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Epstein-barr virus DNAemia monitoring for the management of post-transplant lymphoproliferative disorder

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Abstract

Background. Post-transplant lymphoproliferative disorder (PTLD) is a potentially fatal complication of allogeneic hematopoietic cell transplantation (HCT). Epstein-Barr virus (EBV) reactivation (detectable DNAemia) predisposes to the development of PTLD. *Methods.* We retrospectively studied 306 patients monitored for EBV DNAemia after Thymoglobulin-conditioned HCT to determine the utility of the monitoring in the management of PTLD. DNAemia was monitored weekly for ≥12 weeks post-transplantation. *Results.* Reactivation was detected in 82% of patients. PTLD occurred in 14% of the total patients (17% of patients with reactivation). PTLD was treated with rituximab only when and if the diagnosis was established. This allowed us to evaluate potential DNAemia thresholds for pre-emptive therapy. We suggest 100,000–500,000 IU per mL whole blood as this would result in unnecessary rituximab administration to only 4–20% of patients and near zero mortality due to PTLD. After starting rituximab (for diagnosed PTLD), sustained regression of PTLD occurred in 25/25 (100%) patients in whom DNAemia became undetectable. PTLD progressed or relapsed in 12/17 (71%) patients in whom DNAemia was persistently detectable. *Discussion.* In conclusion, for pre-emptive therapy of PTLD, we suggest threshold DNAemia of 100,000–500,000 IU/mL. Persistently detectable DNAemia after PTLD treatment with rituximab appears to have 71% positive predictive value and 100% negative predictive value for PTLD progression/relapse.

Key Words: *Epstein-Barr virus infections, hematopoietic stem cell transplantation, herpesvirus 4, post-transplantation lymphoproliferative disorders, retrospective studies, rituximab, thymoglobulin*

Introduction

Hematopoietic stem cell transplantation (HCT) recipients are at risk of developing post-transplantation lymphoproliferative disorder (PTLD). Proliferation of Epstein-Barr virus (EBV)-transformed B cells due to insufficient control by EBV-specific T cells causes PTLD [1,2]. The incidence of PTLD among HCT recipients ranges from 0.2–71% depending on the type of transplant, conditioning, graft-versus-host disease (GVHD) prophylaxis and donor type [3–16]. EBV reactivation (detection of EBV DNA in blood) predisposes to the development of PTLD [17].

Treatment options for PTLD include rituximab (anti-CD20), chemotherapy, EBV-specific T cells, unselected donor lymphocytes and reduction of immunosuppression [17–21]. In centers in which EBV-specific T cells are not available, rituximab is used most frequently. Rituximab can be administered pre-emptively (when EBV DNAemia has exceeded a threshold) or therapeutically (after PTLD has been diagnosed). Most centers have moved toward DNAemia monitoring and pre-emptive therapy with rituximab in patients with a risk factor for developing PTLD such as GVHD prophylaxis with antithymocyte globulin (ATG) [22,23].

However, a disadvantage of pre-emptive therapy is that patients who would not develop PTLD get rituximab. This is not only expensive, but also causes toxicities. Moreover, there is no consensus on the EBV DNAemia threshold for initiation of pre-emptive therapy. Centers have developed their in-house quantitative PCR assays for measuring the DNAemia. There is up to 4-log difference in the EBV DNAemia results between two laboratories [24]. Therefore, DNAemia threshold useful at one center (using that center's assay) may lead to too high incidence of PTLD or too many patients getting rituximab unnecessarily if used at

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another center (using the other center's assay) [25]. A great contribution to solving this problem was the recent development of a World Health Organization (WHO) standard [26], followed by the development of a commercially available assay that uses the WHO standard (RealStar EBV PCR, Altona Diagnostics). The RealStar assay reports DNAemia in international units (IU/mL, i.e., standardized EBV DNA copies/mL) instead of crude EBV DNA copies/mL, which vary among in-house assays. However, DNAemia thresholds for pre-emptive therapy in international units per milliliter (IU/mL) are yet to be determined.

At our center, between 2011 and 2015, patients were monitored for EBV DNAemia, but not pre-emptively treated. We used an in-house assay that fortuitously gives results near-identical to the RealStar assay. This gave us a unique opportunity to propose thresholds for pre-emptive therapy in IU/mL, which we present here.

