GENERAL CONSIDERATIONS: A wide array of viruses can infect the kidney. This includes two important RNA viruses, namely, human immunodeficiency virus (HIV) and hepatitis C virus (HCV) and DNA viruses such as polyomavirus BKV (BKV), cytomegalovirus (CMV, adenovirus (ADV) Epstein-Barr virus (EBV) and other viruses of the Herpesvirus group. While tissue tropism is species-dependent, podocytes, parietal capsular epithelium, capillary endothelium, tubular epithelium and interstitial cells can all support viral replication.

Some viruses produce distinctive cytopathic effect, but diagnosis of others may require in-situ hybridization, immunohistochemistry, immunoassays, culture or PCR. Electron microscopy, if combined with measurement of virion diameter, can be used to assign broad viral families, but not individual species. Ultrastructural recognition of viruses is based on size, shape, presence of an envelope, and arrangement in crystalline arrays. The electron microscopist must be cognizant of viral look-alikes such as intranuclear chromatin filaments, nuclear pores cut enface, annulate lamellae, spherical microvesicles, glycocalyceal bodies, and glycogen crystals.

Clinical presentation of viral nephropathies can be quite variable and includes asymptomatic derangements in renal function tests, proteinuria, acute or chronic renal failure, and mass lesions. A brief overview of the principal viral infections encountered in clinical practice follows. Parvovirus (an occasional cause of collapsing glomerulopathy and thrombotic microangiopathy) and rare viral agents such as Ebola virus and Hantavirus will only be mentioned in passing (1, 2).

HIV associated nephropathy (HIVAN): This is the best known viral syndrome associated with HIV infection. The classical description is an African American man with low CD4 counts, acute onset proteinuria without much edema or hypertension, and nephromegaly (3). Microscopic examination reveals collapsing glomerulopathy, tubular microcysts with pale, PAS negative, fuchsins positive protein precipitates, and a lymphocytic interstitial nephritis with a CD4/CD8 ratio of 0.2-0.8. Ultrastructural
examination reveals cylindrical confronting cisternae, nuclear bodies, granulofibrillar transformation of nuclei, and tubulo-reticular inclusions. Atypical forms of HIVAN are being recognized such as those characterized by mild disease, non-collapsing focal segmental glomerulosclerosis, mesangial sclerosis, or podocyte hypertrophy. Patients with HIVAN are surviving longer and some have shown stable renal function for up to 24 months (4). It has been discovered that G1 and G2 variants of the APOL-1/MYH9 increase the risk of HIVAN by 50%. This provides an explanation for the high incidence of HIVAN in sub-Saharan Africa and its rarity in Caucasians. It also opens the way for implementing screening programs that may allow earlier diagnosis of HIVAN and a further improvement in prognosis (5).

The differential diagnosis of HIVAN should include other syndromes directly linked to HIV infection such as IgA nephropathy with p24 or gp41/120 immune complexes, HIV associated thrombotic microangiopathy, and interstitial nephritis not attributable to any other etiology. It is also necessary to keep in mind opportunistic infections, renal toxicity due to highly active anti-retroviral therapy (crystalline nephropathy, mitochondrial tubulopathies, myoglobinuria), and the usual gamut of renal diseases which may occur in HIV infected patients by coincidence.

