Transfusion-related Epstein-Barr Virus (EBV) infection among allogeneic stem cell transplant recipients: a multicenter prospective cohort study (TREASuRE study)

Helen Trottier, Jacques Lacroix, Marisa Tucci, Carolina Alfieri, Chantal Buteau, Nancy Robitaille, Michel Duval, Phil Spinella, et al.
Introduction

• Epidemiology of Epstein-Barr virus (EBV) infection

• Why we need a project on EBV among pediatric stem cell transplant recipients

• Primary objective of this presentation: update on the multicenter cohort study
Epstein-Barr virus (EBV)

- Member of the *Herpesviridae* family; also known as human herpesvirus 4 (HHV-4)

- One of the most common viruses in humans

- 2 EBV genotypes:
  - EBV type 1 and EBV type 2
  - Multiple strains exist for each genotype
    - Being infected with multiple EBV strains is not rare
Epstein-Barr virus (EBV)

- Cause of
  - infectious mononucleosis
  - many clinical more or less severe manifestations
    - neurologic complications, Guillain-Barré syndrome, hepatitis, etc....
  - certain cancers ie Hodgkin’s lymphoma, Burkitt lymphoma and nasopharyngeal carcinoma
  - post-transplant lymphoproliferative disease (PTLD) and other complications in graft recipients (hepatitis, hemophagocytic syndrome, etc.)
Most people are exposed to the virus as children…

Although the reason for this is not understood, mononucleosis usually occurs in older children and young adults, especially college students.

Most primary EBV infections in childhood remain silent (no noticeable symptoms) or are accompanied by mild signs and symptoms (flu-like symptoms).
In countries with higher hygiene standards:

- **for children**: 0 to 70% from birth to 18 years of age (mean ~ 50%)
  - two childhood peaks of EBV seroconversion are known to occur—at ages 2–4 and 14-18 years

- **for adults**: approximately 90-99%

In countries where hygiene practices are less stringent, EBV is generally contracted in early childhood
Transmission of EBV

- Risk factors for infection
  - Sanitation and hygiene
  - Shared saliva
    - this explains the higher incidence of infection with the onset of sexual activity (in adolescence and early adulthood).
  - EBV does not discriminate between sexes or among racial groups.
  - Transfusion....
Healthy EBV seropositive individuals harbor approximately 0.1 to 5 infected B lymphocytes per $10^6$ peripheral blood mononuclear cells (Rocchi et al 1977; Riddler et al, 1994).

This explains the transmissibility of EBV via the leukocyte component of blood.
Prevalence of EBV detected by PCR among 100 randomly selected blood donors (Texas)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-1/2</td>
<td>0 %</td>
</tr>
<tr>
<td>VZV (HHV-3)</td>
<td>0 %</td>
</tr>
<tr>
<td>Kaposi (HHV-8)</td>
<td>0 %</td>
</tr>
<tr>
<td>EBV (HHV-4)</td>
<td>72%</td>
</tr>
<tr>
<td>HHV-7</td>
<td>65%</td>
</tr>
<tr>
<td>HHV-6b</td>
<td>30%</td>
</tr>
<tr>
<td>CMV</td>
<td>1%</td>
</tr>
</tbody>
</table>

EBV transmission via transfusion

- EBV infection acquired via transfusion after open heart surgery in adults:
  - 4 of 5 EBV-seronegative patients (Gerber et al 1969).
  - 6 of 18 EBV-seronegative patients (Henle et al 1970).

- Transfusion-related infectious mononucleosis, 13 days after transfusion in a 19 year old immunocompetent patient (Tattevin et al, 2002).

- EBV transmission from a 21 yr old blood donor to a pediatric solid organ transplant recipient (Alfieri et al, 1996).

- Multiple EBV strains are frequent in hemophilia cohorts
  - Origin ???
Leukoreduction

- Typically, prestorage leukoreduction reduces the number of WBCs per RBC unit from $5 \times 10^9$ to $5 \times 10^6$ (Gibson et al, 2004).

