Update in Hematopathology, Maui Hawaii
09/23-27/2019

Post-Transplant Lymphoproliferative Disorders and EBV-Positive Mucocutaneous Ulcer

Robert W. McKenna, M.D.
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Robert W. McKenna, MD
Lecture Talking Points

• Review of Post-Transplant Lymphoproliferative Disorders
  – Incidence, epidemiology and risk factors
  – EBV viral load monitoring as a surveillance tool
  – Pathology and classification
  – Management and prognosis of PTLD
• EBV-positive mucocutaneous ulcer (EBV MCU)
• EBV MCU in organ transplant recipients
• Reappraisal of immunodeficiency-associated lymphoproliferative disorders
Post-transplant Lymphoproliferative Disorders (PTLD)

- Lymphocytic or plasma cell proliferations resulting from iatrogenic immunosuppression in solid organ (SOT) or allogeneic hematopoietic cell (allo-HCT) transplant recipients
- Range from benign lymphoid hyperplasia to high grade lymphomas
- ~85% of PTLD arise from B lymphocytes
- ~15% from T lymphocytes or NK cells
Epidemiology of PTLD

- Risk of a lymphoproliferative disease following transplant is 8 (adults) to 30 (children) times higher than in the general population for renal transplant recipients
- Most common malignancy in children post-SOT
- In adults, 2\textsuperscript{nd} most common after skin cancers
- In SOT recipients it is the most common cause of cancer related mortality
# PTLD in SOT Versus allo-HCT

<table>
<thead>
<tr>
<th>SOT Recipients</th>
<th>allo-HCT Recipients</th>
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<tbody>
<tr>
<td>• 2 -10% will develop PTLD</td>
<td>• 0.5 - 2% will develop PTLD</td>
</tr>
<tr>
<td>• PTLD of host cell origin</td>
<td>• PTLD of donor cell origin</td>
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<tr>
<td>– donor origin in some liver and lung allografts</td>
<td></td>
</tr>
<tr>
<td>• Median onset 30 to 40 mo. post-transplant</td>
<td>• Peak incidence - 2 to 6 mo. post-transplant</td>
</tr>
<tr>
<td>• Majority of cases are EBV related</td>
<td>• Majority of cases are EBV related</td>
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Epstein-Barr Virus (EBV) in PTLD

• ~ 70 to 75% of PTLD are Epstein-Barr virus (EBV) related (>80% of B cell PTLD)
• PTLD occurring in the first 2 yrs. post-transplant are mostly EBV related
• Late occurring PTLD is mostly EBV-neg.
Risk Factors for Developing PTLD

- Children <10 yrs.; adults >60 yrs.
- EBV seronegative recipient
  - Especially with a seropositive donor
  - 10-76 times greater incidence
- CMV seronegative with seropositive donor
## Risk Factors for Developing PTLD

- **Type of Transplant**

<table>
<thead>
<tr>
<th>Organ Tx</th>
<th>Incidence</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>2.1%</td>
<td>Paraskevas, et al, 2005</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.3%</td>
<td>Singave, et al, 2015</td>
</tr>
<tr>
<td>Liver</td>
<td>2.8%</td>
<td>Singave, et al, 2015</td>
</tr>
<tr>
<td>Heart</td>
<td>6.0%</td>
<td>Singave, et al, 2015</td>
</tr>
<tr>
<td>Lung</td>
<td>10%</td>
<td>Singave, et al, 2015</td>
</tr>
<tr>
<td>Heart-lung</td>
<td>4.6-12.5</td>
<td>Swerdlow et al, 2000</td>
</tr>
<tr>
<td>Small bowel</td>
<td>20.0%</td>
<td>Singave, et al, 2015</td>
</tr>
<tr>
<td>Allo-HCT</td>
<td>0.5-2.5%</td>
<td>Singave, et al, 2015</td>
</tr>
</tbody>
</table>
Immunosuppression as a Risk Factor for PTLD

• Intensity and Duration (cumulative intensity)
  – Solid Organ Transplant
    • OKT3 (21.5%), ATG (4.9%), IL-2 receptor antagonist (7.8%)
  – Allogeneic HCT
    • T-cell depletion of donor marrow or peripheral blood stem cell product (up to 17%)
Risk Factors for Developing PTLD

