Chronic High EBV DNA load carriage among pediatric liver transplant patients

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Introduction

- Epstein-Barr virus (γ-herpesvirus) infection can have various manifestations ranging from asymptomatic viremia to post-transplant lymphoproliferative disorder (PTLD)
- High/increasing EBV viremia is associated with increased risk for PTLD development
- Monitoring EBV DNA loads in whole blood (WB) after Tx can help identify pts at risk for developing PTLD **before the onset of clinical signs**
- Monitoring EBV loads and pre-emptive reduction of immunosuppression lead to decrease in incidence of PTLD*

**Lee TC at al. Am J Transplamt 2005*

Introduction

- Routine long-term EBV load monitoring identified a group of **patients with chronic high viral loads** (CHVL) not accompanied by lymphoproliferations

Table Frequency of CHVL carrier state in paediatric pts by transplant type*

Liver Tx	Intestinal Tx	Heart Tx
18.4% (36/196)	21% (35/166)	28.6% (20/71)

This finding has led to

- Concerns about the clinical significance of CHVL carriage (risk for PTLD?)
- Questions about an optimal management for pts with CHVL

** Lau AH et al., Pediatric Transplant. 2010*

The purpose of the study

The aim of the study was to analyse the **incidence, characteristics and outcome of CHVL carriage** among paediatric liver transplant (LTx) recipients

Material & Methods

- Retrospective analysis of **101** consecutive paediatric pts who underwent LTx at the Children's Memorial Health Institute in Warsaw between January 2013 and December 2015
- Median follow-up period was **25.3 months** (interquartile range, IQR: 16.4 – 32.9)
- EBV DNA load was routinely measured in WB by real-time PCR (*GeneProof EBV PCR Kit*) as a part of surveillance protocol after LTx
- CHVL carriage was defined as the presence of **high EBV loads** (>3.7 $\log_{10}$ copies/mL) for **>50% of samples for > 6 months** following either asymptomatic state or resolution of EBV disease*
- A diagnosis of EBV infection and PTLD was suspected in patients with **unexplained fever, tonsillitis, lymphadenopathy, upper airway obstruction or GI symptoms**

** Gtoh K, Chronic High EBV after Liver Transplant JID 2010:202*

Material & Methods

- The diagnostic evaluation for PTLD included: EBV viral load, quantitative immunoglobulins, flow cytometry of lymphocytes, radiological examination (chest radioraph, CT scan of neck/chest/abdomen/pelvis)
- Biopsy and histologic evaluation of enlarged lymph nodes and other affected tissue was performed. PTLD was defined histologicaly using WHO definitions (2008)*
- **All pts received an universal a/viral prophylaxis** within 10 days after LTx: iv GCV up to 14 days, then valGCV for up to 3-6 months, followed by oral ACV up to 12 months after LTx
- Pts with active CMV infection, high EBV load and suspicion of EBV disease received antiviral treatment with GCV iv (5 mg/kg/day)
- **Pts with high EBV DNA*emia* had IS decreased to the level considered safe and were closely monitored for signs of acute rejection (AR)**

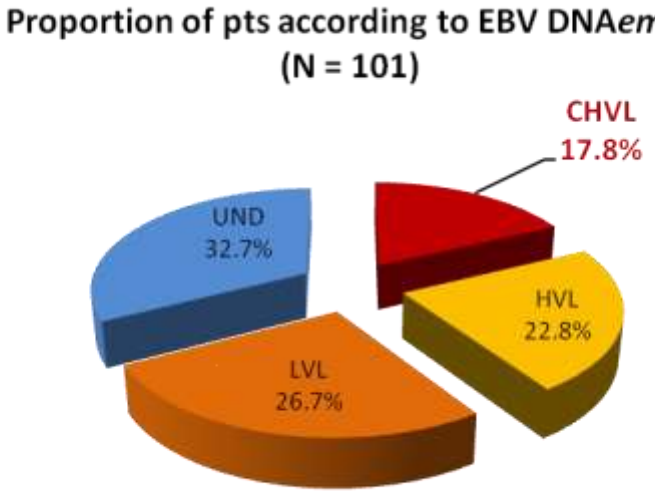
Swerdlow S, et al. WHO Classification of tumours of haematopoietic and lymphoid tissues. 4th edition. Lyon: WHO; 2008. pp. 343

