Journal of Clinical Virology 47 (2010) 189–192

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Short communication

Torquetenovirus viremia kinetics after autologous stem cell transplantation are predictable and may serve as a surrogate marker of functional immune reconstitution

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ARTICLE INFO

Article history:
Received 9 July 2009
Received in revised form
23 November 2009
Accepted 27 November 2009

Keywords:
TTV
Transplantation
Immune reconstitution
Immune deficiency

ABSTRACT

Background: It is common experience that retreating patients too early after a course of intensive chemotherapy predisposes to opportunistic infections despite apparently normal lymphocyte levels.
Objectives: The extent of replication of persistent viruses that cause no obvious disease (and hence need no treatment) might better define when a patient has recovered from functional immune deficiency.
Study design: We used real-time polymerase chain reaction to monitor the kinetics of plasma torquetenovirus (TTV) viremia in hematological patients undergoing autologous hematopoietic stem cell transplantation as support to high-dose chemotherapy (HSCT).
Results: Independently from underlying hematological disease and therapeutic regimen, TTV viremia increased post-HSCT, and this increase paralleled the increase of circulating $CD8^+CD57^+$ T lymphocytes, known to represent an indirect marker of functional immune deficiency. Subsequently, within a matter of months, TTV levels returned to baseline values, at a pace that appeared to be constant over time.
Conclusion: Monitoring of TTV viremia represents a unique opportunity to follow functional immune reconstitution in immunosuppressed patients. Also, the size of the TTV viremia increases resulting from immunosuppressive treatments might be of guidance in determining the appropriate time interval before delivery of a next course of therapy.

1. Background

High-dose chemotherapy supported by autologous hematopoietic stem cell transplantation (HSCT) is a major procedure in the treatment of cancer. Even when patients have recovered a normal absolute lymphocyte count (ALC), opportunistic infections often still remain a serious concern.[1] Monitoring viruses that pre-exist in the patients and produce no obvious disease (and hence require no treatment) might offer a good approximation of real immune reconstitution.

Torquetenovirus (TTV) is a small non-enveloped *Anellovirus*: chronic plasma viremia occurs in over 90% of people. TTV has been shown to reside and multiply in the bone marrow,[2] stimulated peripheral blood mononuclear cells (PBMCs), and possibly other tissues.[3–5] However, it remains a disease-orphan virus, extensive investigations having so far failed to reveal a clear association to any human illness.[6,7]

2. Objectives

Given that TTV levels parallels the increase of $CD8^+57^+$ T lymphocytes in peripheral blood after autologous HSCT,[8] we investigate here whether monitoring TTV plasma levels over time might indeed serve to determining when the patients' immune system has recovered a good level of functionality.

3. Study design

3.1. Patients and specimens

A total of 47 autologous HSCTs for MM or lymphoma (LY) were investigated. The patients were admitted to hospital and

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doi:10.1016/j.jcv.2009.11.027

put on appropriate chemotherapy 2 days (MM) or 7 days (LY) prior to the HSCT. 33 (23 MM and 10 LY) were at their first HSCT, while of the MM patients 13 were at the second and one at the third (time interval from the previous one(s), 143–346 days). Conditioning regimens were as follows: MM patients received high-dose melphalan (70–200 mg/m^2) on day −2 relative to the transplant and granulocyte colony-stimulating factor (G-CSF) 10 mcg/kg on day +1; LY patients were given BEAM polychemotherapy (BCNU 300 mg/m^2 on day −7, etoposide 100 mg/m^2 and ara-C 200 mg/m^2 on days −6 to −3, melphalan 140 mg/m^2 on day −2) and G-CSF on day +1. All but 6 patients received a variable number of heterologous red blood cells and/or platelets units (range: 1–7; mean: 2.8 ± 1.7 transfusions) starting from day +8 post-transplant (PT). Peripheral blood samples were collected from all patients at hospitalization, and on days 0, +40, +70, and +100.

3.2. TTV detection and quantification

DNA extracted from 200 μl of plasma samples was examined for TTV presence and loads by using a single step universal TaqMan real-time PCR assay as previously described.[9,10] All known TTV genotypes were detected because the assay targets a highly conserved segment of the viral untranslated region.[11,12] Reducing the volume of distilled water in which the extracted DNA was diluted and by increasing the volume of input DNA in the PCR reaction allowed to stretch the sensitivity to a lower limit of detection of 2.0 $\log_{10}$ DNA copies/ml of plasma.

