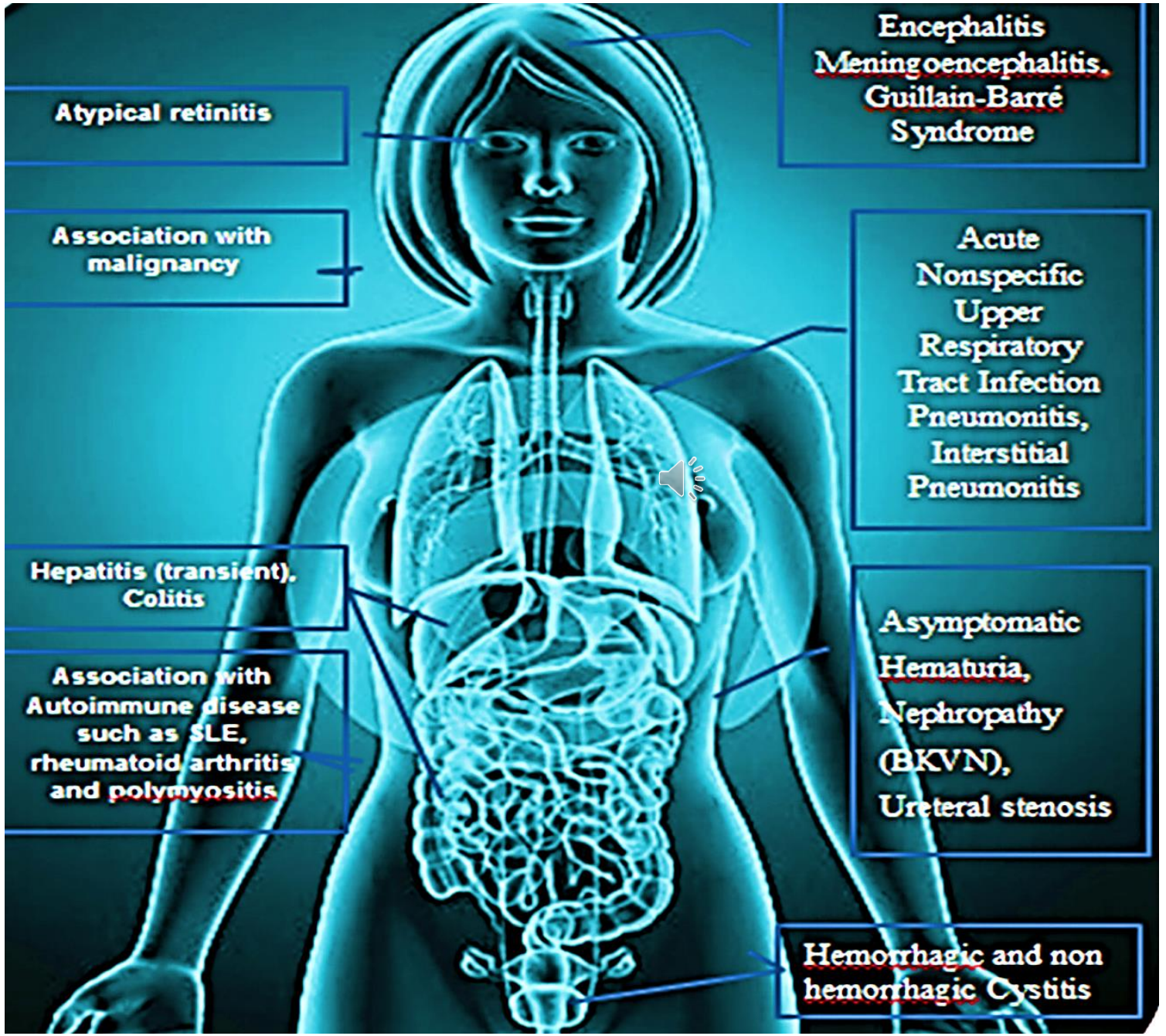
In the Name of God

Diagnosis of Polyoma virus in the Renal Transplantation

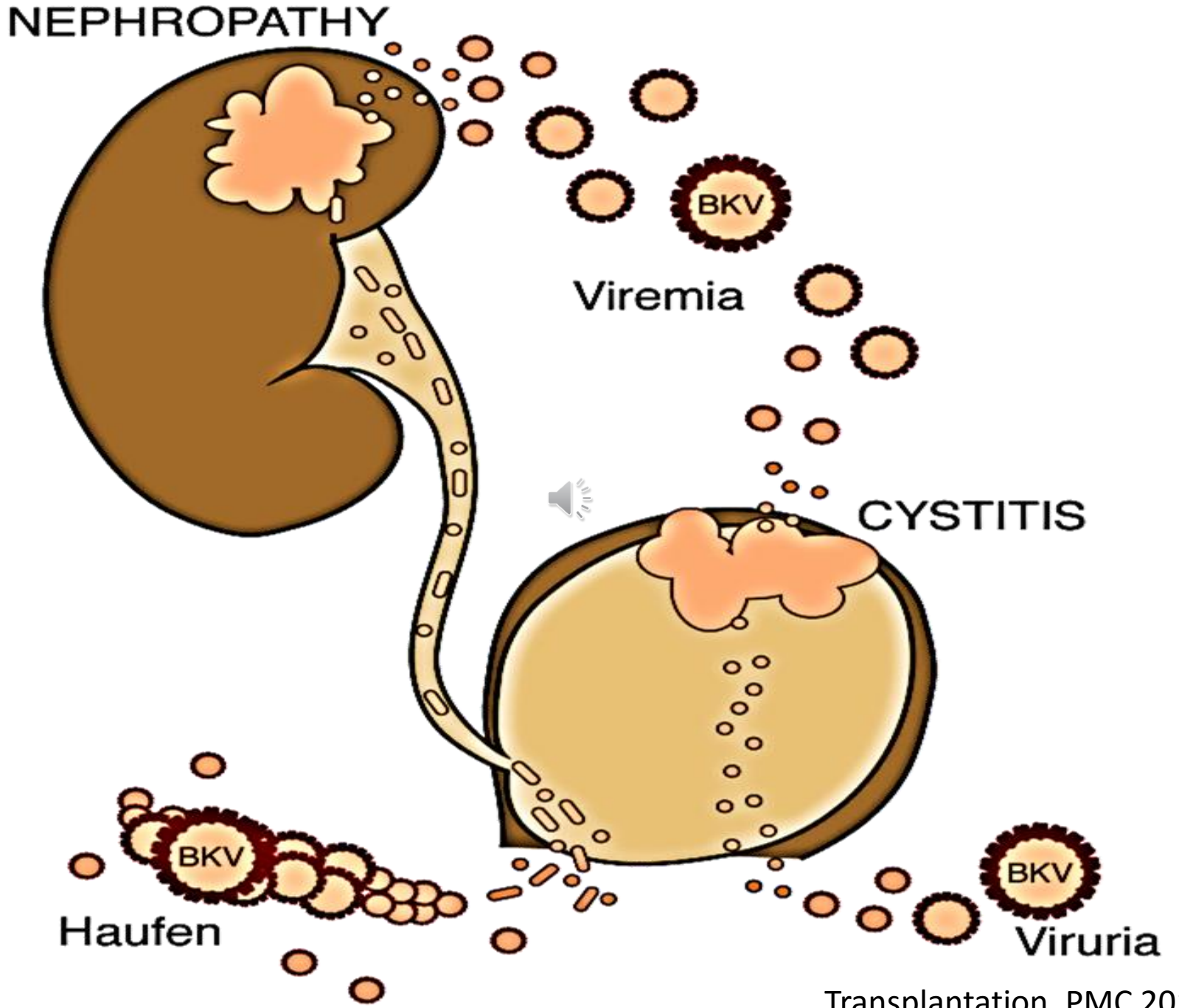
Hassan Argani MD.

Clinical manifestations of BKV in immunocompromised patients



HUMAN POLYOMA VIRUS
Clinical Manifestations

- Asymptomatic viruria
- Pyuria
- Renal dysfunction
- Ureteral stenosis
- Hemorrhagic cystitis



Transplantation. PMC 2017 October 01, 1-15.

Clinical Judgment for BKVN

- Increased cr may be the only manifestation of BKVN
- The incidence of BKVN is highest in the first 2-8 months posttransplant.
- In many cases it can occur years after transplantation.
- The incidence of late BKVN is highest in patients with multi-organ transplants and is possibly related to the more intensive immunosuppressive regimens used for these patients.

BK virus infection in renal transplant recipients: an overview

Fakhriya Alalawi[a,b], Hind Alnour[a,b], Mohsen El Kossi[b,c], John Jenkins[b], Anna Taku[d], Ajay K. Sharma[b,d], Ahmed Halawa[b,e]

Nephrology and Transplantation 2020, 20:127–150

Seroprevalence of BK virus in general public in some countries

Countries	BK-seroprevalence (%)
England	83–91
Finland	60
Italy	83
Germany	71
Switzerland	82
Czech Republic	69
Maryland, United States	69–100
Australia	97
China	45
Japan	80.8
Iran	41.8

Unusual manifestations of BKV

Hemorrhagic cystitis

It is a rare manifestation of BKV. It is commonly reported among hematopoietic cell transplant recipients.

