viral load of more than 1000 IU/mL remained statistically significant (figure 4). We also examined the viral AUC before day 100 as a risk factor for death by 1 year. The median AUC was 1754·2 (IQR 172·0–6358·9) for those patients that died 1 year after haemopoietic stem cell transplantation compared with 700·0 (IQR 95·0–3332·0) for those that survived (p=0·0028). However, in multivariable Cox models, viral AUC was not significantly associated with overall mortality (appendix p 2).

For non-relapse mortality, the adjusted HRs were 1·3 (95% CI 0·9–2·0) for positive viraemia to 500 IU/mL, 2·5 (95% CI 1·3–4·7) for 501–1000 IU/mL, and 4·6 (95% CI 2·8–7·5) for more than 1000 IU/mL, providing evidence of a positive dose-response association (figure 3). Similar findings were reported when viral load was treated as a binary variable at specific

cutoff points (figure 3). However, in these models a potential dose-response association was less apparent because of the characteristics of a binary cutoff point as opposed to a categorical variable.

In patients who survived to day 100 without relapse, the overall 1-year cumulative incidence of non-relapse mortality was 14·0% (95% CI 11·4–16·5). Similar to what was seen with overall mortality, the cumulative incidence estimates varied depending on maximum viral load before day 100 (figure 4). They were 10·7% (95% CI 6·3–15·2) for a viral load of less than 150 IU/mL, 17·0% (11·5–22·4) for a viral load of 151–1000 IU/mL, and 24·2% (14·7–33·7) for a viral load of more than 1000 IU/mL before day 100. Again, in multivariable Cox models, only viral loads of more than 1000 IU/mL remained significant (figure 4) and viral AUC was not significantly associated with non-relapse mortality at 1 year (appendix p 2). Analyses using the methods of Fine and Gray showed similar results to those using Cox analyses (appendix p 3).

Discussion

The results of this cohort study provide several important insights. Our results suggest that higher viral loads carry an increased risk of both death and non-relapse death in the first year after transplantation even when adjusting for the use of pre-emptive therapy and neutropenia that might occur as a result of pre-emptive therapy. Despite the low viral load thresholds used to initiate pre-emptive therapy for our patients (125 IU/mL for most patients), viral loads of more than 500 IU/mL or more than 1000 IU/mL, and their associated increased risk of death, were not completely preventable. Patients who developed viraemia of more than 500 IU/mL had a 20-fold increase in the risk of death by day 60 (figure 2). This risk was significantly diminished, however, after day 60. This is a crucial advance because proof of a definitive association of specific viral load thresholds with important clinical endpoints such as mortality has previously been elusive. To our knowledge, this is the first report to examine this question in a large contemporary cohort with a standardised PCR measure. Other studies have identified cytomegalovirus viraemia as a risk factor for overall mortality and non-relapse mortality.[15,17,18] However, these studies assessed cytomegalovirus viraemia either as a predictor for mortality in non-transplantation settings[17] or analysed it as a binary event.[15,18]