3.3. TTV genogroup determination

For TTV genogrouping, the same DNAs used for TTV detection and quantification were amplified by using 5 distinct nested or heminested PCR protocols, one specific for each genogroup, using the genogroup-specific primers previously described.[13] Nucleotide sequences were aligned with representative TTV sequences existing in GenBank at the time of writing, using the ClustalW algorithm included in the BioEdit program (version 7.04.1).

3.4. Immunophenotyping

Peripheral blood samples were tested with using a panel of monoclonal antibodies directed against CD45, CD3, CD4, CD8, CD16, CD25, CD56, and CD57 (*BD* Bioscience, USA). Data were acquired on a FACS Calibur flow cytometer and analyzed using CellQuest 3.1 (BD Bioscience, USA).

3.5. Statistical analyses

The Pearsons's χ^2-test was applied to evaluate heterogeneity of contingency tables. Differences between means and distributions were evaluated by the two-tailed Student's *t*-test. Associations between variables were determined by applying the Pearson's correlation coefficient. *p* Value was calculated to test the significance of correlation and a value less than 0.05 was considered to be statistically significant.

4. Results

As determined with a universal real-time PCR of high sensitivity (lower limit of detection: 1.0×10^2 DNA copies per ml), at the time of hospitalization (baseline) all but 2 MM and 2 LY patients had demonstrable TTV in plasma (92%) at loads that ranged between 3.1 and 7.8 $\log_{10}$ DNA copies per ml. The viral genogroups present at baseline in a representative number of patients (10 MM and 5 LY) were characterized: most patients (12/16) were found to carry more than one and up to four TTV genogroups (mean ± standard deviation (SD) = 2.0 ± 0.8) (Table 1).

TTV presence and loads were determined again on the day of HSCT and +40, +70, and +100 days PT. Viral loads showed an average increase of approximately 1.5 logs at +100 days PT (Figs. 1A, 2, 3 and Table 1), less pronounced in the patients with high pre-transplant viral load. Indeed, the size of viremia increase was found to correlate negatively with the load of TTV at baseline ($r = -0.530$; $p < 0.001$). However, even the patients with baseline TTV viremia greater than 5.0 $\log_{10}$ (no. 12) exhibited a significant increase of TTV viral loads (mean, 1.5 logs; $p = 0.01$ at +100 days PT). The TTV increases also positively correlated with the dose of melphalan received by the patients ($r = 0.499$; $p < 0.001$).

Table 1
TTV genogroups detected in the plasma of 16 study patients at baseline and at the day of maximum TTV load observed.

Patient and disease	No. HSCT/no. transfusions	Baseline TTV		Post-transplant peak of TTV load	
		Load[a]	Genogroups	Load	Genogroups
Acute myelogenous leukemia					
FA	1/0	5.3	1, 3, 4	7.5[b]	1, 3, 4
Lymphoma					
BN	1/6	3.1	1, 4	8.1	1, 4, 5
BP	1/7	6.7	1, 3	7.5	1, 3, 5
GC	1/5	4.3	1, 4	6.9	1, 3, 4
LM	1/2	6.2	1, 3, 5	7.8	1, 3, 4, 5
MA	1/6	4.4	1, 5	5.4	1, 3, 5
Multiple myeloma					
CA	1/1	4.3	1, 3, 4, 5	7.3[b]	1, 3, 4, 5
CI	1/1	4.3	5	5.0	3, 4
CR	2/0	5.1	1, 3	7.0	3, 5
DLE	1/1	4.0	1, 3	7.5	1, 3
DPG	1/1	5.1	1, 3	6.7	1, 3, 4
FE	3/4	4.4	1	7.5	1
LMG	1/2	4.3	4	6.8	4
MW	1/4	5.7	3	8.5	1, 3, 5
PM	2/3	6.1	1, 4, 5	7.4	3, 4, 5
VS	2/2	3.7	1, 3	5.7	1, 3

[a] $\log_{10}$ DNA copies per ml of plasma.
[b] In this patient, peak TTV load was observed on day +70, in all the others on day +100.