- However, even though leukoreduction significantly reduces the number of EBV genomes in RBC concentrates, it does not completely eliminate EBV-carrying WBCs (Wagner et al, 1995).

- Analogy? Leukoreduction decreases the risk of contracting CMV but does not abolish it.
  - This is why we screen for CMV in at risk populations (such as transplant recipients and immunocompromised patients).
  - Interestingly, we do NOT screen for EBV.
EBV and PTLD / other complications

- EBV causes post-transplant lymphoproliferative disorder (PTLD) which is the result of the outgrowth of EBV-infected cells that would normally be controlled by a functioning immune system.
  - About 3% of pediatric allogeneic hematopoietic stem cell transplant (HSCT) recipients (Ocheni et al, 2008).
  - Life-threatening complication after transplantation (50-80% mortality).
- Related to many other complications (hepatitis, hemophagocytic syndrome, etc).
- Reactivation of EBV may explain PTLD and other complications in seropositive recipients but not in naïve recipients receiving an EBV-negative graft....
Are EBV infected blood products a problem?

- Probably not the case for immune competent individuals
  - Acquiring EBV if immune competent is not a big deal….

- Possibly an issue for immune compromised patients … especially in the pediatric population where EBV seroprevalence is lower than in adults.
  - Pediatric graft recipients receive a very large volume of blood product transfusions during the peri-transplant period.
Preliminary study

- Retrospective cohort study (via chart review) of 422 pediatric patients who have received HSCT at Ste-Justine Hospital from 1993 to 2009
  - EBV antibodies in pre-transplant sera from recipients and donors (VCA, EA, EBNA)
  - Post-transplant EBV PCR results in recipients until 1-year post-transplant
  - Transfusion history and general characteristics of the recipients.
Study results

- 42% (179) female; 58% (250) male
- Mean age at transplantation: 9.1 years (SD=6.1)
- Median age: 8.8 years (IQR:3.6-14.5)
- Transplantation types: autologous (32%), allogeneic-cord blood (27%), allogeneic-other (41%).
- Pre-transplant EBV seroprevalence:
  - 79.8% in recipients; 20% of cohort was naïve for EBV prior to transplantation (missing data=7%)
  - 63.8% in donors excluding patients who received autologous and cord blood grafts (missing data=36%)
Recipients with unknown pre-transplant serologic status (N=10)

Seropositive recipients (N=184)

Naive recipients (N=44)

Figure 1. Kaplan Meier curve for the cumulative incidence of post-transplant EBV infection among recipients according to their pre-transplant EBV serologic status.

- 30 days = 4.6% (1.2-17.0)
- 60 days = 12.7% (5.5-28.1)
- 100 days = 18.8% (9.4-35.7)
- 100 days = 32.6% (25.9-40.4)
Figure 2. Kaplan-Meier curve for the cumulative incidence of post-transplant EBV infection among cord-blood transplant recipients only.

- **Naive recipients** (N=24)
- **Seropositive recipients** (N=70)
- **Recipients with unknown pre-transplant serologic status** (N=6)
Adjusted RR for the association between transfusion of blood products and incidence of post-transplant EBV infection.