Results

Characteristics of studied patients

Pt after LTx	101
Age at LTx, Me (range), years	4.1 (0.1 – 17.8)
Gender (M/F) No.	53/48
Transplant type, n (%)	
LRdLTx	64 (63.4)
Cadaver	37 (36.6)
ABO -incompatible LTx, n (%)	14 (13.9)
Indication for LTx:	
biliary atresia	35 (34.6)
other	66 (65.4)
Primary IS regimen:	
Tacrolimus + MMF	64 (63.4)
Tacrolimus+ MMF + Prednisone	31 (30.7)
Other (tacrolimus-based)	9 (8.9)
Recipient EBV-negative pre-LTx, n (%)	56 (55.4)
EBV D+/R-	41 (40.6)
Recipient CMV-negative pre-LTx, n (%)	42 (41.6)
CMV D+/R-	29 (28.7)
Post LTx outcome:	
Patients with rejection episode(s), n (%)	34 (33.7)
early AR, n (%)	27 (26.7)
Patients with CMV co-infection, n (%)	50 (50.5)
EBV-related PTLD, n (%)	2 (2.0)

18 of 101 (17.8%) pts met definition of CHVL carriers



UND undetectable or <100 copies/mL
LVL low VL (<5000 copies/mL)
HVL transient high VL (>5000 copies/mL)
CHVL HVL in >50% samples for > 6 mo

Results

Comparisons of clinical features - CHVL vs non-CHVL pt

Characteristics	CHVL (n = 18)	Non-CHVL (n = 83)	P -value
Age at LTx; yrs, Me (IQR)	1.24 (0.79 - 1.81)	6.94 (1.24 – 14.10)	**0.0006**
Gender, male; n(%)	7 (38.9)	46 (55.4)	0.203
EBV status R negative/D positive	14 (77.8)	27 (32.5)	**0.0002**
CMV status R negative/D positive	5 (27.8)	24 (28.9)	0.803
Steroids in primary IS scheme	4 (22.2)	33 (39.8)	0.161
Induction therapy (IL-2RA)	2 (11.1)	15 (18.1)	0.474
ABO incompatibility	3 (16.7))	11 (13.2)	0.704
Rejection, any; n(%) early	5 (27.8) 4 (22.2)	29 (34.9) 23 (27.7)	0.560 0.633
Duration of (val)GCV proph, mo; Me (IQR)	5.95(3.97 - 11.8)	5.03 (3.47 - 7.17)	0.323
Active CMV infection	12 (66.7)	38 (45.8)	0.108
CMV DNA*emia* before EBV DNA*emia* onset	4 (22.2)	16 (19.3)*	0.435
Time of EBV DNA*emia* onset, mo; Me (IQR)	2.20 (1.27 - 5.5)	3.15 (1.83 - 5.93)*	0.303
Trough Tac level at EBV DNA*emia* onset 1 mo before the onset	8.73 ± 2.81 8.54 ± 2.01	9.20 ± 4.54* 8.87 ± 2.00*	0.905 0.630
EBV-related PTLD, n (%)	1 (5.6)	1 (1.2)*	0.230

- The median time to CHVL onset was **10.6 mo after LTx** (IQR: 6.5 – 14.6 months)
- CHVL carriage was assocaited with age at LTx and recipient/donor EBV serostatus before LTx

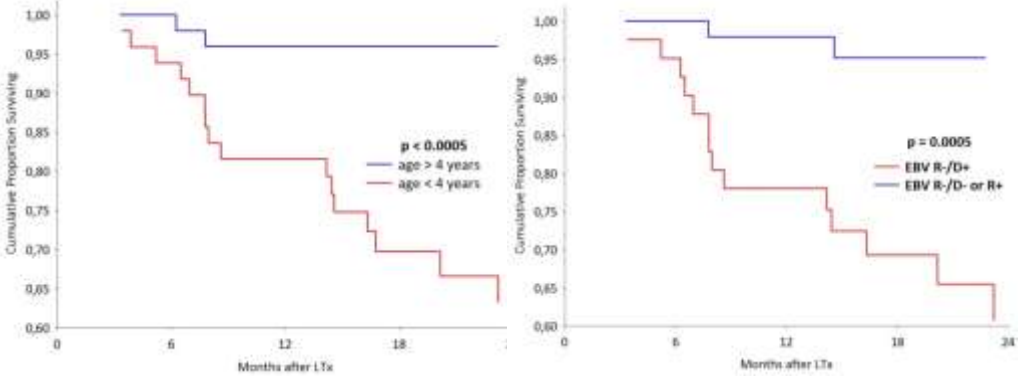


Fig. CHVL-free survival after LTx according to age at LTx (left) or EBV R/D status before LTx (Kaplan–Maier); p-values by log-rank test

* 33 pt from UND group were excluded

Results

EBV loads in WB from patients with CHVL (n =18)