Four degrees of disease severity were recognized:

- grade I: microscopic hematuria;
- grade II: macroscopic hematuria;
- grade III: hematuria with clots;
- grade IV: hematuria with clots, clot retention, and renal failure secondary to obstructive nephropathy

genitourinary cancers

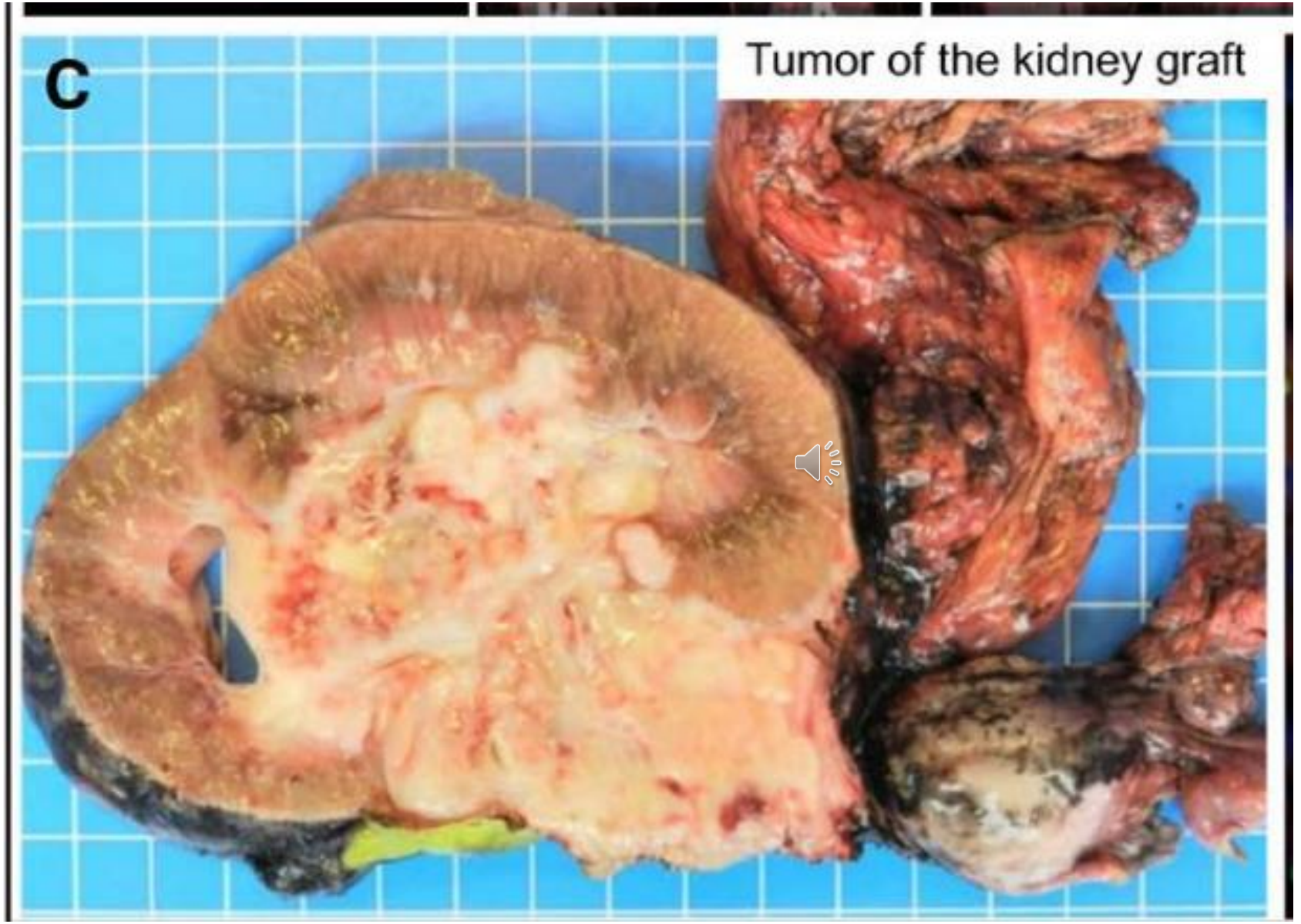
There is a putative link between BKV and the development of genitourinary cancers.

Immunologic Clearance of a BK Virus-associated Metastatic Renal Allograft Carcinoma

Raphael P.H. Meier, MD, PhD,[1,2] Yannick D. Muller, MD, PhD,[2,3] Pierre-Yves Dietrich, MD,[4]
Jean-Christophe Tille, MD, PhD,[5] Sergey Nikolaev, PhD,[6] Ambra Sartori, MSc,[6]
Intidhar Labidi-Galy, MD, PhD,[4] Thomas Ernandez, MD,[7] Amandeep Kaur, MSc,[8]
Hans H. Hirsch, MD,[8] Thomas A. McKee, MD,[5] Christian Toso, MD, PhD,[1]
Jean Villard, MD, PhD,[3,7] and Thierry Berney, MD, MSc[1]

Background. Metastatic carcinoma of a renal allograft is a rare but life threatening event with a difficult clinical management. Recent reports suggested a potential role of BK polyomavirus (BKPyV) in the development of urologic tract malignancies in kidney transplant recipients. **Methods.** We investigated a kidney-pancreas female recipient with an history of BKPyV nephritis who developed a rapidly progressive and widely metastatic donor-derived renal carcinoma 9 years after transplantation. **Results.** Histology and fluorescence in situ hybridization analysis revealed a donor-derived (XY tumor cells) collecting (Bellini) duct carcinoma. The presence of BKPyV oncogenic large tumor antigen was identified in large amount within the kidney tumor and the bowel metastases. Whole genome sequencing of the tumor confirmed multiple genome BKPyV integrations. The transplanted kidney was removed, immunosuppression was withdrawn, and recombinant interleukin-2 (IL-2) was administered for 3 months, inducing a complete tumor clearance, with no evidence of disease at 6-year follow-up. The immunological profiling during IL-2 therapy revealed the presence of donor-specific T cells and expanded cytokine-producing bright natural killer cells but no donor-specific antibodies. Finally, we found persistently elevated anti-BK virus IgG titers and a specific anti-BKPyV T cell response. **Conclusions.** This investigation showed evidence for the potential oncogenic role of BKPyV in collecting duct carcinoma in renal allografts and demonstrated that immunosuppression withdrawal and IL-2 therapy can lead to an efficient antitumor cellular mediated rejection possibly via 3 distinct mechanisms including (1) host-versus-graft, (2) host-versus-tumor, and (3) anti-BKPyV responses.

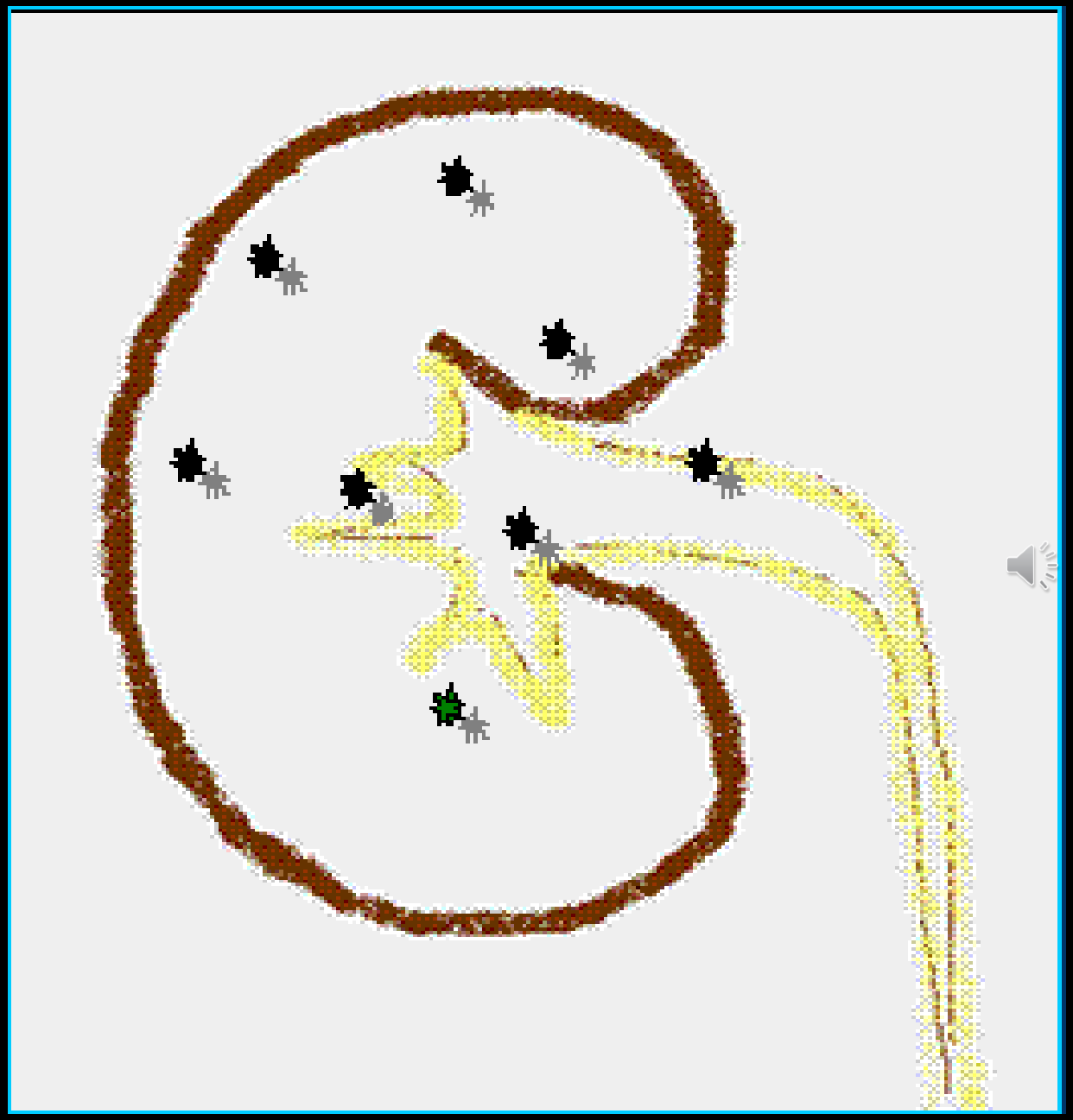
(*Transplantation* 2021;105: 423–429).



Transplantation 2021;105: 423–429).

