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ORIGINAL INVESTIGATION

Kinetics of Alphatorquevirus plasma DNAemia at late times after allogeneic hematopoietic stem cell transplantation

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Abstract

Torque teno virus (TTV) plasma DNA load has been consistently shown to be a surrogate biomarker of immunosuppression in solid organ transplant recipients. It is uncertain whether it may behave similarly in allogeneic hematopoietic stem cell transplant recipients (allo-HSCT). Here, we characterized the dynamics of TTV DNAemia in patients undergoing T-cell replete allo-SCT at late times after transplantation (> day + 100). This retrospective single-center observational study included 33 allo-HSCT patients. Plasma TTV DNA loads were quantified by real-time PCR before initiating the conditioning regimen and at different time points after transplant. Absolute lymphocyte counts (ALC) were measured by flow cytometry. Overall, TTV DNA load increased steadily after engraftment, reaching a peak by day + 90; afterwards, it remained relatively constant until day + 210. TTV DNA loads measured within days + 120 and + 210 correlated inversely with paired ALC, while both parameters did correlate directly within days + 20 and + 60. The median TTV DNA area under a curve between days + 90 and + 210 [$(AUC)_{90–210}$] was significantly higher in patients who received corticosteroids within this time frame for treatment of graft versus host disease (either acute, chronic or both) than in controls ($P = 0.025$). In summary, TTV DNA load may mirror the degree of immunosuppression at late times after allo-HSCT.

Keywords Torque teno virus (TTV) DNAemia · Allogeneic hematopoietic stem cell transplantation · Immunosuppression · Biomarker

Introduction

The genus Alphatorquevirus belongs to the *Anelloviridae*, which includes 29 species (Torque teno virus-TTV-1 to 29) [1]. These are circular, negative sense, single-stranded DNA viruses, that are lymphotropic, ubiquitous and seemingly apathogenic in humans [1, 2]. There is an increasing body of evidence indicating that TTV DNA load in the blood compartment behaves as a surrogate biomarker of immunosuppression in solid organ transplant recipients, thus allowing estimation of the patient's risk of opportunistic infections and allograft rejection [3–10]. Nevertheless, it is currently uncertain whether TTV DNA load could serve the same purpose in allogeneic hematopoietic stem cell transplant recipients (allo-HSCT). The kinetics of plasma TTV DNA load early after allo-HSCT have been precisely characterized [11–17]. In effect, a dramatic drop in plasma TTV DNA load occurs shortly after conditioning, then it increases steadily following engraftment reaching the peak at around day + 90 after transplant. Whether the magnitude of TTV

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DNA load at these early times after allo-HSCT reflects either the degree of overall immunocompetence or the opposite remains a matter of debate [15–17]. In contrast, little is known about both the dynamics of TTV DNA load and its potential clinical value as a marker of immunosuppression late after allo-HSCT (> day + 100). Here, we characterized the dynamics of TTV DNA load within this time frame and investigated whether it is modulated by the use of different immunosuppressive regimens or corticosteroid treatments for graft versus host disease (GvHD).

Materials and methods

Patients

This retrospective single-center study included 33 non-consecutive patients who underwent T-cell replete allo-HSCT at the Hematology Service at the Hospital Clínico Universitario in Valencia between December 2013 and May 2016, as a curative therapy for different hematological cancers (Table 1). Only adult patients (≥ 18 years) with available plasma samples for TTV DNA testing obtained at predetermined time points (see below) were included. As shown in Supplementary Table 1, patients included in the study group were representative of the entire cohort of patients undergoing allo-HSCT at our center during the study period ($n = 88$). The median patient's age at the time of transplantation was 56 years (range 19–70 years). The study period comprised the first 8 months after transplantation. The study was approved by the Hospital Clínico Universitario (INCLIVA Foundation) review board and ethics committee. All the patients gave their written informed consent prior to participating in the study.