<table>
<thead>
<tr>
<th>Type of blood product**</th>
<th>Adjusted relative risk and 95% CI</th>
<th>P-value for trend***</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red blood cells (RBC)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (reference)</td>
<td>--</td>
</tr>
<tr>
<td>Yes</td>
<td>2.37 (0.58-9.70)</td>
<td></td>
</tr>
<tr>
<td>Volume of transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ml</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>&lt;850.0 ml</td>
<td>1.99 (0.47- 8.44)</td>
<td>0.047</td>
</tr>
<tr>
<td>850.0-1890.0 ml</td>
<td>2.40 (0.56-10.24)</td>
<td></td>
</tr>
<tr>
<td>&gt;1890.0 ml</td>
<td>2.86 (0.68-12.11)</td>
<td></td>
</tr>
<tr>
<td><strong>Fresh frozen plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1.00 (reference)</td>
<td>--</td>
</tr>
<tr>
<td>Yes</td>
<td>1.34 (0.62-2.93)</td>
<td></td>
</tr>
<tr>
<td>Volume of transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.00 (reference)</td>
<td>0.079</td>
</tr>
<tr>
<td>&lt;200.0 ml</td>
<td>0.70 (0.22-2.25)</td>
<td></td>
</tr>
<tr>
<td>&gt; 200.0ml</td>
<td>3.16 (1.00-11.17)</td>
<td></td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Yes</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Volume of transfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1260 ml</td>
<td>1.00 (reference)</td>
<td>0.012</td>
</tr>
<tr>
<td>1260-2530 ml</td>
<td>1.65 (0.86-3.18)</td>
<td></td>
</tr>
<tr>
<td>&gt; 2530 ml</td>
<td>2.19 (1.21-3.97)</td>
<td></td>
</tr>
</tbody>
</table>
Many post-transplant EBV infections observed in naïve patients occurred after 2000 i.e., subsequent to implementation of universal pre-storage leukocyte depletion in Canada.

1 seronegative recipient had fatal PTLD:
- received 6825 ml of RBCs and 9790 ml of platelets, respectively (leukocyte-reduced products)
Pilot study supports evidence that EBV is transmitted through blood product transfusion in pediatric HSCT recipients.

Limits of the study: missing data, retrospective design, single center.

Strengths:
- Clinically relevant results
- Number of patients too small to draw definitive conclusions on cause and effect relationship between blood product transfusion and PTLD, but large enough to yield interesting RRs.

We have designed a prospective study in the pediatric HSCT population.
Multicenter cohort study
Objectives

1. To measure the association between transfusion of blood products (RBCs, platelets, plasma) and EBV infection in pediatric recipients of allogeneic hematopoietic stem cell grafts (HSCT);

2. To assess the incidence of post-transplant EBV infection and PTLD (or high and increasing EBV viral load) in this population stratified according to the pre-transplant EBV serostatus of the patient and the EBV status of the graft;

3. To describe other complications related to EBV infection in this population.
Inclusion criteria

- Allogeneic stem cell transplant recipients
- Pediatric population (0 / 20 years)
- First transplantation
Basic design

Pre-transplant period

EBV PCR testing every 1-2 weeks until hospital discharge and at follow-up visit thereafter.

Transplantation and post-transplant period

Description of blood products received (date, type, quantity, units)

Surveillance for PTLD and other EBV complications

Transplantation

Hospital Discharge (~ week 6)

End of the follow-up

Recruitment

-1 0 1 2 6 12

Months

EBV antibody testing (recipients and donors).
Outcome

Primary:
• Post-transplant EBV infection (PCR)

Secondary:
• High/increasing viral load / PTLD
• Other complications (hepatitis, hemophagocytic syndrome, etc.)
  – An adjudicating committee will review data to confirm PTLD and other complications related to EBV.
Case Report Form

- Sociodemographic and pre-transplant indicators such as EBV serology (donors and recipients), primary diagnosis, chemotherapy, etc.
- EBV PCR in blood specimen
- Blood products received (type, volume, date, length of storage, etc)
- PTLD suspected (fever, splenomegaly, adenopathy (with localisation), other visceromegaly (hepatomegaly, nephromegaly), effusion (pleural, pericardial, ascitis, etc), mouth lesion (leukoplakia), tonsil abnormalities, other.
- OTHER: EBV PCR testing in other than blood specimen, Radiology, Biopsy /Fluid tap, Hemophagocytic syndrome, Hepatitis, CMV diseases, GVH, Idiopathic pneumonitis, Encephalitis, others
- List of drugs against EBV/ PTLD (ex: rituximab)
Analysis plan

- Kaplan-Meier curves to estimate the cumulative incidence (95% CI) of
  - EBV infection
  - High/increasing viral load / PTLD
  - Other EBV-complications
    - This will be stratified according to the baseline serologic status of recipients and EBV status of the graft