Stages of BKV infection in the Allograft

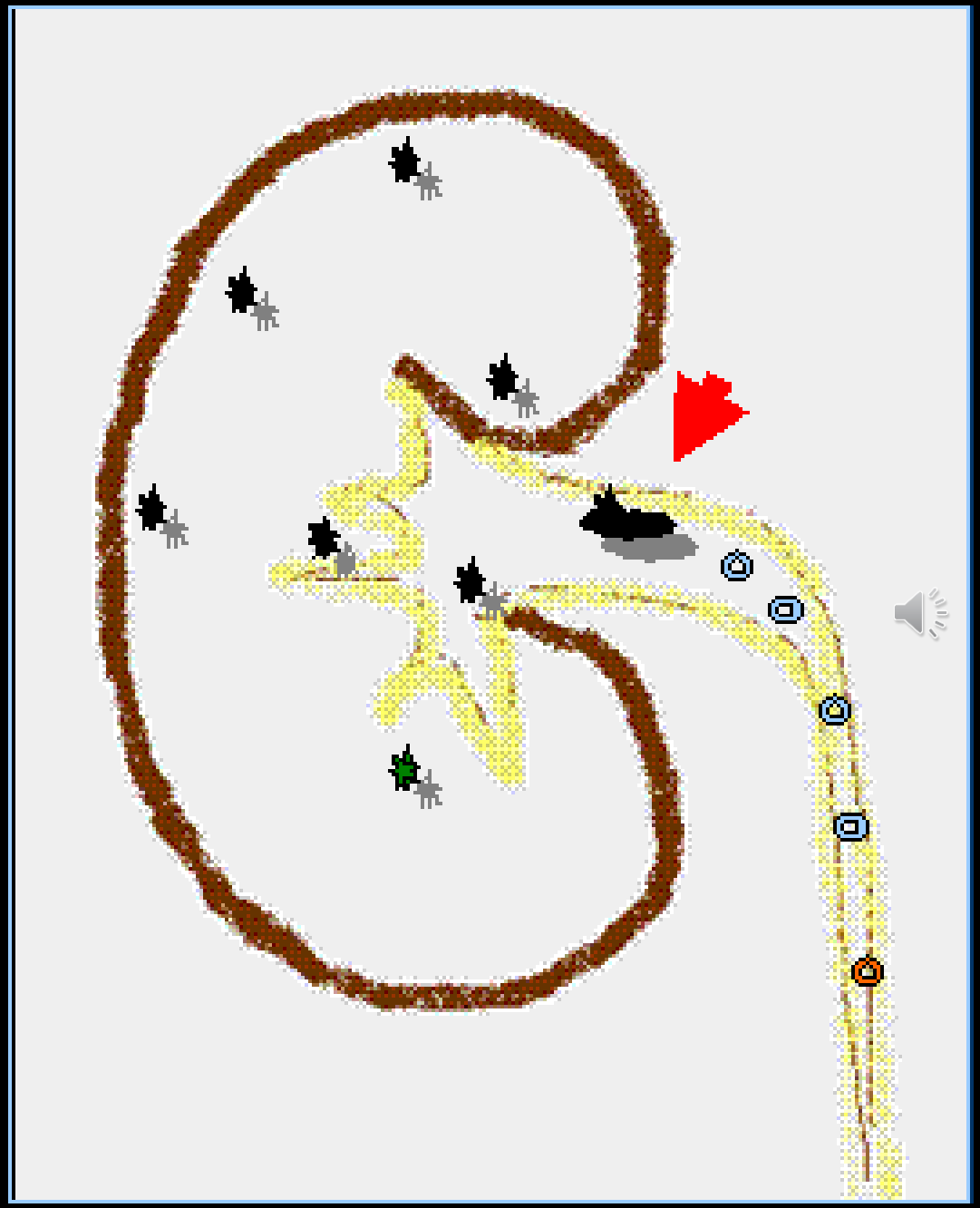
1- Latent infection
2- Limited viral replication
3- High level viral replication with tissue destruction



1) LATENT INFECTION

After primary infection BK and JC remain latent in the kidney and urothelium of ureters and urinary bladder.

- Demonstration by tissue PCR
- No viruria (absence of decoy cell in urine, negative PCR studies in urine)
- No viremia
- No clinical significance (normal renal function)



2) LIMITED (low level) VIRAL REPLICATION

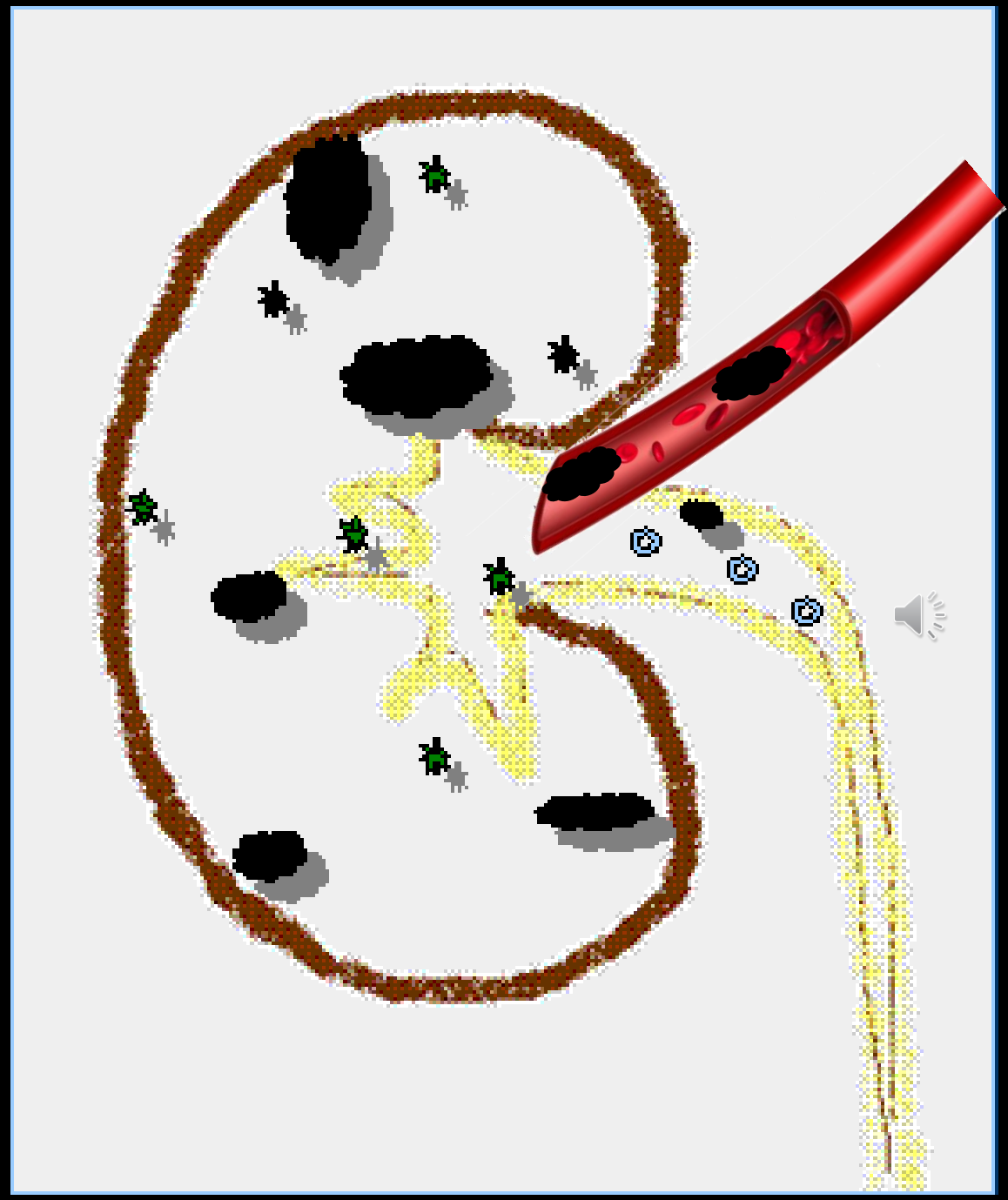
Mostly limited to the urothelium is common in immunosupressed patients and may occur rarely in healthy individuals (i.e. pregnant women).

- 20-65 % of renal transplant patients

- Transient or intermittent viruria
  - Decoy cells on urine cytology
  - Demonstration of viruria with PCR methods

- No viremia

- No clinical significance, normal renal function



3) HIGH LEVEL VIRAL REPLICATION WITH TISSUE DESTRUCTION

POLYOMA VIRUS ALLOGRAFT NEPHROPATHY

- Occurs only in a minority of renal transplant patients (5-10%).
- Persistent viruria
  - Decoy cells on urine cytology
  - Significant viruria by quant. PCR
- Viremia
- Abnormal renal function if parenchymal disease is extensive

Options for intervention in BKVN based on laboratory:

A) Preemptive intervention

- In patients with Viruria and Viremia, progressive and customized decrease in immunosuppression before significant renal scarring has occurred leads to resolution of the infection in 85 to 90% of cases, with long-term preservation of graft function. Risk of acute rejection is low (10 to 15%).
- Intervention is not indicated in the absence of Viremia (ie, Vr only).

B) Obligatory intervention

Late diagnosis and intervention once graft dysfunction is evident decreases the likelihood of viral clearance and is associated with higher rates of premature graft loss up to 30%.

C) Ineffectual, late intervention

- The late stage of BKVN resembles clinically and histologically ESRD from other causes.
- With progressive obliteration of the renal tubules (the primary target of BKVN infection) there is progressive decrease of Viruria and Viremia.

The best policy

- Methods for early screening.
- Early (pre-transplant) risk stratification.

Risk factors for BKVN

- When immunosuppression is more intense
- Transplant from a BV seropositive donor to a seronegative recipient.
- Donor BKV viruria prior to transplant

Meta-analysis

8 risk factors associated with increased risk for BK viremia, ***2*** risk factor for BKV nephropathy

- ***Maintenance therapy regimen including tacrolimus***.
- Allograft from a deceased donor.
- Recipient of male sex.
- History of previous transplant.
- Age at transplantation.
- Ureteral stent use.
- Delayed graft function.
- ***Acute rejection episodes.***

Viruses 2020, 12, 1417;1-21

viruses

MDPI

Review

The Role of HLA and KIR Immunogenetics in BK Virus Infection after Kidney Transplantation

Marija Burek Kamenaric [1,†], Vanja Ivkovic [2,3,†], Ivana Kovacevic Vojtusek [2] and Renata Zunec [1,*]

Protective/or deleterious effect of HLA alleles to BK viremia and/or risk for BKVAN

References	Factor	n	Clinical Effect
	HLA Class I Allele (Classical)		
Bohl et al., 2005 [64]	Cw7 neg	195	In D; in R; in both D and R—↑ BK viremia and BKVAN
Masutani et al., 2013 [18]	A2 pos B44 pos DR15 pos	998	In R—↓ BK viremia In R—↓ BK viremia In R—↓ BK viremia
Teutsch et al., 2015 [65]	A9 pos A2 pos A28/A68 pos	329	In D—↑ BKVAN In R—↑ BKVAN In R—↑ BK viremia
Gheith et al., 2015 [66]	Cw7 neg	5	In D; in R—↑ BK viremia
Dogan et al., 2017 [17]	A24 pos B55 pos	183	R and D matched—↑ BK viremia R and D matched—↑ BK viremia
Wunderink et al., 2018 [63]	B51 pos	407	In R—↓ BK viremia and BKVAN
Kovacevic Vojtusek et al., 2019 [67]	C*07 pos	23	In D—↓ BKVAN
El Husseini et al., 2019 [68]	HLA-A, -B, -C		No association
Kavuzlu et al., 2020 [69]	DRB1*03 pos B*13 pos	232	In R—↑ BKV infection In R—↓ BKV infection
	HLA Class II Allele		
Roark et al., 2016 [70]	DQ5/DQ6 pos	102	In R—↓ BKV viremia In D—no association
Shah et al., 2016 [71]	DQ2/DQ3/DQ4 pos DQ5/DQ6 pos	433	In R—↑ BK viremia In R—↓ BK viremia

Why is early Dx. Mandatory?

