**Banff Draft for Polyomavirus Nephropathy Staging:**

**Stage A** *(early changes)*
- Varying degrees of viral replication with intranuclear inclusion bodies **AND / OR** positive immunohistochemistry (SV-40 T antigen) **AND/OR** in-situ hybridization signals
- No or minimal tubular epithelial cell lysis / cell necrosis
- **No** or minimal denudation of tubular basement membranes caused by viral epithelial cell lysis

(_maximal tubular basement membrane denudation caused by viral epithelial cell lysis in stage A: AT MOST two "lysed/detached" epithelial cells in the most affected tubule or collecting duct_)

- Interstitial fibrosis < 50% of renal cortex (Banff chronicity score < ci3)

**Stage A** *(medulla)* - Changes limited to medulla

**Stage A** *(cortex)* - Changes seen in cortex +/- medulla

**Stage B** *(acute / florid changes)*
- Viral replication (typically readily apparent by LM, IHC and other techniques)
- Marked virally induced tubular epithelial cell necrosis / cell lysis with frank **denudation** of associated tubular basement membranes

(_tubular basement membrane denudation caused by viral epithelial cell lysis in stage B: TBM denudation stretching a length of MORE than two "lysed/detached" epithelial cells in the most affected tubule or collecting duct_)

- Interstitial fibrosis < 50% of renal cortex (Banff chronicity score < ci3)

**Stage B** *(medulla)* - Changes limited to medulla

**Stage B** *(cortex)* - Changes seen in cortex +/- medulla

*Stage A differs from stage B by the DEGREE of virally induced epithelial cell lysis and associated denudation of tubular basement membranes that is no more than minimal in stage A and pronounced in stage B; the extent of viral replication, number of SV40-T antigen expressing cells is not considered for staging.*

**Stage C** *(late sclerosing changes)*
- Viral replication in cortex and medulla (minimal to marked)
- Interstitial fibrosis > 50% of cortex (Banff chronicity score = ci3)

*Stages A and B differ from stage C by the degree of chronic tubulo-interstitial injury.*
PVN staging requires careful assessment of the entire specimen/all available levels. The most affected tubule/collecting duct in the cortex or medulla is used to discriminate between PVN stage A and B.

In order to adequately diagnose PVN or eliminate it from the list of differential diagnoses, 2 biopsy cores INCLUDING medulla are required; comment if samples are suboptimal/inadequate.

Immunohistochemistry to detect polyomavirus replication is most easily performed with antibodies directed against the 'SV40-T antigen'.

In PVN stages A-C interstitial inflammation and tubulitis can vary from Banff scores i0-i3/ t0-t3. Inflammation/ tubulitis is often most pronounced in PVN stage B. Stage A may lack an inflammatory reaction.

PVN and rejection (acute, chronic, cell and/or antibody mediated) can concur. Banff types II and III rejection, chronic T-cell mediated rejection, and acute and chronic antibody mediated rejection (C4d positive) should be diagnosed according to standard criteria, in particular considering "Banff-v" and "Banff-g" lesions.

Concurrent Banff category 4, type I cellular tubulo-interstitial rejection (C4d negative) cannot be reliably distinguished from other forms of interstitial nephritis (due to PVN, ATI etc). If inflammation and tubulitis are seen in areas distant from viral replication, e.g. SV40 positive nuclei (by IHC) or virally altered cells with intranuclear inclusion bodies (by LM) in one of 2 inflammed biopsy cores only, then Banff type 1 rejection might be considered.

Score and report histologic polyomavirus load levels (pvl) additionally (scoring is best performed in IHC (SV-40 T) incubations or by in-situ hybridization); pvl.1-pvl.3 can be seen in all PVN stages.

<table>
<thead>
<tr>
<th>pvl</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>&lt; 1% tubules with viral replication</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 1% - ≤ 10% tubules with viral replication</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 10% tubules with viral replication</td>
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PVN staging is performed in the context of the Banff classification and scoring scheme; it does not replace "Banff". Banff 'acute' and 'chronic' lesion scoring is recorded separately.
General aspects to keep in mind:

- The PVN staging takes **2 major aspects** of polyomavirus induced renal injury into account: 1) virally induced tubular epithelial cell injury, and 2) interstitial fibrosis and tubular atrophy caused by persistent, long lasting (virally induced) ATN.

- Interstitial inflammation can be due to rejection, polyomavirus replication or can have other etiologies; inflammation is not used for PVN staging.

- The degree of "viral load", i.e. the percentage of tubules containing infected cells in the cortex and medulla, is assessed on a scale of 1 - 3 and recorded separately (see footnote 6; the number of infected cells/number of infected tubules is not directly used for PVN staging).

- PVN affects the cortex and/or medulla in a focal fashion. Two biopsy cores with medulla are needed for optimal work-up (see footnote 2).

- It is thought that PVN starts in the medulla and subsequently ascends into the renal cortex. This is the reason why PVN stages A (early) and B (florid) are each subgrouped into "only medullary involvement" versus "cortical involvement".

- PVN staging is based on findings made IN THE ENTIRE tissue sample, cortex and medulla. One tubule with significant virally induced denudation of the TBM can suffice for rendering a diagnosis of "PVN stage B" (see details below).

- Laboratory tests, i.e. clinical signs of viremia or viruria, are not used for PVN diagnosis or staging.

PVN Stage A:

PVN stage A is characterized by "mild early morphologic " signs of polyomavirus replication and ".....not much else...".

