Urinary Polyomavirus-HAUFEN are Ultrastructural Biomarkers of Polyomavirus Nephropathy

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Agenda

2. Evidence that PV-Haufen in the urine, indeed, originate in the kidney with PVN – *Proof of Concept studies.*
3. Clinical Significance of Urinary PV-Haufen detection.
Polyomavirus Nephropathy (PVN)

Definition:

Morphologic evidence of polyomavirus replication in the renal parenchyma (cortex and/or medulla).

- Light microscopy
- Immunohistochemistry (SV40-T expression)
- In-situ hybridization
<table>
<thead>
<tr>
<th>Institution</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columbia University, New York</td>
<td>2.7%</td>
</tr>
<tr>
<td>Cornell University, New York</td>
<td>2.8%</td>
</tr>
<tr>
<td>King’s College, London</td>
<td>2.2%</td>
</tr>
<tr>
<td>University of Arizona, Tucson</td>
<td>3.3%</td>
</tr>
<tr>
<td>University of Basel, Switzerland</td>
<td>0%</td>
</tr>
<tr>
<td>University of Chicago</td>
<td>5.8%</td>
</tr>
<tr>
<td>University of Maryland, Baltimore</td>
<td>9.5%</td>
</tr>
<tr>
<td>University of North Carolina, Chapel Hill</td>
<td>4.2%</td>
</tr>
<tr>
<td>University of Pittsburgh, PA</td>
<td>2.7%</td>
</tr>
</tbody>
</table>

4% (56/1374)
Renal biopsy is the gold standard to diagnose PVN

Can we do better?
Agenda

PV-HAUFEN in the Urine

voided urine sample with polyomavirus cast in PVN
Hypothesis

PV-Haufen are “viral casts” that originate in the diseased kidney (PVN) and their formation follows the same principles of other cast formation.
Intra-renal cast formation

• Tamm Horsfall Protein (THP) is the backbone of intra-renal casts:

• Red blood cell casts: “RBCs leak into tubules & are trapped in THP matrix to form casts.”

(Schrier’s Diseases of the kidney, 9th edition)
• Why do we only see casts in the urine when there is “intra-renal” disease?

• Under what conditions does Tamm-Horsfall protein lead to cast formation?
THP forms a sticky glue like gel only in a high ionic strength environment and is present as a **polymeric form** under these conditions. These conditions exist only within intra-renal tubules.

This is crucial for intra-renal cast formation.
Agenda

• Evidence that PV-Haufen in the urine, indeed, originate in the tubules in cases of PVN.
Observation #1:
In PVN, polyomaviruses aggregate IN renal tubules
Observation #2:
Polyomaviruses aggregate in the setting of high uromodulin / Tamm-Horsfall Protein concentrations present only in renal tubules.
Observation:
PV-Haufen are flushed into the urine
AGENDA

PVN mouse model including THP knockout mouse studies.
MPV ($5 \times 10^7$ Infectious Virions)

Wild type mouse: Black Swiss

Mouse: THP knockout

Experiments performed in collaboration with Satish Kumar, MD (THP knockout mice).
PVN in mice: PVN grade 2, cortex (4 weeks duration)
Observation #6: Mouse Model of PVN
Urinary PV-Haufen are found in mice with PVN

| Immunohistochemistry for SV40 large T antigen with positive nuclear staining in tubular epithelial cell. | Immunohistochemistry for PV capsid VP1 with positive staining in virally injured tubules. |
Mouse Model with PVN: Electron Microscopy of Kidney
PV-Haufen in voided urine from wild type mouse with PVN

PVN Mouse Model: LACKS PV replication and maturation in the urothelium (SV40 T antigen stain, viral capsid protein stain)
Summary from Proof of Concept Studies:

- PV-Haufen are tightly associated with uromodulin / Tamm-Horsfall protein.
- PV-Haufen formation requires a high concentration of Tamm-Horsfall protein; such concentrations only occurs within intra-renal tubules.
- PV-Haufen are found in a mouse model of PVN that lacks any infection of the urothelium.
  - Urinary PV-Haufen do not form in a THP-knockout mouse with PVN.
• Clinical significance of urinary PV-Haufen detection
Can the detection of urinary PV-Haufen accurately separate patients with and without PVN?