Lack of any definite therapy for BKVAN

Diagnostic tests for BKV

- Tests to diagnose infection.
- Tests to diagnose replication.

- Tests to diagnose disease.

PCR

urine cytology

Serology

Histopathology

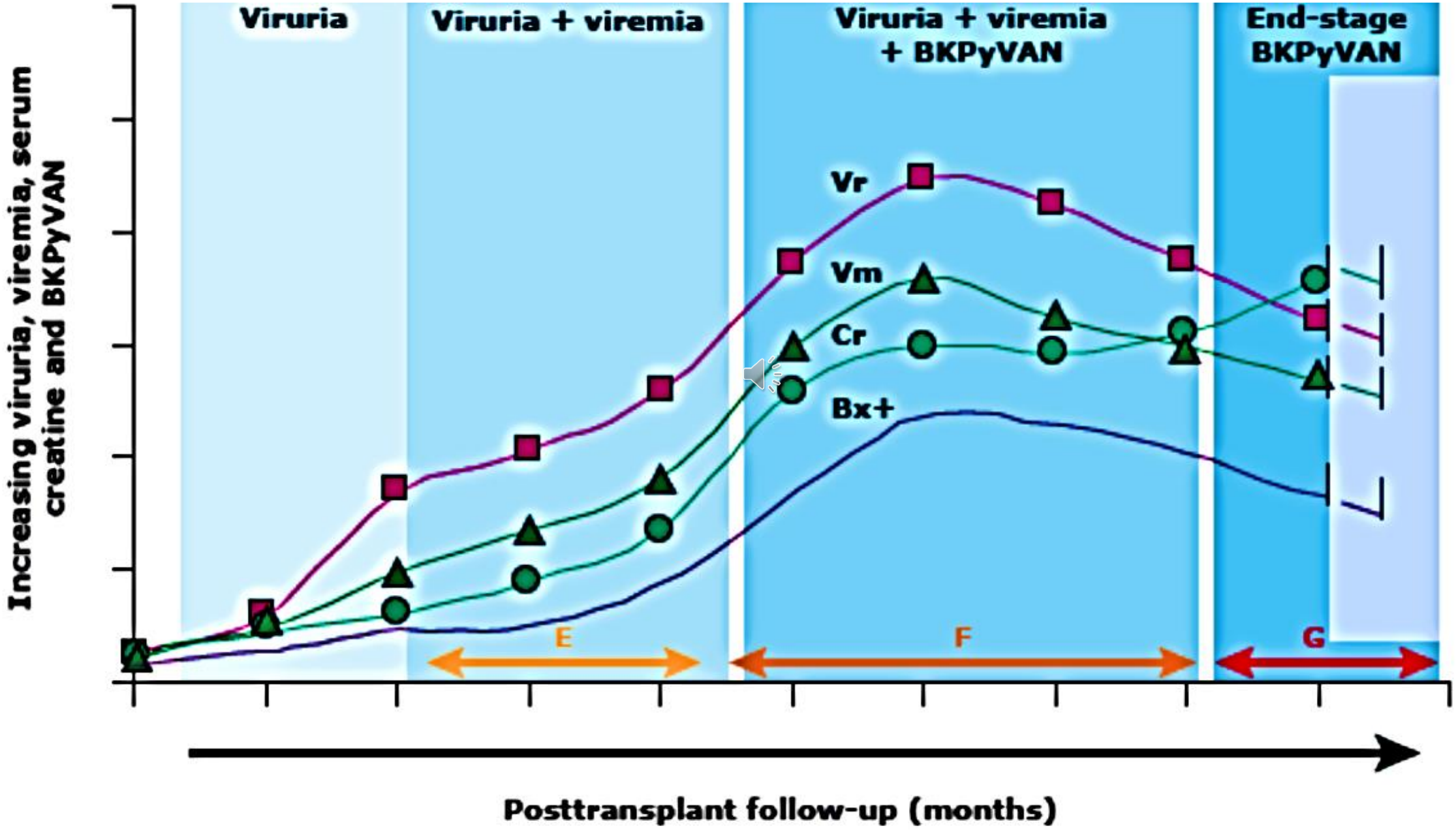
The choice of diagnostic test

12 weeks

Viruria → Viremia → Nephropathy → Allograft loss

Progression of BKVN by months

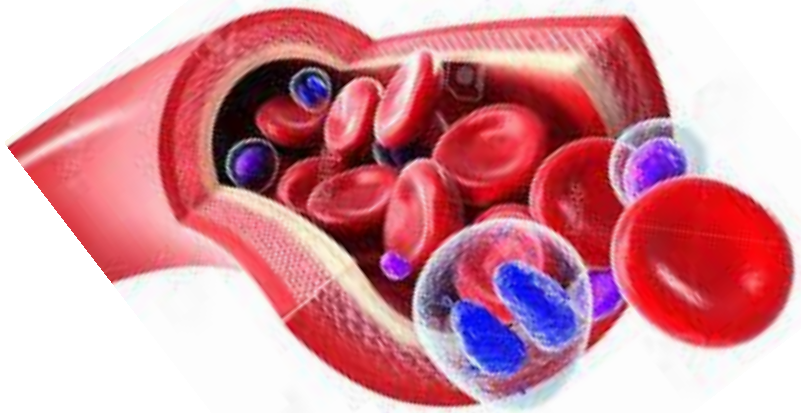
Typical course of viruria, viremia, and BK-induced nephropathy



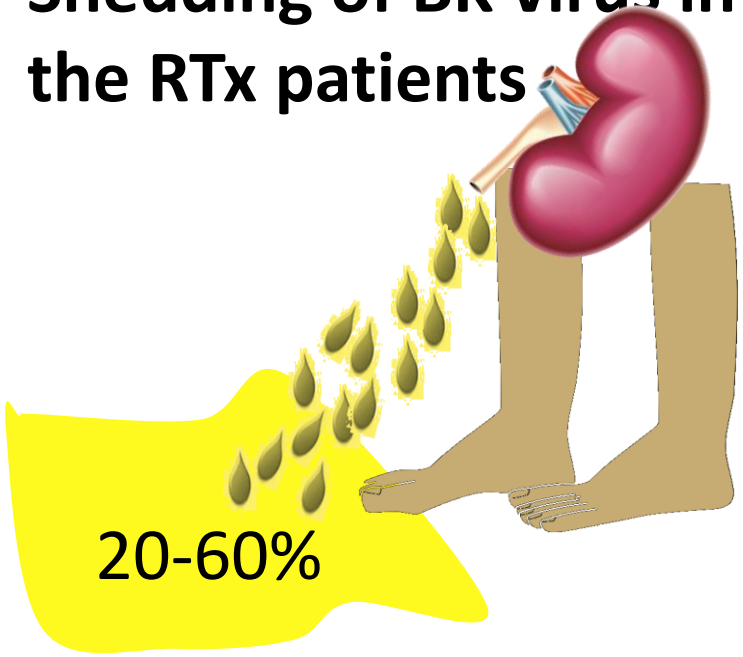
Viruria and viremia

Shedding of BK virus in the immunocompetent persons

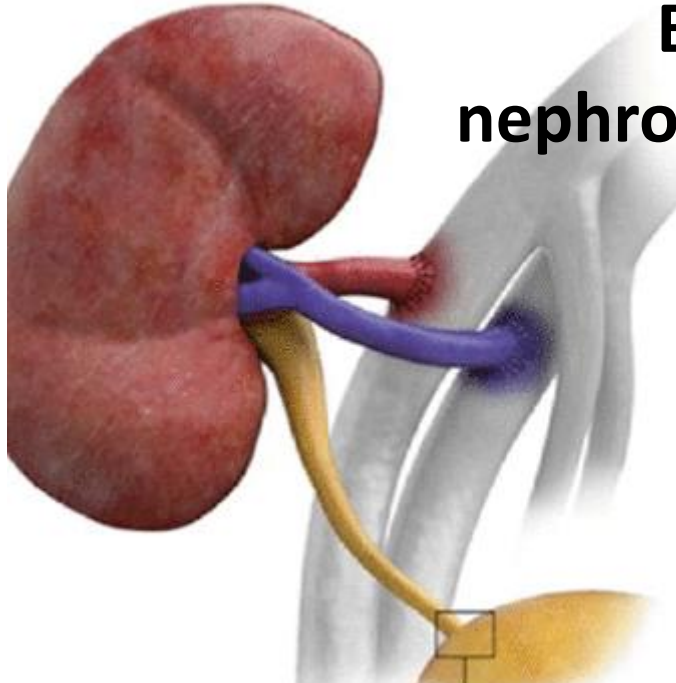
Viremia=13%,



Shedding of BK virus in the RTx patients



BKV nephropathy=8%



Viruria

- Viruria is the earliest manifestation of BKV infection in kidney transplant recipients, affecting 25-30% of patients during the first year following transplantation.
- For most, viruria is asymptomatic, detected only by screening, and does not progress to viremia.
- While viruria is a sensitive marker for progression to BKVN, it is nonspecific.
- urine PCR for BKV is not as useful as plasma PCR for monitoring the response to therapy, since changes in the urine viral load lag behind changes in the plasma viral load upon lowering immunosuppression.