• Allogeneic HCT
  – Degree of HLA mismatch
  – HLA type
    • HLA-A26 and B38 halotypes -- risk factors
  – Severity of GVHD
  – Transplant for primary immunodeficiency
Post-transplant monitoring for EBV Viral Load as a Surveillance Marker for PTLD

Quantitative Epstein-Barr virus shedding and its correlation with the risk of post-transplant lymphoproliferative disorder
Holman CJ, Karger AB, Mullan BD, Brundage RC, Balfour HH.
Department of Laboratory Medicine and Pathology, University of Minnesota
Clin Transplant 2012; 26: 741-747

Pattern Analysis of Epstein-Barr Virus Viremia and Its Significance in the Evaluation of Organ Transplant Patients Suspected of Having Posttransplant Lymphoproliferative Disorders
Young-Uk Cho, MD, PhD, Hyun-Sook Chi, MD, PhD, Seongsoo Jang, MD, PhD, Sang Hyuk Park, MD, and Chan-Jeoung Park, MD, PhD

From the Department of Laboratory Medicine, University of Ulsan College of Medicine and Asan Medical Center, Seoul, Korea.
Am J Clin Pathol 2014;141:268-274
Study Conclusions on Quantitative EBV Monitoring

• Viremic patients developed PTLD ~ 6 times more frequently compared non-viremic patients

• Patients with PTLD vs those without have significantly (p=.002):
  – higher first positive results
  – higher peak levels
  – higher rate of increase in EBV viral load

• EBV PCR with careful attention paid to changes in EBV viremia can lead to earlier diagnosis and treatment of PTLD
EBV DNA viral load monitoring

• A useful surveillance tool in patients at risk of early EBV associated PTLD
  – Good negative predictive value (>90%)
  – Poor positive predictive value (25-65%)
  – Thresholds defining risk and specific trigger points for preemptive intervention are not well defined
  – Most useful for monitoring of
    • Seronegative organ recipients, especially those with a seropositive donor, for 1st year and selected patients beyond the 1st year, eg., children
    • Engrafting Allo-HCT recipients
Diagnosis and Classification of PTLD

• Excisional tissue biopsy is preferred
  – Necessary for assessing architectural features
  – Intralesional heterogeneity in some PTLD
  – Need for sufficient tissue for ancillary studies

• Important ancillary studies
  – Immunophenotyping
    • Flow cytometry and immunohistochemistry
  – EBER-ISH (EBV-encoded small nuclear RNA-in situ hybridization)
  – Cytogenetic/molecular studies
WHO Categories of PTLD (2016)

**Category**
- Non-destructive PTLDs (early lesions)
- Polymorphic PTLD
- Monomorphic PTLDs (classify according to lymphoma they resemble)
- Classical Hodgkin lymphoma PTLD

**Sub-type**
- Plasmacytic hyperplasia
- Infectious mononucleosis
- Florid follicular hyperplasia
- B cell neoplasms*
  - Diffuse large B cell lymphoma
  - Burkitt lymphoma
  - Plasma cell myeloma
  - Plasmacytoma
- T cell neoplasms
  - Peripheral T cell Lymphoma, NOS
  - Hepatosplenic T cell lymphoma

*Indolent B-cell lymphomas such as follicular or MALT lymphomas are designated as in a immunocompetent individual and not PTLD Exception: EBV+ MALT of the skin
## Non-destructive PTLDs (Early lesions) (~10%)

<table>
<thead>
<tr>
<th>Architecture</th>
<th>Preserved, but often a mass lesion</th>
</tr>
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<tbody>
<tr>
<td>Clinical</td>
<td>Younger age</td>
</tr>
<tr>
<td></td>
<td>Solid organ recipients without a</td>
</tr>
<tr>
<td></td>
<td>prior EBV infection</td>
</tr>
<tr>
<td></td>
<td>LN, tonsils and adenoids</td>
</tr>
<tr>
<td></td>
<td>Stage 1E</td>
</tr>
<tr>
<td>Major</td>
<td>Hyperplastic follicles &amp; paracortical expansion</td>
</tr>
<tr>
<td>findings</td>
<td>Small lymphocytes, plasma cells,</td>
</tr>
<tr>
<td></td>
<td>± immunoblasts</td>
</tr>
<tr>
<td>Immuno-phenotype</td>
<td>Polyclonal B-cells, T-cells &amp; plasma cells</td>
</tr>
<tr>
<td></td>
<td>Often EBV+</td>
</tr>
<tr>
<td>Genetics</td>
<td>Polyclonal or oligoclonal</td>
</tr>
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Non-destructive PTLD (IM-Like)