Table 1 Demographic and clinical characteristics of the patients

Parameter	No. (%)
Sex	
Male	16 (48.5)
Female	17 (51.5)
Underlying hematological disease	
Lymphoma	12 (36.4)
Leukemia	12 (36.4)
Myeloma	4 (12.1)
Myelodysplastic syndrome	3 (1.1)
Myelofibrosis	2 (6.1)
Allograft type	
Related	18 (54.6)
Unrelated	15 (45.4)
Human leukocyte antigen-matching	
Matched	17 (51.5)
Mismatched	16 (48.5)
Conditioning regimen	
Myeloablative	6 (18.2)
Reduced intensity	27 (81.8)
Stem cell source	
Peripheral blood	31 (93.9)
Bone marrow	1 (3.0)
Umbilical cord blood	1 (3.0)
Anti-thymocyte globulin use	
Yes	8 (24.2)
No	25 (75.8)
Inmunosuppressive regimens used	
Cyclosporine A-based (with methotrexate or mycophenolate)	19 (57.5)
Tacrolimus and sirolimus	14 (42.5)

Plasma specimens

Cryopreserved plasma samples (at − 80 °C; never thawed before) were retrieved for the analyses described herein. The specimens had been obtained before the initiation of conditioning (median, day − 7; range day − 30 to day − 6) and at different time points after transplantation: day + 20 (range day + 14–day + 24), day + 30 (range day + 25–day + 35), day + 40 (range day + 35–day + 46) day + 50 (range day + 47–day + 55), day + 60 (range day + 56–day + 76), day + 90 (range day + 78–day + 97), day + 120 (range day + 108–day + 141), day + 150 (range day + 142–day + 163), day + 180 (range day + 166–day + 202), and day + 210 (day + 203–day + 256). In all, 333 specimens were available for TTV DNA quantitation. Ten patients had one or more post-transplant missing specimens (total number = 30 samples). Preconditioning specimens were unavailable from 11 patients.

Plasma Torque teno virus DNA load quantitation

TTV DNA load quantification was carried out with a TaqMan real-time PCR targeting a highly conserved segment of the untranslated region (UTR) of the viral genome, thus allowing amplification of all known genetic variants of Alphatorquevirus. Specific details on the procedure have been previously published [14, 15]. All samples from each patient were assayed simultaneously in singlets.

Lymphocyte counts

Enumeration of absolute lymphocyte counts (ALC) was performed by flow cytometry using the BD FACSCanto II Flow Cytometer (BD Biosciences, San Jose, CA, USA).

Statistical analysis

The TTV DNA load area under the curve (AUC) was calculated using the trapezoidal rule with the aid of the STAT GRAPHIC Centurion XVII statistics package (Statpoint Technologies, Inc., Warrenton, VA, USA). Differences between medians were compared using the Mann–Whitney *U* test for unpaired specimens or the Wilcoxon *T* test for paired specimens, as appropriate. Correlations between variables were assessed using the Spearman's rank test. Two-sided exact *P* values are reported; a *P* value ≤ 0.05 was considered to be statistically significant. The statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

Results

Dynamics of plasma TTV DNAemia

All 33 patients had at least one plasma specimen with quantifiable levels of TTV DNA. The kinetics of TTV DNA load in plasma over the study period is shown in Fig. 1. In agreement with previous findings [14], the TTV DNA load was seen to increase steadily (and significantly) from around the time of engraftment (day + 20) until day + 90, when the peak was reached. Afterwards, TTV DNA load remained relatively constant until day + 210, time at which it dropped slightly; in fact, the median TTV DNA load at day + 210 was significantly lower than that quantified at day + 90. Overall, the median TTV DNA load by day + 210 was of greater magnitude than that measured prior to conditioning, although the difference did not reach statistical significance ($P = 0.398$).

TTV DNA load and absolute lymphocyte counts

As shown in Fig. 2, overall, TTV DNA loads measured by days + 120, + 150, + 180 and + 210 correlated inversely with paired ALC (A), while both parameters did correlate directly when measured by days + 20, + 30, + 40, + 50 and + 60 (medians) (panel B).

Effect of the immunosuppressive regimen and corticosteroids use on plasma TTV DNA load late after transplantation

We first assessed whether the immunosuppressive regimen used for the prevention of GvHD had an impact on TTV DNA load at late times after transplantation, using TTV DNA AUC between days + 120 and + 210 ($AUC_{120–210}$) as the end-point. We found that the $AUC_{120–210}$ in patients receiving a cyclosporine A-based regimen (median 7.53 $\log_{10}$ copies × days × ml^{-1}; range 4.68–9.57) was not