Shedding of BKV in the urine (By PCR)

- Healthy older adults
- Pregnancy
- Any immunosuppressive therapy

Urine Sediment in BKVN

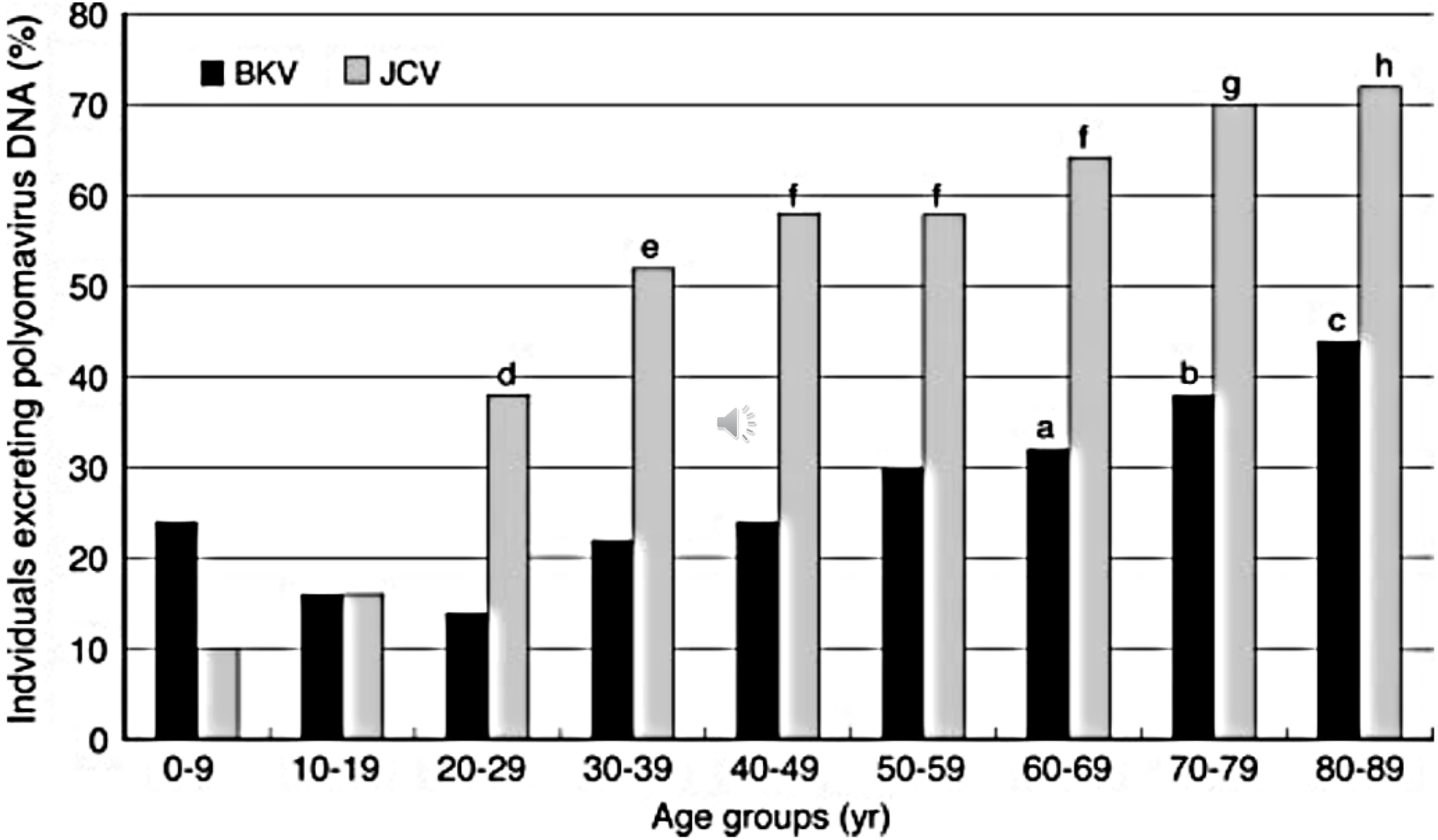
- **Pyuria**
- **Hematuria**
- **Cellular casts (WBC and epithelial)**
- **Decoy Cells**
- **Normal**

JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2007, p. 193–198 Vol. 45, No. 1
0095-1137/07/$08.00+0 doi:10.1128/JCM.01645-06

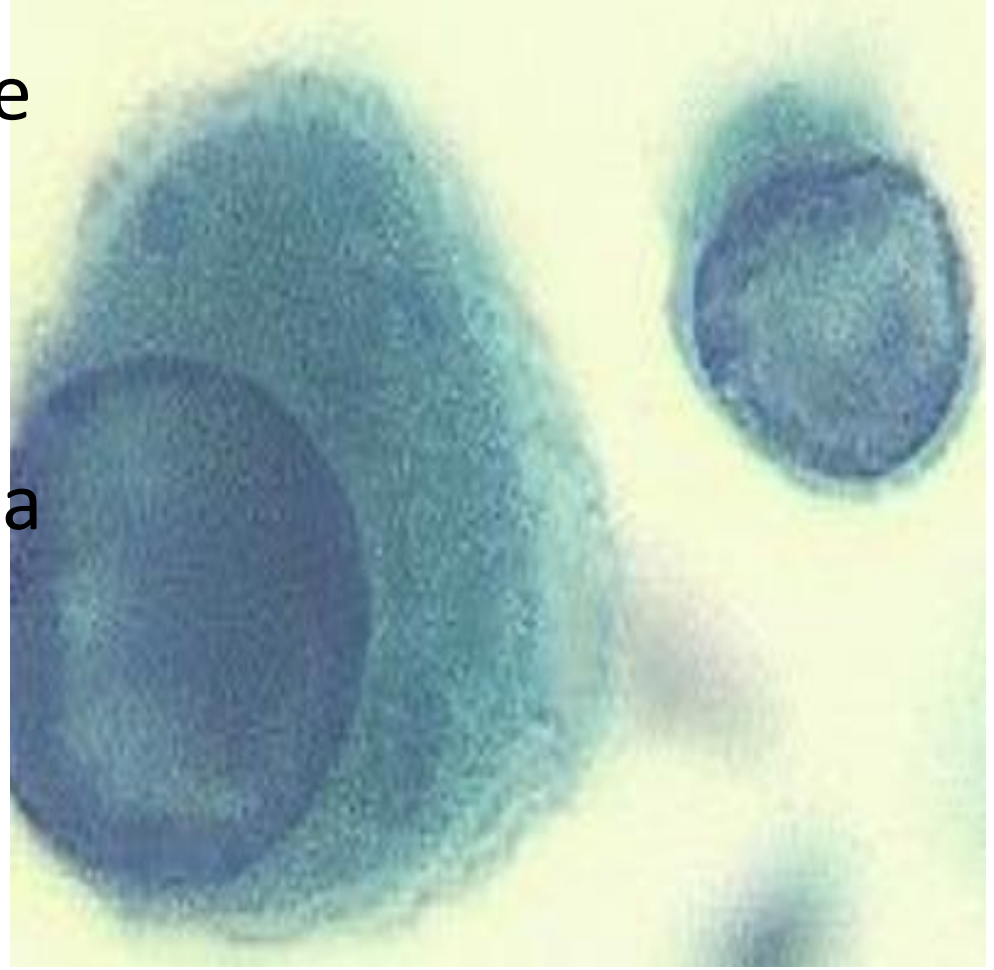
Age-Related Urinary Excretion of BK Polyomavirus by Nonimmunocompromised Individuals▽

Shan Zhong,[1] Huai-Ying Zheng,[1] Motofumi Suzuki,[1] Qin Chen,[1] Hiroshi Ikegaya,[2] Naoto Aoki,[3] Shuzo Usuku,[4] Nobuyoshi Kobayashi,[5] Souichi Nukuzuma,[6] Yukiharu Yasuda,[7] Noboru Kuniyoshi,[8] Yoshiaki Yogo,[1]* and Tadaichi Kitamura[1]

Department of Urology, Faculty of Medicine, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655, Japan[1]; National Research Institute of Police Science, Kashiwa, Chiba 277-0882, Japan[2]; Sakura Hospital, Hachinohe, Aomori 039-110, Japan[3]; Yokohama City Institute of Health, Yokohama, Kanagawa 235-0012, Japan[4]; Yokohama Laboratory, Japan Frozen Foods Inspection Corporation, Yokohama, Kanagawa 236-0004, Japan[5]; Department of Microbiology, Kobe Institute of Health, Kobe, Hyogo 650-0046, Japan[6]; Yasuda Children's Clinic, Machida, Tokyo 194-0032, Japan[7]; and Nagareyama Central Hospital, Nagareyama, Chiba 270-0114, Japan[8]


Individuals excreting polyomavirus DNA (%)
80
70
60
50
40
30
20
10
0
BKV
JCV
d
e
f
f
a
f
b
g
c
h
0-9
10-19
20-29
30-39
40-49
50-59
60-69
70-79
80-89
Age groups (yr)

- “Urine Decoy cells”, characterized by large viral nuclear inclusions that replace the normal chromatin (Papanicolaou stain).
- They do not have a significantly greater prognostic value for progression to viremia than detection of viruria by PCR.
- Urothelial origin
- Sign of viral activation
- Not diagnostic for BKVN
- Normal renal function

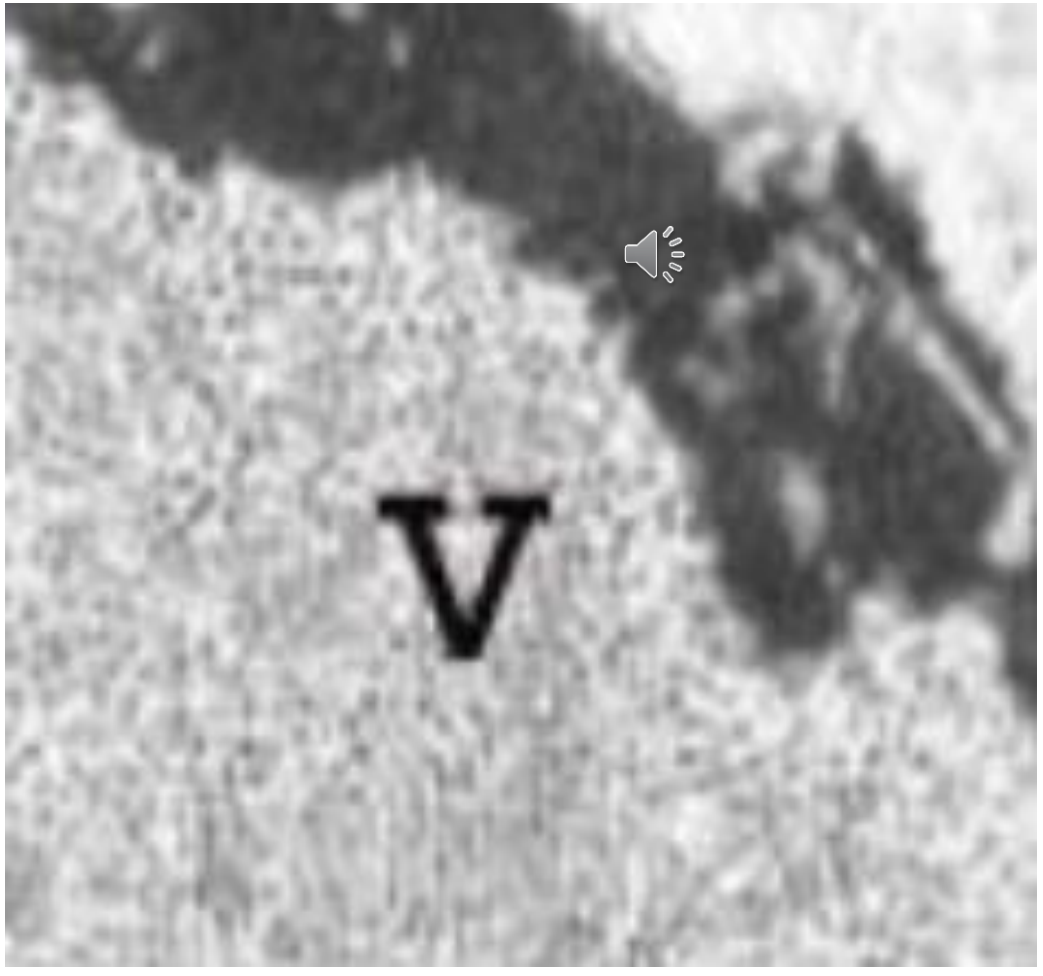


PPV=27%

NPV=100%

Electron microscopy

Shows the contrast between the thousands of newly formed virions inclusions (V) in the nucleolus.