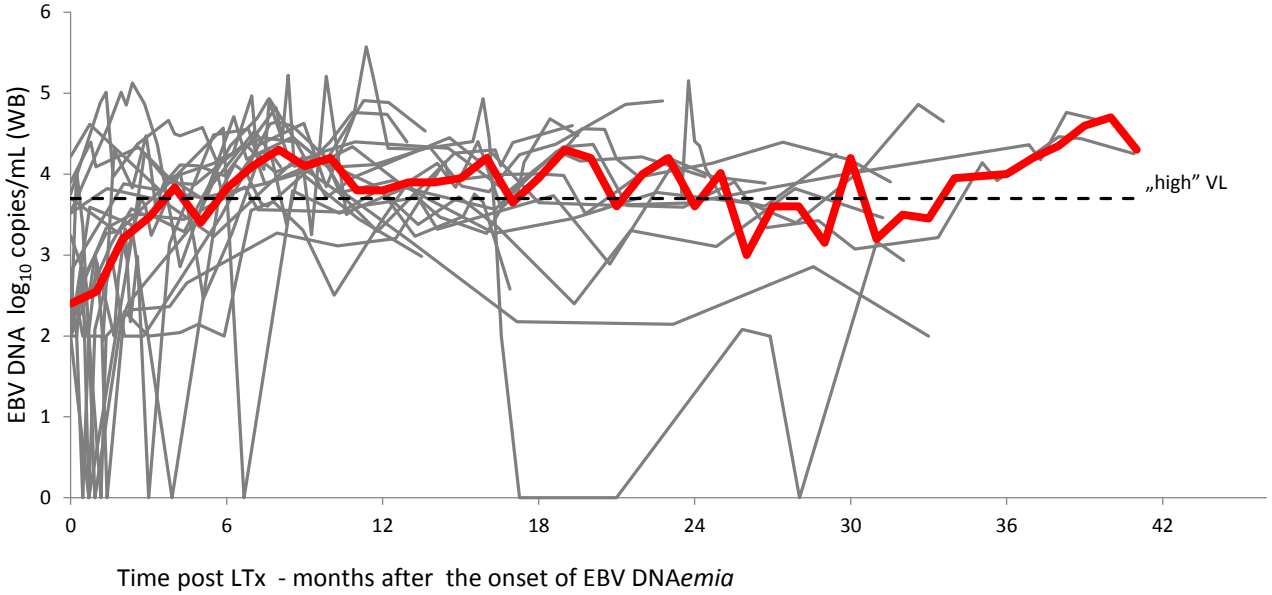


Fig. Kinetics of EBV viremia in pts with CHVL

Individual (gray lines) and median (thick red line) EBV loads in whole blood (n = 18)

ME (IQR) peak EBV DNA: 4.8 $\log_{10}$ copies/mL (4.5 – 5.1)
66 160 copies/mL (34 000 – 133 380)

Results

CHVL – the outcome

In all pt with CHVL immunosupression (i.e. TAC dose) was decreased to the level considered safe

- The CHVL carrier state **resolved without progression to PTLD** in 7/18 (38.9%) pts*, while CHVL continued to **persist <u>without evidence of lymphproliferations</u>** in 10 (55.6%) pts.
- The median time to resolution in those who resolved their CHVL carrier state was **10 months** (range 6.7 – 17.3 months).
- The 10 patients who continued CHVL carriage experienced their carrier state for a median time of **12.6 months** (range 6.5 – 25.8 months) during the study period.

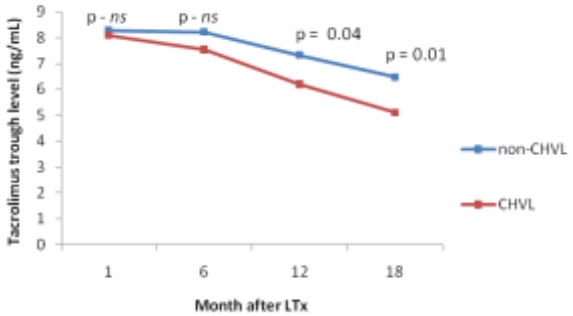


Fig. The average trough TAC levels in patients with CHVL compared to non-CHVL
p-values by Mann-Whitney U test

*Four children experienced more than one episode of CHVL carriage

Results

CHVL outcome - pt with PTLD

- One CHVL pt (5.5%) developed biopsy-proven PTLD (PH) at 16.4 mo after LTx (8.6 mo after CHVL-onset)
- Treatment: RIS (↓FK506) + rituximab (anty-CD20) + GCV (iv)
- **Outcome***:* viral infection resolved, good allograf function was mainteined (AR episode at 26 mo after LTx)