- Cox Proportional-Hazards regression models to measure the association between EBV infection and the transfusion of blood products
  - Plasma, RBCs, Platelets.
Determining the source of EBV in severe EBV infections

- Genotype the EBV strain from patients with high/increasing EBV viral load (expected ~3%)

- Blood units administered to these patients will be traced back to the donors who in turn (with consent) will be serologically assessed for EBV, and all seropositive donors will have their EBV strain genotyped for comparison to the patient’s strain.
Sample size

- Sample size have been estimated using preliminary data (published from our pilot study, Trottier et al, 2012) and PASS v.12 software

- Realistic sample size expected: 400 allogeneic HSCT
1) Association between plasma and EBV infection

Two Independent Proportions (Null Case) Power Analysis

Numeric Results of Tests Based on the Odds Ratio: O1 / O2
H0: O1/O2=1. H1: O1/O2=OR1<>1. Test Statistic: Z test with pooled variance

<table>
<thead>
<tr>
<th>Sample Prop</th>
<th>H1 Prop</th>
<th>O.R. if H0</th>
<th>O.R. if H1</th>
<th>Target Alpha</th>
<th>Actual Alpha</th>
<th>Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>P1</td>
<td>1.000</td>
<td>2.500</td>
<td>0.0500</td>
<td>0.1999</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>P2</td>
<td>0.5172</td>
<td>0.3000</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: exact results based on the binomial were only calculated when both N1 and N2 were less than 100.

Based on an 100-day cumulative incidence of EBV of 30%, a proportion of recipients exposed to plasma of 12% (sample allocation ratio=0.13) and an expected RR of ~2.5 (assumptions made with data provided by Trottier et al, 2012).
2) Association between RBCs and EBV infection

Based on a 100-day cumulative incidence of EBV of 30%, a proportion of recipients exposed to RBCs of 89% (sample allocation ratio = 8.1) and an expected RR of ~2.5.
### 3) Association between Platelets and EBV infection

Based on an 100-day cumulative incidence of EBV of 30%, a proportion of recipients exposed to low level of platelets of 50% (sample allocation ratio=1) and an expected RR of ~2.5 (assumptions made with data provided by Trottier et al, 2012).

#### Two Independent Proportions (Null Case) Power Analysis

Numeric Results of Tests Based on the Odds Ratio: $O_1 / O_2$

- $H_0: O_1/O_2=1$. $H_1: O_1/O_2=OR1<>1$. Test Statistic: Z test with pooled variance

| Sample Size | Sample Prop| H1 | Prop | O.R. | O.R. | Target | Actual
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Grp 1</td>
<td>Grp 2</td>
<td>Trtmnt</td>
<td>Control</td>
<td>if H0</td>
<td>if H1</td>
<td>Alpha</td>
<td>Alpha</td>
</tr>
<tr>
<td>N1</td>
<td>N2</td>
<td>P1</td>
<td>P2</td>
<td>OR0</td>
<td>OR1</td>
<td>0.0500</td>
<td>0.0501</td>
</tr>
<tr>
<td>81</td>
<td>81</td>
<td>0.5172</td>
<td>0.3000</td>
<td>1.000</td>
<td>2.500</td>
<td>0.1954</td>
<td></td>
</tr>
</tbody>
</table>

Note: exact results based on the binomial were only calculated when both N1 and N2 were less than 100.
4) Estimation of the 30-day cumulative incidence of EBV per group

1) Seronegative recipients receiving negative graft.

<table>
<thead>
<tr>
<th>Confidence Level</th>
<th>Sample Size (N)</th>
<th>Target Width</th>
<th>Actual Width</th>
<th>Proportion (P)</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Width if P = 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.950</td>
<td>120</td>
<td>0.100</td>
<td>0.100</td>
<td>0.070</td>
<td>0.031</td>
<td>0.131</td>
<td>0.185</td>
</tr>
</tbody>
</table>

->expected 30-day cumulative incidence of EBV of 7% (pilot data)

2) Seronegative recipients receiving positive graft.