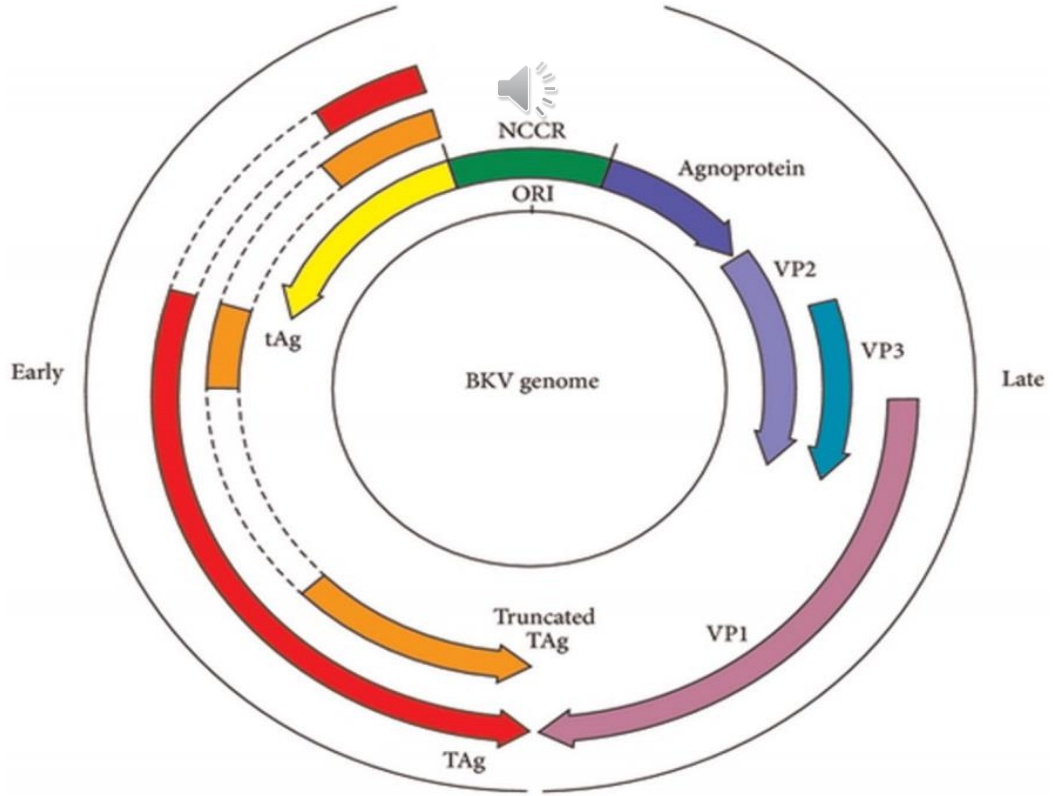


0041-1337/02/7407-987/0
TRANSPLANTATION

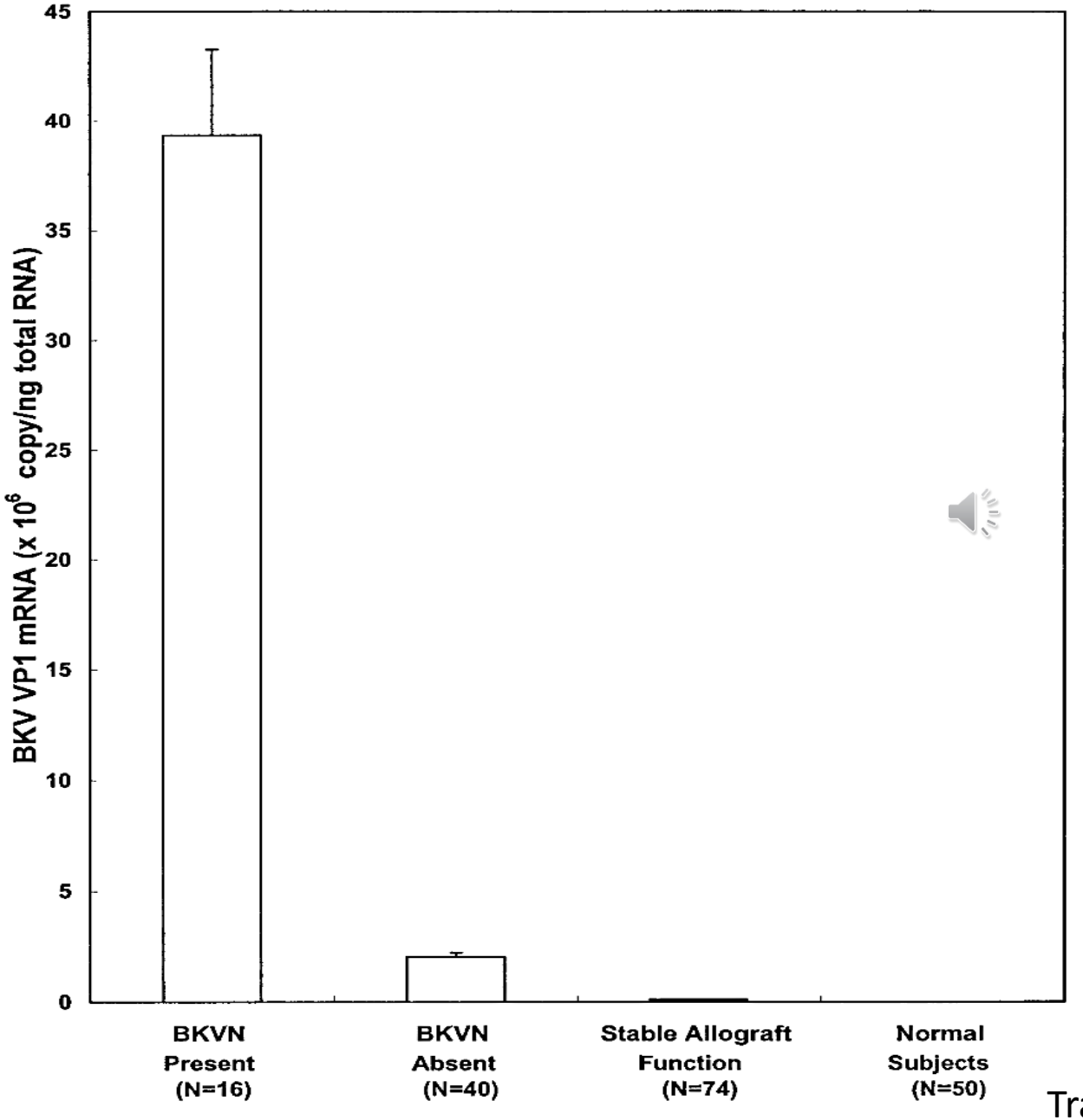
Vol. 74, 987–994, No. 7, October 15, 2002
Printed in U.S.A.

NONINVASIVE DIAGNOSIS OF BK VIRUS NEPHRITIS BY MEASUREMENT OF MESSENGER RNA FOR BK VIRUS VP1 IN URINE[1]

RUCHUANG DING,[2] MARA MEDEIROS,[2] DARSHANA DADHANIA,[2] THANGAMANI MUTHUKUMAR,[2] DAVID KRACKER,[2] JIN M. KONG,[2] SUSANNA R. EPSTEIN,[2] VIJAY K. SHARMA,[2] SURYA V. SESHAN,[3] BAOGUI LI.[2] AND MANIKKAM SUTHANTHIRAN[2,4]



Levels of BKV VP1 mRNA in urinary cells



Conclusions

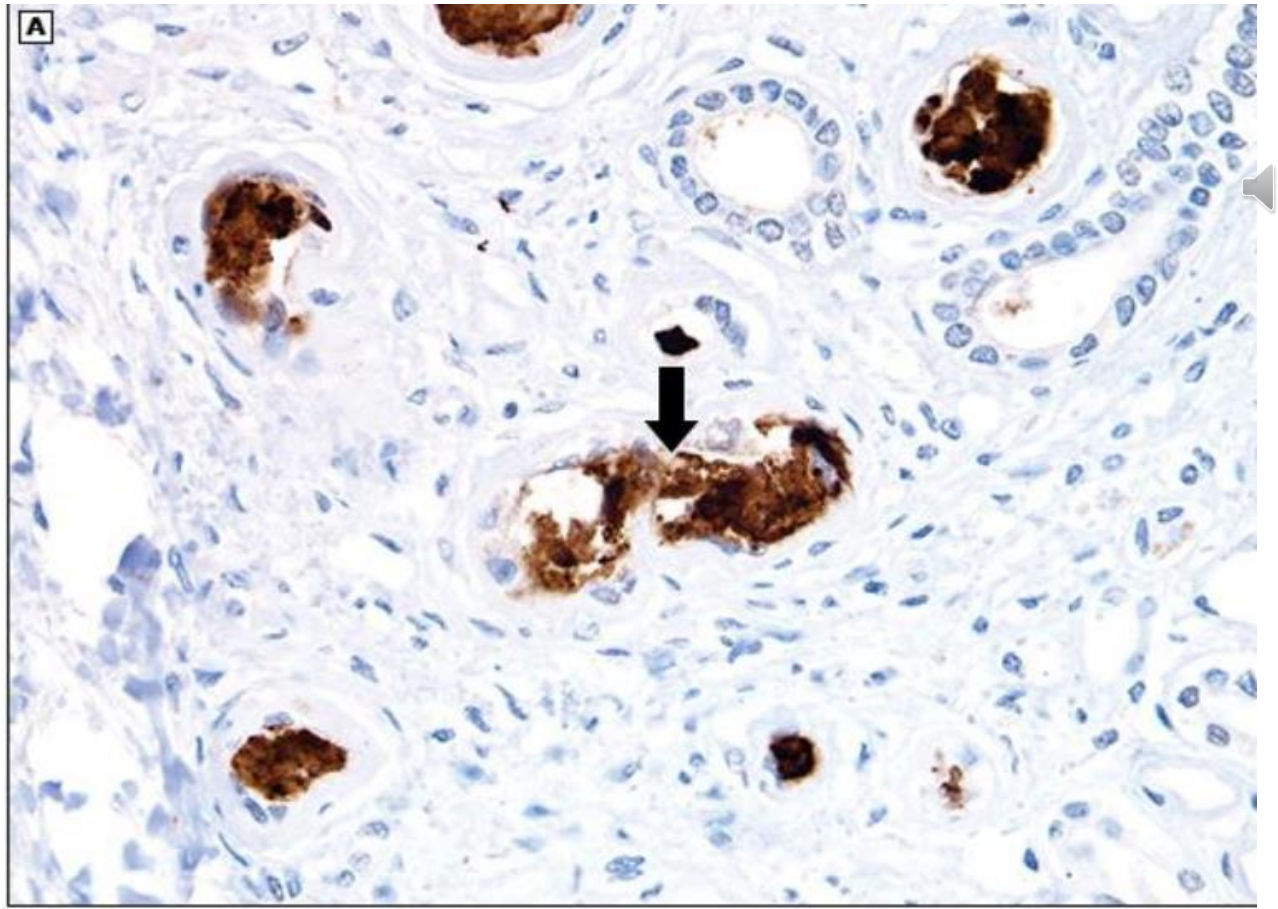
Measurement of BKV VP1 mRNA in urinary cells offers a noninvasive and accurate means of diagnosing BKV nephritis.