In addition, we monitored patients who developed PTLD and thus were treated with rituximab. This gave us an opportunity to determine whether DNAemia monitoring post-rituximab could distinguish between PTLD patients achieving sustained remission versus developing progression or relapse of PTLD. Thus, here we also present data on the utility of monitoring after therapy with rituximab.

Patients and methods

Patients and transplantation

A total of 306 first allogeneic HCT recipients were studied. For details, see Table I and Supplementary file 1.

EBV serology and DNAemia assays

EBV serology for viral capsid antigen immunoglobulin (Ig)G and EBV nuclear antigen 1 (EBNA1) IgG was determined using Trinity Captia assays until June 1, 2015; after that the Architect EBV assay (Abbott Diagnostics) was used. A patient or donor was considered EBV-seropositive if positive for viral capsid antigen IgG or EBNA1 IgG.

For EBV DNAemia, an in-house assay was used for the patients characterized in Table I. DNA was extracted from whole blood. Quantitative polymerase chain reaction (PCR) was run using primers and probes targeting EBNA1 gene. The limit of detection was approximately 100 copies/mL. The dynamic range for this assay was 500–100,000,000 copies/mL whole blood.

EBV DNAemia was monitored routinely once a week till 12 weeks post-transplantation for all patients. After 12 weeks, DNAemia ordering was at the discretion of the attending physician.

Table I. Patient characteristics.

Patient characteristics	N = 306
Median patient age (range)	51 (16–67)
Median donor age (range)	34 (13–69)
Gender (donor→recipient) (%)	
M→M	119 (39)
M→F	77 (25)
F→M	60 (20)
F→F	50 (16)
Disease stage[a] (%)	
Good risk	141 (46)
Poor risk	165 (54)
EBV serostatus (%)	
D-R-	3 (1)
D + R-	11 (3.5)
D-R +	30 (10)
D + R +	242 (79)
Unknown/indeterminate[d]	20 (6.5)
Graft type (%)	
Bone marrow	3 (1)
Cord blood	11 (3.5)
Peripheral blood stem cells	292 (95.5)
Conditioning (%)	
Flu + Bu + TBI + ATG	270 (88)
Flu + Bu + ATG	27 (9)
Other chemotherapy/TBI[c] + ATG	9 (3)
Donor type (%)	
HLA-matched sibling	106 (35)
8/8[b] Unrelated donor	133 (43)
7/8 Unrelated donor	55 (18)
6/8 Unrelated donor	1 (0.3)
6/6 Cord blood	1 (0.3)
5/6 Cord blood	0 (0)
4/6 Cord blood	10 (3)

Flu, fludarabine; Bu, busulfan; TBI, total body irradiation.
Percentages are rounded to zero decimal point, except if ≤1%, in which case the percentages are rounded to one decimal point.
[a]Good risk disease was defined as primary acute leukemia (Acute Myeloid leukemia, Acute lymphoblastic leukemia, biphenotypic) in first remission, chronic myeloid leukemia in first chronic or accelerated phase, myelodysplasia with <5% marrow blasts or aplastic anemia. All other diseases/disease stages were considered poor risk (including all patients with myelofibrosis, chronic myelomonocytic leukemia lymphoma, multiple myeloma).
[b]Refers to matching donor and recipient in HLA-A, B, C and DRB1, except for cord blood (refers to matching in HLA-A, B and DRB1).
[c]Other chemotherapy/TBI included myeloablative combinations of VP16 (Etoposide), Melphalan, Cytarabine, Fludarabine, Busulfan or TBI.
[d]Unknown/intermediate EBV serostatus in patient and/or donor.

To be able to convert the results of the in-house assay to IU/mL [26], between March 2016 and June 2016, EBV DNAemia in 57 HCT recipients (Supplementary Table 1) was determined by both the in-house assay (results in genome copies/mL) and the internationally standardized RealStar assay (results in IU/mL). Of 319 blood specimens in which DNAemia was determined by both assays, DNAemia was quantifiable (500–100,000,000 copies/mL by in-house assay,