• Changes associated with IM in the normal host

• May be difficult to distinguish from polymorphic PTLD in tonsils

• Must exclude partial involvement by a monomorphic PTLD in some cases

# Polymorphic PTLD (20-30%)

| Architecture          | Effaced  
|-----------------------|----------
|                       | Destructive, usually extranodal masses |
| Clinical              | Most common in children, usually after EBV-infection |
|                       | Lung, GI, LN, tonsils, Stage 1E-IV |
| Major findings        | Full spectrum of lymphoid maturation |
|                       | Often geographic necrosis |
|                       | Scattered atypical immunoblasts |
|                       | RS-like cells |
| Immuno-phenotype      | Poly or monoclonal B-cells |
|                       | Admixed T-cells |
|                       | RS-like cells: CD30+, CD20+, CD15-EBER-positive |
| Genetics              | Clonally rearranged: monoclonal B-cells (75%), non-clonal T |

Courtesy of Elizabeth Courville, MD.
Polymorphic PTLD
Courtesy of Elizabeth Courville, MD.
Monomorphic PTLD (~60%)

<table>
<thead>
<tr>
<th>Architecture</th>
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<tbody>
<tr>
<td>Clinical</td>
<td>Extranodal masses; Older age (56 yrs.) LN, BM, skin, soft tissue Stage IIIE, IV</td>
</tr>
<tr>
<td>Major findings</td>
<td>Monomorphic, high grade lymphoma Some have bizarre or multinucleated cells resembling RS cells</td>
</tr>
<tr>
<td>Immuno-phenotype</td>
<td>Clonal B-cells (CD20, CD79a, CD19) Most are non-GC type (CD10-, BCL6-, MUM1+, CD138-) EBV positive in 70%</td>
</tr>
<tr>
<td>Genetics</td>
<td>Clonal B-cells or T-cells Somatically mutated IGH, p53, RAS, MYC rearrangements, BCL6 somatic hypermutation (90%), multiple chromosomal gains (trisomies 2, 7, 9, 11, 22, X), losses, breaks (1q region)</td>
</tr>
</tbody>
</table>
### Monomorphic PTLD, T/NK-cell type (<15%)

<table>
<thead>
<tr>
<th>Architecture</th>
<th>Effaced</th>
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</table>
| Clinical     | Usually extra-nodal  
              Poor outcome, 60% dead at 2 months from Dx |
| Major findings | Peripheral T-cell lymphoma NOS  
                   Hepatosplenic T-cell lymphoma (10% of T cell PTLD)  
                   Aggressive NK/T cell lymphoma (rare)  
                   May resemble polymorphic PTLD |
| Immunophenotype | Loss of T-cell antigens  
                  1/3 EBER-positive |
| Genetics     | Clonal TCR rearrangement  
                   i7(q10), +8 (HSTL)  
                   P53 and other oncogene mutations common |
# PTLD, classical Hodgkin lymphoma (<1-8%)

<table>
<thead>
<tr>
<th>Architecture</th>
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</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>Most common in young adults following renal transplant 5yrs. post TX; 30M:1F Stage 1-2, nodal 75% survival</td>
</tr>
<tr>
<td>Major findings</td>
<td>Fulfills criteria for CHL (MC&gt;NS&gt;LD)</td>
</tr>
<tr>
<td>Immuno-phenotype</td>
<td>~100% EBER-positive</td>
</tr>
<tr>
<td>Genetics</td>
<td>Conality is not easily demonstrable No other genetic abnormalities</td>
</tr>
</tbody>
</table>

Must full fill both morphologic and immunophenotypic criteria for CHL

Jaffe E et al. Hematopathology. 1st edn 2011
Classification Issues

• Intraleisonal variability
• Variation between different sites of disease
• Overlapping histologic features
  – No absolute guidelines for dealing with borderline lesions
    • IM from polymorphic PTLD
    • Polymorphic from some DLBCL monomorphic PTLD
• Significant variation in classification among “experts”
EBV Negative PTLD
(25-35% of cases)