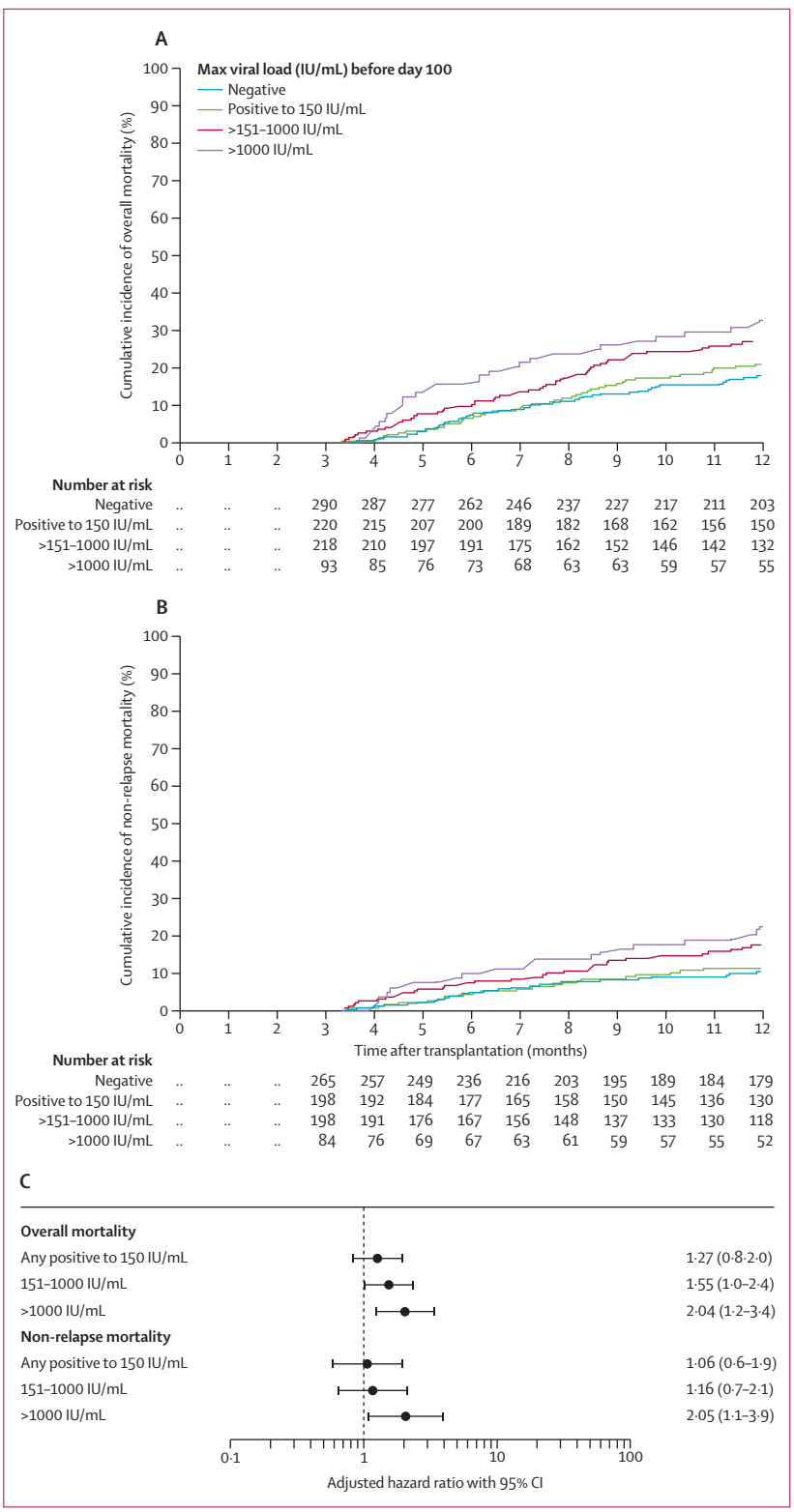


Figure 4: **Cumulative incidence of overall mortality (A) and non-relapse mortality (B) at 1 year after haemopoietic cell transplantation in survivors at day 100 (n=832) stratified by maximum cytomegalovirus viral load before day 100 and multivariable Cox proportional hazard models assessing maximum cytomegalovirus viral load before day 100 as a risk factor for overall and non-relapse mortality (C)**

Covariates for overall mortality models were age, donor relation, transplantation year, underlying disease, disease risk, haemopoietic stem cell transplantation-specific comorbidity index score, neutropenia before day 100, and cytomegalovirus viraemia after day 100 (time-dependent). Covariates for non-relapse mortality were age, donor relation, transplantation year, disease risk, haemopoietic stem cell transplantation specific comorbidity index score, acute graft-versus-host disease, chronic graft-versus-host disease, neutropenia before day 100, and cytomegalovirus viraemia after day 100 (time-dependent).

These data establish the suitability of using cytomegalovirus viral load as a surrogate clinical endpoint for clinical trials and might provide evidence to preclude the collection of additional clinical endpoint data after fast track approval, as is presently required by some regulators. Our data provide strong epidemiological evidence that drugs or biological therapies that can prevent high viral loads early after haemopoietic stem cell transplantation might reasonably be expected to have an effect on mortality, even if they cannot completely prevent viraemia or the initiation of pre-emptive antiviral therapy. In 1993, Kojima and colleagues[19] predicted that using PCR to measure HIV-1 RNA copy number in the plasma of infected patients was "likely to be built into every clinical trial of anti-HIV-1 therapy in the near future." It has been 20 years since the landmark studies[20–22] correlating HIV-1 viral load with disease progression were published, changing the regulatory environment to allow the use of a virological endpoint, and thereby fostering the development of dozens of new antiretroviral agents.