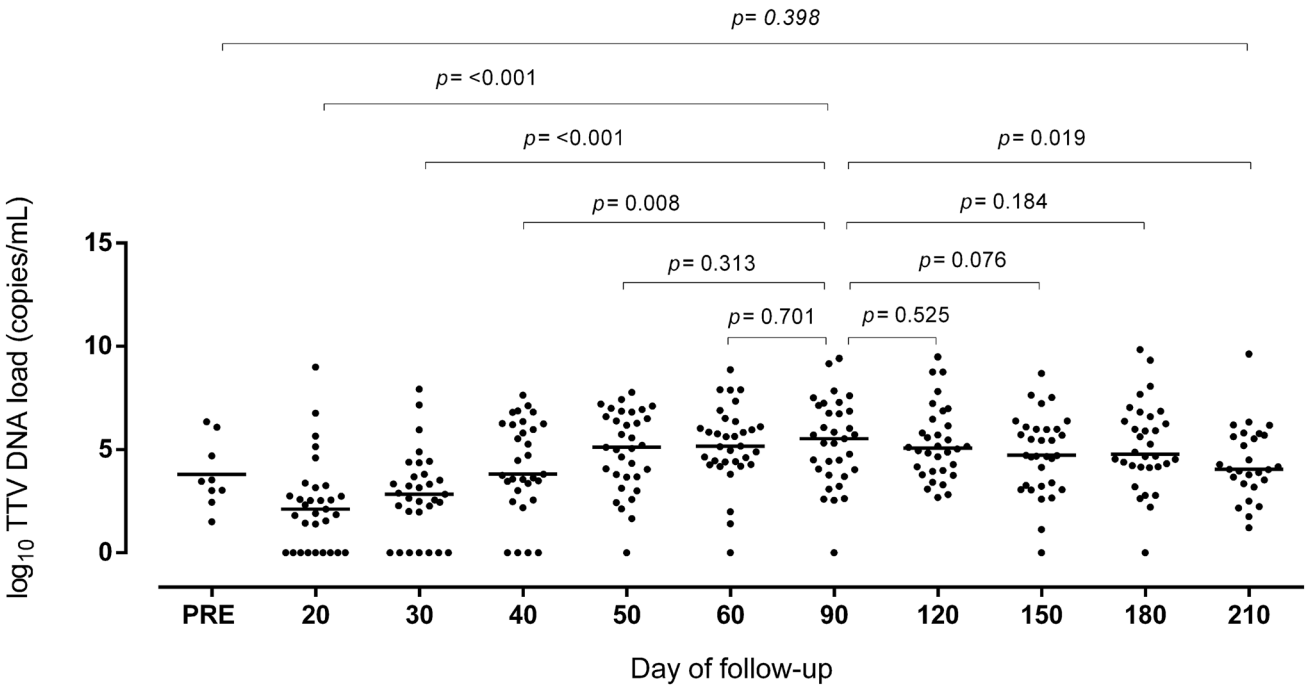

Fig. 1 Dynamics of plasma TTV DNAemia in allo-HSCT recipients. TTV DNA loads were quantified prior to conditioning (PRE) and at different time points following allo-HSCT. Bars indicate the median values and the standard deviations. Comparative *P* values (Wilcoxon *T* test) are shown

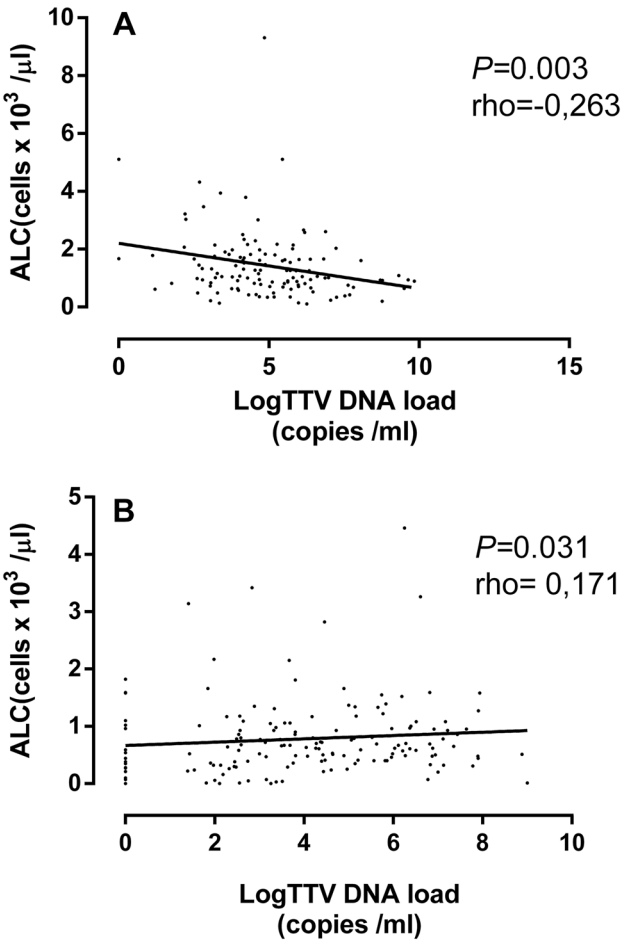

Fig. 2 Correlation between plasma TTV DNA loads and absolute lymphocyte counts (ALC) measured at all time points within days + 120 and + 210 (**a**) and days + 20 and + 60 (**b**). Spearman rank correlation coefficients (rho) and *P* values are shown