• Technical difficulties in detecting EBV

• EBV lost in transformation (hit and run theory)
  – Chromosomal translocations and point mutations supplanted EBV as a driving force

• Other oncogenic viruses (? HHV8, CMV, etc)

• Chronic antigenic stimulation
# Comparison of EBV(+) and EBV(-) PTLD

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EBV + PTLD</th>
<th>EBV - PTLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>40 years</td>
<td>50 years</td>
</tr>
<tr>
<td>Median time from Tx PTLD in 1st yr. post-Tx</td>
<td>10-23 months 42%</td>
<td>50-60 months 15%</td>
</tr>
<tr>
<td>Morphology</td>
<td>Polymorphic 36% 43%</td>
<td>Monomorphic 7% 65-90%</td>
</tr>
<tr>
<td>Immunophenotype (GC type)</td>
<td>15%</td>
<td>60%</td>
</tr>
<tr>
<td>Prognosis (DOD) (Nelson) 5 yr. survival (Heil)</td>
<td>20% 47%</td>
<td>40% 51%</td>
</tr>
</tbody>
</table>

Treatment of PTLD

• Goals of treatment:
  – Eliminate the PTLD
  – Preserve the transplanted graft
Monomorphic B-cell PTLD: Treated as systemic polymorphic PTLD
Prognosis for PTLD

• Highly variable depending on pathology and other risk factors
  – 25 to 60% mortality rate
• 3-year overall survival (OS) rates >60%
  – 73% OS in one large recent study with rituximab use
EBV Positive Mucocutaneous Ulcer—A Study of 26 Cases Associated With Various Sources of Immunosuppression

Stefan D. Dojcino, MD, FRCPath,* Girish Venkataraman, MD,†
Mark Raffeld, MD,† Stefania Pittaluga, MD, PhD,† and Elaine S. Jaffe, MD†


• An entity characterized by isolated well-circumscribed, ulcers that occur in mucocutaneous locations
• Majority of lesions occurred in oral cavity (16), the skin (6) or gastrointestinal tract (4) in patients with:
  • Iatrogenic immunosuppression (MTX or AZA, CYA induced): n=8
  • Age related immunosuppression (63-101 years): n=17
  • Hematopoietic stem cell transplant patient: n=1
Morphologic Characteristics of EBV MCU

Polymorphous infiltrate

- Lymphocytes and immunoblasts
- Scattered plasma cells, histiocytes, eosinophils, and dispersed apoptotic cells
- Scattered large pleomorphic cells reminiscent of Hodgkin cells, often R-S-like cells
- R-S-like cells co-express B-cell antigens CD20 (88.5%), CD30 (100%) and some CD15 expression (43%)
- Background necrosis and ulceration

*Am J Surg Pathol 2010;34:405–417*
Disease Course and Follow-up

- All iatrogenic immunosuppressed patients achieved complete remission with reduction in immunosuppression
- 30% of age related EBV-MCU received aggressive therapy
- No disease associated death over a median follow-up of 22 months (3-72 months)

- Conclusion: EBV MCU is a clinicopathologic entity with Hodgkin like features and a self limited indolent course responding well to conservative management
EBV-positive Mucocutaneous Ulcer in Organ Transplant Recipients
A Localized Indolent Posttransplant Lymphoproliferative Disorder

Melissa Hart, MD, Beenu Thakral, MD, Sophia Yohe, MD, Henry H. Balfour, Jr, MD, Charanjeet Singh, MD, Michael Spears, MD, and Robert W. McKenna, MD

EBV+ MCU in Transplant Recipients

• 7/56 (13%) SOT recipients with EBV-positive PTLD had isolated mucosal ulcerative lesions
  – 4 had oral lesions (lip, gingiva, buccal mucosa, tongue)
  – 3 had GI lesions (1 each, esophagus, ileum, rectum)

• Median age was 61 yrs. (range 18-70); 5 were male

• The duration of immunosuppressive therapy prior to symptoms was 0.6 -13 years (median = 6.3 yrs.)
Esophageal ulcer (2X)
EBV MCU resembling monomorphic PTLD, large cell lymphoma

Ulcer on the lip of a 70 yr. old man
10 yrs. post-renal transplant
EBV MCU resembling monomorphic PTLD, large cell subtype

Small Bowel ulcerative lesion
In all cases the large B-cells were CD20, CD30 and EBER positive, R-S-like cells were present in 5/7
CD3 and CD8 immunostain
Whole Blood EBV DNA quantification

• None of the patients had EBV DNA detectable in blood at diagnosis or follow-up (median 16.5 mo.)