The association of cytomegalovirus viraemia with increased risk of death might be a result of the immunomodulating effects of cytomegalovirus infection. Cytomegalovirus has been implicated in the pathogenesis of invasive bacterial and fungal infections and graft-versus-host disease,[11,23] and our data show that most of the non-relapse deaths were caused by these complications, either alone or in combination. The association of high level cytomegalovirus viraemia with death was seen both for overall and non-relapse mortality, thus providing no evidence that a putative protective effect of cytomegalovirus reactivation on relapse of the underlying malignancy would affect survival.[12,24–26]

Although the association of cytomegalovirus viraemia with cytomegalovirus disease was not the focus of this study, our data also showed that viral load is associated with an increased risk of disease only during times when patients were not receiving pre-emptive therapy. This finding confirms the excellent efficacy of pre-emptive therapy as shown in recent clinical trials.[1–3,27]

The study has several strengths, including large sample size, uniform management of patients, and thorough diagnostic approach for suspected cases of cytomegalovirus disease. We also consider the use of the WHO standard (IU/mL) for quantifying cytomegalovirus viral load a strength, although full commutability has not yet been achieved and measurements might still vary roughly two-fold between assays.[10] A few limitations should be noted. First, because the mechanisms of cytomegalovirus reactivation and its secondary effects are not yet known and could not inform more accurate assumptions, we assumed that the risks associated with viraemia began as soon as the viraemia occurred and continued even after treatment lowered the viral load. Second, the high-risk patients such as those who would receive steroids were included in the analyses because it was not known at the time of transplantation who would require treatment for graft-versus-host disease. We attempted to control for this by adjusting the models for acute and chronic graft-versus-host disease. Additionally, variation in management of cytomegalovirus and transplantation techniques that might result in differences in the rates of viraemia or death could not be accounted for in this single-centre study. However, the rates of cytomegalovirus reactivation and pre-emptive therapy use in this study are similar to those in other published cohorts.[1,2,25,28] Karnofsky scores were not consistently collected for the patients; however, their addition would be expected to have little additional effect on mortality and non-relapse mortality after adjusting for the haemopoietic stem cell transplantation specific comorbidity index.[29] Duration of underlying disease was also not assessed because it was not thought to have an effect on the association between cytomegalovirus viraemia and the endpoints of interest and the underlying health of the patient was already taken into account with both the haemopoietic stem cell transplantation-specific comorbidity index score and disease risk, which incorporates disease stage.

Overall, in this large cohort of patients undergoing allogeneic haemopoietic stem cell transplantation who were monitored with a PCR-based pre-emptive therapy strategy, we have identified that cytomegalovirus viral load is associated with an increased risk of overall and non-relapse mortality in the first year after transplantation even after controlling for the use of pre-emptive therapy. There is some evidence of a dose-response association with higher viral loads associated with higher risk of death and, for overall mortality, high viral loads have an increased effect early after transplantation. In view of these data, it seems reasonable that cytomegalovirus viral load should be acceptable as a surrogate clinical endpoint for clinical trials going forward.

Contributors

MLG, WL, TCM, YC, KRJ, MAM, and MB were responsible for the design of the study and interpretation of the data. MLG, WL, and HX analysed the data and created the figures. Data were collected by MLG, BMS, MLS, SG, SÖ, JY, FS, and LEK. All authors contributed to the writing and revision of the manuscript and approved the final version.

Declaration of interests

MLG reports grants from Merck and Co during the study and grants and personal fees from Astellas outside the submitted work. WL and HX received grants from Merck and Co for the conduct of the study. TCM, YC, and MAM are employees of, and own stock in, Merck and Co. MLS reports personal fees from Jazz Pharmaceuticals, outside the submitted work. MB reports grants and personal fees from Merck and Co during the conduct of the study; grants and personal fees from Astellas, Shire, Roche/Genentech, Gilead, and Chimerix; and personal fees from Clinigen and Microbiotix, outside the submitted work. BMS, SG, SÖ, FS, LEK, KRJ, and JY declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

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Cytomegalovirus viral load and mortality after haemopoietic cell transplantation in the era of pre-emptive therapy: a retrospective cohort study

Supplementary Appendix

Table 1. Results of multivariable Cox proportional hazards models evaluating CMV viral load as a time-dependent risk factor for overall mortality with viral load as a categorical variable day 61-365 after HCT (n=926)