significantly different ($P = 0.1$) from that seen in patients treated with tacrolimus and sirolimus (median 8.58 $\log_{10}$ copies × days × ml^{-1}; range 3.93–9.56). We next aimed at assessing the impact of the use of corticosteroid drugs on TTV DNA load late after allo-HSCT. We chose the TTV DNA AUC between days + 90 and + 210 ($AUC_{90–210}$) as the analytical end-point; the AUC allows the estimation of the actual magnitude ("exposure") of a given parameter over time when measurements are conducted at discrete moments. Out of the 33 patients, 6 were excluded from these analyses because of early relapse of the underlying disease ($n = 4$) or premature death ($n = 2$). Out of the remaining 27 patients, 12 did not receive corticosteroids at any time during the study period (control group) and 15 did so (test group) because of either, acute GvHD ($n = 5$), chronic GvHD ($n = 7$) or both ($n = 3$). All these latter patients were under corticosteroids therapy within days + 90 and + 210 (Table 2). As shown in Fig. 3, the median TTV DNA $AUC_{90–210}$ was significantly higher in patients treated with corticosteroids than in controls ($P = 0.025$), yet no significant correlation was seen between this parameter and the total cumulative dose of corticosteroids prescribed, although a trend was noted (rho, 0.364; $P = 0.182$). No significant differences ($P = 0.502$) were seen between control and test patients regarding the immunosuppressive regimens used (cyclosporine A-based, eight patients in the control group and seven patients in the test group; tacrolimus plus sirolimus: four patients in the control group and eight patients in the test group).

Discussion

The potential use of TTV DNA load as a surrogate marker of immune competence in SOT recipients is supported by an increasing body of evidence [2–10]. Nevertheless, demonstration of a comparable clinical utility in the allo-HSCT setting remains elusive, likely reflecting the profound biological differences between these two transplant modalities. Namely, we recently suggested that the magnitude of TTV DNA load early after engraftment may behave as marker of immune system reconstitution, as it was found to predict protection from high-level plasma cytomegalovirus (CMV) DNAemia [15]. In contrast, data from two other studies pointed to the opposite idea, whereby plasma TTV DNA would actually be a surrogate marker for immunosuppression [16, 17]. Notwithstanding, we also provided some evidence indicating that the degree of immunosuppression after transplant may modulate the level of TTV replication [14]; in fact, in that previous study [14], we found that patients with severe aGvHD receiving corticosteroids at high doses experienced increases in TTV DNA load between days + 20 and + 60 of greater magnitude than controls (patients with grades 0-I aGvHD), regardless of ALC counts, a phenomenon also reported by Masouridi-Levrat and colleagues [13]. In light of these latter findings, we postulated that once TTV DNA levels reach the plateau phase, concurrently with full lymphocyte repopulation, at late times after allo-HSCT, it may actually behave as a biomarker of immunosuppression [14, 15], an assumption with which other authors did concur [17]. The data presented herein seemed to support this idea.

In our setting the dynamics of TTV DNA load over the first 8 months after transplantation followed a previously recognized pattern [17]; increasing TTV DNA levels were seen from around the time of engraftment peaking by day + 90; then relatively stable levels until day + 210 were observed, time at which they dropped slightly, but remained above those measured prior to conditioning. The scarce number of cases in this series precluded any meaningful subgroup analysis evaluating the impact of the type of malignancy or the conditioning regimen on TTV DNA load kinetics as performed in another study [17].