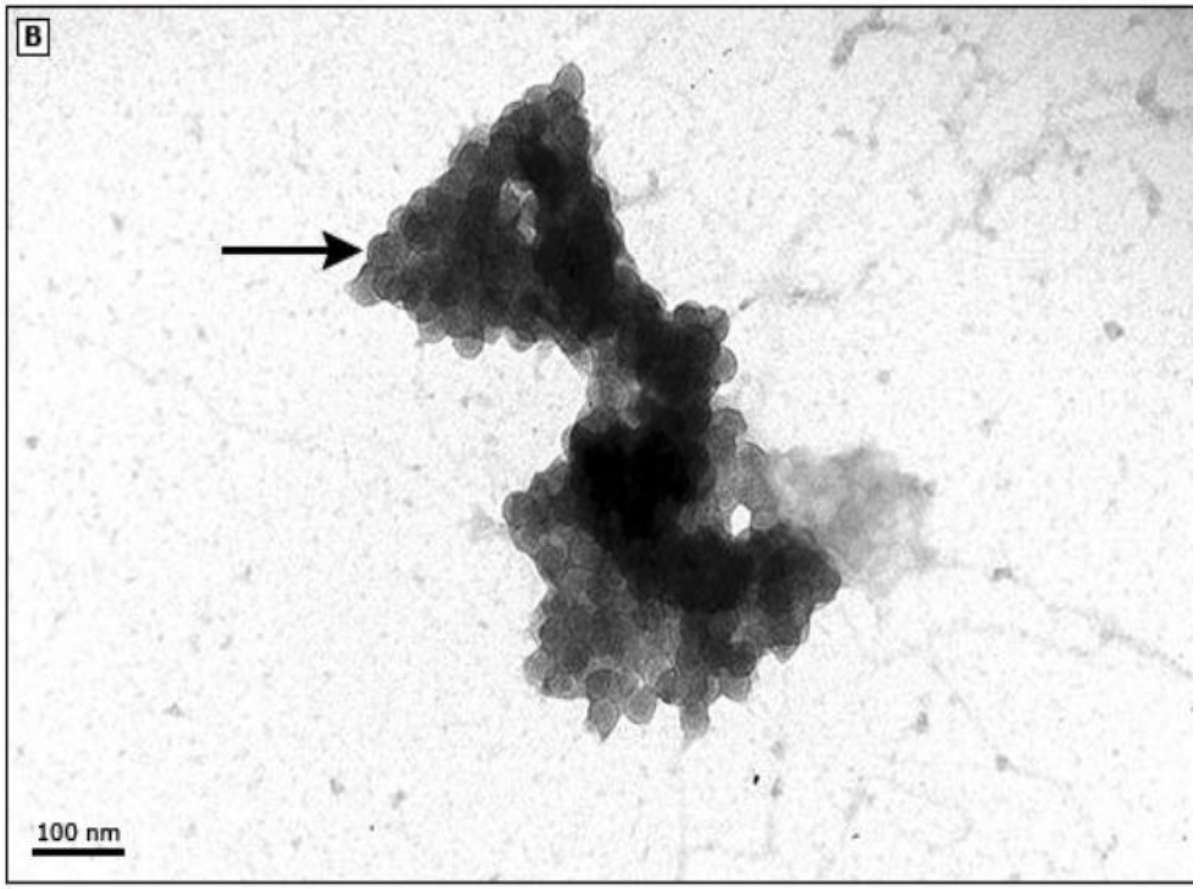
Transplantation. Vol. 74, 987–994, No. 7, October 15, 2002

Haufen Bodies

Aggregate of BKV particles and Tamm-Horsfall protein

Corresponds to upper levels of BK viremia (Usually> 1 million copies/ml)





Valuable of the Haufen Bodies

- Sensitivity, specificity for BKVN> 95 percent.
- It may be used if renal allograft biopsy cannot be safely performed.
- It may be used in children.

Experimental and Clinical Transplantation (2021) 2: 104-109

ARTICLE

Comparing Urine and Blood Screening Methods to Detect BK Virus After Renal Transplant

Kevin McGann,[1] David DeWolfe,[2] Marie Jacobs,[3] David Wojciechowski,[3] Martha Paplakis,[2] C. Sabrina Tan[1,4]

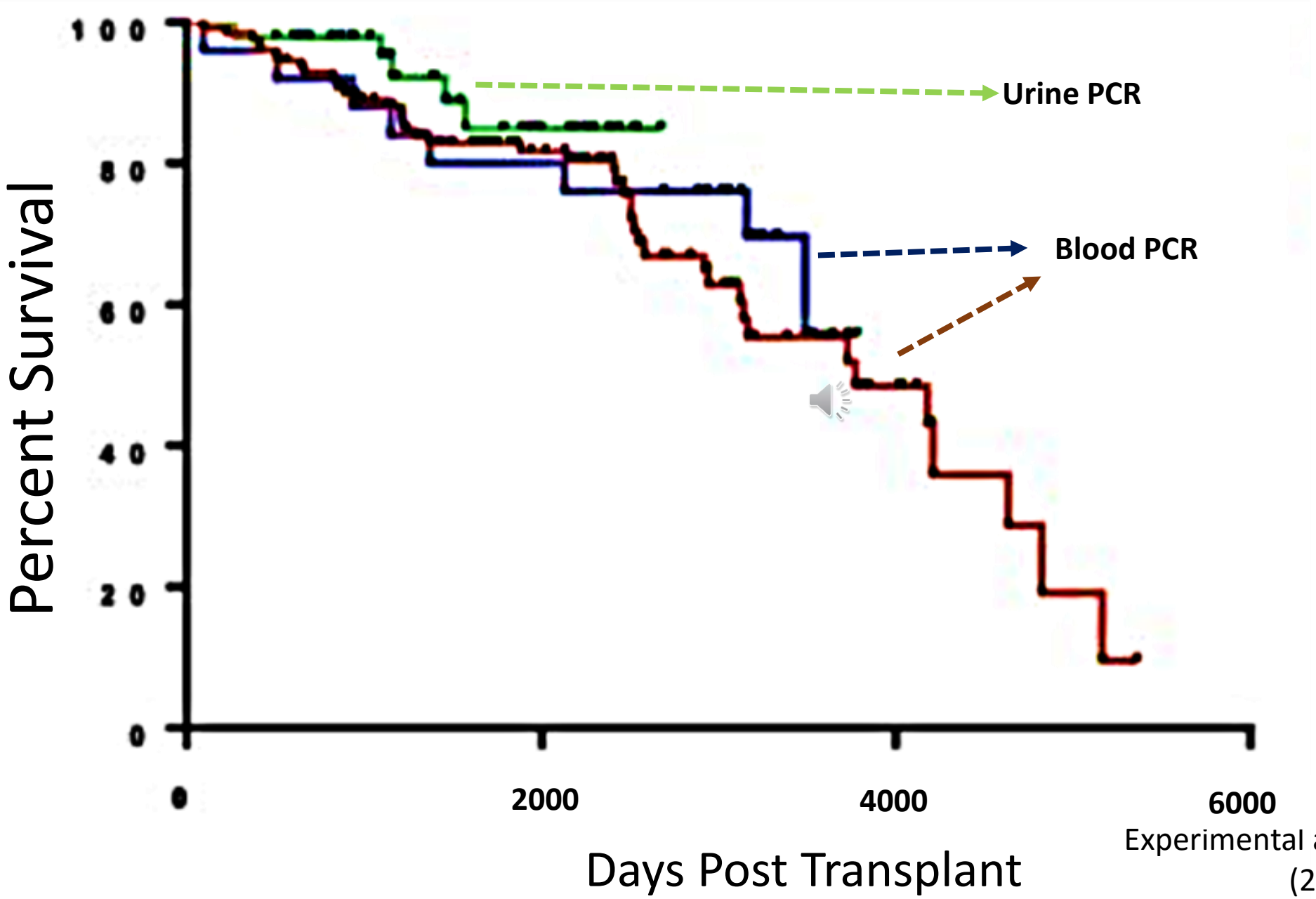
209 patients with BK polyomavirus reactivation after kidney transplant at 2 different institutions from 2008 to 2018. BK polyomavirus reactivation in blood was detected earlier if the patient was screened by urine screening protocol.