Risk factor		Adjusted Hazard Ratio	95% CI
Patient Age	0-40 years	1	
	41+ years	1·4	1·1-2·0
Transplant year	Continuous	0·9	0·8-0·9
Underlying disease	Acute leukemia	1	
	Chronic leukemia	0·6	0·4-0·9
	Lymphoma	0·6	0·4-0·9
	Other diseases	0·6	0·5-0·9
Disease risk	High	1	
	Intermediate	0·5	0·3-0·8
	Low	0·5	0·3-0·6
HCT-CI score	Low	1	
	Intermediate	1·3	0·8-2·2
	High	2·0	1·3-3·2
Acute GVHD	Grade 0-2	1	
	Grade 3+	2·5	1·8-3·3
Neutropenia	No	1	
	Yes	5·7	4·0-8·0
Preemptive Therapy	No	1	
	Yes	0·02	0·0-0·1
CMV viral load	No viremia	1	
	Any positive-500 IU/ml	1·4	1·0-2·0
	501-1000 IU/ml	1·7	1·0-3·0
	>1000 IU/ml	3·1	2·1-4·7

Table 2. Results of multivariable Cox proportional hazards models evaluating CMV viral load as a time-dependent risk factor for non-relapse mortality with viral load as a categorical variable by 1 year after HCT (n=926)

Risk factor		Adjusted Hazard Ratio	95% CI
Patient Age	0-40 years	1	
	41+ years	1·4	1·1-2·0
Graft source	HLA-matched, related	1	
	Haploidentical	1·3	0·6-2·6
	Cord blood	3·12	1·8-5·7
	Unrelated/mismatched	1·6	1·0-2·4
Disease risk	High	1	
	Intermediate	0·8	0·4-1·4
	Low	0·5	0·3-0·7
HCT-CI score	Low	1	
	Intermediate	1·2	0·6-2·4
	High	1·8	1·0·3·4
Acute GVHD	Grade 0-2	1	
	Grade 3+	3·4	2·4-4·9
Chronic GVHD	Other	1	
	Extensive	1·9	1·2-2·9
Neutropenia	No	1	
	Yes	7·5	4·7-12·0
Preemptive Therapy	No	1	
	Yes	0·03	0·0-0·1
CMV viral load	No viremia	1	
	Any positive-500 IU/ml	1·3	0·9-2·0
	501-1000 IU/ml	2·5	1·3-4·7
	>1000 IU/ml	4·6	2·8-7·5

Table 3. Results of multivariable Cox proportional hazards models evaluating CMV viral load as a time-dependent risk factor for overall mortality with viral load as a categorical variable by 1 year after HCT stratified by CMV donor patient serostatus (n=926)

Adjusted HR (95%CI)	**Entire cohort (n=926)**	**CMV D+/R+ (n=333)**	**CMV D-/R+ (n=459)**	**CMV D+/R- (n=134)**
Overall mortality Day 60-365				
Any positive- 500 IU/ml	1·4 (1·0-2·0)	1·8 (0·9-3·9)	1·0 (0·6-1·6)	2·3 (1·1-5·1)
501-1000 IU/ml	1·7 (1·0-3·1)	1·7 (0·6-5·3)	1·3 (0·6-2·8)	1·7 (0·2-13·5)
>1000 IU/ml	3·1 (2·1-4·7)	4·5 (1·9-10·7)	2·1 (1·2-3·7)	2·1 (0·6-7·5)

Table 4. Results of multivariable Cox proportional hazards models evaluating CMV viral load as a time-dependent risk factor for overall mortality and non-relapse mortality with viral load as a categorical variable by 1 year after HCT, excluding recipients of cord blood grafts

Adjusted HR (95%CI)	**Entire cohort (n=926)**	**Cord blood excluded (n=824)**
Overall mortality Day 60-365		
No CMV viremia	1	
Any positive- 500 IU/ml	1·4 (1·0-2·0)	1·6 (1·1-2·2)
501-1000 IU/ml	1·7 (1·0-3·1)	2·0 (1·1-3·6)
>1000 IU/ml	3·1 (2·1-4·7)	3·7 (2·4-5·7)
Non-relapse mortality by Day 365		
No CMV viremia	1	
Any positive- 500 IU/ml	1·3 (0·9-2·0)	1·4 (0·9-2·2)
501-1000 IU/ml	2·5 (1·3-4·7)	2·7 (1·3-5·4)
>1000 IU/ml	4·6 (2·8-7·5)	4·9 (2·9-8·4)

