Detection of Multiple Double-Stranded DNA Viruses after Cord Blood Transplantation is Frequent and Persistent

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Background: Latent viral infections remain an important cause of morbidity following cord blood transplantation (CBT). The cumulative incidence, risk factors, and kinetics of reactivation of multiple double-stranded DNA viruses after CBT are unknown. This lack of understanding limits development of strategies for broader prophylaxis.

Methods: Weekly plasma samples through 100 days post-CBT were retrospectively tested by quantitative PCR for HHV-6B, HHV-6A, BK, adenovirus (ADV), and EBV; twice-weekly tests for CMV were performed prospectively. Patients with ≥1 year of follow up and availability of >60% of samples while alive, with <14 days between samples, were included. We identified a cohort of 125 CBT recipients from 2007-2014 with a median of 13 samples per patient (range, 2-14). Cumulative incidence curves of any detection of ≥1, ≥2, ≥3, or ≥4 viruses within 100 days were created, risk factors were analyzed in Cox models, and reactivation dynamics were characterized. Most patients with CMV reactivation were treated with antivirals; this was not accounted for in these data.

Results: Characteristics of the cohort are presented in **Table 1**; 95% of patients had ≥1 virus. Detection rates were: CMV, 58%; HHV-6B, 74%; HHV-6A, 0%; BK, 62%; ADV, 10%; and EBV, 3% (**Table 2**). Detection of multiple viruses at any time and concurrently was frequent (**Fig. 1**); ≥3 viruses were detected in 39% of the cohort at any time and in 14% concurrently. The proportion of patients with detection of any and multiple viruses peaked by wk 4 and persisted through wk 14. Risk factors for ≥2 viruses: *pending analysis*.

Median time to detection was similar for all viruses (3-4 wks) except EBV (7.6 wks; **Table 2**). Median time to peak viral load from first detection was longest for BK (7 wks) and shortest for HHV-6B (3 days). Despite preemptive therapy for CMV, median time to peak viral load was 3 weeks. Among patients with ≥5 samples tested, the median proportion of positive samples after first detection was <50% for each virus except BK with a median of 100% (**Fig. 2**).

Median viral loads after reactivation ranged from 1.9 (CMV) to 3.4 (BK) log_{10} copies/ml; CMV was mitigated by treatment. Max viral loads were significantly higher than first for all except EBV; mean differences ranged from 0.1 (EBV) to 1.6 (BK) log_{10} copies/ml (**Table 2, Fig. 3**). Viral load did not markedly differ if viruses were detected alone or concurrent with other viruses (**Fig. 3**).

Conclusions: We demonstrate frequent and persistent detection of multiple double-stranded DNA viruses through day 100 after CBT. BK demonstrated the greatest and most sustained expansion, whereas HHV-6B reached max levels soon after first detection. These findings provide the rationale to study the impact of multiple virus reactivations on organ disease, health care utilization, and mortality in larger cohorts. This data will be critical to design trials using novel, safer therapies (e.g. CMX001 [brincidofovir], multi-virus-specific T cells) for broad prevention of viral reactivation.

Table 1. Demographic and clinical characteristics

	Overall n=125 n (%)	1 virus n = 28	2 viruses n = 49	≥3 viruses n = 42
Age				
≤20	22 (18)	4 (14)	10 (20)	8 (19)
21-40	37 (30)	5 (18)	12 (24)	20 (48)
41-60	45 (35)	13 (46)	18 (37)	11 (26)
>60	21 (17)	6 (21)	9 (18)	3 (7)
Female	66 (53)	13 (46)	27 (55)	22 (52)
High risk disease	17 (14)	4 (14)	8 (16)	3 (7)
Myeloablative conditioning	60 (48)	12 (43)	21 (43)	26 (62)
Double CBT (vs single)	113 (90)	23 (82)	44 (90)	40 (95)
Grade 3-4 GVHD	21 (17)	6 (21)	9 (18)	6 (14)