- Polyomavirus replication is indicated by: intranuclear viral inclusion bodies in tubular and/or parietal epithelial cells.

- intranuclear expression of SV40-T antigen (by IHC or IF)

- detection of polyomavirus DNA (by in-situ hybridization)

- detection of polyomavirus capsid antigens (by IHC or IF)
detection of intracellular virions of 40-50 nanometer in diameter by electron microscopy

Very early stages of PVN may lack light microscopically detectable intra nuclear inclusion bodies and only show crisp intra nuclear staining signals for SV40-T antigen by immunohistochemistry (the T-antigen marks viral replication - it does not mark virus particles). Any 3+ or greater intranuclear staining signal for SV40-T antigen (on a scale of 0-5 +) in one (or more) epithelial cell nuclei (with or without viral inclusion bodies by LM) is considered abnormal and evidence of "polyomavirus replication" / PVN.

Note:
- THE DEGREE OF VIRALLY INDUCED ACUTE TUBULAR INJURY is minimal with no more than 2 lysed epithelial cells in the most affected tubule or collecting duct.
- Polyomavirus load levels (pvl; see footnote 6) can range in PVN stage A from pvl.1 - pvl.3.
- Interstitial inflammation is frequently inconspicuous; the degree of interstitial inflammation is not used for staging.
- Fibrosis involves per definition less than 50% of the cortex.

**PVN Stage A (tubular cross section with virally induced injury):**

Tubule/collecting duct (cortex or medulla) most affected by viral injury shows epithelial cell lysis AND denudation of the TBM over a length of AT MOST two cells per circumference.

*The number of cells with viral intranuclear inclusions is NOT considered for staging.*
**PVN Stage A:**

Tubules in this particular example do not show easily discernible intranuclear viral inclusion bodies by light microscopy. Epithelial cells demonstrate crisp intranuclear expression of SV40-T antigen (staining intensity "4-5" on a scale of "0-5"). There is no evidence of host tubular epithelial cell lysis and denudation of tubular basement membranes is lacking. The interstitium is without significant inflammation or fibrosis.

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**PVN Stage A:**

There is marked SV40-T expression in many tubular epithelial cell nuclei/tubules (staining intensity 5 on a scale of 0-5). In addition also sloughed cells in tubular lumens express SV40-T. However, frank tubular epithelial cell lysis and denudation of corresponding tubular basement membranes is not seen. Such changes -if representative for the entire biopsy sample- are categorized as "PVN-stage A". The interstitium shows inflammation without significant fibrosis.

*The number of cells with viral intranuclear inclusions is NOT considered for staging.*
PVN Stage B:

PVN stage B is characterized by "florid" signs of polyomavirus replication.

In PVN stage B, polyomavirus replication results in severe, virally induced acute tubular injury. In order to be classified as "disease stage B", THE MOST AFFECTED TUBULE/ COLLECTING DUCT in the cortex or medulla shows TBM denudation over a length of MORE than two "lysed/detached" epithelial cells per circumference.

Note:
- Polyomavirus load levels (pvl; see footnote 6) can range in PVN stage B from pvl.1 - pvl.3.
- Interstitial inflammation and tubulitis is often present; the degree of interstitial inflammation is not used for staging.
- Fibrosis involves per definition less than 50% of the cortex.

PVN Stage A:

In PVN stage A, only "minimal" tubular epithelial cell lysis (arrows) and corresponding denudation of tubular basement membranes is found not exceeding 2 cells per cross section in the most affected tubule / collecting duct. The image on the left shows the "maximal change" that is still categorized as disease stage A with early detachment of 2 necrotic cells from the TBM.
**PVN Stage B (tubular cross section with virally induced injury):**

Tubule/collecting duct (cortex or medulla) most affected by viral injury shows epithelial cell lysis AND denudation of the TBM over a total length of MORE THAN two cells per circumference.

*The number of cells with viral intranuclear inclusions is NOT considered for staging.*
**PVN Stage B:**

High power examination shows tubular cross sections with intranuclear viral inclusion bearing epithelial cells, epithelial cell lysis (thin arrow) and denudation of the tubular basement membrane over a length of more than 2 cells (thick arrows and arrow heads) marking these 2 cases as "PVN-stage B. In this particular case, the interstitium shows marked inflammation, edema, and associated tubulitis that are not used for PVN staging. There is no fibrosis.

**PVN Stage B:**

High power examination shows tubular cross sections with relatively severe virally induced epithelial cell injury, viral inclusion bodies, and denudation of the TBM (arrows) over a length of > 2 cells per cross section. In this particular case, interstitial fibrosis was ci-2. Note: sloughed intra tubular / - luminal epithelial cells are not per se indicative of disease stage B if the corresponding TBM segments are not denuded.
PVN Stage C:

PVN stage C is characterized by varying signs of polyomavirus replication, ranging from minimal to marked (pvl.1 - pvl.3), and varying degrees of tubular injury. Interstitial fibrosis is, per definition, > 50%, i.e. Banff ci-3 (typically accompanied by Banff ct-3).

PVN Stage C:

The samples show diffuse interstitial fibrosis and tubular atrophy. Some tubular cells demonstrate intranuclear viral inclusion bodies (arrows). If more than 50% of the renal cortex demonstrates fibrosis (Banff ci-3 lesion) in the setting of polyomavirus replication (polyomavirus load levels pvl.1 - pvl.3), then PVN is classified as PVN-stage C.