Table 1. Demographic Comparison of BK Polyomavirus Viremia Cohorts

	BIDMC Blood Cohort	BIDMC Urine Cohort	MGH Blood Cohort	*P* Value
Total patients, No.	27	50	132	
RTR age at transplant, y	58 (51-63)	54 (41-64)	58 (49-65)	.343
Donor age, y	54 (41-61)	43 (29-55)	50 (35-57)	.082
RTR sex, No.				.250
Male	16 (59%)	37 (74%)	99 (75%)	
Female	11 (41%)	13 (26%)	33 (25%)	
RTR/donor sex mismatch, No.	10 (37%)	28 (56%)	52 (39%)	.249
RTR race/ethnicity, No.				.001
White	22 (82%)	34 (68%)	94 (71%)	
Black/African American	3 (11%)	14 (28%)	16 (12%)	
Asian	2 (7%)	2 (4%)	3 (2%)	
Hispanic/other	0 (0%)	0 (0%)	19 (14%)	
Graft source, No.				.544
Living	15 (56%)	22 (44%)	58 (44%)	
Deceased	12 (44%)	28 (56%)	74 (56%)	
Induction therapy, No.				.002
rATG	20 (74%)	40 (80%)	110 (83%)	
RTRs with prior renal transplant, No.	2 (7%)	9 (18%)	20 (15%)	.332

Experimental and Clinical Transplantation (2021) 2: 104-109

Graft Survival in Group Screened by ***Urine*** compared with ***Blood*** PCR



Experimental and Clinical Transplantation (2021) 2: 104-109

CONCLUSION

- Urine screening performed in the early post-transplant period may be effective for early detection of BKV reactivation.

Allow lowering of immunosuppression and affect graft outcome.

Experimental and Clinical Transplantation (2021) 2: 104-109

Conclusion for Urine test

Urine Screening tests for BK polyomavirus-associated nephropathy

Method	Sensitivity* (%)	Specificity* (%)	PPV*	NPV*	Advantage	Disadvantage
Urine quantitative PCR	100	78	Moderate	High	▪ Precedes BKPyV viremia by 6 to 12 weeks ▪ Earlier identification of patients at risk for subsequent BKPyVAN	▪ Limited utility for monitoring response to therapy (ie, immunosuppression reduction) ▪ May remain persistently positive
Urine decoy cells	100	71	Low	High	▪ Lower cost	▪ Decoy cells identification needs experience ▪ Does not distinguish among polyomaviruses (ie, JCPyV versus BKPyV)
Urine Haufen	100	99	High	High	▪ High PPV ▪ Might be useful in settings where allograft biopsy is not feasible	▪ Requires electron microscopy ▪ Not widely available

Viremia

- Viremia in 10%-30% of recipients in first six months post-Tx. and in 5% to 10% thereafter.
- As with viruria, viremia is typically asymptomatic.
- Viremia has greater PPV than viruria for progression to BKVN.
- Viremia is present in nearly ***all*** patients with BKVN and has a positive predictive value of approximately ***40 to 65 percent*** to development of BKVN.
- Viremia is an indication to reduce immunosuppression in kidney transplant recipients.
- Higher viral loads and sustained viremia have greater predictive value for concomitant or progression to biopsy-confirmed BKVN.

The threshold of plasma BKV

- Levels >1000 copies/mL are considered positive.
- Levels >10,000 copies/mL correlate with biopsy-confirmed BKVN.

Serial estimate of viremia

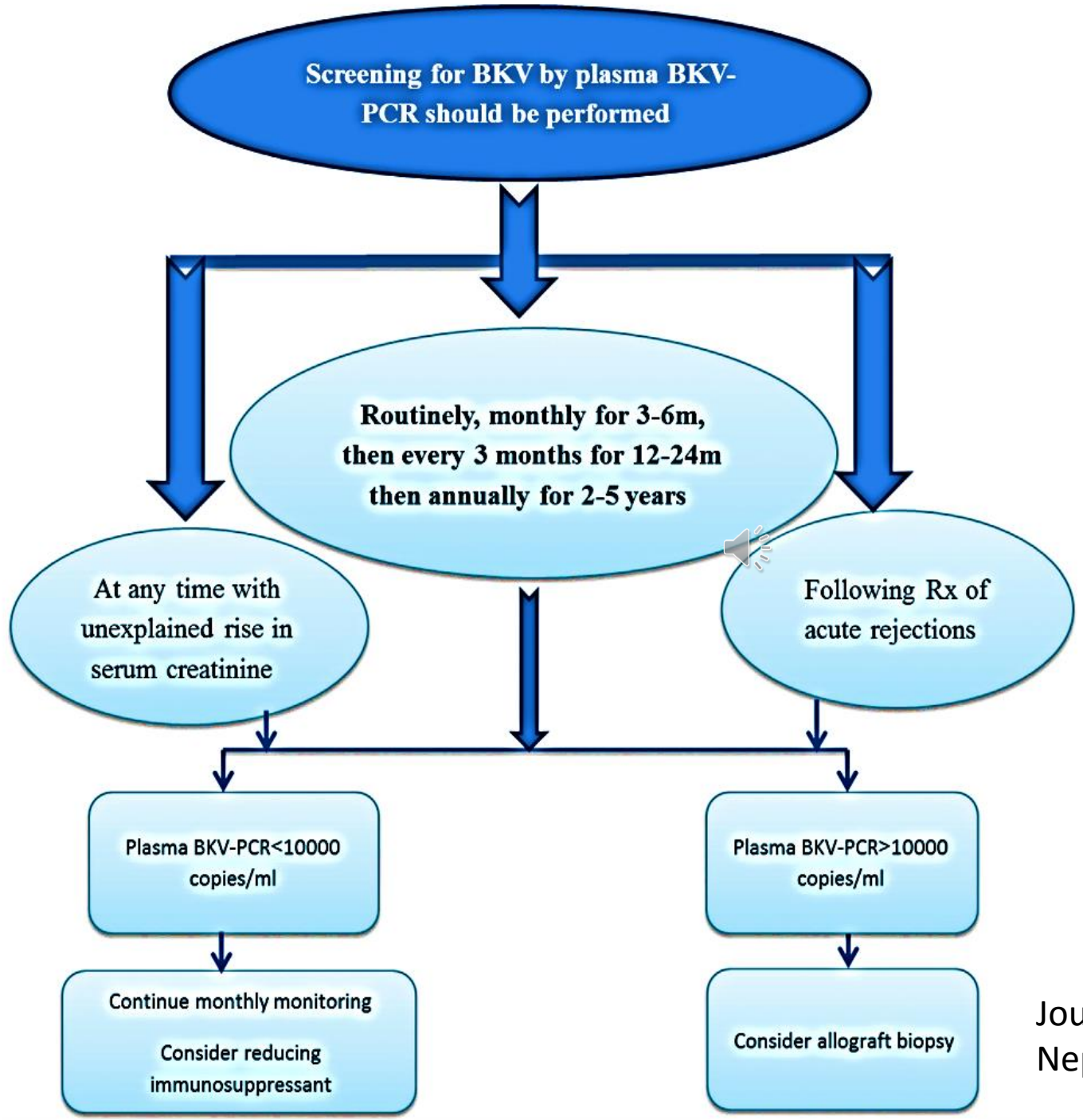
- The best technique to demonstrate resolution of BK activity following reduction of immunosuppression.
- Significant, sustained Viremia (>5000 BKV copies/mL for 3 consecutive weeks) characterizes patients with high, uncontrolled viral replication, leading to parenchymal injury.
- It is required to follow-up patients who lost their allograft because of BKVN and considered for Re-transplantation

KDIGO guidelines for kidney transplant recipients :

Screening all recipients of BKV using quantitative plasma PCR at least ***monthly*** for the first 3 to 6 months after Tx. then ***every 3 months*** until the first year, and subsequently whenever there is an unexplained rise in serum creatinine and after treatment for acute rejection.

AST guidelines for kidney transplant recipients:

Recommend quantitative PCR ***monthly*** up to month 9 post-transplant followed by testing ***every 3 months*** up to 2 years or at the time of surveillance and indication of an allograft biopsy.



Journal of The Egyptian Society of Nephrology and Transplantation 20:127–150

False negative of PCR?

Most quantitative PCR primers designed against BK genotype I (Dunlop) strain as a reference. Using this genotype I strain as a reference might be as much as four-fold less sensitive for different strains (limit to detect 10/000copies/µl for the other strains compared with 10 copies/µl for genotype I), which is risky as uncommon BKV subtypes are often associated with BKVN, and such assays are unable to detect them at low viral levels

Plasma Screening tests for BK polyomavirus-associated nephropathy

Method	Sensitivity* (%)	Specificity* (%)	PPV*	NPV*	Advantage	Disadvantage
Plasma quantitative PCR¶ (preferred)	100	88Δ	Moderate	High	▪ High PPV for BKPyVAN if VL ≥10,000/mL plasma ▪ Ability to monitor response to therapy (ie, reduction in immunosuppression)	▪ Relatively expensive ▪ Nonstandardized significant variability among assays ▪ Rare reports of biopsy-confirmed BKPyVAN without concomitant viremia/DNAemia

¶ Plasma qualitative PCR is also available but has limited clinical value and should generally not be used.

Δ Specificity increases to 90% if viral load is greater than 10,000 copies/mL of blood with >3 weeks.

Indication of Renal biopsy in the BKVN

- If the cause for renal allograft dysfunction is uncertain.
- If kidney dysfunction and/or viremia fail to resolve despite reducing immunosuppression.

When IS. should be reduced?

Stepwise reduction of immunosuppressive medications is recommended when BKV plasma PCR is persistently (for 3 weeks and longer) greater than 1000 copies per milliliter (mL)

SUMMARY-1

- BKV replication typically progress through three stages.
  - Asymptomatic viruria occurs in 1/4 to 1/3 of patients during the first posttransplant year.
  - Viremia follows viruria in approximately one-half of patients.
  - In a subset of viremic patients, viral replication progresses leading to damage to renal tubular epithelium and BKVN.
- Like viruria and viremia, BKVN is typically asymptomatic, and a rise in serum creatinine may be the sole presenting sign.

SUMMARY-2

- presumptive diagnosis is often made based upon the presence of significant viremia (plasma BKV load ≥10,000 copies/mL).
- For patients with viremia>1000 copies/mL and normal allograft function, we typically reduce immunosuppression and monitor the viral load every two to four weeks thereafter to ensure that it is down trending.
- Renal allograft biopsy is the gold standard for diagnosing BKVN, assessing its severity, and evaluating for concomitant processes.

SUMMARY-3

- Without control of viral replication, allograft loss can ensue within a period of months.

- Acute rejection should be suspected in patients with BKVN whose serum creatinine levels increase after immunosuppression has been decreased.

RECOMMENDATION

We recommend routine screening for BKV for all kidney transplant recipients in the early posttransplant period by plasma PCR monthly for the first six months following transplant, then every three months until 2 years and then annually for five years.