Table 2. Characteristics of viral detection

Virus	No. (%)	First detect, wk (IQR)	Max detect, wk (IQR)	Max viral load, (IQR)[a]	Median viral load, (IQR)[a,b]	Mean viral load difference, max-first[a,c]	Median proportion positive (range)[d]
CMV	73 (58)	4 (2, 6.9)	7 (4.9, 10)	2.1 (1. 8, 2.7)	1.9 (1.6, 2.1)	0.5, p <0.001	0.3 (0.04, 1)
HHV-6B	92 (74)	3 (2.4, 4)	3.4 (3, 4.8)	3.5 (3, 4.2)	3 (2.7, 3.3)	0.4, p <0.001	0.25 (0.08, 1)
EBV	4 (3)	7.6 (5.6, 10)	8.6 (6.4, 11.1)	2.5 (2.3, 2.6)	2.3 (2.2, 2.4)	0.1, p = 0.1	–
BKV	77 (62)	4 (2, 5)	11 (7.7, 12.9)	4.5 (3.8, 5.1)	3.4 (3, 3.9)	1.7, p <0.001	1 (0.11, 1)
ADV	12 (10)	4.6 (2.6, 8.1)	9.4 (5.4, 12.3)	3.3 (2.1, 4.5)	2.8 (2.1, 3.1)	1.1, p = 0.02	–

[a]Log_{10} copies/ml

[b]Median of mean viral load for each patient after reactivation

[c]Average maximum viral load minus first positive viral load; p values computed by paired T-test

[d]Proportion of positive samples after 1st positive, restricted to patients with ≥5 samples; EBV and ADV not included due to limited data

