

Clinical Application of Real-time Quantitative Epstein-Barr Virus (EBV) Polymerase Chain Reaction Assay to Posttransplant Infection

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1. REVISED ABSTRACT

Background: Quantitative monitoring of EBV DNA is often done routinely posttransplant but relationships between the risk of EBV disease by transplant (tx) type and the amount and change in viral load are imprecise. **Methods:** Our laboratory developed a TaqMan real-time quantitative PCR assay (qEBV) that targets a 71-bp portion of the highly conserved EBNA-1 gene with reliable detection of 10 copies of EBV per reaction. We have used qEBV to monitor posttransplant EBV infections since 2003. Data are expressed as copies/mL. **Results:** 8,919 qEBV assays were done on 1,385 patients (pts) after tx. The majority of tests (>95%) were on whole blood samples. EBV DNAemia was documented in 303 tx recipients (22%). Proportions of positive pts differed by tx type: lung, 27/74 (36%); heart, 26/77 (34%); liver, 37/148 (27%); renal, 129/544 (24%); pancreas, 27/139 (21%); hematopoietic cells (HCT), 78/432 (18%). The proportion of pts with DNAemia posttransplant was significantly greater in lung (P <0.001; Fisher exact text) and heart tx (P = 0.003; Fisher exact text) as compared with HCT. The distribution of peak viral loads was: 147 pts (49%), $10^3 - 10^4$; 121 pts (40%), $10^4 - 10^5$; 22 pts (7%), $10^5 - 10^6$; 13 pts (4%) at least 10^6 . Of the 13 pts with at least 10^6 copies/mL blood, 12 (92%) had EBV-related disease; 8 of them had posttransplant lymphoproliferative disorder (PTLD). Viral loads of $10^5 - 10^6$ were less predictive of EBV-related disease: 55% of these pts (12/22) were diagnosed with a symptomatic EBV-related illness. Among all 1,385 tx pts, 45 (3%) had PTLD. At the time of PTLD diagnosis, qEBV values were available for 27 (60%) of these pts: 25 (93%) were positive whereas 2 had no EBV DNA detected. Of the 25 pts diagnosed with PTLD whose qEBV was positive, 21 (84%) had their highest qEBV at the time of diagnosis. 19/25 (76%) had subsequent qEBVs and 17/19 decreased with therapy. **Conclusions:** The likelihood of a positive qEBV varied with tx type. It was significantly higher in lung and heart recipients as compared with HCT. Although we could not define an exact quantitative threshold for tissue-invasive EBV disease, the qEBV provided guidance on risk of disease for pts with higher amounts of EBV DNAemia. EBV disease occurred in 92% of pts with qEBV at least 10^6 and 55% of pts with qEBV at least 10^5 .

2. INTRODUCTION

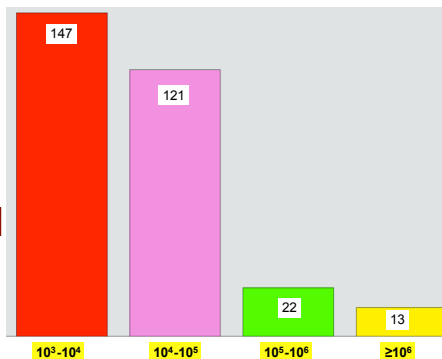
Quantitative monitoring of EBV DNA (qEBV) is often done routinely posttransplant but relationships between the risk of EBV disease by transplant (tx) type, the presence, the amount, and the rate of change in viral load are imprecise. We have maintained a FileMaker Pro database of qEBV results since introducing this test into clinical use in October 2003. An analysis of the data collected from 10/1/03 to 4/27/09 are presented in the Results section of the poster. This study is approved by the University of Minnesota Research Institutional Review Board (0707E13223).

Table 1. Proportion of Patients with EBV DNAemia

Type of Transplant	No. of Patients	No. Positive (%)	P Value vs HCT*
All transplant patients	1,385	303 (22%)	
All solid organ patients	953	224 (24%)	0.03
Lung	74	27 (36%)	< 0.001
Heart	77	26 (34%)	0.003
Liver	148	37 (27%)	NS
Renal	544	129 (24%)	0.03
Pancreas	139	27 (21%)	NS
≥ 2 solid organs	107	28 (26%)	NS
Hematopoietic cells (HCT)	432	78 (18%)	

*Fisher exact test, 2-sided.

Fig. 1. Distribution of Peak Positive Whole Blood EBV Loads in Transplant Patients



3. RESULTS

Fig. 2. Patterns of EBV DNAemia Posttransplant

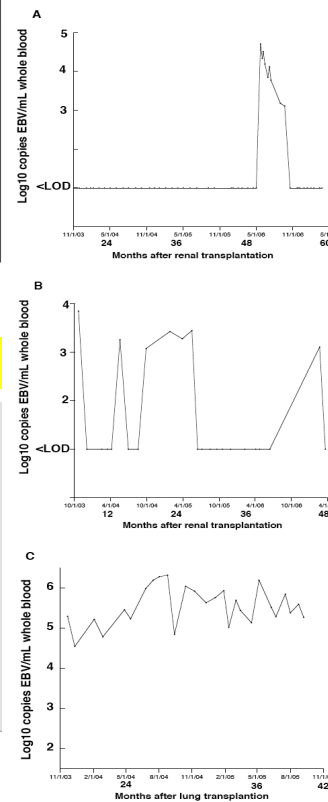


Table 2. Posttransplant Lymphoproliferative Disorder

	No. of patients	%
Transplant patients with PTLD	45/1385	3%
PTLD patients with qEBV at diagnosis	27/45	60%
PTLD patients positive for EBV DNA at diagnosis	25/27	93%
PTLD patients with highest qEBV at diagnosis	21/25	84%
PTLD patients with subsequent qEBVs post-diagnosis	19/25	76%
PTLD patients with decreasing qEBVs with therapy	17/19	90%

4. DISCUSSION AND CONCLUSIONS

The likelihood of a positive qEBV posttransplant varied with tx type (Table 1). It was significantly higher in lung and heart recipients as compared with HCT. Peak qEBV $\geq 10^5$ are not common (Fig. 1) but the incidence of EBV disease in these groups was associated with the quantity of EBV. Disease occurred in 92% of pts with qEBV $\geq 10^6$ versus 55% with qEBV $\geq 10^5$ (P=0.03, Fisher exact test). We have identified 3 patterns of posttransplant EBV DNAemia (Fig. 2) and are prospectively investigating their association with tissue-invasive EBV disease. 45 (3%) of 1,385 tx pts developed PTLD (Table 2). qEBV was available for 27/45 pts and 25 (93%) of 27 pts were positive; 21 (84%) of the 25 pts had their highest blood viral loads at the time of PTLD diagnosis. 19 pts were monitored after diagnosis and 17 (90%) experienced a decrease in whole blood viral load with therapy. Although we could not define an exact quantitative threshold for tissue-invasive EBV disease, the qEBV provided guidance on risk of disease for pts with higher amounts of EBV DNAemia.

5. REFERENCES

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