**Hepatitis C virus infection:** Systemic symptoms of hepatitis C virus infection usually (but not always) precede kidney involvement. These include liver disease, gastrointestinal tract symptoms, porphyria cutanea tarda, and autoimmune disorders such as Sjogren syndrome, hypothyroidism, and diabetes mellitus (6). The commonest lesion associated with hepatitis C virus in the kidney is cryoglobulinemic glomerulonephritis(7, 8). Less often, biopsies may show non-cryoglobulinemic membranoproliferative glomerulonephritis, membranous nephropathy, leukocytoclastic vasculitis, fibrillary/immunotactoid glomerulonephritis, post-infectious glomerulonephritis or incidental disease. Chronic transplant glomerulopathy, TMA and interferon-α therapy precipitated acute rejection have been described in the allograft kidney. Epidemiologic associations between hepatitis C virus infection and
proteinuria in large scale population studies suggest that the spectrum of hepatitis C virus associated disease in the kidney is wider than currently appreciated (9). Hepatitis B infection can produce the same spectrum of pathology in the kidney as hepatitis C virus, although the most common finding in biopsies is membranous nephropathy, rather than membranoproliferative glomerulonephritis. Laboratory investigations have enhanced our understanding of the pathogenesis of hepatitis C virus associated clinical syndromes. It has been shown that the virus binds to and activates B-cells by interaction with the CD81 or TLR4 receptors. Activated B-cells secrete antibodies which cross link with rheumatoid factor and form viral RNA containing immune complexes with the property of cryoglobulins. Hepatitis C virus antigens processed by dendritic cells generate Th1 cells which produce interferon-\(\gamma\), and activated macrophages, which produce a variety of cytokines (IL-1\(\beta\), tumor necrosis factor-\(\alpha\)), chemokines, proteolytic matrix metalloproteases, and reactive oxygen species that damage the endothelium and produce vasculitis. Production of autoantibodies and cryoglobulins has been attributed to molecular mimicry with host matrix proteins and immunoglobulin molecules.

**Infections with viruses of the Herpesvirus family:** Cytomegalovirus nephritis is primarily described in the allograft kidney, and has become extremely uncommon in this era of intense viral monitoring and prophylactic or pre-emptive ganciclovir therapy (10). Most often biopsies performed in the context of CMV viremia show occasional virus infected cells in the peritubular capillary lumen or tubular epithelium. Some of the intra-capillary cells may simply reflect circulating neutrophils rather than a true nephritis. Epstein-Barr virus (EBV) is occasionally encountered in kidney transplant biopsies. It may cause a banal interstitial nephritis or florid post-transplant lymphoproliferative disease. Clinical presentation includes a rejection like graft dysfunction, mass lesion, hydronephrosis, or lymphocele. Histologic clues to distinguish post-transplant lymphoproliferative disease from acute rejection include an expansile nodular
infiltrate, serpiginous necrosis, and lymphocyte atypia, although some forms of the disease show only mature lymphocytes and plasma cells (11). The large majority of cases will consist of CD20 positive B-cell rice infiltrates that express Epstein-Barr virus encoded EBER RNA, although EBV positive T-cell lesions, and EBV negative lymphomas also need to be kept in the differential diagnosis.

Herpesvirus 6 can infect the kidney and produce rejection-like infiltrates, but the extent to which it produces kidney dysfunction is not clear. Progress in the field is hampered by non-availability of antibodies that can reliably detect viral antigens in formalin fixed tissue. Herpesvirus 8 can result in Kaposi's sarcoma lesions within the kidney. Infection of the kidney with Herpes simplex or Varicella is exceedingly uncommon.

**Adenoviral nephritis:** This disease occurs more commonly after hematopoietic stem cell transplantation rather than kidney transplantation, presumably because of more severe immune depletion. The spectrum of disease includes asymptomatic viruria, acute tubular necrosis, granulomatous and necrotizing interstitial nephritis, and pyelonephritis. While using immunohistochemistry or in-situ hybridization to establish the diagnosis (12). It is important to keep in mind that commercially available reagents may not detect the more than 50 viral genotypes that have now been recognized. Ancillary testing such as urine culture or PCR may be necessary to establish the diagnosis. Specific anti-viral therapy is not available. Cidofovir is probably the most effective anti-viral agent available today, although the therapeutic index is quite low.