• 35 of 44 (80%) SOT patients with systemic PTLD during the same observation period exhibited EBV DNA in blood specimens (P<0.001)
EBV + MCU: a Unique Lymphoproliferative Disorder

- Unlike most other EBV driven lymphoproliferative disorders that are usually a response to a generalized systemic EBV infection
- EBV MCU develop in patients with apparent sufficient immune response to control a systemic EBV infection but
- A level of immune dysregulation that allows for a localized self-limited lymphoproliferative disorder
Clinical Course

• All lesions resolved with
  – Reduction of immunosuppression (7/7)
  – Rituximab (3/7)

• Five patients are living
  – 4 healthy; 1 awaiting second renal transplant

• Two patients died 17 and 60 mos. following resolution of EBV MCU

• No patients recurred with EBV MCU or other PTLD
  (Follow up = 8 to 111 months—median 16.5 months)
EBV+ MCU in Transplant Recipients

• EBV MCU appears to be a distinctive form of EBV-related PTLD
  – Pathologic features of a destructive aggressive PTLD
  – Clinically behaves like a reactive or non-destructive PTLD
  – Not associated with increased whole-blood EBV DNA
  – Likely to resolve with conservative management

• EBV MCU should be considered in the differential diagnosis of post-transplant mucosal ulceration

• Clinicopathologic correlation and staging with modern imaging are essential to exclude systemic PTLD
Immunodeficiency-associated lymphoproliferative disorders: time for reappraisal?
Natkunam Y1, Gratzinger D1, Chadburn A2, Goodlad J3, Chan JK4, Said J5, Jaffe E6, de Jong D7.

Abstract
Immunodeficiency-associated lymphoproliferative disorders (IA-LPDs) are pathologically and clinically heterogeneous. In many instances, similar features are shared by a spectrum of IA-LPDs in clinically diverse settings. However, the World Health Organization (WHO) classifies IA-LPDs by their immunodeficiency setting largely according to the paradigm of posttransplant lymphoproliferative disorders but with inconsistent terminology and disease definitions. The field currently lacks standardization and would greatly benefit from thinking across immunodeficiency categories by adopting a common working vocabulary to better understand these disorders and guide clinical management. We propose a 3-part unifying nomenclature that includes the name of the lesion, associated virus, and the specific immunodeficiency setting for all IA-LPDs. B-cell lymphoproliferative disorders (LPDs) are usually Epstein-Barr virus (EBV)+ and show a spectrum of lesions, including hyperplasias, polymorphic LPDs, aggressive lymphomas, and, rarely, indolent lymphomas. Human herpes virus 8-associated LPDs also include polyclonal and monoclonal proliferations. EBV− B-cell LPDs and T- and NK-cell LPDs are rare and less well characterized. Recognition of any immunodeficiency is important because it impacts the choice of treatment options. There is an urgent need for reappraisal of IA-LPDs because a common framework will facilitate meaningful biological insights and pave the way for future work in the field. Blood. 2018 Nov 1;132(18):1871-1878.

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3-Part Unifying Nomenclature

• Name of lesion
  – B-cell hyperplasia (eg, plasmacytic hyperplasia)
  – Polymorphic B-cell lymphoproliferations (eg, MCU)
  – Lymphoma (WHO terminology) (eg, DLBCL)

• Viral status
  – eg, EBV⁺/⁻, HHV8⁺/⁻

• Specific immunodeficiency setting
  – eg, Posttransplant (solid organ), iatrogenic (methotrexate), immune senescence
Examples of Proposed Unifying Nomenclature

- Plasmacytichyperplasia, EBV+, posttransplant (solid organ)
- Polymorphic B-LPD, EBV+, iatrogenic (methotrexate)
- MCU, EBV+, Primary immunodeficiency (CHARGE syndrome)
- Diffuse large B-cell lymphoma, EBV-, HIV infection
- Diffuse large B-cell lymphoma, T-cell/histiocyte-rich, EBV+, immune senescence
- Primary effusion lymphoma, HHV8+, EBV+, HIV infection