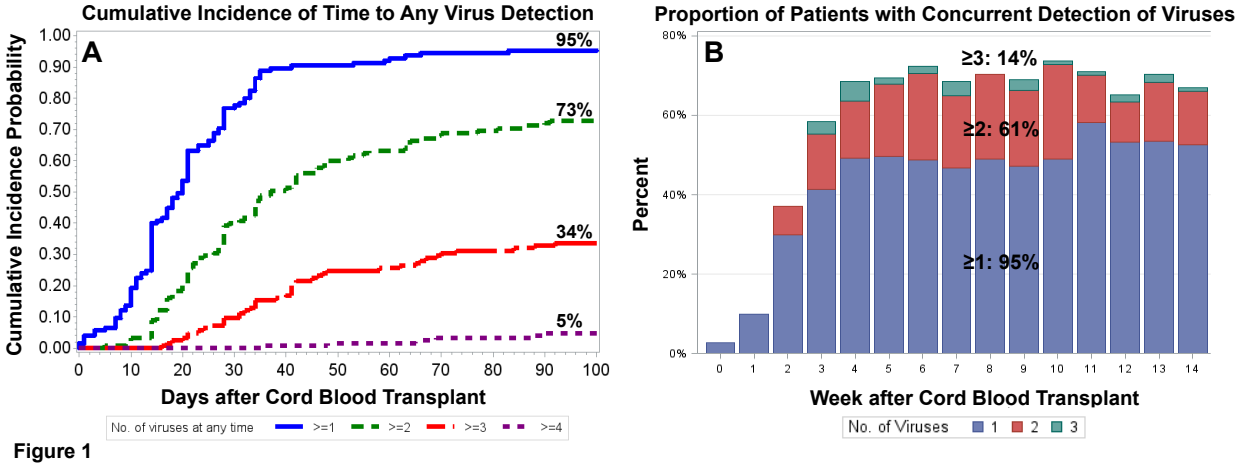

Figure 1

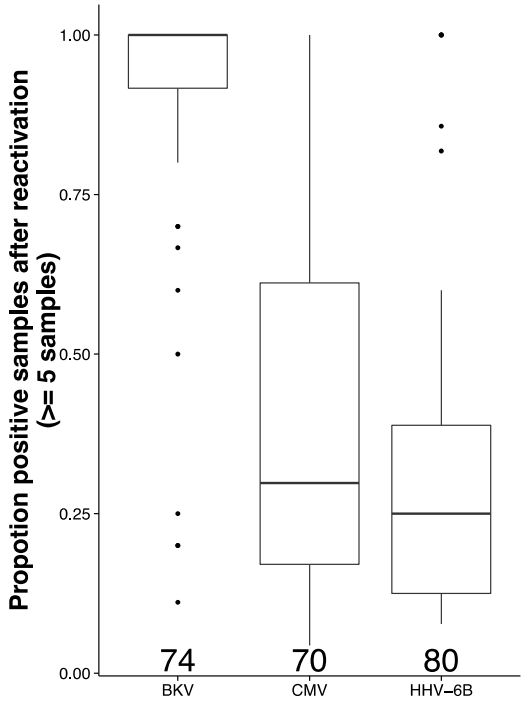

Figure 2. Numbers indicate number of included patients

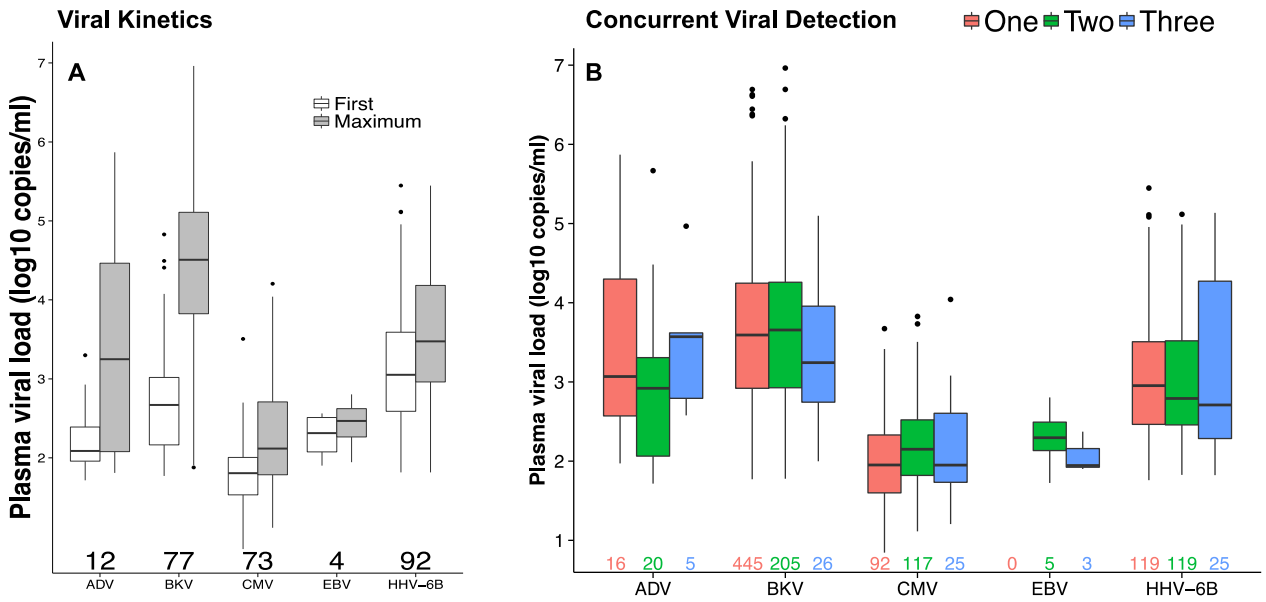

Figure 3. A) Bars represent the distribution of first and max viral load per virus, per patient; numbers indicate # of patients. **B)** Bars represent the viral load per virus in the population when detected alone, with 1 other virus, or with 2 other viruses; numbers indicate # positive samples.