Table 5. Results of multivariable Cox proportional hazards models evaluating maximal CMV viral load and viral area under the curve (AUC) before day 100 as a risk factor for overall mortality and non-relapse mortality 1 year after HCT among patients who survived to day 100 (n=832)

Endpoint	**CMV viral load measure**	**Adjusted HR**	**95% CI**
Overall mortality	Maximal viral load[1]		
	No viremia	1	
	Any positive-150 IU/ml	1·3	0·8-1·9
	>150-1000 IU/ml	1·5	1·0-2·4
	>1000 IU/ml	2·0	1·2-3·4
	Viral AUC[2]		
	No viremia	1	
	Below lower quartile	1·0	0·6-1·6
	Lower quartile- median	0·9	0·5-1·7
	Median- upper quartile	1·0	0·5-1·9
	> upper quartile	0·9	0·4-1·8
Non-relapse mortality	Maximal viral load[3]		
	No viremia	1	
	Any positive-150 IU/ml	1·1	0·6-1·9
	>150-1000 IU/ml	1·2	0·7-2·1
	>1000 IU/ml	2·0	1·1-3·9
	Viral AUC[4]		

No viremia	1	
Below lower quartile	0·9	0·4-1·9
Lower quartile- median	1·0	0·4-2·3
Median- upper quartile	1·1	0·4-2·8
> upper quartile	1·2	0·5-2·9

[1] Adjustment factors for this model were: age at transplant, donor relationship, transplant year, underlying disease, disease risk, HCT-CI score, neutropenia, and CMV viremia after day 100.
[2] Adjustment factors for this model were: age at transplant, donor relationship, transplant year, underlying disease, disease risk, neutropenia, and preemptive therapy prior to day 100.
[3] Adjustment factors for this model were: age at transplant, donor relationship, transplant year, disease risk, acute GVHD, chronic GVHD, HCT-CI score, and neutropenia.
[4] Adjustment factors for this model were: age at transplant, donor relationship, transplant year, disease risk, preemptive therapy before day 100.

Table 6. Comparison of results of multivariable Cox proportional hazards models and competing risk regression models evaluating CMV viral load as a risk factor for CMV disease and non-relapse mortality

Endpoint	**Cox model**		**Fine and Gray**	
CMV Viral load threshold	Adjusted HR	95% CI	Adjusted HR	95% CI
CMV disease[1]				
Any positive vs. Negative (no PET)	6·0	2·4-15·0	6·0	2·4-15·0
Any positive vs. Negative (PET)	0·2	0·1-1·3	0·5	0·1-1·7
>250 vs. ≤ 250 IU/ml (no PET)	2·5	1·3-4·7	2·1	1·1-4·1
>250 vs. ≤ 250 IU/ml (with PET)	1·4	0·6-2·1	1·0	0·5-1·9
>500 vs. ≤ 500 IU/ml (no PET)	2·1	1·0-4·2	1·7	0·8-3·6
>500 vs. ≤ 500 IU/ml (with PET)	1·0	0·6-1·8	1·0	0·5-1·8
>750 vs. ≤ 750 IU/ml (no PET)	2·9	1·5-6·0	2·3	1·1-4·9
>750 vs. ≤ 750 IU/ml (with PET)	0·9	0·5-1·7	0·9	0·5-1·7
>1000 vs. ≤ 1000 IU/ml (no PET)	3·2	1·6-6·7	2·5	1·2-5·5
>1000 vs. ≤ 1000 IU/ml (with PET)	0·9	0·5-1·7	0·8	0·4-1·7
Non-relapse mortality[2]				
Any positive vs. Negative	1·8	1·2-2·6	1·7	1·1-2·6
>250 vs. ≤ 250 IU/ml	2·9	2·0-4·1	2·7	1·8-4·0
>500 vs. ≤ 500 IU/ml	3·2	2·2-4·6	3·1	2·1-4·7
>750 vs. ≤ 750 IU/ml	3·7	2·5-5·3	3·5	2·2-5·4
>1000 vs. ≤ 1000 IU/ml	3·6	2·4-5·4	3·3	2·1-5·4

[1] Cox model results correlate to Figure 2 in the manuscript.
[2] Cox model results correlate to Figure 3d in the manuscript.