**Polyomavirus BKV:** Polyomavirus BK (BKV) is linked to polyomavirus-associated nephropathy (PyVAN) in 1%-10% of kidney transplant patients. The definitive diagnosis of PyVAN requires a biopsy demonstrating cytopathic changes and a positive immunohistochemistry or in-situ-hybridization test. There is considerable inter-laboratory variation in staining intensity and assessment of percentage of infected cells, but the binary classification of biopsies into virus positive and negative
is fairly reliable. A minimum of 2 biopsy cores should be taken, given the focal nature of PyVAN and the possibility of sampling error in up to 37% of cases (13). The histological findings PyVAN should be semi-quantitatively assessed. Extent of fibrosis and tubular atrophy may be the most important predictor of a poor outcome. The 2009 Banff conference formulated a working proposal in which primary emphasis was placed on the extent of viral cytopathic effect. Application of this system can result in an identical stage being assigned to biopsies which differ markedly in the degree of inflammation and associated unfavorable outcome (14).

The diagnosis of acute rejection concurrent with PyVAN should only be made in the presence of endarteritis, fibrinoid vascular necrosis, glomerulitis, or C4d deposits along peritubular capillaries. Trying to assess occurrence of tubulitis away from areas of obvious viral cytopathic effect is not helpful, since tubules damaged by virus can be expected to release cytokines which would cause inflammation and tubulitis in a much larger surrounding area. It is our experience that in the setting of persistent viruria (even without viremia or nephropathy) biopsies with putative episodes of acute rejection that satisfy Banff criteria for diagnosis do not always respond well to steroids (15). This suggests that the spectrum of virus associated disease in the kidney is wider than currently appreciated. In PyVAN, C4d or immune complex deposits have been observed in the tubular basement membranes, but not peritubular capillaries. MHC class II up-regulation by the tubular epithelium has been proposed as a marker of rejection but this idea is not supported by all workers in the field (16).

The American Society of Transplantation recommends that screening for BKV replication should be performed at least every 3 months during the first 2 years post-transplant, and then annually until the fifth year post-transplant. Using this strategy, at least 80-90% patients at risk for PyVAN can be identified before significant functional impairment of the renal allograft occurs. Acceptable techniques for screening include urine cytology for ‘decoy cells’ and urine or plasma PCR. Testing
for BKV viruria allows a high negative predictive value to rule out BKV nephropathy of 6 to 12 weeks before viremia and nephropathy. It can also identify a subgroup of patients with persistent viruria and increased risk for recurrent episodes of rejection-like graft dysfunction (17). Testing for BKV viremia has a higher positive predictive value than urine but with a shorter window period of 2 – 6 weeks. Plasma screening is preferred over urine in many centers as it is felt to detect clinically more significant replication. In patients with sustained plasma BKV DNA and loads of >4 log_{10} cp/mL, a diagnosis of “presumptive PyVAN” should be made in absence of demonstrable BKV replication in biopsies. An important caveat to remember in interpreting PCR tests is that no international standard is available as a reference calibrator. In addition, quantitation of viral load can be seriously compromised in the presence of uncommon mutant strains. Detection of three-dimensional viral aggregates in urine (so-called Haufen) by electron microscopy has been reported to have high positive and negative predictive values for biopsy proven BKV nephropathy (18). However, histologic disease is no longer considered the desired end point for therapeutic intervention, since nephropathy diagnosed by a biopsy performed in the setting of a rising serum creatinine has worse outcome. This situation is analogous to cytomegalovirus infection where viremia alone justifies anti-viral therapy, and it is not required to demonstrate tissue invasion.

Other polyomaviruses: While BKV accounts for the vast majority of nephropathies polyomavirus JC (JCV) or SV40 should be considered in cases where biopsies shown obvious viral inclusions, but PCR testing for BKV is negative. Immunohistochemistry for polyomavirus antigens is usually positive in these instances since the antibodies are not species specific and cross react with all three viruses. A negative PCR test for BKV may also be negative if we are dealing with a mutant virus: this issue can be resolved by using an alternate set of PCR primers directed against a different part of the viral genome. A new human polyomavirus
designated as HPyV9 has recently been amplified from the plasma of kidney transplant patients. It is not yet know whether it can cause renal dysfunction.

References