<table>
<thead>
<tr>
<th>Confidence Level</th>
<th>Sample Size (N)</th>
<th>Target Width</th>
<th>Actual Width</th>
<th>Proportion (P)</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Width if P = 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.950</td>
<td>81</td>
<td>0.100</td>
<td>0.100</td>
<td>0.040</td>
<td>0.009</td>
<td>0.109</td>
<td>0.226</td>
</tr>
</tbody>
</table>

->expected 30-day cumulative incidence of EBV of 4% (pilot data)
4) Estimation of the 30-day cumulative incidence of EBV per group (con’t)

3) Seropositive recipients receiving negative graft.

<table>
<thead>
<tr>
<th>Confidence Level</th>
<th>Sample Size (N)</th>
<th>Target Width</th>
<th>Actual Width</th>
<th>Proportion (P)</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Width if ( P = 0.5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.950</td>
<td>55</td>
<td>0.100</td>
<td>0.099</td>
<td>0.020</td>
<td>0.001</td>
<td>0.100</td>
<td>0.276</td>
</tr>
</tbody>
</table>

->expected 30-day cumulative incidence of EBV of 2% (pilot data)

4) Seronegative recipients receiving positive graft.

<table>
<thead>
<tr>
<th>Confidence Level</th>
<th>Sample Size (N)</th>
<th>Target Width</th>
<th>Actual Width</th>
<th>Proportion (P)</th>
<th>Lower Limit</th>
<th>Upper Limit</th>
<th>Width if ( P = 0.5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.950</td>
<td>81</td>
<td>0.100</td>
<td>0.100</td>
<td>0.040</td>
<td>0.009</td>
<td>0.109</td>
<td>0.226</td>
</tr>
</tbody>
</table>

->expected 30-day cumulative incidence of EBV of 4% (pilot data)
Proposal submission…

- Proposal has been submitted to CIHR on September 17th
- Budget estimated at 882 000 (Can $)
- CRF and Site Report Form ready
- Letters of collaboration provided by 6 centers (Canadian & American) for the proposal submission
Minimal requirements

- EBV Serology testing pre-transplantation
  - 10-30 days before transplantation (both recipients and donors)

- EBV PCR testing post-transplantation
  - At least once / 1-2 week during hospitalisation,
  - Twice / month from discharge to 6 month post-transplant
  - Once a month (if possible) from 6 month to 12 months

- 1 year follow-up post-transplantation for PTLD surveillance (and other EBV complications).
<table>
<thead>
<tr>
<th>Centers</th>
<th>Expected number for 2 year*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Canada</strong></td>
<td></td>
</tr>
<tr>
<td>CHU Sainte-Justine (Montreal)</td>
<td>66</td>
</tr>
<tr>
<td>Children's Hospital (Winnipeg)</td>
<td>18</td>
</tr>
<tr>
<td>Hospital for Sick Children (Toronto)</td>
<td>90</td>
</tr>
<tr>
<td><strong>United States</strong></td>
<td></td>
</tr>
<tr>
<td>St. Louis Children’s Hospital</td>
<td>36</td>
</tr>
<tr>
<td>Masonic Cancer Center (Minneapolis)</td>
<td>136</td>
</tr>
<tr>
<td>Medical College of Wisconsin (Milwaukee)</td>
<td>54</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>400</td>
</tr>
</tbody>
</table>

* With a 90% participation rate
Acknowledgements

Dr. Phil Spinella
- Thank you !!!

Dr. Steiner

All sites who accepted to provide a letter of collaboration
- Dr. Verneris (US)
- Dr. McArthur (US)
- Dr. Spinella (US)
- Dr Duval (Canada)
- Dr. Egeler (Canada)
- Dr. Cuvelier (Canada)

Big special thanks to the blinded reviewers from the Blood Net for the comments
Loss on follow up?

- Not expected in the peritransplant period as patients will be hospitalized.

- However, loss on follow-up are possible for patients transferred in other centers after hospitalization.
Ethical considerations

- Possibly waived consent as the study is observational.
- Consent will be required for severe cases and their traced blood donors (genotyping).