Table 2 Clinical characteristics of patients who underwent corticosteroid treatment and did not relapse or die within the study period

Patient	Corticosteroid use (prednisone)			aGvHD (day of diagnosis after allo-HSCT)	cGvHD (day of diagnosis after allo-HSCT)
	Day of initiation after allo-HSCT	Day of cessation after allo-HSCT	Cumulative dose through day 210 (mg)		
1	116	> 365	5240	116	–
2	120	> 365	3735	–	107
3	97	202	4980	94	265
5	49	104	2940	49	172
6	97	173	3115	–	97
8	24	103	1085	24	–
10	204	266	360	–	170
11	46	213	4470	46	–
12	42	> 365	3361	42	–
14	192	> 365	1080	–	150
15	154	240	1505	–	154
16	28	93	2090	23	–
17	45	195	3265	26	45
18	120	> 365	3775	–	120
19	133	248	1050	–	96

aGvHD acute graft-versus-host disease, *cGvHD* chronic graft-versus-host disease, *allo-HSCT* allogeneic hematopoietic stem cell transplantation

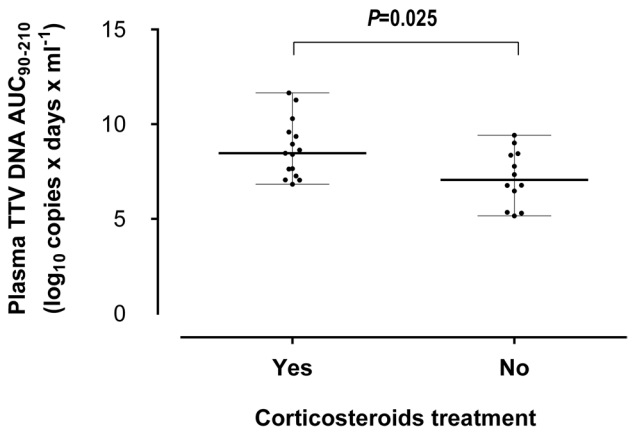

Fig. 3 Area under the curve (AUC) for $\log_{10}$ TTV DNA loads quantified between days + 90 and + 210 in patients either undergoing or not corticosteroid therapy for graft-versus-host disease. Comparative *P* value is shown

Two observations supported the idea of TTV DNA load may behave as a marker of immunosuppression after day + 100 after allo-HSCT. First, TTV DNA loads measured within days + 120 and + 210 correlated inversely with paired ALC. In a recent study [17], an inverse correlation was observed between these two parameters; in such analysis, nevertheless, all measured values over the entire follow-up period were taken into consideration, and thus no separate subanalyses for early and late times after allo-HSCT were performed. Second, the use of corticosteroids, known to impair immune functionality, had a profound impact on TTV DNA dynamics; in fact, median TTV DNA $AUC_{90–210}$ was significantly higher in patients treated with prednisone for GvHD (acute, chronic or both) than in controls and a trend towards a correlation between this TTV DNA kinetics parameter and the cumulative dose of corticosteroids used was seen. Of interest, the type of immunosuppressive regimen used to prevent the occurrence of GvHD did not differ between patients undergoing corticosteroid treatment and those not doing so. In line with our observations, Wohlfarth and colleagues [17] reported that patients with acute GvHD requiring systemic corticosteroids at high doses showed a trend towards higher TTV DNA levels at 4–5 months after transplantation.

The inverse correlation between TTV DNA loads and paired ALC measured between days + 120 and + 210 can be explained, at least in part, by the effect of corticosteroids on lymphocyte redistribution (resulting in lymphopenia) and functionality [18], as T-cell immunity is likely to play a major role on the control of TTV replication. This finding also favors the idea that T lymphocytes are not the only cell target for TTV replication [2]. In support of this assumption, we previously reported that, overall, TTV DNA loads were significantly higher in saliva than in plasma specimens and that they correlated to a lesser extent with ALC than TTV DNA loads quantitated in plasma following engraftment [19].

The current study was an exploratory one that was simply aimed at characterizing the kinetics profile of TTV DNA load at late times after allo-HSCT, and in this sense was not undertaken to assess the potential clinical value of TTV

DNA load as a biomarker for predicting post-transplant immune-related complications. Prospective studies are best suited to this purpose and are underway. In addition to its retrospective and unicentric design, main limitations of this study are the limited size of the cohort, which undermined the robustness of statistical analyses, and the impossibility of having all time point specimens from all patients for TTV DNA quantitation, although the actual number of missing specimens represented less than 10% of those planned to subject to analysis.

In sum, our data suggest that TTV DNA loads measured at late times after allo-HSCT may reflect the net state of immunosuppression of patients. Nevertheless, further longitudinal and prospective studies to validate this hypothesis are warranted. Whether TTV DNA load measurements at late times after allo-HSCT may be helpful to predict the occurrence of certain clinical events such as CMV DNAemia recurrences, late CMV disease or post-transplant lymphoproliferative disease, which frequently occur after day 100, remains to be investigated.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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