(Qualitative PV-Haufen detection)

### Shedding of Haufen in Patients with PVN versus Control Patients (PV activation only)

<table>
<thead>
<tr>
<th></th>
<th>Urinary PV-HAUFEN present</th>
<th>Urinary PV-HAUFEN absent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PATIENTS WITH Biopsy proven PVN (n = 53)</strong></td>
<td>53/53 (100%)</td>
<td>0/53 (0%)</td>
</tr>
<tr>
<td><strong>PATIENTS WITHOUT Biopsy evidence of PVN (n=277)</strong></td>
<td>3/277 (1%)</td>
<td>274/277 (99%)</td>
</tr>
</tbody>
</table>
The predictive value of urinary PV-Haufen shedding compared to PCR and cytology assays
<table>
<thead>
<tr>
<th></th>
<th>Haufen</th>
<th>Decoy cells</th>
<th>Plasma PCR</th>
<th>Urine PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kappa Statistic</td>
<td>0.98</td>
<td>0.25</td>
<td>0.62</td>
<td>0.34</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>100%</td>
<td>72%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99%</td>
<td>36%</td>
<td>88%</td>
<td>47%</td>
</tr>
<tr>
<td>Positive Predictive</td>
<td>97%</td>
<td>40%</td>
<td>74%</td>
<td>44%</td>
</tr>
<tr>
<td>Value for PVN</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Negative Predictive</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Value for PVN</td>
<td></td>
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Does urinary PV-Haufen shedding mirror the time course of PVN?
Agenda

• PV-Haufen in the setting of bone marrow transplantation
University of North Carolina Bone Marrow Transplantation Unit:

• 45 urine samples from 15 patients with clinical evidence of hemorrhagic cystitis (median time: 3 months; range 2-9 months).

All urine samples PV-Haufen negative

Multicenter Study in Children with Bone Marrow Transplantation

- Children’s Hospital of Philadelphia
- Cincinnati Children’s Hospital Medical Center
Summary

- Urinary PV-Haufen originate in kidneys with PVN.
- The genesis of urinary PV-Haufen is similar to the formation of other renal casts.
- Presence and absence of urinary PV-Haufen accurately reflects the time course of PVN.
- The degree of urinary PV-Haufen shedding reflects the severity of intra-renal PVN.
Agenda

• Negative Staining EM- brief technique used for detection of PV-Haufen
How easy is urine PV-Haufen testing by negative staining EM?
Negative Staining EM for the detection of Urinary PV-Haufen

1. Sample Clarification
   (250g x 5 minutes)
   Supernatant from Step #1

2. Sample Concentration
   (20,000g x 30 minutes)
   Pellet removed for grid preparation

3. Grid Preparation
   (15 minutes)
Grid Staining

Specimen 1: Concentrated Pellet for 5 minutes, then wash for 10 seconds each, and stain for 30 seconds.

Specimen 2: Concentrated Pellet for 5 minutes, then wash for 10 seconds each, and stain for 30 seconds.

Specimen 3: Concentrated Pellet for 5 minutes, then wash for 10 seconds each, and stain for 30 seconds.
Haufen Shedding in Urine

EM Grid

urine concentrate
Collaborators

University of North Carolina at Chapel Hill

- **Electron Microscopy:**
  - Victoria Madden
  - Robert Bagnell

- **Division of Nephrology and Hypertension:**
  - Randy Detwiler
  - Karen True
  - Eddie Fuller
  - Vimal Derebail

- **Division of Transplantation Surgery:**
  - Tomasz Koslowski
  - Valerie Buchholz

- **Division of Bone Marrow Transplantation:**
  - Terrence Comeau
  - Julia Whitley

- **Division of Nephropathology:**
  - Volker Nickeleit
  - Bruna Brylawski
  - Lauraine Rivier

**Centers for Disease Control (CDC):**
- Charles Humphrey

**Children’s Hospital of Philadelphia:**
- Benjamin Laskin

**Cincinnati Children’s Hospital Medical Center:**
- Stella Davies
- Sonata Jodele