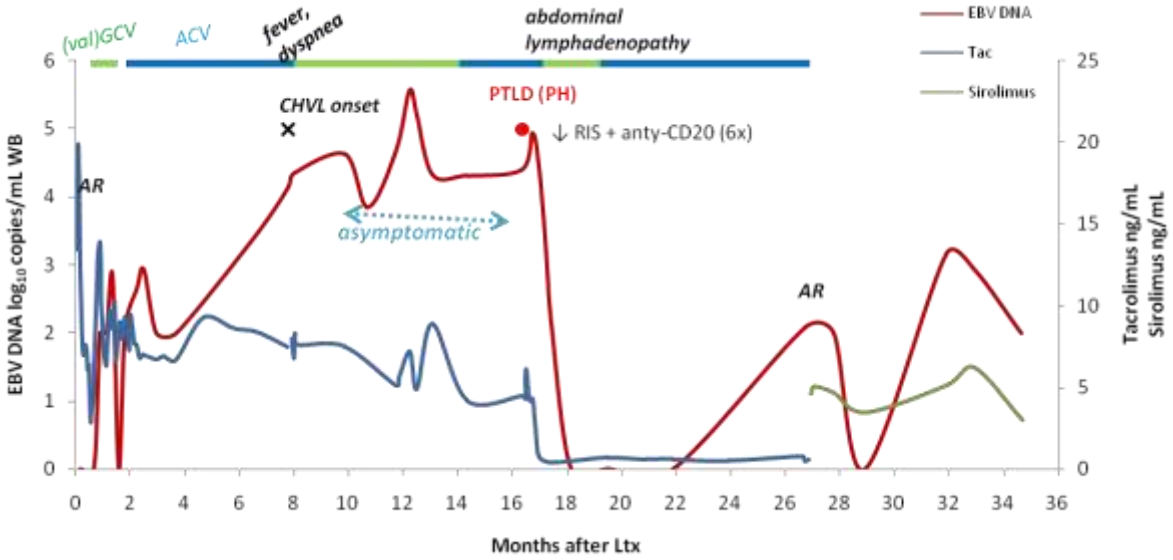


Fig. Longitudinal EBV load and immunosupressive drugs levels (Tacrolimus, Sirolimus) in WB in CHVL pt progessed to PTLD*

* Biopsy of enlarged lymph node (mesentery LN) – plasmatic hyperplasia (Ki67 index 30%, CD20+, EBER+), hypergammaglobulinemia, Monoclonal IgG κ and λ

Results

Comparisons of clinical features - CHVL vs transient high EBV DNA*emia*

Characteristics	CHVL (n = 18)	HVL (n = 23)	P-value
Age at LTx, yrs; Me (IQR)	1.24 (0.79 – 1.81)	1.10 (0.57 – 2.53)	0.687
Gender, male; n(%)	7 (38.9)	13 (56.5)	0.262
LTx type, cadaveric ; n (%)	1 (5.6)	5 (21.7)	0.146
EBV status R negative/D positive	14 (77.8)	16 (69.6)	0.411
CMV status R negative/D positive	5 (27.8)	12 (52.2)	0.104
Steroids in primary IS scheme	4 (22.2)	9 (39.1)	0.248
Induction therapy (IL-2RA)	2 (11.1)	3 (13.0)	0.851
ABOi; n(%)	3 (16.7)	5 (21.7)	0.684
Rejection, any; n(%) early	5 (27.8) 4 (22.2)	10 (43.5) 7 (30.4)	0.300 0.556
Duration of (val)GCV prophylaxis, mo; Me (IQR)	5.95 (3.97 - 11.8)	5.27 (3.47 - 8.4)	0.745
Active CMV infection	12 (66.7)	16 (69.6)	0.843
Period from LTx to EBV DNAemia onset, mo; Me (IQR)	2.20 (1.27 - 5.5)	3.37 (1.73 - 3.8)	0.612
Trough Tac level, (ng/mL) at EBV DNAemia onset	8.73 ± 2.81	10.21 ± 5.12	0.366
1 mo before the onset	8.54 ± 2.01	8.92 ± 2.22	0.572
Peak EBV load, copies/mL; Me (IQR)	$\log_{10}$ 4.8 (4.53 - 5.1)	$\log_{10}$ 4.0 (3.8 - 4.46)	**0.00004**
Period from LTx to peak EBV load, mo; Me (IQR)	11.7 (9.3 - 23.1)	12.8 (6.7 - 17.6)	0.412
Symptomatic EBV infection; n(%)	7 (38.9)	10 (43.5)	0.716
PTLD	1 (5.5)	1 (4.3)	0.858

- Pts with transient HVL and CHVL **did not differ significantly** with regard to baseline characteristics, IS degree (i.e. through Tac levels) before/at the onset of EBV DNA*emia*, occurence of graft rejection or active CMV infection
- Peak EBV loads are significantly higher in WB of CHVL carriers

Conclusions

The results of this study indicate that:

- CHVL carriage is frequent among paediatric pts after LTx
- Younger and EBV-negative prior LTx children are more likely to be CHVL carriers
- Asymptomatic pts with CHVL can develop PTLD
- EBV DNA monitoring and RIS is **crucial especially in high risk pt after LTx**

Thank you for your attention

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